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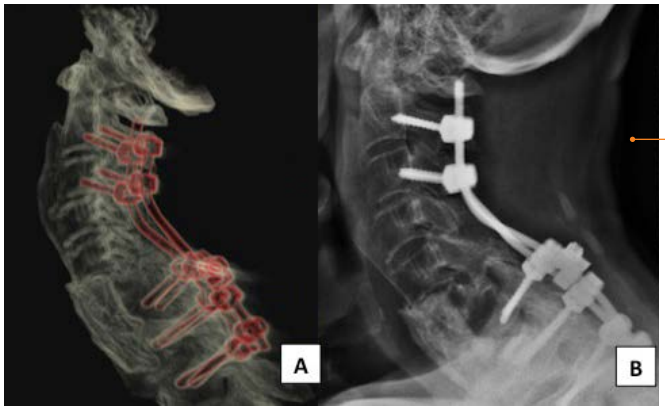
ARTICLES



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SUMMARY

SECTIONS

ARTICLES

ORIGINAL ARTICLES

The important role of academic leagues (extensions) in Brazilian medical education

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With a centennial history of success, academic extensions in Brazilian medical education stand for a major aspect of undergraduate students' knowledge. Their significant role in our medical schools and in our society started in 1920 with the *"Liga de Combate à Sífilis"* (in English, League for the fight against Syphilis) [1] of the University of São Paulo Medical School. It established a cutting-edge change: from that moment, students could get together in groups under medical supervision, to offer assistance to those in need. It truly has altered paradigms in Brazilian health care.

A Medical Academic League or Extension (MAL) is a group of mainly undergraduate medical students that aim to study a subject according to the core values of the university [2]. Those students, with a common interest, can get together, invite a supervising physician, and develop essential skills based on learning, assistance, and research. Essentially, a MAL is an opportunity for students to be closer to their communities, being part of health promotion and social change processes, leading them to be better doctors and notably better citizens [3].

There are some guiding principles for a MAL to help students improve their skills. As a matter of fact, without a basic set of principles, a group of students cannot even be called a league. Health education, advocacy, communication strategies, decision-making, and leadership are crucial features for a MAL to help build a highly-skilled physician integrated into a multi-professional team, which is precisely one of the things that can enhance the quality of health assistance in our country [4]. More importantly, being part of a MAL is not a mandatory activity. It shows self-motivation from the students to pursue a better education.

The European Academy of Teachers in General Practice and Family Medicine (EURACT) has listed seven essential roles for the medical professional [5], and they all can be developed and exercised through the participation in a MAL: medical expert [6] (evidence-based medicine discussions while offering assistance to the population); health advocate (being closer to the surrounding community can help understand their problems); manager [7] (leadership skills are necessary for managing students who organize MAL activities); communicator (MAL helps students to communicate better with patients, peer-to-peer communication and reporting their status to their supervisors); collaborator [8] (working in a mul-

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tidisciplinary team); scholar (research and medical education are essential characteristics of a MAL); professional (exercising ethical assistance to the population).

MALs have a history of almost a hundred years of success in building better doctors and, in the end, making a better society since these health professionals can offer improved assistance for our population. This country has serious issues when it comes to health services, especially in the public system. Nevertheless, MALs should be considered an innovative way to offer education for students and well-structured health care for the population in need. Therefore, medical schools should support this kind of initiative fomenting this path of citizenship access in Brasil.

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Laparoscopic renal biopsy

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The Guidelines Project, an initiative of the Brazilian Medical Association, aims to combine information from the medical field in order to standardize producers to assist the reasoning and decision-making of doctors.
The information provided through this project must be assessed and criticized by the physician responsible for the conduct that will be adopted, depending on the conditions and the clinical status of each patient.

INTRODUCTION

The histological evaluation of the renal tissue is particularly useful for investigating and treating several types of kidney pathologies. The histopathological diagnosis provides crucial information for determining the prognosis and the ideal treatment. Amongst the general indications for kidney biopsy are kidney diseases of unknown etiology, nephrotic syndrome, proteinuria and systemic diseases with suspicion of compromised kidney function, such as systemic lupus erythematosu[1,3]. Although there several ways of obtaining samples of the renal tissue, the percutaneous renal biopsy (PRB) is the most widely used, since it is reliable, minimally invasive, and can be performed under local anesthesia. However, some conditions represent a contraindication for PRB, such as bleeding diathesis, morbid obesity, solitary kidney, polycystic kidney, multiple kidney cysts, uncontrolled hypertension, and previous failed attempts of PRB[4-6]. Under those circumstances, kidney biopsy can be performed using alternative methods, such as guided by computed tomography (CT)[7], with a laparoscopic approach (transperitoneal or retroperitoneal)[4], and transjugulars[8]. The laparoscopic renal biopsy (LRB) provides a minimally invasive approach to open biopsy, providing a direct view of the kidney.

TECHNICAL ASPECTS

Retroperitoneal operative technique

Surgery is performed traditionally under general anesthesia. Before beginning, the orogastric and vesical tubes are inserted. The patient is positioned on the right or left lateral decubitus, with the contralateral lower limb flexed and the ipsilateral extended. The upper body and limbs are immo-

bilized with tape over the thoracic region, hip, and lower limb in order to prevent movement during the procedure. The operating table can be bent, or a small cushion can be placed under the subcostal area to improve the exposure of the kidney during the procedure. A 1.5 cm incision is made, inferior and distally to the 12th rib, carefully dissecting the tissues until reaching the retroperitoneum. Through that opening, the blunt digital dissection of the retroperitoneal fat to facilitate the introduction of the expander balloon, which enlarges the dissection area while achieves the hemostasis of the retroperitoneal space. A 10 mm trocar is inserted through the incision and, once the optical system is coupled, the insufflation of CO2 begins until reaching a 12-15 mmHg pressure. This procedure can be simplified by using a optical exchanger (consisting of a optical shutter with a transparent window and blunt in the distal end), which, assisted by a 0 or 30-degree lens, enters the retroperitoneum under direct view. Then, the retroperitoneal space is dissected and, with the optics, the dissection area can be enlarged towards the lower pole of the kidney. Once there is enough room under direct view, a second trocar, now of 5 mm, is positioned to assist the procedure. The kidney dissection begins by exposing the lower pole using a Maryland instrument and scissors. After the biopsy site is chosen, with the help of laparoscopic biopsy forceps, the cold removal of a sample of about 0.5 cm is conducted and sent to the pathologist in saline solution. In case of doubt, the freezing analysis is used to confirm if the sample is representative. After the biopsy, the hemostasis is conducted by putting pressure on the site with gauze or bag for a few minutes. If needed, an electric or argon scalpel can be used to cauterize the incision, or the area can be covered with a homeostatic material (gelatin sponge or oxidized cellulose) or stitched using homeostatic absorbable thread. When using an argon scalpel, it is important to open the CO2 exit portal, since the flow of argon gas can increase abdominal pressure. If necessary, a third trocar can be placed to assist with the procedure. After 5 minutes, the retroperitoneum pressure is reduced to 5 mmHg to verify the effectiveness of the hemostasis. If no active bleeding is observed, the incisions are sutured, and there is no need for drainage. Finally, the vesical and orogastric tubes are removed.

Transperitoneal operative technique

As with the retroperitoneal technique, all positioning and pre-op preparations are duly followed. After positioning the patient on lateral decubitus at 45 degrees on a table that provides lateralization, the table is positioned so that the patient is on horizontal decubitus. A small incision is made on the periumbilical region and, with the help of a Veress needle, the pneumoperitoneum is obtained. After reaching a pressure of 15 cmH2O, a 10 mm trocar is introduced to house the camera. Then, the peritoneal cavity is analyzed. Next, the table is repositioned to the usual position, and two other 5 mm trocars are introduced under direct view: one at the iliac fossa area and the other at the mid-1/3 between the xiphoid appendix and the umbilical scar. Using dissection tweezers, scissors, and an electric laparoscopy scalpel, a small dissection of the retroperitoneum is conducted, exposing the lower pole of the kidney. Then, a sample of the kidney tissue is obtained using laparoscopy tweezers or a puncture needle under direct view. After the procedure, all measures previously described concerning homeostasis and to suture are meticulously observed.

When choosing an access route, trans or retroperitoneal, for a kidney biopsy, some aspects need to be taken into account, such as the preference of the surgeon, difficulty in obtaining surgical access (particularly relevant in patients with previous surgeries) and the need for concomitant evaluation of other intraperitoneal organs. Each surgical access has its advantages. As presented, when using retroperitoneal access there is no violation of the cavity and no need for mobilization of the bowels. The transperitoneal access, on the other hand, provides a larger surgical site, and the existence of references makes the procedure easier. Whatever the route is chosen, it is important to observe all the precautions to minimize the risks of the procedure, specially concerning kidney bleeding, muscle bleeding, surgical site infections, hematuria, inadvertent lesions of the intestines and other organs, and other complications, such as the absence of kidney tissue (in case of doubt, conduct a freezing biopsy). After the surgery, most patients are released from hospital within 24 hours. Patients who require oral, subcutaneous anticoagulants can receive it 24-48 hours after the procedure and, in case of intravenous therapy with heparin, the patient must be carefully observed for the possibility of bleeding at the biopsy site. Patients

with a persistent of significant drop in hematocrit and signs of hypovolemia during the postoperative period must be evaluated using computed tomography to measure the bleeding.

METHOD

Amongst the general indications for kidney biopsy are kidney diseases of unknown etiology, nephrotic syndrome, proteinuria and systemic diseases with suspicion of compromised kidney function, such as systemic lupus erythematosus. The purpose of this guideline is to gather medical information that can assist in decision making for patients to whom the only option to obtain a viable kidney tissue sample is under direct view. It was conducted from a systematic review of the literature with no time restriction on the Medline database using the following descriptors: P: Patients to whom the only option to obtain a viable kidney tissue sample is under direct view. Laparoscopic renal biopsy (transperitoneal or retroperitoneal), C: Kidney biopsy through open access and O: Benefit or damage. The search strategy was structured as follows: (Kidney OR Kidney Diseases) AND (Biopsy OR renal biopsy OR kidney biopsy) AND (Laparoscopy OR Laparoscopic-assisted renal biopsy OR Endoscopy OR Retroperitoneal Space OR retroperitoneal laparoscopic renal biopsy) AND (Diagnosis/Broad[filter]), retrieving 1,770 papers, out of which nine were selected to answer the clinical question: Is laparoscopic renal biopsy effective and safe? The recommendations were designed by the review authors with the initial characteristic of synthesis of evidence and were submitted to validation by all authors who participated in the creation of the guidelines. The grades for recommendation used comes directly from the power available in the studies included according to Oxford[16] and the Grade[18] system.

RESULTS

We found no comparative studies and selected 13 series of cases; in three studies[9-11](C), the access used was transperitoneal and in six, retroperitoneal[4,5,12-15](C).

Transperitoneal laparoscopic renal biopsy

In a series of cases with children (average age 8 years, [1 year and 10 months to 13 years and 7 months] with bleeding disorders or previous percutaneous biopsy failure, they were submitted to percutaneous renal biopsy assisted by transperitoneal laparoscopy (three portals). Hemostasis was performed through simple compression with gauze. The average surgery time was 35 minutes and hospitalization time was 33.5 hours. There were no intraoperative complications, and the material was considered sufficient for diagnosis in all cases by the pathologist. The ultrasonography showed no evidence of kidney bleeding or bruising[9](C).

In 21 patients (14 males, 7 females, average age 58 years [21-83 years]) the method of choice for kidney biopsy was the transperitoneal technique assisted by three portals and simple compression with gauze (5-20 min. interval). The average bleeding volume was 5.5 ml and the surgery time was 65-120 minutes. The sample was adequate in all patients, who were able to feed and ambulate on the following day.

There were no postoperative hemorrhagic complications, and a hernia at the site of the trocar was reported three months after the surgery on a patient with multiple abdominal surgeries[10](C).

In another series of cases, the transperitoneal laparoscopic technique was used to conduct diagnosis biopsies on two patients with morbid obesity (BMI 51.6 and 35 kg/m²) and one with obese (BMI 31 kg/m2), all with a solitary kidney. All biopsy samples were adequate, and the patients were discharged from hospital after 1.3 days on average with no significant complications[11](C).

Retroperitoneal laparoscopic renal biopsy

A series included 40 patients with coagulopathy (30%), polycystic kidney, multiple renal cysts (30%), solitary kidney (12.5%), and morbid obesity (10%), who underwent retroperitoneal laparoscopic renal biopsy (LRB). All the biopsies were conducted using a Trucut needle. The average intervention time was less than 1 hour, and the sample for histopathological examination was considered adequate in all cases. There was a low rate of surgical complications (7.5%) according to the Clavien classification (little bleeding at the portal site [two cases] and post-biopsy kidney hemorrhage requiring embolization [one case])[12](C).

In a series of cases, LRB was conducted on 17 patients with an average age of 8.1 years (2-12 years) using two or three trocars. The LRB was successfully performed on 15 patients (88%), and om two cas-

es there was a rupture of the peritoneum requiring conversion (one to open and the other to transperitoneal). There was one case of perirenal hematoma that was spontaneously resolved. The complication rate was 17.6% (3/17 cases). The average surgery time was 65 minutes, and the average estimated blood loss was 52 ml, and the average hospital stay was 2,2 days[13]**(C)**.

The authors performed 53 kidney biopsies using a retroperitoneoscopic approach; 28 patients were males and 25 females, aged between 13 months and 19 years (average of 4 years old). The biopsies were requested after the following diagnosis: nephrotic syndrome, hemolytic-uremic syndrome, hematuria, idiopathic purpura, proteinuria[14]**(C)**. Ten patients had kidney failure, and all biopsy samples were adequate. There was conversion to open surgery on one case due to kidney bleeding; in 51 cases, the estimated blood loss was lower than 20 cc. The average hospitalization time was 48 hours for the first 20 cases and between 24-36 for the others[14]**(C)**.

In a series of cases, the authors reported the results of laparoscopic kidney biopsies using retroperitoneal access performed on a single center with a total of 32 patients[4]**(C)**.

Later, there was a new publication with a slight change in protocol and the addition of 42 new LRBs to this series. The operative technique remained the same, but the conventional measures were evaluated to confirm the kidney tissue and ultrasonography was used during surgery in difficult cases. Sixty-four patients (29 males and 45 females, average age 45 years [3-79]) underwent LRB with the two-portal technique, due to several indications: morbid obesity, solitary kidney, coagulopathy, failed percutaneous biopsy, kidney at a high position, and low visibility with ultrasound. The average surgery time was 123 (9-261) minutes, and the bleeding was 67 (5-2000) ml; the samples obtained were considered adequate for 96% of the patients. A total of 58% of the patients were discharged from hospital in less than 24 hours; as complications (13.5% of patients) there was significant bleeding on three patients (two during and one after surgery) and a seromuscular colon lesion[5]**(C)**.

Using a retroperitoneal approach, the authors performed laparoscopic kidney biopsies with two portals in 20 patients aged between 2-18 years (average of 9.7 years) in whom the percutaneous needle approach was not possible due to uncontrolled hypertension, bleeding disorders, anticoagulant us, and anatomical alterations. The biopsy site was cauterized using bipolar cautery. The biopsy was performed successfully in all cases, except in one, which was converted to an open procedure. The average surgery time was 40 minutes, blood loss was minimal, and average hospital stay was 1.2 days in the postoperative period, with a return to everyday activities after 3-5 days. In one case there was a peritoneal rupture without post-operative consequences[15]**(C)**.

Recommendation

- Laparoscopic renal biopsy (transperitoneal or retroperitoneal) can be the first option for cases in which there is a contraindication for kidney biopsy with a percutaneous needle. **(C)**
- The kidney sample from the laparoscopic biopsy is adequate for the histopathological diagnosis at a rate that varies from 96% to 100%. **(C)**

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POINT OF VIEW

Observations on multi-generational interactions in academic surgical practice and education

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SUMMARY

Although there is a natural passage of responsibilities and duties from one generation to the next in any organization, academic or otherwise, managing intergenerational differences is challenging. In academic surgery, in which there are duties to patients, institutional administrators, faculty colleagues, resident trainees, and medical students are faced with multi-generations of individuals who have their own perspectives of what is "required" of them and what is "fulfilling" to them. The purpose of this essay is to relate our observations of the challenges and opportunities to manage these relationships from the perspective of North and South America in all levels involved with surgical care and teaching.

KEYWORDS: *Education, medical. Intergenerational relations. Teaching. General Surgery.*

INTRODUCTION

Although there is a natural passage of responsibilities and duties from one generation to the next in any organization, academic or otherwise, managing intergenerational differences is challenging. In academic surgery, in which there are duties to patients, institutional administrators, faculty colleagues, resident trainees, and medical students are faced with multi-generations of individuals who have their own perspectives of what is "required" of them and what is "fulfilling" to them. The purpose of this essay is to relate our observations of the challenges and opportunities to manage these relationships from the perspective of North America (the United States) and South America (Brasil). Moreover, it does not intend to offend or criticize anyone or any generation but to show how the mores of the world in 2017 are influencing surgical practice in all levels and how doing the possible before the impossible may help to create a better medicine.

THE GENERATIONS

The division of individuals into "generations" is artificial, and some individuals may not fit the stereotypical characterization of the group, but there are some qualities common to most members[1,2].

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The Western world (America, Europe, Oceania) has more or less characteristic generations, as described below. Some local variations may occur due to local conditions, such as the generations that came after a country becomes independent like what occurred in Armenia and Poland. There are no good studies on whether African and Asian societies experience similar trends and patterns in generations.

Traditional (Veterans, the Silent Generation, and the Greatest Generation). This is the group born before 1946 and has endured a great depression and a world war. This group respects loyalty and authority. They value stability and are reluctant to question the "system."[3]

Baby Boomers. This is the group born approximately between 1946 to 1964. In the United States, this group lived through the cold war and Vietnam War and in Brazil a dictatorship government. They are driven, self-centered, and "live to work." However, they tend to be judgmental of views different than their own[4].

Generation X. This is the group born between 1964 and 1980. This group saw the AIDS crisis, women's rights, gay rights, single-parent homes, and dissemination of the personal computer. They are technologically literate, adaptable, self-reliant, but "work to live." They are not intimidated by authority and can be cynical[5].

Millennials (Generation Y). This is the group born between 1980 and 1996. In both the United States and Brazil, this group grew up with the Internet. They are used to having technology as part of their daily lives. Being the children of Baby Boomers, many have been raised with extensive parental involvement. They want to make a difference in the world and expect to have meaningful, fulfilling work. They may be impatient with "climbing the ladder" and think that they have much to teach older generations[6].

Generation 2020 (Z). This is the group born after 1996. In both the United States and Brazil, powerful hand-held devices with multiple ways to communicate, send and receive information are viewed as mere extensions of themselves. They are now just entering the workforce or higher education. What they will be like is yet to be determined[7].

Needless to say, these characterizations are woefully simplistic. Nevertheless, this is a good foundation to discuss our observations.

MEDICAL STUDENTS

Although there are a few "older" medical students from Generation X, most are now Millennials. Dr. Robin McLeod in her presidential address to the Society for Surgery of the Alimentary Tract (SSAT) in 2014 recommended that the profession of surgery should adapt to the medical students and resident trainees from generation Y ("Millennials")[8]. A generation gathers its characteristics from the way the previous generation raised their descendants and from the environment and social status quo. Generation Y was born in a highly technological society and "has also been nurtured, pampered, and programmed by their helicopter parents who made sure they attended the best schools and were given the best opportunities. They have been told they are special and they can achieve anything. They have been rewarded not only for excellence but also for participation. Moreover, they have come to believe that they should be rewarded for what they do"[8]. The Millennials are characterized by "much idealization and few realizations," "I have rights, and my right is your duty" and an introspective (even selfish) behavior. Although this generation occasionally or frequently clashes with the expectations of Baby Boomers, one must not forget that the previous generation created Generation Y, created the environment for Generation Y and role-modeled their lifestyle.

Moreover, if we consider civilization is improving along time, generations are getting better. Millennials are better than previous generations in a series of characteristics and they have to deal with the changes in pressures--economic, regulatory, social and personal-- that this generation faces compared to prior generations. However, it may be not better in ALL aspects. Perhaps medicine, as we have come to know it, may not ideally absorb this new culture.

Medicine is different from many other professions. There are child prodigies in different arts and sciences but not in medicine (especially surgery). It is not a natural gift, not self-taught, and it demands experience, physical presence, hard work and, sometimes bitter, self-reflection. This generation's high level of confidence without experience, the need for immediacy and belief that everything is accessible online may be detrimental to surgery.

Millennial medical students have different expectations and attitudes in regards to learning, their chosen profession and their future at work and at home. The proportion of female students has increased.

Women have been the majority of medical students since 2009 in Brazil[9], while in the United States, 47% are women[10].

Medical students show a lack of interest in face-to-face learning. It is not uncommon to have nearly empty lecture halls with students preferring to attend "remotely." There is the occasional air of disregard for professors and the profession since they believe one can upload knowledge to the mind and surgical skills to the hands by simply connecting to the computer. Students are frequently late, physically absent or absentminded in theoretical classes. Attention is usually caught only by realistic simulators or professors who treat them more as equals than apprentices.

lLeft-Wing ideology is common because helicopter parents fully supported this generation, why should not the University and the government continue supporting them? In Brazil, protests and strikes are frequent now. Not only fighting for local conditions but also for national medical policies.

A lack of interest in the future is common since a high level of confidence in thriving is omnipresent. Social media contributes to this concept since all posts of peers show achievements, never failures. This led to 20% of Brazilian medical students not applying for residency immediately after medical school in order to give themselves "a break" and have an opportunity to "pursue happiness."

SURGICAL RESIDENTS

Surgical residents (most of the generation Y individuals with fewer and fewer from Generation X) are similar to medical students. Women represented 47% of the applicant pool for surgical residency in the US in 2017[11]. There is a definite shift in priorities for both men and women.

We evaluated the attitudes, experiences during training and professional expectations of surgical residents in 2011[12] based on the adaptation of a questionnaire[13]. The main findings were a high satisfaction with the specialty, but sizeable financial concern and conflicting opinions about the future. After 5 years we surveyed new residents again. Interestingly, the findings now pointed out that only 65% of the residents are satisfied working with patients and they present lower job satisfaction and more criticism of teaching techniques compared to the initial study.

In Brazil, a surgical residency program encompasses 2 years of rotation in various surgical specialties, which is a requirement for the following years in specific specialties[14]. Similarly to medical students, 20% of residents did not apply to the continuation of residency for surgical specialties immediate after finishing the 2 years rotation in order to dedicate themselves to non-medical activities for a while. A newspaper article, somewhat curiously, called this behavior as "the generation that found success after being fired"[15]. It told anecdotal cases of young graduates from prominent Universities that were fired or quit their resident training jobs due to the economic crises and radically changed their lifestyle to live a simple life.

FACULTY

Faculty includes mostly Traditionals at the most senior level (although these are becoming fewer and fewer), Baby Boomers at the mid to senior level and now more and more Generation X'ers at the junior and mid-levels. This "mixing" of generations can lead to conflict as, on the whole, each generation has a different expectation as to what they put into work and what they get from work.

Research faculty is very influenced by the technological advances of the world in 2017. Technology made distances shorter and communication easier. The world is interconnected and immediate. A prospective study with an extended follow-up may not be appealing. This is seen by a decrease in the number of prospective studies in medical meetings and journals. Making multi-generational research groups work in an era where the junior faculty is fluent in forms of communication which are not as familiar to the senior faculty is increasingly challenging.

As funding is becoming harder in both North and South America, the natural tendencies of each generation may be brought into more sharp relief. Traditionals and Baby Boomers will not necessarily question the system that has been the basis of their careers, but instead, focus resources towards more popular subjects. Gen-Xer's with their natural disregard for authority, cynicism and less internalization of work as part of their identity, may adapt, but will not feel good about it. Millennials want mentors to guide them through the process but feel that success should always come. In an environment where research is believed to be associated with a significant

reduction in clinical work and clinical reimbursement[16] although it is possible to excel in both[17]. It is becoming easier to work with non-physicians[18].

In addition to the actual work that is performed, the workplace cultural norms and mores are changing. Professors are now guided by a loss of spontaneity to avoid misinterpretations of speech which may be considered offensive. As Millennials consume information through their devices in a non-traditional way, older faculty who are used to the didactic form of teaching view members of the audience focused on their devices rather than the lecture are disappointed by what appears to be a lack of interest. Unfortunately, financial interests are now surpassing the priesthood of teaching. A "restraint of trade" (I will not teach everything I know to my future competitors) is becoming notorious among some educators.

PATIENTS

Patients, of course, come from all generations. The generational difference affects how physicians communicate with their patients. Because of cultural sensitivities, physicians are facing a loss of spontaneity with Gen-X, Millennials and Gen 2020 patients. Similar to what happens in teaching, words, in the context of Traditionals or Baby Boomers speaking to one another, may be misunderstood as offensive by younger generations. We have experienced patients wishing to record the consultation for their own records. Also, there are anecdotal reports of consultations being recorded secretly. Awareness of being recorded changes physician's behavior resulting in defensive practice whereby doctors order more investigations, write more prescriptions or refer for more specialist opinions[19]. An additional concern is with the ability to readily upload video or voice data to the internet, such recordings of privileged exchanges on social media may cause distress, upset or embarrassment to one or both parties[19].

Technology also allows patients to search for information online before a consultation. Although access to high-quality information will lead to a better-educated patient, we have also had the experience of patients consuming unscientific, unreviewed and even fake data, thereby making informed consent all but impossible. With Generation-Xer's and Millenial's tendency to question authority, such access to dubious information makes the job of counseling the patient all the more difficult.

Medical technology also has two sides. On the one hand, technology allows for new or less aggressive procedures; on the other, some physicians can be enslaved by technology. Part of the Brazilian Engineers oath could be added to our medical vow since Hippocrates did not anticipate this: "I swear that during my duty as Engineer I will not allow me to be blinded by the bright light of technology, as I should not forget that I work for the benefit of the mankind not for the benefit of the machine."

With respect to medical care, having patients take ownership of their health can be challenging. We have encountered patients having the attitude of "my health is YOUR problem, not mine." Attending surgeons have to deal with wishes and expectations of patients and their good will to comply with the proposed treatment or not.

We have seen different phases of medical practice over our careers:

Physician medicine: physicians did whatever they wanted without much involvement of patients. This was the period where patients were from the Traditional and Baby Boomer generations. These generations tended to have more respect for authority and physicians were considered authority figures.

Legal medicine: an increasing number of patients, mostly from the Gen-X with their suspicion of authority, participated in their own care decisions, but with the caveat that an adverse event was due to some physician error. This lead to more "defensive" practices by physicians to inoculate themselves from potential legal action. This occasionally evolved to a pleasing medicine when physicians allowed patients to choose how to manage their care and avoided conducts to limit the quality of life even if care was compromised.

At present, we face a drive-thru medicine: (a) there is little concern about punctuality. Patients want to be seen as soon as possible even if they are late or early; (b) a personal relationship with the service provider is not pursued; (c) consultation should be as brief as possible due to a busy agenda; (d) the customer chooses the product, not the provider. Patients search for information online and come looking for a specific procedure or medication. Medical care is summarized by an exchange of money for a product but instead of fast food, it is medical care.

HOW SHOULD WE, AS EDUCATORS, RESPOND?

Millennials - who comprise current medical students and residents and the near-future workforce – have different expectations and attitudes from previous generations. Educators must learn how to communicate and understand them. Personal beliefs and opinions, conduct in front of patients, dress codes, attitude towards pets, etc. may be more important to create or destroy a teacher-pupil relationship than medical knowledge and educational skills. This relationship is necessary because they must be guided and mentored. This generation faces a delay in the rites of passage[8]. They must be taught that hierarchy is important in surgery. Not like in the past when residents had to bow before professors, but the hierarchy necessary to coordinate a surgical procedure in which multiple operators perform according to a determined grading. They must be taught that happiness can be found in the passion for operating and treating patients, as showed by Dr. Nathaniel Soper in his 2017 SSAT presidential address[20]. They must be taught that resilience is necessary to thrive, citing another SSAT president[21]. If the 10.000-hours rule[22] is valid for most professions, it is undoubtedly more valid for surgery. Finally, they may be shown that perhaps those who take the best of their generation but ignores the worse are successful individuals.

Previous generations are affected by the *status quo* too. Technology must be used beneficially. It means that those unfamiliar with it must catch up. It also means that it is a good communication channel with youngsters. Previous generations must extract the best from this moment but are also responsible for keeping ancient knowledge alive.

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PALAVRAS CHAVE: *Educação médica. Relação entre gerações. Ensino. Cirurgia geral.*

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The overexpression of lncRNA H19 as a diagnostic marker for coronary artery disease

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SUMMARY

OBJECTIVE: *Our study aimed to investigate the diagnostic value of lncRNA H19 for coronary artery disease (CAD) and to explore its possible mechanisms. Methods: A total of 30 CAD patients and 30 healthy individuals, as well as patients with different cardiovascular diseases, were included in this study. Blood was drawn from each participant to prepare serum samples, and the expression of lncRNA H19 was detected using qRT-PCR. The ROC curve analysis was used to analyze the diagnostic value of H19 for CAD. The effects of patients' basic information and lifestyle on H19 expression were analyzed. The plasma level of TGF-β1 was measured by ELISA. The H19 overexpression in the human primary coronary artery endothelial cell (HCAEC) line was constructed, and the effects of H19 overexpression on the TGF-β1 expression were analyzed using Western blot. The results of H19 expression were specifically upregulated in patients with CAD but not in healthy individuals and patients with other types of cardiovascular diseases. The ROC curve analysis showed that the H19 expression level could be used to predict CAD accurately. Gender, age, and patients' lifestyle had no significant effects on H19 expression, but H19 expression was higher in patients with a longer course of disease in comparison with the controls. H19 expression was positively correlated with the serum level of TGF-β1, and H19 overexpression significantly increased TGF-β1 protein level in HCAEC. Conclusion: H19 overexpression participates in the pathogenesis of CAD by increasing the expression level of TGF-β1, and H19 expression level may serve as a diagnostic marker for CAD.*

KEYWORDS: *Coronary artery disease. Diagnosis. RNA, Long Noncoding. Transforming Growth Factor beta1.*

INTRODUCTION

Coronary artery disease (CAD) is the leading type of heart disease, and it develops when major blood vessels responsible for supplying oxygen, blood, and nutrients to the heart become diseased or damaged after the formation of plaques [1]. Despite the high mortality of other severe diseases, such as malignant tumors, CAD is still considered to be a major cause of deaths worldwide [2]. In America, CAD causes one death every 1 minute and is responsible for one-third of all deaths among people older than 35 years [1]. The condition is even worse in China. A recent study has shown that more than 40 % of all deaths in China are caused directly by CAD or its complications [3]. Angioplasty and bypass surgery are the two main methods

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used in the treatment of CAD, but their effects are not lasting [4,5]. As a non-invasive method, drug treatment is still the most widely used treatment for CAD; however, drug tolerance can be developed during long-term treatment, and adverse side effects lead to unsatisfactory treatment outcomes [6]. Therefore early diagnosis and treatment are vital in improving treatment outcomes of CAD.

Serum biomarkers, which are relatively easy to be obtained, have been widely used in the diagnosis of various human diseases [7,8]. Long non-coding RNA (lncRNA) are RNAs without protein-coding ability. LncRNAs contain more than 200 nucleotides, which is significantly longer than miRNA, siRNA and other shorter RNAs [9]. Previous studies have shown that lncRNAs are involved in almost every aspect of key biological or even pathological processes in the human body [10], and abnormal expression of many lncRNAs in serum has been proved to predict a variety of human diseases accurately. A recent study has shown that lncRNA H19 is significantly correlated with the increased risk of CAD. However, its diagnostic value for CAD and mechanism are still unclear.

In this study, the expression of H19 in the serum of patients with CAD and other types of cardiovascular diseases was detected. The diagnostic value of H19 for CAD was explored, and the effect of H19 overexpression on TGF-β1 was also investigated.

METHODS

Patients

A total of 30 patients with CAD and 30 healthy individuals (control group) were selected and enrolled from January 2016 to January 2017 in the Renmin Hospital of the Wuhan University. CAD diagnosis was performed in strict accordance with the diagnostic criteria established by the American College of Cardiology/American Heart Association (AHA). Exclusion criteria: (1) patients with a malignant tumor or other major organ diseases; (2) patients with a serious infection over the 6 weeks before the beginning of this study; (3) patients with active chronic inflammatory disease; (4) patients with mental disease. A total of 30 patients were included (14 females and 16 males) and their age ranged from 25 to 76 years, with an average age of 46±10.7 years. The control group included 15 males and 15 females, and the age ranged from 24 to 71 years, with an average age of 47±9.6 years. There were 8 cases of hypertension, including 4 males and 4 females (22 to 78 years old, with an average age of 49±14.3 years); 9 cases of type I diabetes, including 4 males and 5 females (29 to 72 years old, with an average age of 46±10.1 years); 10 cases of type II diabetes mellitus, including 5 males and 5 females (31 to 77 years old, with an average age of 48±9.4 years); 7 cases of abnormal aoritic aneurysm, including 3 males and 4 females (24 to 79 years old, with an average age of 51±14.1 years.); 8 cases of valvular disease, including 4 males and 4 females (33 to 73 years old, with an average age of 51±12.4 years); 17 cases of dilated cardiomyopathy, including 8 males and 9 females (31 to 72 years old, with an average age of 45±17.1 years); 6 cases of viral myocarditis, including 3 males and 3female (31 to 77 years old, with an average age of 50±11.4 years); 10 cases of atrial fibrillation, including 6 males and 3 females (31 to 66 years old, with an average age of 48±13.2 years); and 12 cases of peripheral artery disease, including 7 males and 7 females (23 to 61 years old, with an average age of 43 ±13.1 years). There were no significant differences in the background information of patients with different diseases. The Ethics Committee of the Renmin Hospital of the Wuhan University approved this study. All patients signed informed consent.

Blood extraction and serum preparation

Whole blood (100 ml) was collected from each patient. Blood was kept at room temperature for 2 h, followed by centrifugation at 2500 rpm/min for 15 min and. The supernatant (serum) was then collected. Serum samples were maintained at 4 °C before usage.

Cell culture

Human primary coronary artery endothelial cells (HCAEC) were purchased from ATCC (ATCC® PCS-100-020™) and were cultured in strict accordance with the instructions provided by ATCC. Cells were harvested during the logarithmic growth phase for subsequent experiments.

ELISA to measure the serum level of TGF-β1

The ELISA-Quantikine kit (R&D Systems, Minneapolis, MN, USA) was used to measure the levels of TGF-β1 in serum in strict accordance with the manufacturer's instructions. The detection range for TGF-β1 was 100–1200 ng/L.

Establishment of lncRNA H19 overexpression cell lines

The HCAEC cell was cultured overnight to reach 80-90 % confluence. H19 cDNA was inserted into the GV299 lentiviral vector according to the methods described previously. After transfection, the cells were cultured overnight at 37 °C before collection.

Real-time quantitative reverse transcription PCR

Total RNA was extracted from the cells and serum using a Trizol reagent (Invitrogen, USA). The RNA samples were tested using a UV spectrophotometer, and only the ones with OD260/OD280 ratio between 1.8 and 2.2 were used in the reverse transcription with SuperScript IV Reverse Transcriptase (Thermo Fisher Scientific, USA) to synthesize cDNA. The PCR reaction system was prepared using SYBR® Green Real-Time PCR Master Mixes (Thermo Fisher Scientific, USA). The following primers were used: 5'- ATCGGTGCCTCAGCGTTCGG-3' (sense) and 5'- CTGTCCTCGCCGTCACACCG-3' (antisense) for lncRNA H19; 5'-CCCAGCATCTGCAAAGCTC-3' (sense) and 5'-GTCAATGTACAGCTGCCGCA-3' (antisense) for TGF-β1; 5'-GACCTCTATGCCAACACAGT-3' (sense) and 5'-AGTACTTGCGCTCAGGAGGA-3' (antisense) for β-actin. CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, USA) was used to carry out PCR reaction. Reaction conditions were: 95 °C for 30 s, followed by 40 cycles of 95 °C for 10 s and 65 °C for 30 s. The $2^{-\Delta\Delta CT}$ method was used to process Ct values, and the expression level of each gene was normalized to endogenous control β-actin.

Western-blot

Total protein was extracted from cells using cell lysis solutions (Thermo Fisher Scientific, USA) and then it was quantified by BCA assay. 20 μg of protein from each sample was subjected to 10 % SDS-PAGE electrophoresis, followed by transmembrane to PVDF membrane. After washing, the membranes were blocked with 5 % skimmed milk at room temperature for 1 h, followed by incubation with corresponding primary antibodies, including rabbit anti-TGF-β1 antibody (1: 1000, ab92486, Abcam) and rabbit anti-β-actin antibody (1: 1000, ab8226, Abcam) overnight at 4 °C. After washing, the anti-rabbit IgG-HRP secondary antibody (1: 1000, MBS435036, MyBioSource) was added and incubated with the membranes at room temperature for 4 h. After washing, signal detection was performed using ECL detection reagent (Sigma-Aldrich, USA). The expression level of TGF-β1 was normalized to endogenous control β-actin using image J software.

Statistical analysis

SPSS19.0 (SPSS Inc., USA) software was used. The normal distribution data were recorded (ˆx±s), and comparisons between the two groups were performed using the t-test. Comparisons among multiple groups were performed using ANOVA. Non-normal distribution data were analyzed using the non-parametric Mann-Whitney U test. Fisher's exact probability test or chi-square test was used to analyze the correlations between H19 expression and various clinical data. The correlation between the serum level of H19 and TGF-β1 was analyzed using Pearson correlation analysis. $P < 0.05$ was considered to be statistically significant.

RESULTS

The serum level of lncRNA H19 in patients with CAD

QRT-PCR was used to detect the expression of H19 in serum samples of 30 CAD patients and 30 healthy individuals. The results showed that the expression level of H19 was significantly higher in CAD patients than in the control group (Fig. 1A), which indicates that an increased expression level of H19 is very likely to be involved in the pathogenesis of CAD. The diagnostic value of H19 for CAD was analyzed using a ROC curve. As shown in Fig. 1B, the area under the ROC curve was 0.9367 ($p < 0.001$) with a 95 % confident interval of 0.8797 to 0.9936, suggesting that H19 is a promising biomarker for CAD.

Differential expression of lncRNA H19 in patients with different types of cardiovascular diseases

H19 has been proved to be involved in the pathogenesis of various cancers. However, the expression pattern of H19 in diabetes mellitus, as well as in different types of cardiovascular diseases, still has not been reported. Therefore, in this study, the expression level of H19 in the serum of patients with CAD, type 1 diabetes, type 2 diabetes and various cardio-

vascular diseases including hypertension, abnormal aortic aneurysm, valvular disease, dilated cardiomyopathy, viral myocarditis, atrial fibrillation, and peripheral artery disease was detected (Fig. 1C). Compared with control groups and patients with other types of cardiovascular diseases, the expression level of H19 was explicitly and significantly increased in the serum of patients with CAD ($p < 0.05$). There were no significant differences in the expression level of H19 between the control and patients with other diseases ($p > 0.05$). Using CAD, other heart diseases, gender, age and individual's living habits (smoking, drinking and vegetarian) as independent variables and serum H19 as a dependent variable, linear regression analyses were performed. The results showed that only CAD was a potential predictor [regression coefficient (B)=−0.299; p=0.000], and other variables provided a p-value higher than 0.05. These results suggest that the increased expression of GAS5 can potentially serve as a specific diagnostic biomarker for CAD.

Correlation between the expression level of H19 and basic information of CAD patients

Above data have shown that H19 may potentially serve as a diagnostic marker for CAD. However, lncRNA expression can be induced or regulated by patients' living habits, such as smoking and drinking, which in turn affect the diagnostic value. Therefore, the effects of the background and living habits on the expression of H19 in CAD patients were investigated. According to the median expression level of H19, 30 CAD patients were divided into a high expression group and a low expression group, with 15 patients in each. Gender, age, smoking, drinking, and vegetarian diet had no significant effects on H19 expression, while the expression level of H19 was significantly higher in patients with a duration of disease >5 years than in patients with a duration of disease <=5 years. These data suggest that an increased H19 expression level can accurately diagnose CAD for patients with different backgrounds and lifestyles, especially those with longer duration of disease.

Correlation between serum level of H19 and serum level of TGF-β1

An increased TGF-β1 level in the blood has been proved to be closely correlated with the development of CAD. Therefore, the serum level of TGF-β1 in both CAD patients and healthy individuals was detected using ELSIA. As shown in Fig. 2a, the serum level of TGF-β1 was significantly higher in CAD patients compared with healthy control individuals. Also, the Pearson correlation analysis showed that serum expression level of H19 was positively correlated with the serum level of TGF-β1 ($R=0.0119$, $p < 0.00001$). These results suggest that H19 expression is positively correlated with TGF-β1 expression.

H19 overexpression increased the expression level of TGF-β1 in HCAECs

Studies have shown that H19 is related to CAD. While its mechanism is still unknown, it is well known that H19 can interact with TGF-β1, and that TGF-β1 is related to the development of CAD. Therefore, the interactions between H19 and TGF-β1 were investigated through H19 overexpression. As shown in Fig. 3a, compared with control cells and negative control cells (empty virus vector transfection), the expression of H19 was significantly increased after the transformation with H19 expressing vector, indicating the successfully established H19 overexpression cells line. After H19 overexpression, the expression level of TGF-β1 was significantly increased at both mRNA level (Fig. 3b) and protein level (Fig. 3c). These data suggest that H19 can promote the expression of TGF-β1 to participate in CAD.

DISCUSSION

CAD is responsible for more than one-third of all deaths worldwide [1, 3]. With the development of modern society and the changes in people's diet structure as well as lifestyle, the incidence of obesity, which is an independent risk factor for CAD, has increased dramatically, leading to a significant increase in the incident of CAD. So far, CAD treatment is challenged by poor outcomes as well as adverse effects. Therefore, early diagnosis and treatment may be a promising way to improve treatment outcomes. The development of diseases, such as malignant tumors and inflammatory diseases, is usually accompanied by changes in serum levels of certain substances. Therefore, serum biomarkers, which are relatively easy to be obtained, have been widely used in the diagnosis of various human diseases. In a recent study, Zhang et al. reported that serum omentin-1 levels were significantly reduced

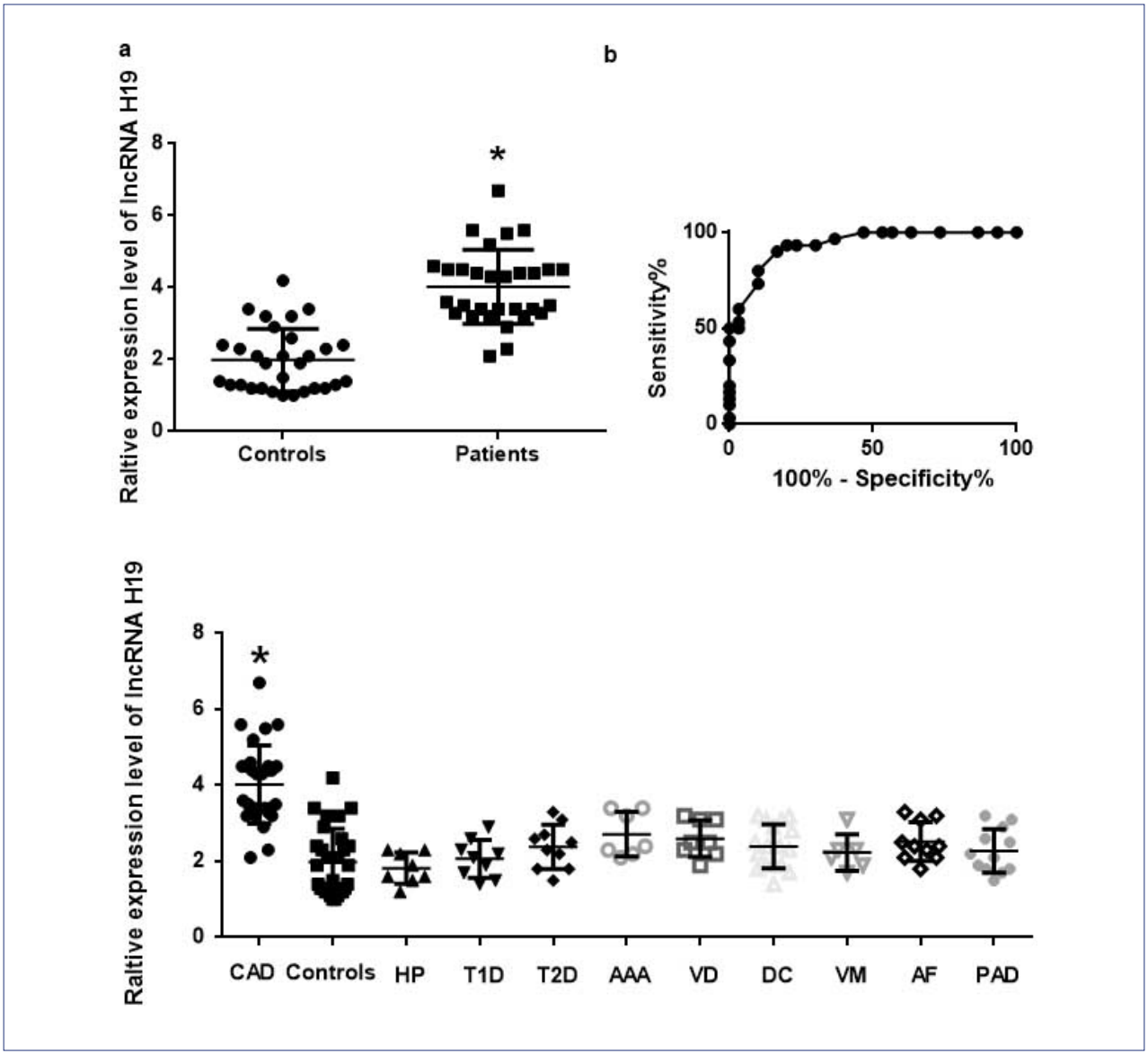

FIGURE 1 LNCRNA H19 EXPRESSION IN PATIENTS WITH CAD AND THE DIAGNOSTIC VALUE.

A Relative expression of lncRNA H19 in 30 patients with CAD; B R Diagnostic value of lncRNA GAS5 for CAD analyzed using ROC curve analysis; (Notes: *compared with the control group, p<0.05.)

C Differential expression of lncRNA H19 in patients with different types of cardiovascular diseases (Notes: *compared with the control group or patients with other types of disease; HP, hypertension, T1D, type 1 diabetes; T2D, type 2 diabetes; AAA, abnormal aortic aneurysm; VD, valvular disease; DC dilated cardiomyopathy; VM, viral myocarditis; AF, atrial fibrillation; PAD peripheral artery disease.)

in patients with acute coronary syndrome and stable angina pectoris, which were two subtypes of CAD, indicating that low serum omentin-1 level may serve as an indicator for CAD. In another study, an increased serum level of visfatin and decreased serum level of vaspin were detected in asymptomatic patients with CAD, and the reduced serum levels of vaspin were found to be correlated with the severity of CAD.

LncRNAs have been proved to be involved in almost every aspect of all key biological and pathological processes in the human body [10]. Previous studies have shown that the development of certain human diseases is usually accompanied by changes of some lncRNAs in serum. Therefore, the application of serum lncRNAs in the diagnosis of human diseases has become increasingly popular. However, the application of lncRNAs as a biomarker in the

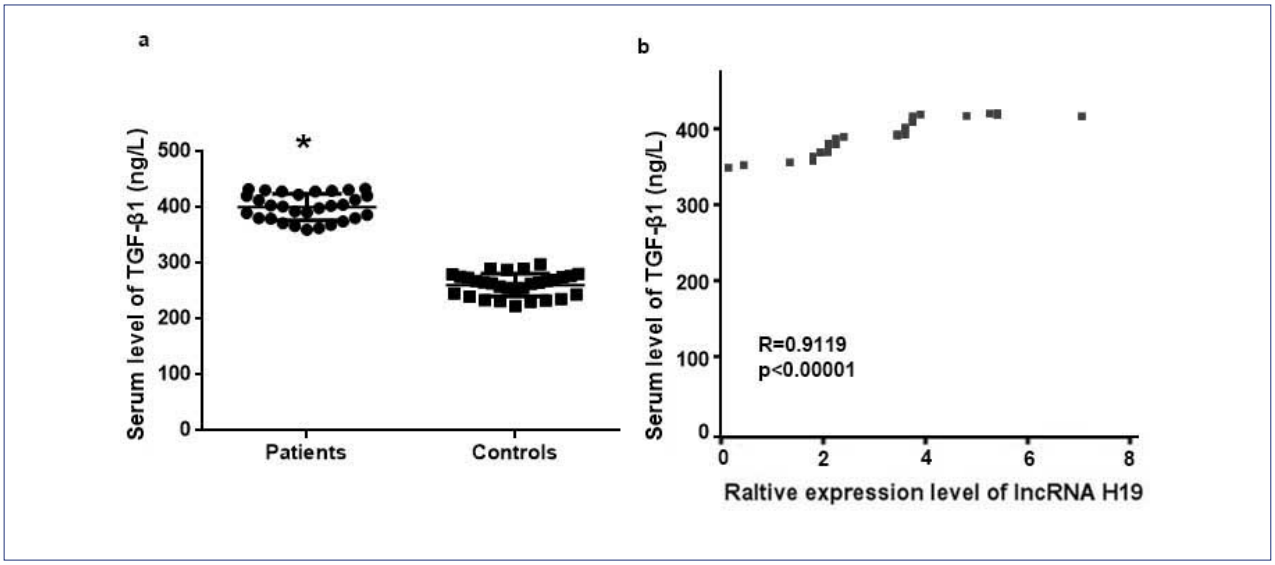

FIGURE 2 CORRELATION BETWEEN THE SERUM EXPRESSION LEVEL OF H19 AND THE SERUM LEVEL OF TGF-β1

a Serum level of TGF-β1 in CAD patients and healthy control individuals. b Correlation between the serum expression level of H19 and the serum level of TGF-β1analyzed using the Pearson correlation analysis.
Notes: *compared with the control group, p<0.05

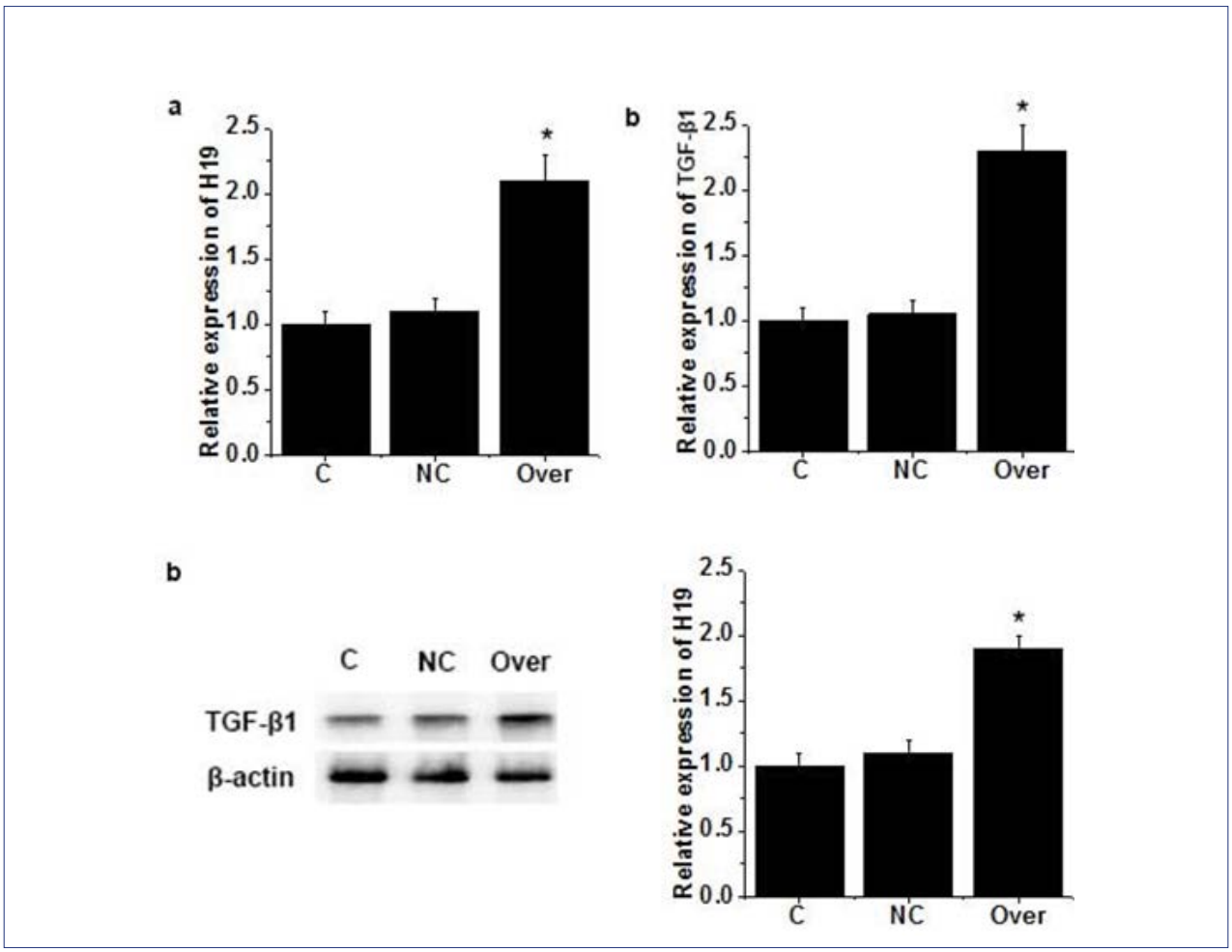

FIGURE 3 H19 OVEREXPRESSION INCREASED THE EXPRESSION LEVEL OF TGF-β1 IN HCAECS

a Expression of H19 in HCAECs with different treatments; b Expression of TGF-β1 mRNA in HCAECs with different treatments; c Expression of TGF-β1 protein in HCAECs with different treatments.
Notes: *compared with the control group or negative control group, p<0.05.

diagnosis of CAD has not been well studied, except for one recent study which found that plasma lncRNA CoroMarker was significantly increased in the plasma of CAD patients but not in patients with other types of cardiovascular diseases, and the decreased expression level of this lncRNA could be used to predict CAD accurately. The functionality of lncRNA H19 is extensive in different disease models. Especially in cancer, lncRNA H19 is an oncogene that is usually overexpressed, and circulating lncRNA H19 in blood has been proved to be a biomarker for different types of cancers such as breast cancer and gastric cancer. In a recent study, Zhang et al. reported that the serum level of lncRNA H19 was significantly increased in the plasma of patients with CAD, and the increased expression level of lncRNA H19 was significantly correlated with the increased risk of CAD in the Chinese population. Consistent with previous studies, in our study, the serum level of H19 was specifically increased in patients with CAD but not in healthy individuals or patients with other types of cardiovascular diseases. Besides that, the ROC curve analysis also showed that serum H19 could also be used to accurately predict CAD, indicating that serum H19 is a specific and accurate diagnostic marker for CAD. Stability is critical for biomarkers. It is known that the expression of some lncRNAs can be induced or regulated by patients' living habits, such as smoking and drinking. In our study, age, gender, and patients living habits showed no significant effects on H19 expression, while the H19 expression level was increased with the prolonged course of the disease. These data suggest that serum H19 is a promising biomarker for CAD.

Although H19 has been proved to correlate with CAD, its mechanism is still unclear.

It is well known that H19 can interact with TGF-β1, and polymorphism and the expression of TGF-β1 are related to the development of CAD. In our study, the serum level of H19 was found to be positively correlated with the serum level of TGF-β1. Besides that, H19 overexpression also significantly increased the expression level of TGF-β1 in HCAEC. These data suggest that the upregulation of H19 expression may play a role in CAD by increasing the expression level of TGF-β1.

CONCLUSION

In conclusion, the serum level of H19 was higher in patients with CAD than in healthy individuals or patients with other types of cardiovascular diseases. Serum H19 is a stable, specific and accurate diagnostic marker for CAD. The serum H19 expression level was positively correlated with the plasma level of TGF-β1, and H19 overexpression significantly increased the TGF-β1 protein level in HCAEC. Our study is still limited by the small sample size. Future studies with more significant sample sizes may be needed to confirm our conclusions further.

Acknowledgments

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RESUMO

OBJETIVO *Nosso estudo teve como objetivo investigar o valor diagnóstico do lncRNA H19 para doença arterial coronariana (DAC) e explorar os possíveis mecanismos. Métodos Um total de 30 pacientes com DAC e 30 pessoas saudáveis, bem como pacientes com diferentes doenças cardiovasculares foram incluídos neste estudo. O sangue foi extraído de cada participante para preparar amostras de soro e a expressão de lncRNA H19 foi detectada por qRT-PCR. A análise da curva ROC foi utilizada para analisar o valor diagnóstico de H19 para DAC. Efeitos da informação básica dos pacientes e estilo de vida na expressão de H19 foram analisados. O nível plasmático de TGF-β1 foi medido por ELISA. A linha de células endoteliais da artéria coronária primária (HCAEC) humana de sobre-expressão de H19 foi construída e os efeitos da sobre-expressão de H19 na expressão de TGF-β1 foram analisados por Western blot. Resultados A expressão de H19 foi especificamente regulada positivamente em pacientes com DAC, mas não em pessoas saudáveis e em pacientes com outros tipos de doenças cardiovasculares. A análise da curva ROC mostrou que o nível de expressão de H19 pode ser usado para prever com precisão a DAC. Sexo, idade e estilo de vida dos pacientes não têm efeitos significativos sobre a expressão de H19, mas a expressão de H19 foi maior em pacientes com curso mais longo da doença em comparação com os controles. A expressão de H19 correlacionou-se positivamente com o nível sérico de TGF-β1 e a superexpressão de H19 aumentou significativamente o nível de proteína de TGF-β1 em HCAEC. Conclusão A superexpressão de H19 participa da patogênese da DAC aumentando o nível de expressão de TGF-β1 e o nível de expressão de H19 pode servir como marcador diagnóstico de DAC.*

PALAVRAS-CHAVE: *Doença da artéria coronariana. Diagnóstico. RNA Longo não Codificante. Fator de Crescimento Transformador beta1.*

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APnet, an innovative multidisciplinary medical information platform for acute pancreatitis

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SUMMARY

The APnet information platform aims at assisting patients suffering from acute pancreatitis, health professionals and patients' relatives in the acute pancreatitis care pathway by providing an integrated information system. The system consists of a mobile platform and a Clinical Information System. The system is currently on the formal operation phase focusing on addressing the needs of citizens of China.

KEYWORDS: *Pancreatitis. Pancreatitis, acute necrotizing. Smartphone. Cell phone. Health information systems.*

INTRODUCTION

With the increasing number of acute pancreatitis (AP) patients in China, the limited financial input and low medical technical level in primary clinics can hardly satisfy patients' demand for medical services. Moreover, follow-up and management for discharged AP patients are neglected by most hospitals at present, which can lead to recurrence and severe complications of AP. Meanwhile, with the rapid development of internet technology and the multiplying growth of broadband speed, the use of information/communication technologies and web service in medical service are growing increasingly popular, providing remote communication or services that assist with certain health-care activities, thus lowering health-care costs and improving health-care quality [1-3]. Therefore, it is urgent to build a information platform to facilitate communication between doctors and AP patients.

In the present study, we built a multidisciplinary medical information platform named APnet for AP to help Chinese clinicians who need to make difficult clinical decisions concerning their patients and increase consultancy channels with doctors, providing them with knowledge of the latest developments in pancreatology, facilitating the two-way referral of patients, and providing convenient consultation for AP patients after discharge.

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PLATFORM ARCHITECTURE

The architecture of the APnet platform can be found in figure 1. With all core elements modularized, the APnet platform focuses on the establishment, linkage, and integration of various services. As far as the server is concerned, data-sets are managed by MySQL RDBMS and used by the Apache server. HTML and PHP5 languages which were included in Apache are used in interaction with the MySQL RDBMS for the display of APnet web statistics in real-time and/or updated. It interacts through a "reverse proxy" with the associated Windows server. As far as data exchange is concerned, it adopts the service-oriented architecture standard for the message exchange interface and the HL7/XML standard for the message delivery formats among systems. As far as the client is concerned, an application launched by the web browser will enable access to its web pages depending on user profiles. As far as security is concerned, APnet platform is hosted on a secure Windows server operating system protected by a firewall. The data and messages in the message exchange interface must follow WS-Security regulations to include the encryption setting and the signature in order to ensure the non-repudiation and consistency of the data and messages. The computer languages used are HTML/XHTML, JAVA, PHP, and Visual C++.

The APnet platform contains six modularized core elements: (1) medical service resources registration, (2) remote medical information transmission, (3) electronic healthcare record management, (4) service log and system monitoring, (5) healthcare institutions interface, and (6) information security management.

The information platform can play the role of different medical institutional healthcare services as well as the role of an e-counter website and Web Service Provider portal. Through service resources, registration, and integration of other healthcare service resources, it can internally link up with the various model health service e-counters and externally interface with the available healthcare resources to provide assistance requested by different types of users.

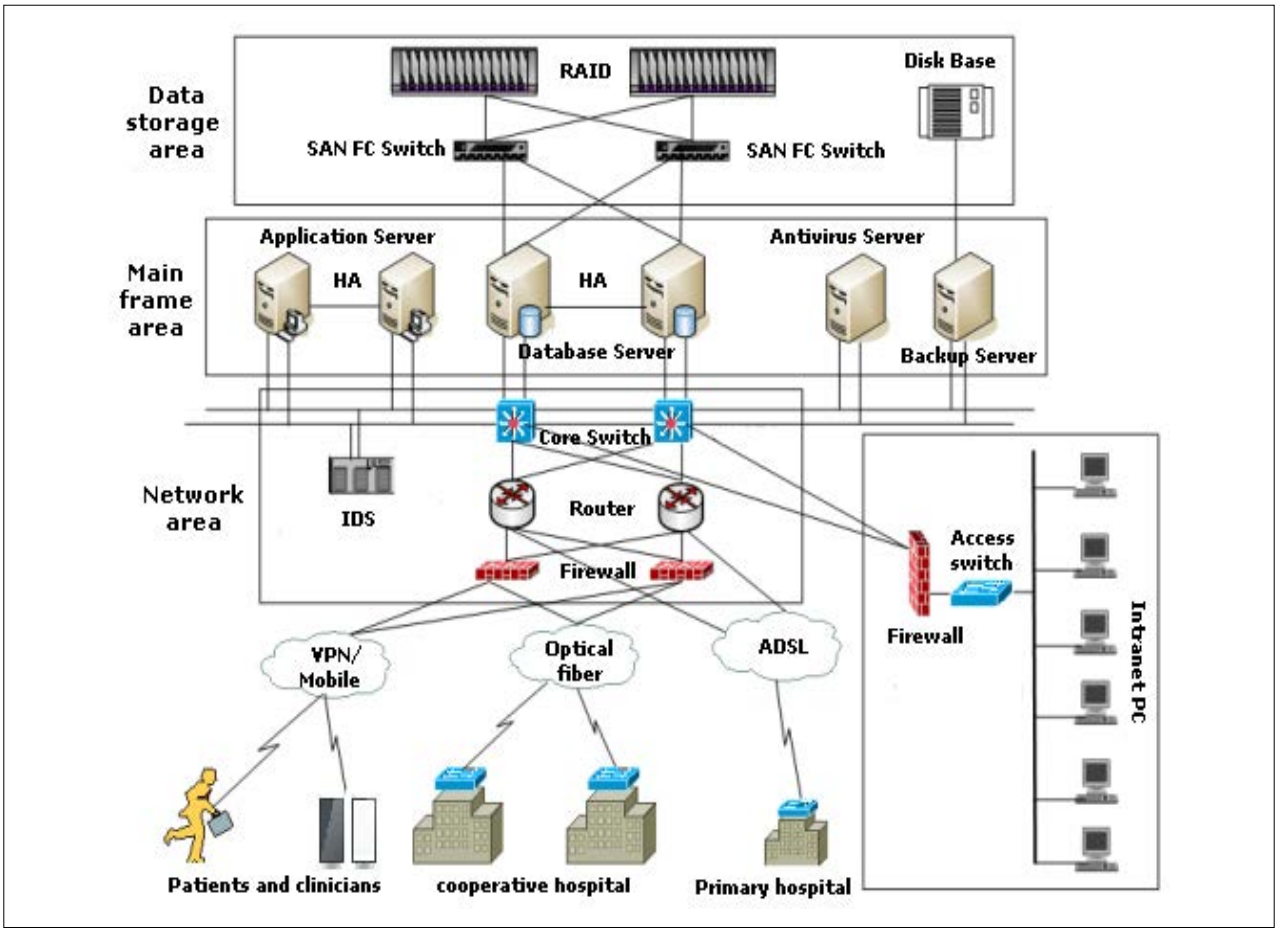

FIGURE 1 - THE ARCHITECTURE OF THE APNET INFORMATION PLATFORM

SYSTEM FUNCTION DESIGN

The APnet platform is divided into 7 functional architectural areas, as shown in Figure 2 : (1) service portal; (2) medical service resources registration; (3) electronic healthcare record service; (4) remote clinical and physiological information transmission system; (5) healthcare resources interface service; (6) remote health education; (7) information platform core operation services.

The service portal utilizes a single service window that provides healthcare information and resources inquiry and performs operations for public and e-counter attendants. It provides an integrative, continuous, and shared digital healthcare service media and acts as a regional healthcare and living resources information integration center too. The service resources registration application is comprised of a healthcare service resources repository which includes professional healthcare and living support by concentrating service resources information provided by various healthcare modes. It also acts as a media providing member service requirements to allow for a healthcare and living resources sharing environment. Moreover, the various healthcare service modes can provide essential information of service content through registered items of the remote healthcare information platform and be displayed in the service resources usage interface of the platform portal to provide inquiry of such resources by the users. The electronic healthcare record is comprised of an electronic healthcare record index and electronic healthcare record summary. Presently, the remote healthcare information platform mainly uses the identity card number to authenticate member identity. Aside from being an electronic healthcare record index with all the necessary information of the member, it also simultaneously stores the member's healthcare service log in various healthcare modes. The detailed factual healthcare information will not be stored in the information platform but in the information systems of various healthcare service modes. If needed, the information may be searched and read through the data exchange mechanism between systems and displayed afterward in an electronic healthcare record summary. The remote clinical and physiological information transmission system can assist the various healthcare service modes by carrying out clinical information or physiological signal measurement and management to record, store, and transfer the various clinical information or physiological signals obtained with measuring instruments at the member end. The various healthcare service modes may also obtain member hospital discharge preparatory information by interfacing with the remote healthcare information platform and the long-term healthcare information network, thereby establishing continuous healthcare service. Then we can provide appropriate requirements and personalized healthcare service in line with its healthcare requirements after discharge; healthcare service can effectively achieve healthcare information sharing and minimize the waste of unnecessary healthcare resources. Users certified by single

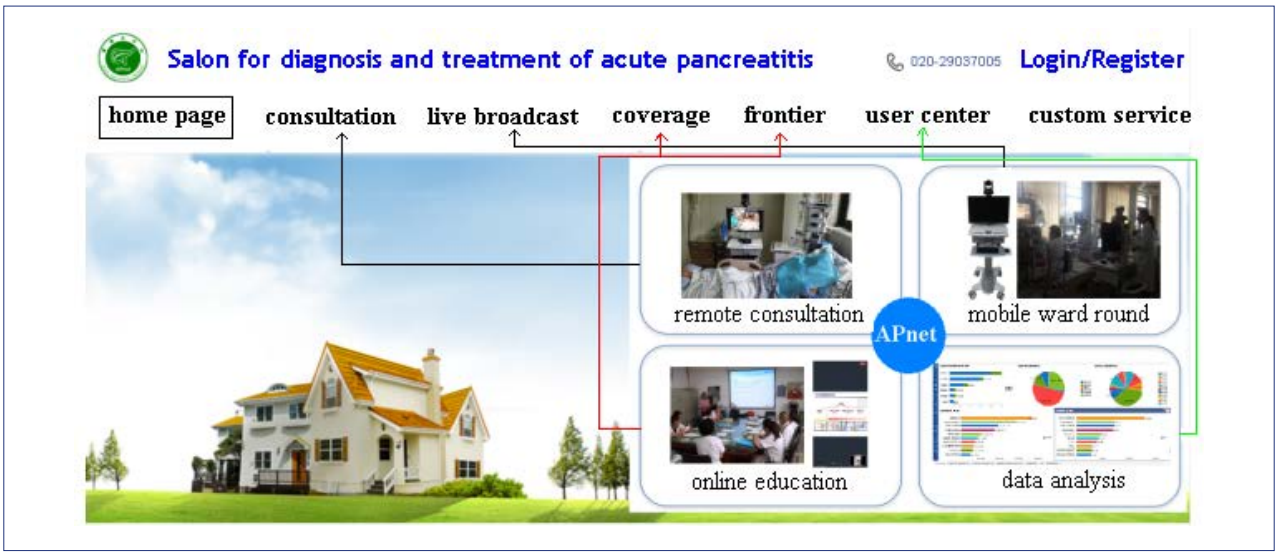

FIGURE 2 - THE HOMEPAGE OF APNET INFORMATION PLATFORM WEBSITE

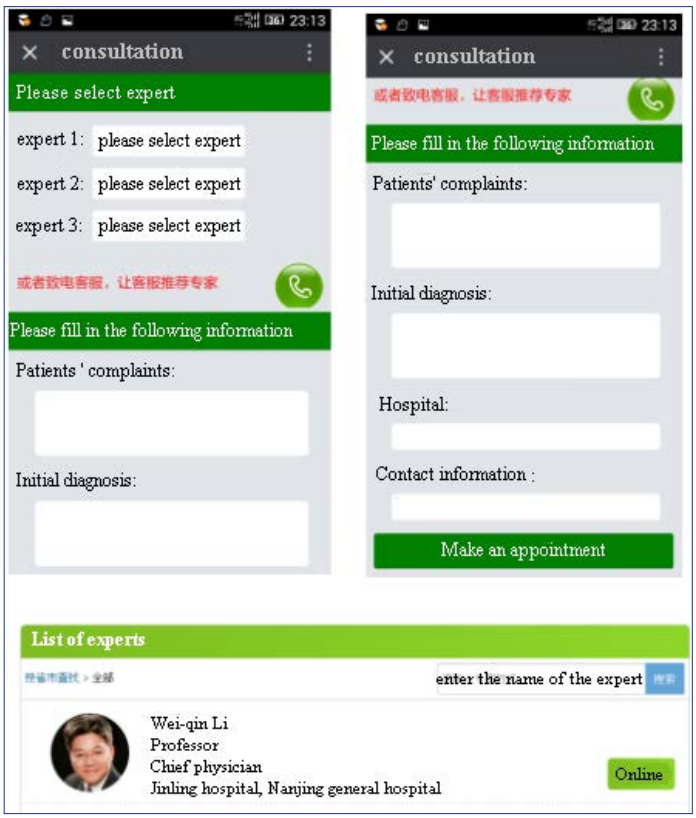

FIGURE 3 – REMOTE CONSULTATIONS APPLICATION BY APNET PLATFORM

sign-on via the remote healthcare information platform may also use remote health education services. Remote health education services may also be useful to members of various healthcare service modes. In remote health education learning services, the information platform disseminates health education content to a member's personal computer or mobile phone.

The information platform core operation provides two mechanisms for the entire information platform: single sign-on mechanism and information security exchange mechanism. The information security exchange mechanism is comprised of the Public Key Infrastructure and WS-Security. To ensure the completeness, confidentiality, authentication, and non-repudiation of the healthcare service information, the APnet platform utilizes PKI technology and employs different public and private keys to carry out the electronic signature mechanism.

SYSTEM EVALUATION AND DEPLOYMENT

On system deployment, the remote healthcare information platform, user, health administration, and social administration healthcare resources are connected through the internet. Meanwhile, the provision of linkage and authorization via the IDC computer room service platform can facilitate the sharing of various healthcare resources as shown in Figure 2. The information platform is placed in the co-location computer room (IDC computer room) of the Internet Service Provider (ISP). The FTTB optical fiber broadband's dedicated line is connected to the internet, ensuring transmission speed and system quality of the data channel. On internet security prevention deployment, all internet access and inquiry requests must be confirmed by the preventive firewall, the intrusion prevention system (IPS), and anti-virus security prevention before being allowed to connect to the internal application system internet.

Regarding the backup mechanism of the application system service, a two-set design is installed regardless of the internet security protection equipment or application server to ensure non-interrupted system service. The internet security protection equipment will accommodate the redundant requests of the application server and the back-end service support of the application server request. Under emergency and necessary circumstances, the backup server will replace the original server as a system backup.

The deployment of the APnet information platform in public hospitals, doctors' and patients' mobile phone terminals is currently in progress. Analysis of the system performance and comparison with the existing health-care services is expected to demonstrate its clinical acceptance and effectiveness in diagnosis and managing AP.

The APnet platform was officially launched on November 26th, 2016. Up to July 1st, 2017, the information platform had more than 2,000 users. Through the platform, there were 87 remote consultations (Figure 3), 32 bidirectional referrals, 58 remote teaching, eight live academic conferences about AP, and 120 latest progress of AP. The APnet platform includes more than 7,000 patients with AP. In the future, prospects for the development of the APnet platform is worth users' expectation. More functional modules will be incorporated into it, such as an AI-based decision support system and a pancreatitis biological sample database. Coverage in the country will be further expanded [4-6].

RESUMO

A plataforma de informações APnet tem como objetivo auxiliar pacientes que sofrem de pancreatite aguda, profissionais da saúde e familiares de pacientes no percurso de cuidados para a pancreatite aguda, oferecendo um sistema integrado de informações. O sistema consiste de uma plataforma móvel e um Sistema de Informações Clínicas. Ele atualmente se encontra na fase de operação formal, focado em atender às necessidades dos cidadãos da China.

PALAVRAS CHAVE: *Pancreatite. Pancreatite necrosante aguda. Smartphone. Telefone celular. Sistemas de informação em saúde.*

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A pancreatic hemorrhagic pseudocyst with pseudoaneurysm and the role of doppler ultrasonography: a case report

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SUMMARY

Hemorrhagic pseudocysts with pseudoaneurysms are a rare and fatal complication of chronic pancreatitis due to the erosion of pancreatic to peripancreatic arteries. The timing of the rupture cannot be accurately predicted, but prompt diagnosis and management are essential to prevent further bleeding.

We describe the case of a 68-year-old man who presented acute epigastric pain and anemia and had a history of chronic pancreatitis with a pseudocyst. A biliary and pancreas MRI showed an enlarged size of a known pancreatic pseudocyst with internal high signal intensity material. Color-Doppler ultrasonography showed pulsating signals in the pseudocyst, and our final diagnosis was a pseudoaneurysm in the pancreatic hemorrhagic pseudocyst. The pseudoaneurysm was successfully treated with coil embolization of the feeding artery.

We report this case of a rare complication of chronic pancreatitis to show that color-Doppler ultrasound is a non-invasive and effective diagnostic tool for pseudoaneurysm, which enables early detection and prompt treatment without the need for invasive diagnostic modalities.

KEYWORDS: *Pancreas. Aneurysm, false/diagnostic imaging. Pancreatic pseudocyst. Embolization, therapeutic.*

INTRODUCTION

Hemorrhagic pseudocysts with pseudoaneurysms are a rare and fatal complication of chronic pancreatitis[1]. The following three mechanisms account for pseudoaneurysms related to pancreatitis: 1) severe inflammation and enzymatic autodigestion of a pancreatic or peripancreatic artery producing arterial disruption with pseudoaneurysm formation; 2) an established pseudocyst eroding into a visceral artery, thereby converting the pseudocyst into a large pseudoaneurysm; and 3) a pseudocyst eroding the bowel wall with bleeding from the mucosal surface [2]. It has been reported that a pseudoaneurysm following pan-

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creatitis occurs most commonly at the splenic artery, followed by the gastroduodenal, pancreaticoduodenal, left gastric, and common hepatic arteries [3]. Mortality rates due to bleeding pseudoaneurysms can be up to 40% depending on patient clinical status, site, and characteristics of the bleeding lesion and surgical procedure employed[2]. The timing of rupture is unpredictable; therefore early diagnosis and correct management are essential in preventing the fatal outcomes[4]. In our case, the patient had a pancreatic hemorrhagic pseudocyst with pseudoaneurysm and color Doppler imaging could speed the diagnosis with prompt management.

CASE

A 68-year-old man visited our outpatient clinic for epigastric pain without other gastrointestinal symptoms such as vomiting, diarrhea, or GI tract bleeding. The patient had been diagnosed with chronic pancreatitis with a pseudocyst 2 years ago outside the hospital. Also, he was diagnosed with portal vein thrombosis and treated with Warfarin for a month. At the time of admission, the patient's blood pressure was 110/70mmHg. Hemoglobin (Hb) was decreased by 9.0 g/dL, and hematocrit (Hct) was 29.3%. Prothrombin time (PT) was prolonged to 36.3 sec and activated partial thromboplastin time (aPTT) was also prolonged to 49.9 sec

Biliary and pancreas dynamic T1-weighted axial MR image revealed an enlarged known pseudocyst in the pancreas head filled with high signal intensity contrast material in the lesion (Fig.1). Also, pancreas showed diffuse parenchymal swelling with peripancreatic fat infiltration.

Ultrasonography was performed for further characterization of the lesion. Grayscale ultrasonography showed about a heterogeneously hyperechoic lesion of approximately 4cm at the pancreas head, which correlated with hemorrhagic pseudocyst on previous biliary and pancreas MRI. There was a hypoechoic sac of about 1.3cm located at the anteroinferior portion of the pseudocyst. In the sac, Color Doppler ultrasonography showed a typical swirling signal called "yin-yang" sign and arterial flow was detected on duplex Doppler ultrasonography (Fig. 2A & 2B). Based on the findings of the color Doppler imaging, pseudoaneurysm in pancreatic pseudocyst was considered. The physician decided to perform embolization for pseudoaneurysm treatment. A selective

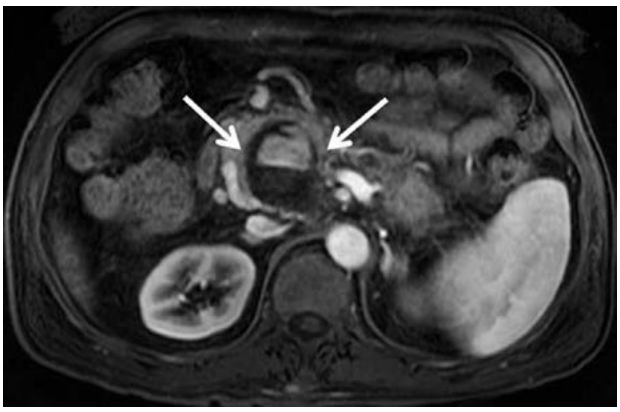
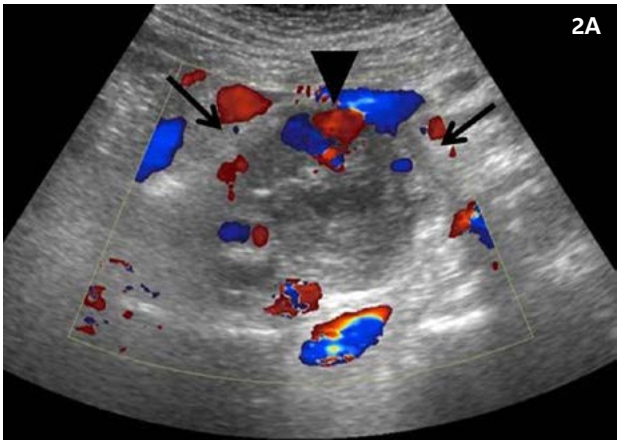


FIGURE 1. Biliary and pancreas dynamic T1-weighted axial MR image shows a contrast-filled pseudocyst of about 4.4x3.8cm at the pancreas head portion, which is suspected to be a hemorrhagic pseudocyst (arrows).



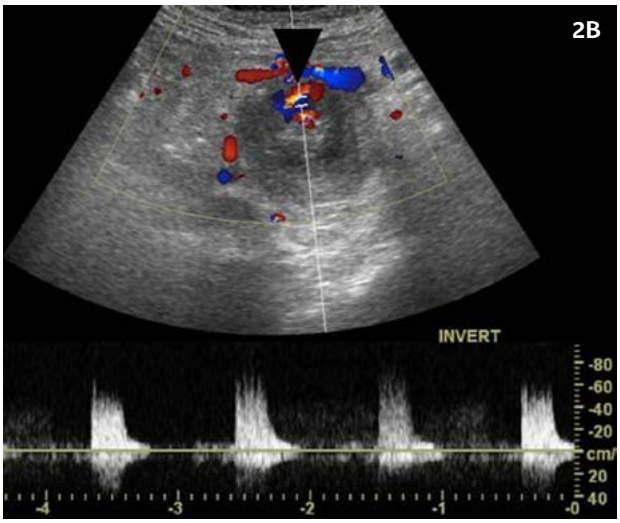


FIGURE 2A. Color Doppler ultrasonography shows a heterogeneously hyperechoic pseudocyst of about 4cm at the pancreas head and an anechoic sac located at the anterior portion of the pseudocyst wall. The typical swirling signal called "yin-yang" sign is seen in the sac (arrowhead).
FIGURE 2B. Duplex Doppler ultrasonography depicts prominent arterial flow in the sac.

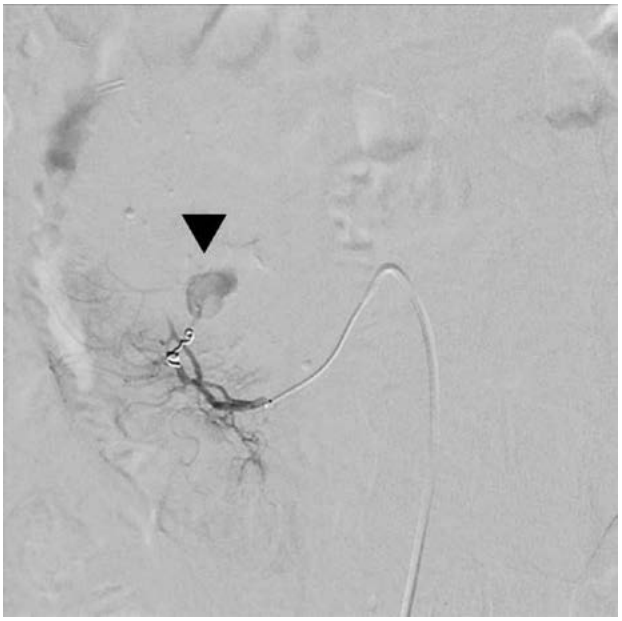


FIGURE 3. Digital subtraction angiogram shows the pseudoaneurysm (arrowhead) during coil embolization of pancreaticoduodenal artery of superior mesenteric artery.

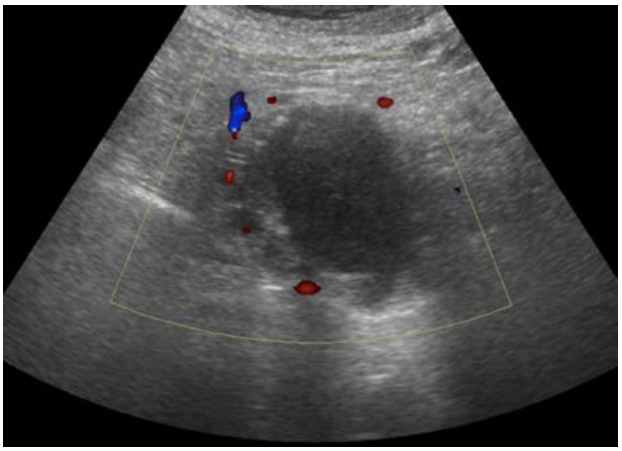


FIGURE 4. Follow-up color Doppler ultrasonography 6 days after the interventional treatment shows no more vascularity within the pseudocyst.

angiogram of the superior mesentery artery showed the pseudoaneurysm was being fed by the 1st branch of the superior mesenteric artery. Coil was deployed and on post coil embolization angiogram, the pseudocyst was not excluded on arterial flow. Besides the first branch of the superior mesenteric artery, other feeding vessels were suspected. a glue-lipiodol mixture was injected into the pseudoaneurysm and control angiography confirmed total exclusion of the pseudoaneurysm (Fig.3). 6 Days later, follow up abdominal ultrasonography, and color Doppler imaging was performed, and vascularity was no longer seen in the pseudocyst (Fig. 4).

DISCUSSION

Hemorrhage is uncommon in pancreatic pseudocysts, but it is a potentially life-threatening complication. According to the literature, the incidence of pancreatic pseudoaneurysm in patients with chronic pancreatitis is less than 10%, and spontaneous hemorrhage arising from a pancreatic pseudocyst reportedly ranges from 1.4 to 8.4%. When bleeding occurs, it can be potentially lethal, as mortality rates can reach up to 40% [5]. Therefore early detection of pseudoaneurysm and appropriate treatment is required in preventing massive bleedings. The combination of angiographic embolization and surgery is thought to be the most appropriate management.

The diagnosis of bleeding into a pancreatic pseudocyst should be suspected in the presence of any of the following conditions: (1) sudden enlargement of previously present pseudocyst; (2) development of a bruit over a pseudocyst in a patient with or without abdominal pain; (3) upper gastrointestinal bleeding in a patient with a known pseudocyst and no other source seen on upper gastrointestinal endoscopy; or (4) a sudden decrease in hematocrit with no evidence of gastrointestinal bleeding in a patient with a pancreatic pseudocyst [6].

The diagnostic modalities of hemorrhagic pseudocyst include contrast-enhanced CT scan, ultrasonography, and angiography. On CT scan, pseudoaneurysms are readily diagnosed as rapidly enhancing intracystic lesions, with attenuations similar to those of the aorta and other larger splanchnic arteries. Ultrasonography is a valuable tool for diagnosis of pseudoaneurysms, and it has been widely utilized as a noninvasive imaging modality for investigation of vascular disease. The gray-scale US illustrates pseudoaneurysm as a hypoechoic cystic structure nearby a supplying artery; however, its findings are accompanied by other clinical conditions such as hematomas and cystic masses. Therefore color Doppler US can be used for confirmation for pseudoaneurysm [7]. Blood flow within a cystic structure is characterized by a typical swirling motion called the "yin-yang sign," however this pattern of flow can also be seen in a saccular aneurysm, so diagnosis made from this finding alone may prove to be inaccurate. The hallmark of the diagnosis is the demonstration of a communicating channel between the sac and the feeding artery with a "to-and-fro" waveform at the duplex Doppler US. Conventional angiography remains the

standard reference for diagnosis but it is an invasive procedure, and noninvasive diagnostic modalities should be included in the initial work-up if possible [8].

In our case, color Doppler ultrasonography allowed a quick diagnosis of pancreatic hemorrhagic pseudocyst with pseudoaneurysm and prompt treatment before massive hemorrhage could occur. Post-embolization doppler imaging also showed a change in vascularity within the pseudocyst, which confirmed the complete exclusion of the pseudoaneurysm. Therefore, Doppler ultrasonography is a safe and effective diagnostic tool and can be useful to follow up pseudoaneurysms without the need for invasive diagnostic modalities.

Acknowledgments

This work was supported in part by the Soonchyunhyang University Research Fund."

PALAVRAS-CHAVE: *Pâncreas. Falso aneurisma/diagnóstico por imagem. Pseudocisto pancreático. Embolização terapêutica.*

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Non-ketotic hyperosmolar hyperglycemic chorea

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KEYWORDS: *Chorea. Dyskinesias. Hyperglycemia.*

INTRODUCTION

Chorea is a type of hyperkinesia characterized by the presence of involuntary, brief and unsustained movements caused by irregular sequential muscle activation that flows continuously in a disorderly and unpredictable way.[1] It can be a manifestation of a primary neurologic genetic disorder, such as Huntington disease, or may occur as a neurologic complication of a systemic, toxic or metabolic cause, e.g., hypo/hypercalcemia or hyperglycemia.[2] Bedwell[3], in 1960, was the first author to describe the rare clinical syndrome of nonketotic hyperosmolar hyperglycemic (NKHH) chorea. Although rare, it is a treatable condition and, therefore, should be recognized.

OBJECTIVES

To describe the clinical presentation and neuroradiologic findings of a typical case of NKHH chorea.

CASE

An 80-year-old man was admitted after the abrupt onset of involuntary movements that affected his whole body three months before admission. Medical history revealed diabetes mellitus and systemic arterial hypertension with irregular follow-up and poor glycemic control.

Neurological examination showed orofacial dyskinesias and gait associated choreoatheteroid move-

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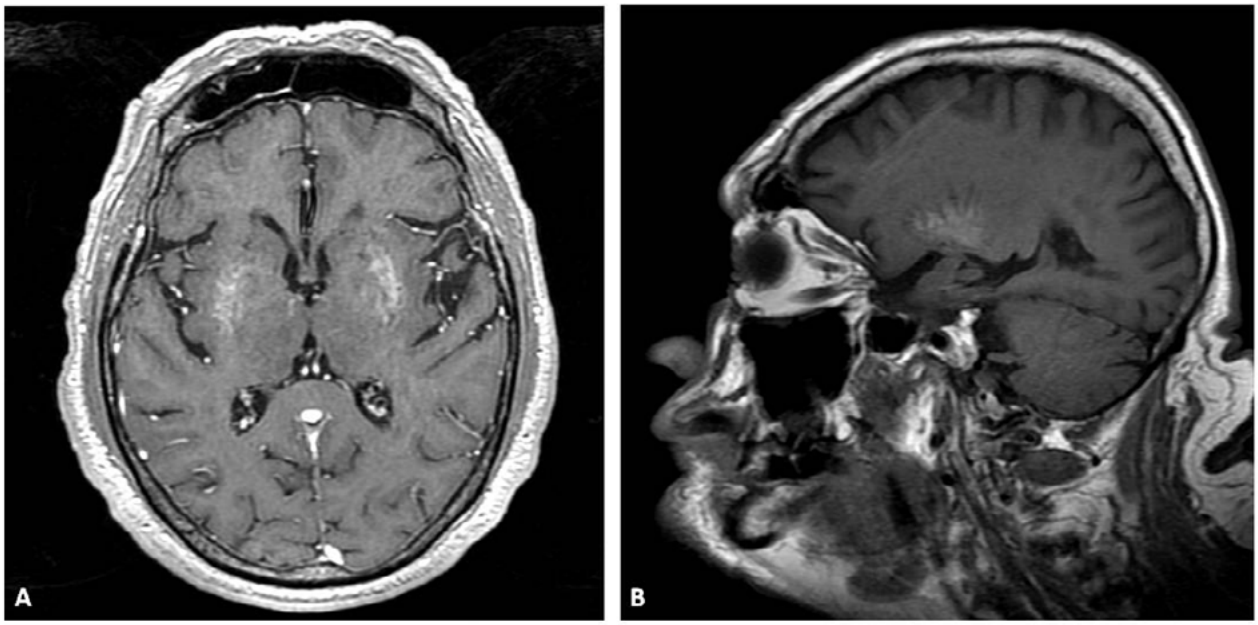

FIGURE. AXIAL (A) AND SAGITTAL (B) T1-WEIGHTED IMAGES SHOWING BILATERAL SPONTANEOUS HYPERINTENSE AREAS IN THE BASAL GANGLIA

ments affecting mainly the left lower limb. Hemoglucotest was 462mg/dL on admission and brain MRI showed non-specific hyperintense areas in the basal ganglia on T1 (FIGURE).

The diagnosis of chorea secondary to nonketotic hyperosmolar hyperglycemic state was established through clinical and imaging findings, and the patient was managed with intensive diabetes control and haloperidol 5mg orally twice daily. There was a remarkable improvement in the next few days, and the patient was subsequently discharged with almost no symptoms.

DISCUSSION

Chorea pathophysiology is still not widely understood. However, unlike in parkinsonism and dystonia, intracortical inhibition of the motor cortex is normal.[4] Semiquantitative analysis of single photon emission computed tomography in patients with hemichorea due to various causes suggests that there is an increase in activity in the contralateral thalamus, possibly due to disinhibition as a result of loss of normal pallidal inhibitory input.[5]

Non-ketotic hyperglycemia-induced chorea occurs more often in women and is usually associated with very high blood glucose[6]. The exact pathophysiology of NKHH chorea remains unclear. However, many hypotheses as blood hyperviscosity, petechial hemorrhage, depletion of gamma-aminobutyric acid (GABA) and cerebral vascular insufficiency have been suggested.[7] The correction of the metabolic abnormality usually is curative, but it can rarely continue for months after resolution of hyperglycemia.[8] Striatal permanent vascular changes may mean persistence of chorea for long periods.[9]

Many of the metabolic choreas are associated with abnormalities on MRI scans. Nevertheless, the etiology of the MRI changes is not fully understood.[10] Hepatocerebral degeneration and hyperglycemic chorea are often associated with high signal intensity on T1-weighted MRI involving the striatum and pallidum.[8]

Chu et al.[11], in a report of two patients with hyperglycemic hemichorea-hemiballism, found high signal intensities on T1- and T2-weighted images as well as on diffusion-weighted MRI accompanied by a reduction in diffusion coefficient, suggestive of hyperviscosity, rather than petechial hemorrhages, as the mechanism of edema in the striatum. This is also corroborated by another study of seven patients with hyperglycemic choreoathetosis using MRI and MR spectroscopy.[12] Interestingly, the presence of high counts of acanthocytes may predispose patients with diabetes to develop hyperglycemic chorea.[13]

CONCLUSION

A thorough physical examination and compatible clinical history and imaging are essential tools for diagnosing and treating metabolic chorea. In this case of symptoms secondary to diabetes decompensation, glycemic control added to a central dopaminergic inhibitor were effective.

Authors Disclosures

No conflict of interest to disclose.

PALAVRAS CHAVE: *Coreia. Discinesias. Hiperglicemia.*

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Non-puerperal uterine inversion associated with myomatosis

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SUMMARY

Uterine inversion is an uncommon complication of the puerperium and it is an even rarer complication of the non-puerperal period. In this way, uterine inversions are classified into two groups, being of puerperal origin due to obstetric problems and non-puerperal origin due to gynecological problems. In general, a non-puerperal uterine inversion occurs as a possible complication of a sub mucosal leiomyoma, after an expansive process, a dilation of the cervix occurs and thus its protuberance over the vaginal canal.

KEYWORDS: *Uterine inversion. Uterine diseases. Leiomyoma.*

INTRODUCTION

In general terms, uterine inversions are divided into two groups: those of puerperal origin and non-puerperal inversions[1-3]. As a rule, most cases published to date report puerperal uterine inversion as more frequent and common[2-4].

Non-puerperal uterine inversion is scarcely recorded in the literature and, among the reported cases, that caused by submucosal leiomyoma is the most frequently described condition and, generally, in black women[1,4-6].

Thus, the differential of the expelled myomas is associated with the capacity of the myoma to distend the endometrial cavity (by the increase in size and location), whereas this process triggers an inflammatory reaction on the uterine wall, leading to a contraction of response as an attempt to expel the tumor, justifying the nomenclature as expelled myoma[7].

As for the diagnosis of non-puerperal uterine inversion, it is taken from chronic signs (which mainly

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include irregular transvaginal bleeding, anemia and sensation of mass being externalized by the vaginal introitus) and from acute signs (which include pelvic pain and intense vaginal bleeding)[1,3,4,8,9]. Some studies highlight the occurrence of ureterovaginal fistula and acute urinary retention, and, as a rule, urinary events are justified by several reports that describe extrinsic urethral compression as a closely associated factor[1,4,6-9].

In time, it is still valid to mention that complaints of pelvic pain and transvaginal bleeding are characterized as the main manifestations recorded in the literature[7]. Scientific considerations also describe that the rectal examination is a complementary diagnostic examination of great value in order to confirm the absence of the uterus in the pelvis and to rule out elemental diagnostic doubts in more severe uterine inversions[7].

Complementary imaging tests, such as nuclear magnetic resonance and ultrasonography are mentioned in the literature as tools to support the diagnosis because they help with any doubts found in the physical examination, as well as support surgeons for the establishment of surgical planning and approach[7].

The treatment to be instituted may vary according to the patient's current clinical situation and previous history[6,8,9]. Abdominal or vaginal hysterectomies are commonly recommended for women with established offspring[4-6]. The option of vaginal myomectomy may be indicated for cases in which the malignancy is ruled out; as for cases of uterine inversion in which the malignancy is installed, the literature supports radical abdominal hysterectomy[1,2,6,9].

CASE DESCRIPTION

MPR, 55 years old, female, black, stay-at-home mom, GIIIPIIIA0 (two NB and one caesarean section), was admitted to the Gynecology outpatient care of Hospital Universitário Materno Infantil da UFMA (HUMI-UFMA) with complaints of lumbar pain, urinary incontinence for six months, with uterus externalized by the vaginal introitus, characterizing the diagnostic impression of uterine prolapse. The clinical state also included abdominal pain in the Right Iliac Fossa (RIF), irregular menstrual cycles with increased flow and average duration of three days.

At physical examination: blood pressure: 130x80 mmHg, heart rate: 82 bpm, respiratory rate: 18 bpm, regular general state, paleness 2+/4+, hydrated, acyanotic, anicteric, afebrile. Abdomen: hydro-aerial sounds present (RHA+), tympanic to percussion, absence of palpable masses or visceromegaly, superficial pain to palpation in RIF, negative decompression in the whole abdomen, Pfannenstiel incision scar visualized in the abdomen. On genital examination: total uterine prolapse, with visible genital bleeding; atrophic uterine body with mucosal dryness; uterine cervix undetermined, not being possible to determine its anatomical limits. Pap smear (06/16/2016 - negative for epithelial lesions).

Personal morbid antecedents: diabetes mellitus type II and systemic arterial hypertension; makes use of glyphage 500 mg and olmesartan + hydrochlorothiazide 40 mg + 12.5 mg. Laboratory exams (Complete Blood Count, Creatinine, Urea, Glycaemia and Urine Culture) and imaging (Pelvis CT) were requested, then she was admitted to the service for diagnostic investigation and treatment.

During hospitalization, the patient developed a good general condition, with some episodes of transvaginal bleeding and elimination of yellowish fetid odor secretion, with prolapse being hydrated with AGE on alternate days. Complete blood count presented: Hemoglobin (Hb) at 6.70 g/dL, Hematocrit (Ht) at 26%, Leukocytes at 8,600/uL without left shift, platelets 319,000/uL, Creatinine at 0.8mg/dL, Urea at 23mg/dL, Urine 1 normal and negative Urine Culture. As practice, three red blood cell concentrates were prescribed to control anemia, as well as Metronidazole EV of 8/8h for seven days for bacterial vaginosis.

TVUS showed uterine prolapse with uterine myoma (Figure 1A).

Pelvis CT pelvis showed complete uterine prolapse; bladder with irregular contours and spaced walls, especially on its floor, with an apparent low bladder component; no filling failure ureters, with low insertion ureteral meatus; absence of free fluid or lymph node enlargement in the pelvis; absence of adnexal masses; bone structures without particularities and abdominal wall without abnormalities (Figures 1B, C, D).

After stabilization of the condition (Hb: 9.5 g/dL and Ht: 38%), with volume expansion of crystalloids and blood transfusion, she presented a favorable opinion from anesthesiology and cardiology, followed by a surgical procedure on the 16th Day.

Surgical report: Proposed surgery: Vaginal hys-

terectomy + bilateral anexectomy. Patient in lithotomy position, under spinal anesthesia, asepsis and antisepsis. Surgical findings: Uterine prolapse, with inverted uterus and uterine fundus externalizing under the expelled myoma (Figures 2A, B), there was no significant intraoperative blood loss without intercurrences during the surgical procedure, opening of the posterior wall of the uterus (Figure 2C) with visualization, section and bilateral ligature of the round and annexes ligaments (Figure 2D), lowering of the bladder, clamping, sectioning and ligature of the uterine arteries (Figure 3A), of the cardinal and uterus ligaments bilaterally (Figure 3B), removal of surgical piece (uterus and annexes) (Figure 3C), bilateral adnexectomy was performed with revision of hemostasis and closure of the vaginal vault (Figure 3D), the excisional material was referred for anatomopathological analysis. Bladder drainage: functioning and with clear diuresis. Rectal touch without alteration and cleansing of the perineum. The patient leaves the operating room in good condition.

On the 17th Day and 1st Post-op Day, she complained of moderate intensity pelvic pain, with prescription of tramadol 100 mg + 250 ml SF0, 9%, satisfactory diuresis and no transvaginal bleeding.

On the 18th Day and 2nd Post-op Day, she presented good general condition, without complaints, communicative, acyanotic, anicteric, afebrile.

She was discharged on the 19th Day and 3rd Post-op Day, with no complaints, with preserved physiological eliminations, good general condition, lucid, oriented in time and space, paleness +1/+4, hydrated, acyanotic, anicteric, afebrile, eupneic in ambient air, abdomen flaccid, rounded, painless to superficial and deep palpation, RHA+, without visceromegaly, lower limbs without edema, well perfused, without transvaginal bleeding. She received return orientations for outpatient consultation in 15 days with result of anatomopathological examination and US of pelvis for follow-up.

In the outpatient return, she was in good general condition, lucid, oriented in time and space, colored, hydrated, anicteric, afebrile, absence of transvaginal bleeding and secretions, anatomopathological report without criteria for malignancy, presenting in the uterine body: intramural leiomyomas and a larger submucosal, adenomyomas. In cervix: chronic cervicitis with erosion and ectocervical mucosa presenting irregular acanthosis and common parakeratosis of uterine prolapse. US: no change worthy of note.

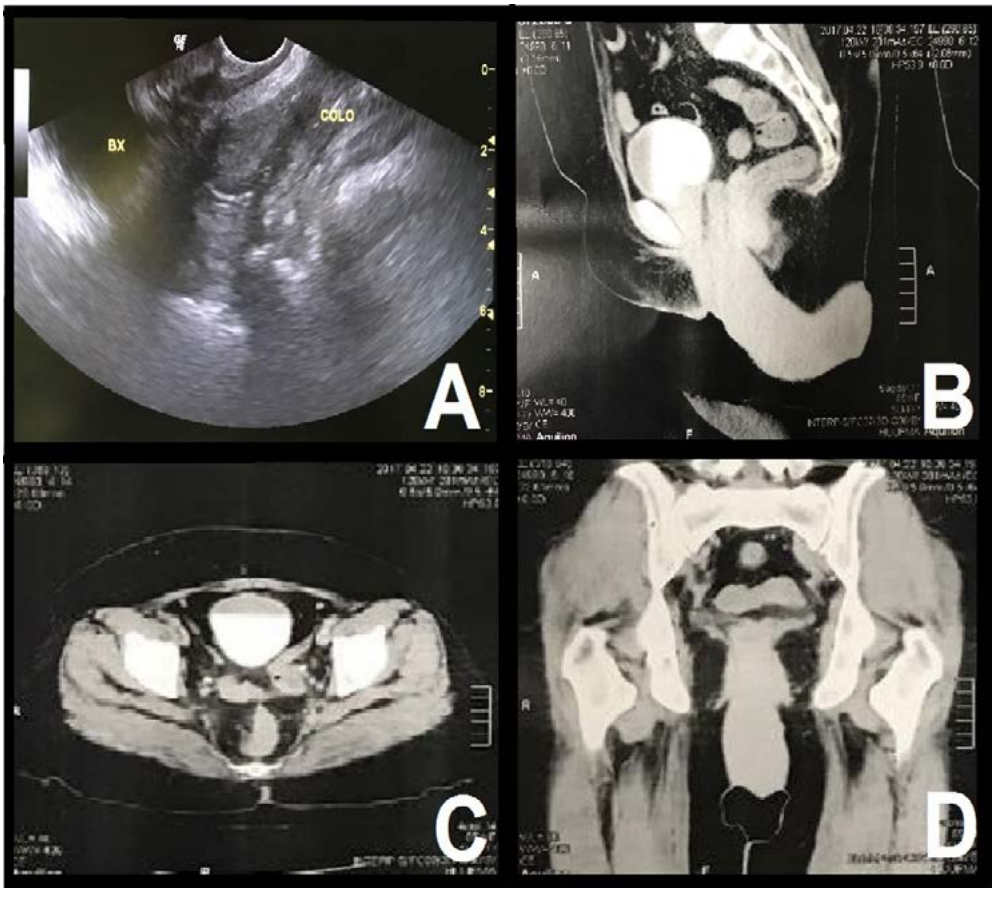

FIGURE 1. Imaging exams. A: TVUS with uterine myoma in uterine prolapse - B: Pelvic CT sagittal cut - C: Pelvic CT axial cut - D: Pelvic CT coronal cut. Vieira, GTB. São Luís - MA, 2018.

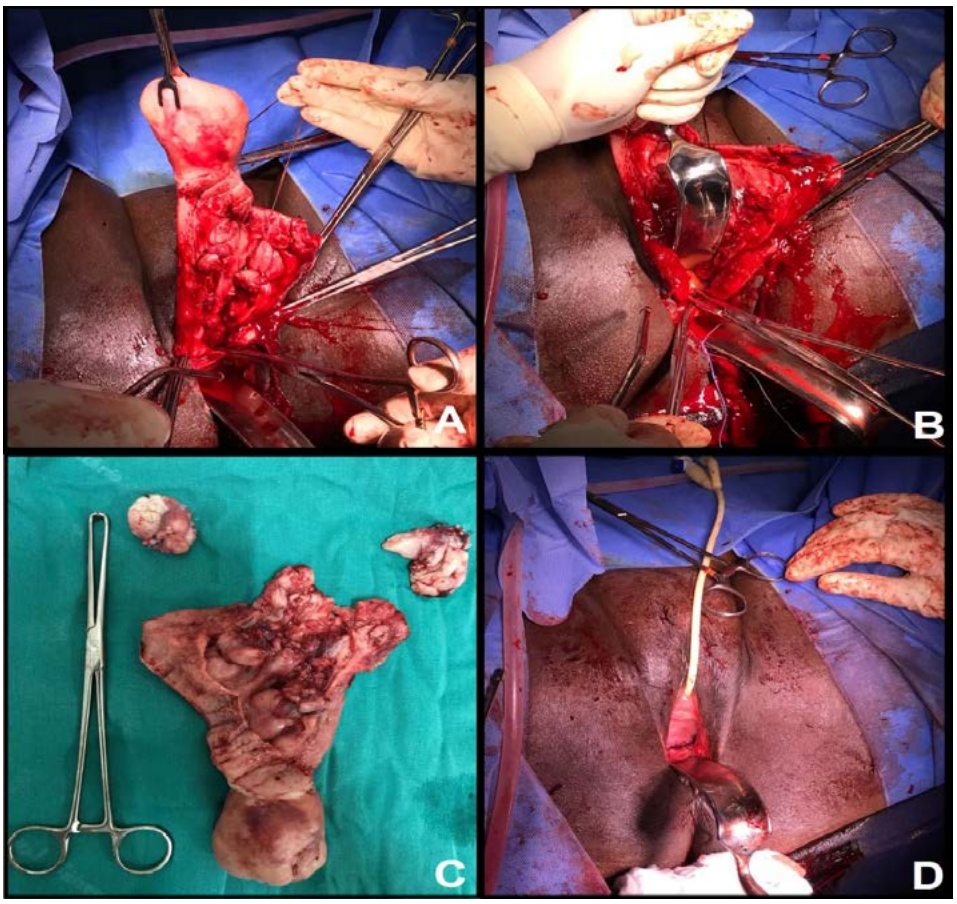

FIGURE 2. A: Uterine prolapse with inverted uterus - B: Mobilization of the surgical piece - C: Opening of the posterior wall - D: Ligature of the round ligament and annex. Vieira, GTB. São Luís - MA, 2018.

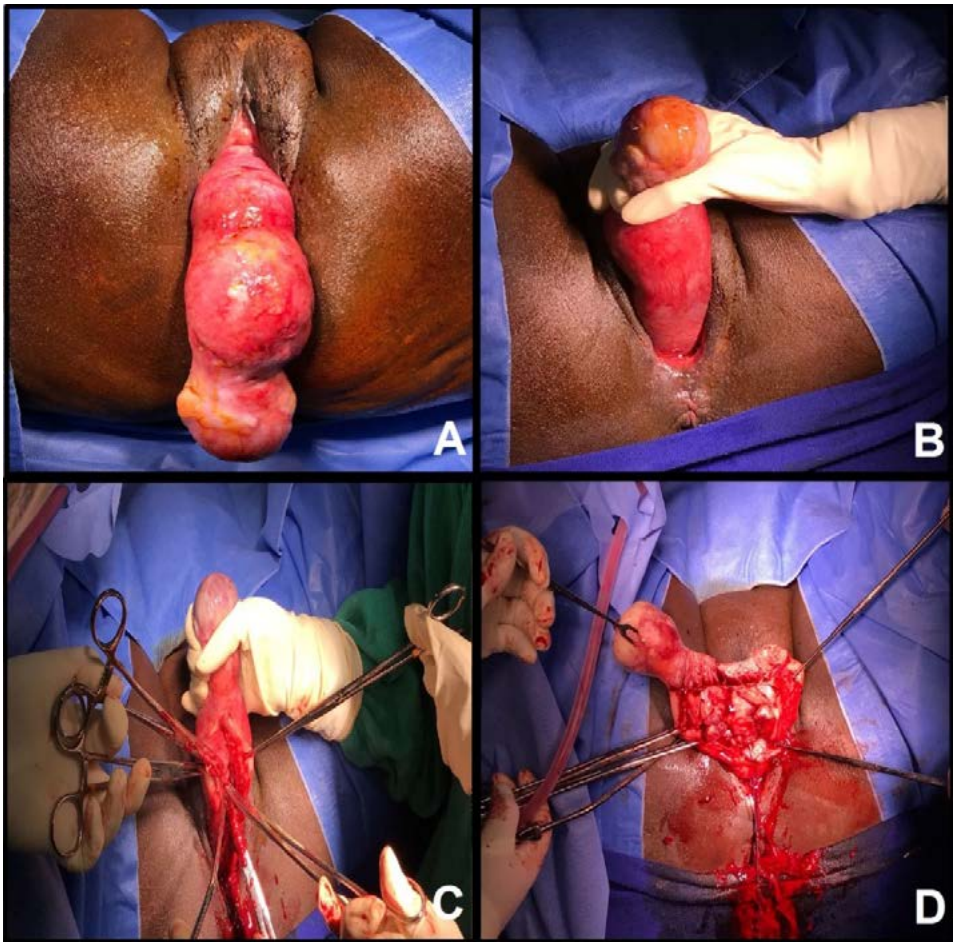

FIGURE 3. A: Ligature of the uterine arteries - B: Ligature of the cardinal and uterosacral ligaments - C: Surgical piece (uterus and annexes) - D: Closure of the vaginal vault. Vieira, GTB. São Luís - MA, 2018.

Thus, the outcome of the clinical case continues discharged from gynecological surgery, with improvement of the patient's body perception and, consequently, quality of life, in aspects related mainly to sexual activity.

DISCUSSION

Uterine inversion is an uncommon complication of the puerperium and is an even rarer complication of the non-puerperal period[1]. Thus, uterine inversions are classified into two groups, being those of puerperal origin due to obstetric problems and inversions of non-puerperal origin due to gynecological problems[2,3].

The uterine inversion of non-puerperal origin reported more frequently in the literature stands out for those caused by benign submucosal leiomyoma and prevalent in black women, often of African origin[1-5].

Non-puerperal uterine inversion occurs less frequently in postmenopausal women and its pathophysiology is associated with the traction effect of lesions in benign masses[2,6].

In young women of reproductive age, non-puerperal uterine inversion is frequently associated with leiomyosarcoma, rhabdomyosarcoma, malignant mixed müllerian tumor and endometrial polyp[1,8].

Leiomyomas are considered the most common gynecological disorders found in the gynecological specialty; however, non-puerperal uterine inversion by leiomyoma is classified as an extremely rare pathology[1].

The literature indicates that there is a great relationship of approximately 90% of non-puerperal uterine inversion with tumors, of which approximately 70% were leiomyomas and 20% malignant tumors, which justifies anatomopathological research in all situations[8].

In general, the etiology of uterine inversion is not clearly defined. The most plausible explanations are justified by a thin uterine wall, disordered growth of the tumor mass, size of the mass, its location, fixation of the mass in the uterine wall by only a single pedicle, dilation of the uterine cervix due to distension of the uterine cavity and dehiscence of tumor mass[1,5,8,9].

In general, non-puerperal uterine inversion occurs as a possible complication of a submucosal leiomyoma: after the expansion process, dilation of the uterine cervix occurs and, thus, its protrusion on the vaginal canal[1,3-5,9].

Its diagnosis can be very complex. Thus, as a rule, the patient may present several clinical aspects, which are fundamental for the clinical diagnosis of non-puerperal uterine inversion[1,2,6]. In this way, the diagnosis is organized into signs and symptoms of chronic and acute order[5].

The chronic signs that stand out are intense/irregular vaginal bleeding, iron deficiency (anemia) and sensory perception of a bulky mass coming down the vagina[1,5,8].

The acute signs that stand out are an intense pain in the pelvic floor and intense vaginal bleeding[5]. Routinely, some literature also highlights intermittent acute urinary retention, presentation of ureterovaginal fistula and vaginal secretion[5,9].

In summary, the main symptoms highlighted in non-puerperal uterine inversion are anemia due to irregular vaginal bleeding, vaginal (foul-smelling) secretion, abdominal or pelvic floor pain, mass in the vagina and urinary retention due to obstruction of the urethra[1,9].

The literature describes a classification for the treatment of uterine inversion in which the option is according to the stage of the inversion[2]. In stage 1, the inversion of the uterus is said to be incomplete and is restricted to the intra-uterus with the fundus occupying the cavity[2]. In stage 2, the complete inversion of the fundus of the uterus through the fibromuscular colon is observed[2]. Stage 3 refers to total inversion with the fundus located in the vaginal vulva[2]. Finally, stage 4 is characterized by the involvement of the vagina in the inversion[2].

The delay in the treatment of uterine inversion can be very compromising, besides leading to constriction (strangulation) ring formation, cervical edema and tissue necrosis. In several studies, vaginal myomectomy followed by vaginal hysterectomy were instituted as definitive treatment, being the gold standard for those women who are perimenopausal, menopausal or postmenopausal[4-6,8].

However, depending on the situation, certain surgical approaches may be defined for the treatment of non-puerperal uterine inversion[8]. Thus, we can classify the approaches in two, with the abdominals using the Huntington and Haultain techniques, and the vaginal ones including the techniques of Kustner and Spinelle[6,8,9].

The literature suggests that patients who present

in stage 1 of the inversion are those in which the repositioning of the uterus should often be evaluated and performed if feasible; however, for patients who present inversion in stages 2, 3 and 4, hysterectomy would be the best indication[2].

It is worth mentioning that in those patients in whom there is a desire to gestate or who are nulliparous, one must, whenever possible, opt for the abdominal approach and preservation of the uterus; otherwise, vaginal myomectomy followed by routine vaginal hysterectomy should be recommended[1,2,4,5,8].

CT, although it is not the gold standard diagnostic test for pelvic masses, may substitute the MRI, when conditions do not allow its use.

Finally, the therapeutic effect achieved with the use of the vaginal hysterectomy and bilateral anexectomy surgical intervention, in view of the improvements of the symptoms related to non-puerperal uterine inversion with myomatosis, is mainly regarding symptoms of urinary incontinence, increased sexual satisfaction and better perception of the image body.

CONCLUSIONS

The case reports a non-puerperal uterine inversion with myomatosis, which is a rare condition and, if not properly treated, it can evolve with major complications such as infection, anemia and hypovolemic shock.

As already mentioned, in the cases reported to date, the diagnosis was concrete regarding the visual inspection of externalized mass in the vaginal introitus, however, it is highlighted that the anatomopathological diagnosis is essential for patient follow-up.

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RESUMO

A inversão uterina é uma complicação incomum do puerpério e é uma complicação ainda mais rara do período não puerperal. Dessa forma, as inversões uterinas são classificadas em dois grupos, sendo as de origem puerperal decorrentes de problemas obstétricos e as inversões de origem não puerperal decorrentes de problemas ginecológicos. Em geral, a inversão uterina não puerperal decorre como uma possível complicação de um leiomioma submucoso — após o processo expansivo, ocorre a dilatação do colo uterino e, dessa forma, a sua protusão sobre o canal vaginal.

PALAVRAS-CHAVE: *Inversão uterina. Doenças uterinas. Leiomioma.*

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Primary infratentorial diffuse large b-cell lymphoma: a challenging diagnosis in an immunocompetent patient

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SUMMARY

We describe the case of a female patient, 52 years old, with dizziness and left motor incoordination for 2 weeks. Brain MRI magnetic resonance imaging) revealed a hyperintense lesion on T2-weighted images, without restricted diffusion, in the left middle cerebellar peduncle. Spectroscopy demonstrated peak of lipids and perfusion did not show any elevation in relative cerebral blood volume (rCBV). The patient underwent an open biopsy and resection, and the diagnosis of diffuse large B-cell lymphoma (DLBCL) was established. The patient received intravenous dexamethasone with symptoms remission, followed by four cycles of methotrexate plus cytarabine. After 3 months, the patient returned with decreased consciences level and a new MRI revealed a right superior frontal gyrus lesion with features suggesting a lymphomatous lesion. The patient died five days after her relapse.

KEYWORDS: *Cerebellum. Lymphoma, Large B-Cell, Diffuse. Magnetic Resonance Spectroscopy. Middle cerebellar peduncle.*

INTRODUCTION

Primary central nervous system lymphoma (PCNSL) is an unusual form of extranodal non-Hodgkin lymphoma that may affect the brain, leptomeninges, spinal cord or eyes, representing 3% of all intracranial neoplasms [1,2]. It is less frequent in immunocompetent patients, and the average age of diagnosis in these patients is 50-65 years [1,2]. Regarding location, only 9% of PCNSL is found in the cerebellum, and it usually presents as a single lesion (60-70%) [2-4]. Microscopic features of the lesions show diffuse large B-cell lymphoma (DLBCL) in 90% of the cases [5]. On neuroimaging, lymphomas tend to have an iso to low signal on T2-weighted images and restricted diffusion due to high cellularity. When these

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features are not observed, as in our case, infratentorial PCNSL may be a challenging diagnosis, and other differential diagnoses, such as metastatic tumors, malignant gliomas, demyelination and inflammatory disorders including neurosarcoidosis should be considered [3,6]. Herein we report a case of dizziness and ataxia, in which neuroimaging shows an expansive lesion in left middle cerebellar peduncle, with the final diagnosis of diffuse large B-cell lymphoma.

CASE REPORT

A 52-year-old Caucasian woman with no significant past medical history presented with symptoms of dizziness and left motor incoordination for 2 weeks at the Hematology and Hemotherapy Center of the University of Campinas. The initial physical and neurological examination revealed ataxic gait and dysdiadochokinesia. She underwent a brain computed tomography (CT) which revealed an expansile lesion of the posterior fossa. Brain magnetic resonance imaging (MRI) showed a heterogeneous lesion in the left middle cerebellar peduncle, with hyperintensity on T2-weighted and intense enhancement on T1-weighted after contrast (Figures 1A and B). Spectroscopy showed a peak of lipids on 1.3 ppm, without choline elevation and perfusion did not demonstrate an elevation in rCBV (Figure 1C). She was seronegative for HIV 1 and 2, HBsAg and anti-HCV antibodies. The cerebrospinal fluid examination was unremarkable. No additional lesions were seen in a CT of the chest, abdomen, and pelvis, and the bone marrow biopsy showed no evidence of malignancy. The differential diagnoses included inflammatory demyelinating and infectious conditions versus lymphoproliferative malignancies, as the lesion had low perfusion and peak of lipid, features observed in both conditions.

The patient underwent an open biopsy of the tumor and resection; histological examination showed diffusely infiltrative large cells with scant cytoplasm, evident nucleoli, and frequent mitotic figures. Immunohistochemical profiling revealed a diffuse large B cell lymphoma, non-germinal center (Figure 2). Bone marrow involvement was negative. The diagnosis of stage IE (Ann Arbor staging system) DLBCL of the cerebellum was established.

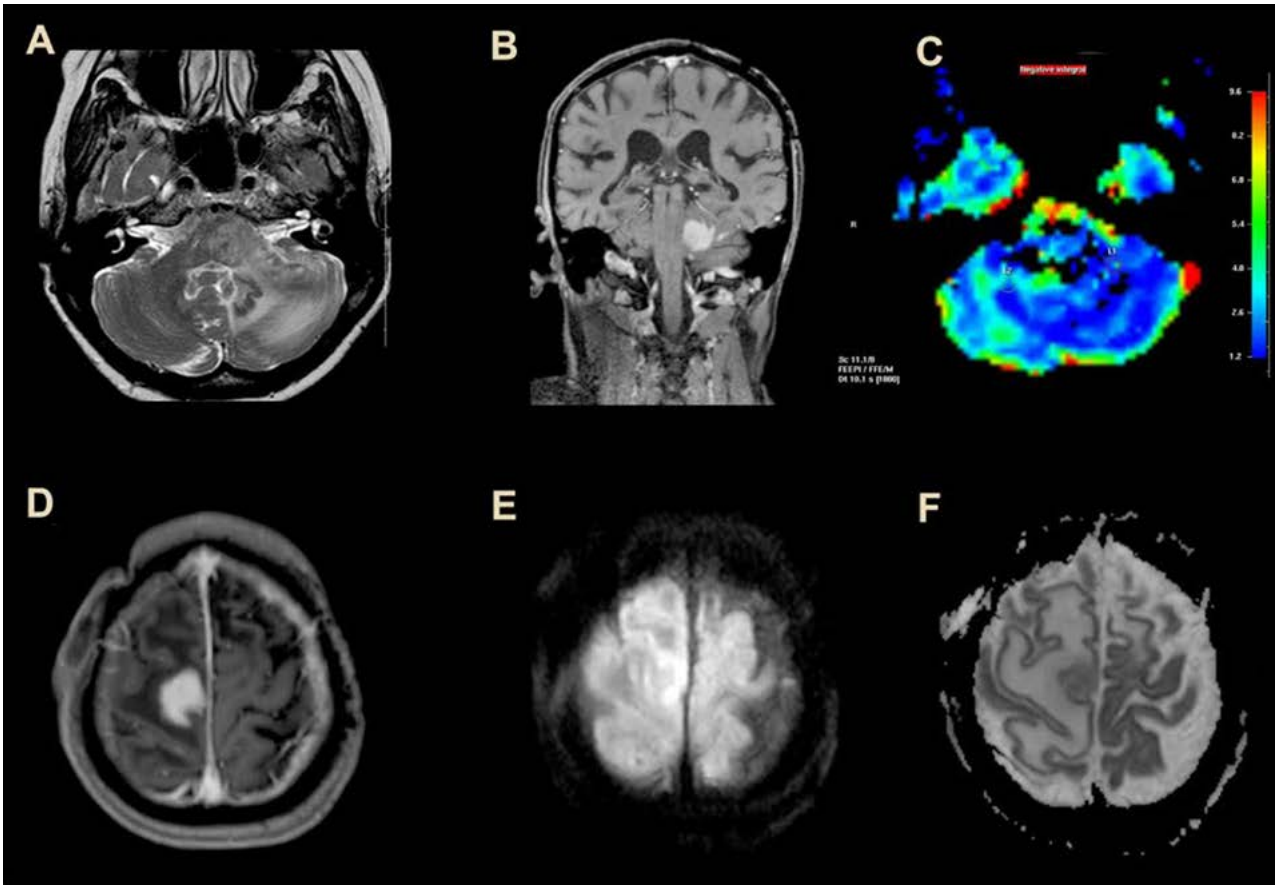

FIGURE 1

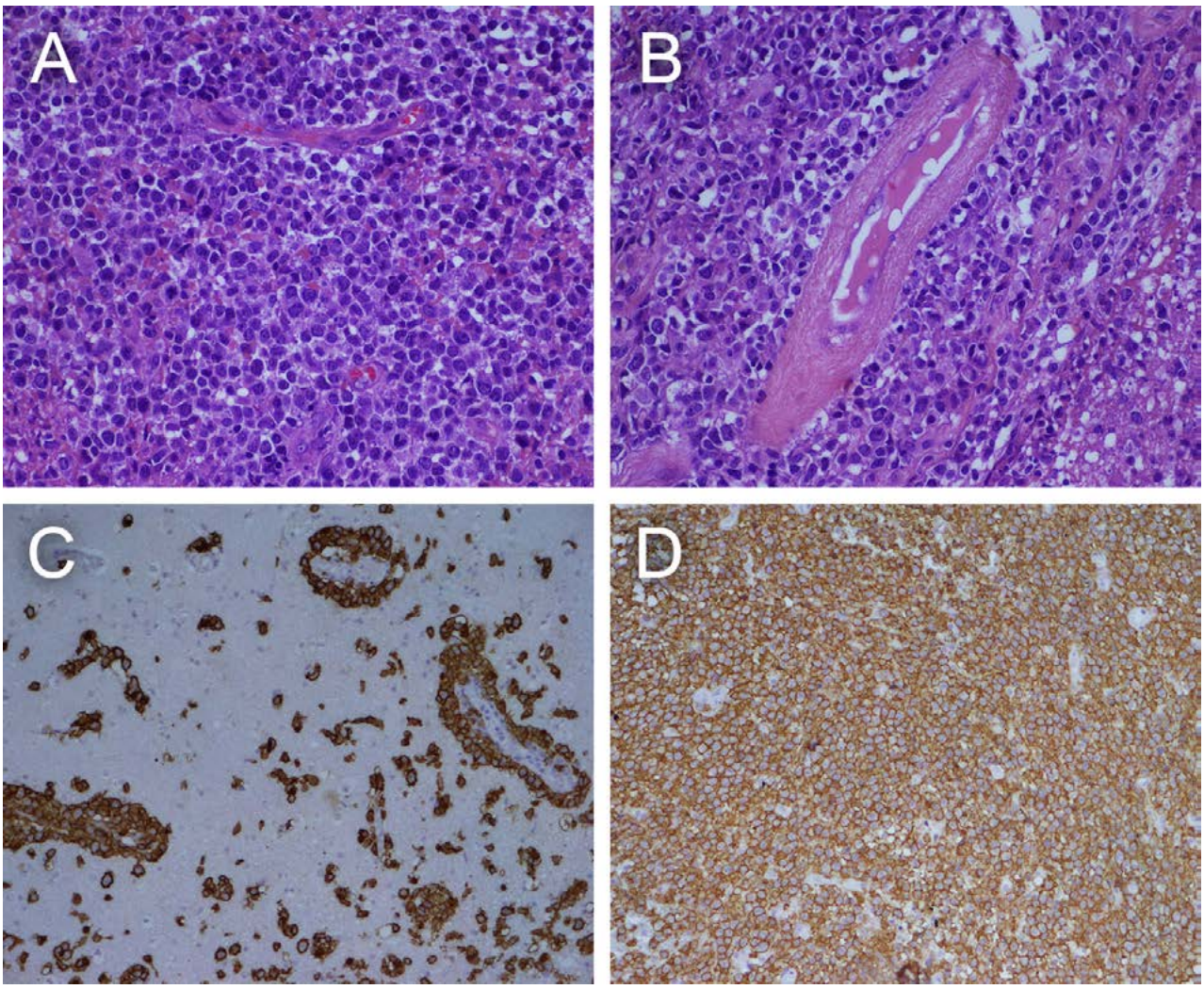

FIGURE 2

Intravenous dexamethasone was initiated, and the patient had symptoms remission. After that, the patient received four cycles of high-dose methotrexate and cytarabine (IELSG 20 Protocol) [7]. During the treatment, she experienced hematologic toxicity grade 3 and 4, febrile neutropenia and received antibiotic therapy. The patient had improvement after the fourth cycle of chemotherapy. MRI was used for control and presented complete remission of the initial tumor. Cranial radiotherapy was not performed. She remained with motor sequels, left hemiparesis and reduced muscle strength. After 3 months, she returned with decreased consciences level, sleepily, two episodes of seizures and difficulty in swallowing. A new MRI showed homogeneous nodular lesion in the right superior frontal gyrus (Figure 1D) with restricted diffusion (Figures 1E and F) and a peak of lipids on spectroscopy; the aspects were suggestive of lymphoma in the frontal convexity, but a new biopsy was not performed. The patient died after five days.

DISCUSSION

PCNSL is rare among intracranial neoplasms (only 3%), with a yearly incidence of 0,5 case per 100.000 people [2,3]. Only a small proportion (13%) is located in the infratentorial brain, of which 9% affect the cerebellum [2,3,8], with the involvement of the middle cerebellar peduncle being even rarer. In immunocompetent patients, as the case described, the lymphoma mostly affects those in the fifth decade of life [9].

The involvement of the cerebellum and other deep structures (periventricular regions, basal ganglia, and brainstem) is considered by some prognostic scores as independent image criteria associated with poor outcome and reduced survival [10], which explains the importance of the site of the lesion.

As for neuroimaging, PCNSL typically presents as an iso to hypointense lesion on T2-weighted, in some cases with perilesional edema. Diffusion restriction is observed in the solid components of the lesion (due to high cellularity), and intense and homogeneous

enhancement is frequently found [3]. Central necrosis is not usually found in immunocompetent patients, it is more common in immunocompromised [6].

The pattern of central nervous system lymphomas on spectroscopy is similar to that of other high-grade tumors, and there is usually increased choline, reduced myoinositol and a peak of lipids and lactate in the solid portion on spectroscopy. In this case, there was no choline elevation, and only lipids were observed in the spectra.

Neuroimaging is very important to suggest, through certain characteristics as described, the nature of the injury. However, in some atypical cases as this, some differential diagnosis such as metastatic lesions, gliomas, and inflammatory processes, particularly granulomatous disease, should be considered.

In the case of our patient, the first MRI showed an infiltrative lesion in the left middle cerebellar peduncle, characterized by a heterogeneous signal on T2 and extension to adjacent cerebellar deep nuclei (Figure 1A and 1B). In the sequences of perfusion, hypoperfusion (low rCBV) was noted (Figure 1D), suggesting that the main differential diagnosis could be lymphoma, inflammatory and demyelinating processes (common etiology of middle cerebellar peduncle). Low perfusion in PCNSL is observed due to the low induction of neovascularization and destruction of the elements of the blood-brain barrier [4].

Neurosarcoidosis, infectious granulomatous diseases (such as tuberculosis and paracoccidioidomycosis) and vasculitis should be considered in these lesions especially if it shows enhancement [11]. Granulomatous disease lesions can be of single/multiple iso or hypointense on T1-weighted and hypointense on T2-weighted, usually with low perfusion. Vasculitis and neurosarcoidosis usually involve younger patients, and infratentorial gliomas are more frequent in pediatric patients. Metastases can occur in this region and in this age group, usually iso or hyporintenseon T1-weighted and hyperintense on T2-weighted and FLAIR (hypointense if the primary neoplasm is from the gastrointestinal tract). However, they are hyperperfused on rCBV. Demyelination diseases are characterized by a hyperintense signal in T2-weighted and FLAIR, showing low perfusion when compared to lymphoma, and usually occurs in younger patients.

Due to the atypical MRI pattern suggesting an etiology, a biopsy was performed, and the diagnosis of DLBCL was established.

The patient underwent 4 cycles of high-dose methotrexate and cytarabine (IELSG 20 protocol) [7] and obtained complete remission, as previously reported. She was not submitted to cranial radiotherapy. Later, another lesion with typical lymphoma features was diagnosed in the supratentorial brain.

Infratentorial DLBCL is a rare entity, and may also be misdiagnosed in immunocompetent patients, in the context of atypical MRI lesions.

The prognosis is poor, and there is scarce knowledge about its cause and optimal clinical management.

RESUMO

Descrevemos o caso de uma paciente do sexo feminino, de 52 anos, apresentando história de tontura e perda da coordenação motora do lado esquerdo há duas semanas. A RM (ressonância magnética) de crânio revelou uma lesão hiperintensa nas imagens ponderadas em T2, sem restrição à difusão, localizada no pedúnculo cerebelar médio esquerdo. A espectroscopia demonstrou pico de lipídeos, sem elevação do volume sanguíneo cerebral relativo (rCBV) à perfusão. A paciente foi submetida à biópsia a céu aberto, estabelecendo o diagnóstico de linfoma difuso de grandes células B (DLBCL). Houve remissão dos sintomas após o início do tratamento com dexametasona endovenosa, seguida de quatro ciclos de metotrexato associado à citarabina. Após três meses, a paciente retornou apresentando rebaixamento do nível de consciência, e a RM de crânio revelou uma nova lesão de origem linfomatosa no giro frontal superior direito. A paciente faleceu após cinco dias.

PALAVRAS-CHAVE: *Cerebelo. Linfoma difuso de grandes células B. Espectroscopia de ressonância magnética. Pedúnculo cerebelar médio.*

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ORIGINAL ARTICLE

Determination of Mirabegron in rat plasma by UPLC–MS/MS after oral and intravenous administration

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SUMMARY

Mirabegron is a kind of β3 adrenergic receptor agonist which is an effective drug for the treatment of overactive bladder. In this research, a UPLC–MS/MS method is developed and validated for the study of mirabegron pharmacokinetic in rats. A protein precipitation method is applied for sample preparation with acetonitrile. m/z 397.3→379.6, m/z 326.4→121.0 for mirabegron, tolterodine (IS), respectively in the positive ion mode was performed for quantitation. The method is reliable and reproducible in our study (intra-day precision≤11.06%, inter-day precision≤11.43%) with concentration curves linear from 5 to 2500 ng/mL(R2>0.999). Stability studies demonstrated that mirabegron was stable under a variety of storage conditions. This method was successfully applied for determining mirabegron in rats after oral and intravenous administration.

KEYWORDS: *Urinary bladder, overactive. Adrenergic beta-3 receptor agonists. Plasma. Administration, intravenous. Administration, oral. Rats.*

INTRODUCTION

Overactive bladder (OAB) is a kind of syndrome characterized by symptoms of urinary urgency, frequency, nocturia and urge incontinence[1-3]. It is estimated that in 2018 there were 546 million people suffering from OAB worldwide. Irwin et al.[4] revealed that the prevalence of OAB worldwide is estimated as being greater in women than in men in 2008 (11.6% vs. 9.7%, respectively), 2013 (11.7% vs. 9.8%) and 2018 (11.9% vs. 10.0%) (Irwin and others 2011).

Mirabegron, namely (2-(2-amino-1,3-thiazol-4-yl)-N-[4-(2-{[(2R)-2-hydroxy-2-phenylethyl]amino} ethyl)phenyl]acetamide), trade name Myrbetriq meer-bet-trick in the US and Betmiga in Europe, is a novel, effective and highly selective β3 receptor agonists for the treatment of OAB[5,6]. It was approved in the United States in July 2012[7]. Astellas Pharma Inc. found it is an agonist of the human β3-adrenoceptor[1]. The activation of the β3 adrenergic receptor in the detrusor can relax the muscle in the bladder and increase bladder capacity. Takusagawa et al.[8] suggested that CYP3A4 and CYP2D6 are involved in the oxidative metabolism of mirabegron in vitro, although Sawamoto et al.[9] indicated that these isozymes play a limited role in vivo. Before further pharmacological and pharmacokinetic research is fully possible, it is necessary to first develop an an-

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alytical method for the effective determination of Mirabegron in biological fluids.

A literature survey reveals that several pieces of literature reported the analytical methods for the determination of this β3 adrenoceptor agonist mirabegron in biological fluids have been established. Raymond van Teijlingen et al.[10] developed and validated a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for determining mirabegron and its metabolites. Zhou et al.[11] was the only group using Liquid Chromatographic (LC) to Separate Mirabegron Enantiomers on a Chiralpak AY-H Column. However, no piece of literature focused on the ultra performance liquid chromatography-tandem mass spectrometry (UPLC–MS/MS) method for determining mirabegron in biological fluids. Compared with LC and LC-MS/MS, UPLC–MS/MS shows a dramatic enhancement in speed, sensitivity, selectivity, robustness, resolution as well as sample throughput.

In the present study, the development of a sensitive and rapid method for estimating mirabegron in plasma was achieved and successfully applied to the pharmacokinetic study of mirabegron after oral and intravenous administration. Acetonitrile precipitation was applied in our work. Separation and quantitation of mirabegron in plasma samples were performed with UPLC-MS/MS. The column was packed with C18 particles of 1.7 µm, which contribute to higher column performance, efficient separation and short analysis time. The total run time for each sample was shorter than 3min (2.5min). Our method behaved faster and more sensitively compared with that of previous studies[10,11].

EXPERIMENTAL PROCEDURE

Chemicals

Mirabegron (Cat: BD 256310, Purity>99%) was obtained from Toronto Research Chemicals, Inc. (Toronto, Ontario, Canada). Tolterodine (internal standard, IS) (Cat: CLS-BD17869, Purity>95%) was purchased from J&K Scientific Ltd. (Beijing, China). Acetonitrile was obtained in HPLC grade from Merck KGaA (Darmstadt, Germany), as well as methanol. Formic acid, obtained from Tedia Company (Cincinnati, OH, USA) was HPLC-grade. Millipore Milli-Q purification system (Bedford, MA, USA) was used to prepare ultra-pure water. All other reagents used in the whole experiment were of analytical or HPLC grade.

Ultra performance liquid chromatography and tandem mass spectrometry conditions

UPLC-MS/MS with ACQUITY UPLC H-Class and XEVO TQD triple quadrupole mass spectrometer (Waters Corp., Milford, MA, USA) equipped with electrospray ionization (ESI) interface were used to analyze mirabegron and tolterodine. Data were processed using Masslynx 4.1 (Waters Corp., Milford, MA, USA) software. Water (containing 0.1% formic acid) was chosen as the aqueous portion of the mobile phase, and acetonitrile was selected as the organic phase in our study. Separation was achieved in 2.5 min by gradient elution using a UPLC® BEH C18 column (2.1mm × 50 mm, 1.7 µm). The column was kept at 40°C with a flow rate of 0.35 mL/min. The injection volume was of 2 µL. Elution was in a linear gradient. In the progress of elution, acetonitrile content was changed from 45 to 95% between 0.3 and 1.3 min. Organic phase acetonitrile was maintained at 95% for 1 min. Then in the next 0.2 min, it was decreased to 45%. The positive ionization mode was used for compound ionization with nitrogen (flow rate of 800 L/h, cone gas of 50 L/h) as the desolvation gas. Argon was used as the collision gas. Table 1 showed the main working parameters of the mass spectrometer. The MRM transitions were m/z 397.3→379.6 and m/z 326.4→121.0 for Mirabegron and IS, respectively (Fig.1).

Standard solutions, quality control (QC) sample, and calibration standards

A total of 10 mg Mirabegron and tolterodine were dissolved respectively in methanol to obtain a concentration of 2.00 mg/mL and 1.0mg/mL standard stock solution. The stock solutions of the standards were further diluted in methanol to produce combined standard working solutions at a series of concentrations. The concentrations of Mirabegron QC samples in rat plasma were 25, 500, 2000 ng/mL. The IS working solution was diluted from the 1.00 mg/mL tolterodine stock solution to make the final concentration at 250 ng /mL. The lower limit of quantitation (LLOQ) was defined as the lowest concentration on the calibration curves. The LOD was defined as a signal/noise ratio > 3. The concentration levels of plasma calibration standards were 5, 10, 50, 100, 250, 500, 1000, and 2500 ng/mL. They were prepared by spiking 10 µL working standard solutions into 90µL blank plasma (vortexed for 30 s). All stock solutions were kept at −40 °C until to use.

TABLE 1. MASS-TO-CHARGE (M/Z) VALUES FOR PROTONATED MIRABEGRON AND TOLTERODINE OBTAINED BY ESI+ AND MASS TRANSITIONS USED FOR QUANTIFICATION IN THE MRM MODE.

Drug	[M+H]+	Mass transition	Capillary	Cone voltage	Collision
	(m/z)	(m/z to m/z)	(KV)	(V)	energy
Mirabegron	397.34	397.34→379.67	0.7	35	15
Tolterodine	326.47	326.47→121.07	1	50	30

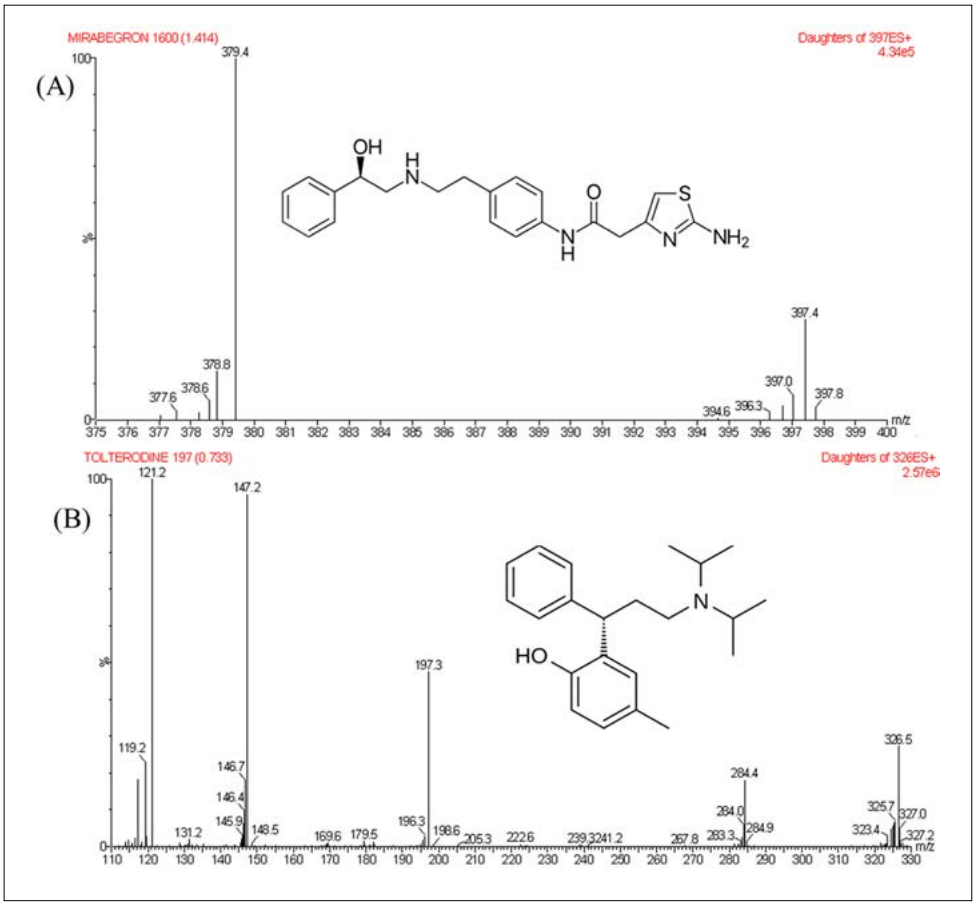

FIGURE 1. The mass spectrum and chemical structures of Mirabegron and IS in the present study: (A) Mirabegron; (B) Tolterodine (IS).

Sample preparation

Deproteinization using acetonitrile was applied as a reliable and straightforward technique for sample preparation. All frozen plasma samples were thawed and vortex-mixed before analysis. Plasma 100 µL followed by 30µL IS (50 ng /mL) and 200 µL acetonitrile were added into fresh 1.5 mL clean EP tubes. The tubes were vortexed thoroughly for 2.0 min to mix well and then spun in a centrifuge at 13000 rpm for 10 min. The upper organic phase 100 µL was carefully transferred into 0.5mL clean glass tubes and was diluted with an equal volume of water. Then 2 µL supernatant was put for analysis by UPLC–MS/MS system after vortex mixed.

Method validation

According to the Guidance for Industry, Bioanalytical Method Validation of the Food and Drug Administration (USFDA)[12] and European Medical Agency (EMA) guidelines[13] of bioanalytical method validation, selectivity, linearity, precision, accuracy, recovery, stability and matrix effect were the items of validation.

Mixed blank plasma from ten rats was used for the evaluation of the selectivity of the method towards endogenous plasma matrix. The chromatograms of a blank sample, a blank plasma sample spiked with Mirabegron and IS, and a rat plasma sample 15 min after oral administration of a single dosage of 20.0 mg/kg Mirabegron are shown in Fig.2.

To evaluate the linearity, concentrations with seven points of Mirabegron were generated using the analyte to IS peak area ratios by weighted ($1/x^2$) least squares linear regression on three consecutive days.

The precision and accuracy of the method were assessed by determining QC samples in rat plasma at different concentrations (25, 500, 2000 ng/mL for Mirabegron in rat plasma) on three separate days. Precision was expressed by the intra- and inter-day relative standard deviation (RSD), required to be less than 15%. Accuracy was expressed as the relative error (RE = measured value/true value-1) with an acceptance criterion of±15% for all QC samples.

Peak area ratios of Mirabegron from plasma samples spiked with a known concentration of unextracted samples and the extraction of the blank plasma at the same concentration at three QC levels (25ng/mL, 500ng/mL, 2000ng/mL) were used as the percent extraction recoveries.

Stability was examined in all matrices at 3 QC concentrations (n=5) under different conditions: at room temperature for 12 h, in the autosampler at room temperature for 12 h, on storage at −40 °C for 30 days, and through three complete freeze-thaw cycles. All of the stability testing QC samples were determined by using the calibration curve of freshly prepared standard samples.

The matrix effect (ME) was evaluated by comparing the ratio (A/B *100%) of peak response of Mirabegron containing an equivalent amount both extracting from blank plasma (A) and dissolved in pure standard solution (B).

Application to a pharmacokinetic study

The pharmacokinetic study was carried out in ten healthy male Sprague-Dawley rats (250 ± 20 g) obtained from the Laboratory Animal Center of Wenzhou Medical University (Wenzhou, China). All ani-

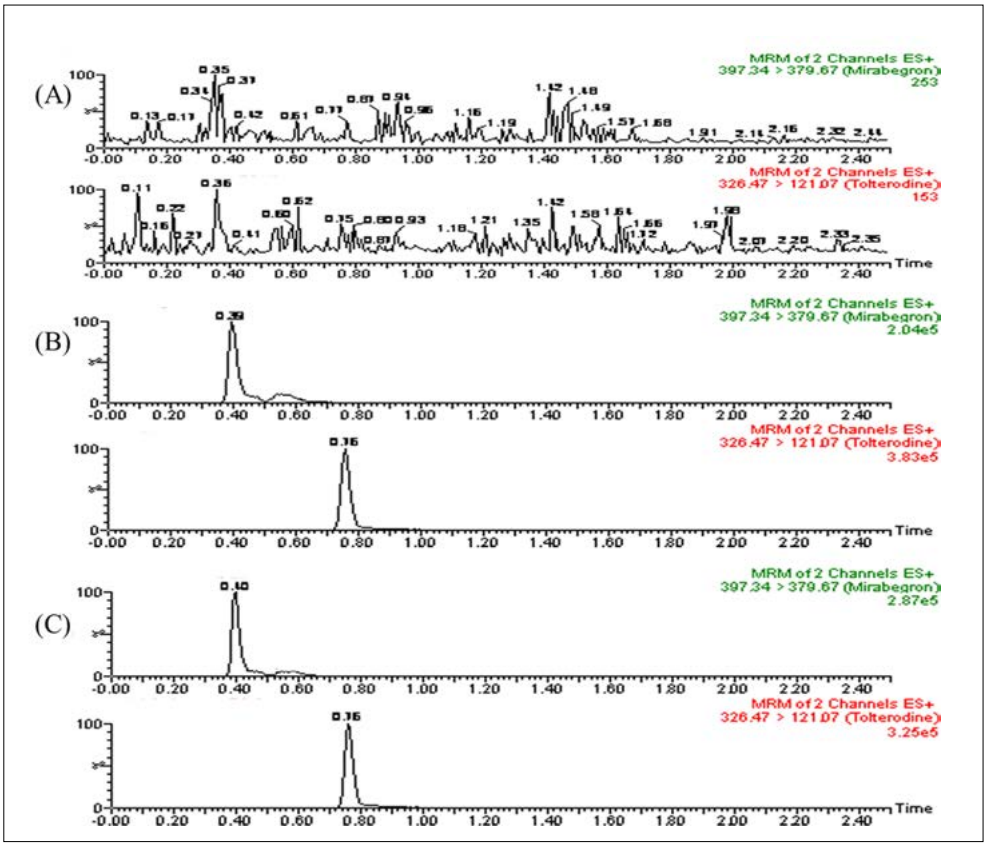

FIGURE 2. Representative chromatograms of Mirabegron and IS in rat plasma samples. (A) a blank plasma sample; (B) a blank plasma sample spiked with 50 ng/ml Mirabegron and IS; (C) a rat plasma sample 15 min after oral administration of a single dosage of 20.0 mg/kg Mirabegron.

TABLE 2. PRECISION, ACCURACY, AND RECOVERY FOR MIRABEGRON OF THE QUALITY CONTROL SAMPLE IN RAT PLASMA (N = 6).

Analyte	Concentration(ng/mL)	Found(ng/mL)	CV(%)		Accuracy RE(%)	Recovery(%)
			Intra-Day	Inter-Day		
Mirabegron	25	23.8573±2.75	11.06	11.43	-4.61	84.95
	500	495.50±14.71	2.98	3.83	-0.90	92.81
	2000	2001.82±12.54	0.97	0.81	0.09	93.26

mals were housed in an environmentally controlled room which was maintained at a temperature of 20 ± 5°C and a relative humidity of 50-60%, with natural light-dark cycles. The rats were allowed to adapt to these conditions for at least one week. All experimental procedures and protocols were reviewed and approved by the Animal Care and Use Committee of Wenzhou Medical University and followed the Guide for the Care and Use of Laboratory Animals. All rats were randomly divided into two groups (n=5 per group): Group A (oral administration of mirabegron at 20 mg/kg), Group B (intravenous administration of mirabegron at 10 mg/kg). Rats fasted for at least 12 h before mirabegron administration, but water was freely available. The blood samples(0.5 ml) were collected into heparinized tubes from the tail vein at 0, 0.083, 0.167,0.333, 0.5, 1, 2, 3, 4, 6, 8, 12 and 24h after oral or intravenous administration of mirabegron (dissolved in normal saline, homogenized at 36°C for 30 min). The samples collected were then centrifuged at 13000 rpm for 10 min immediately. Plasma samples separated from whole blood were transferred into 0.5 mL clean centrifuge tubes as soon as possible and stored at -40 °C until analysis. The pharmacokinetic parameters were evaluated by non-compartmental modeling using DAS version 3.0 (Bontz Inc., Beijing, China).

RESULTS AND DISCUSSION

Method development and optimization

Different columns, such as UPLC® BEH C18 (2.1 mm × 50 mm, 1.7μm), UPLC BEH C18 Column(2.1 mm × 100 mm, 1.7 μm), UPLC® BEH HILIC (2.1 mm × 50 mm, 1.7 μm) were compared for chromatographic separation. The UPLC® BEH C18 (2.1mm × 50 mm, 1.7 μm) column demonstrated satisfactory chromatographic results with minimal matrix effects and proper retention time for mirabegron and tolterodine over other columns. The mobile phase played a critical role in achieving good chromatographic behavior and appropriate ionization[14,15]. To produce the best response, sensitivity, separation efficiency, and appropriate ionization, several mobile phase systems were tested to identify the optimal mobile phase. The mobile phase systems of acetonitrile-water and methanol-water at various ratios were tested, and different buffers including formic acid, acetic acid, and ammonium acetate were evaluated. Finally, a mobile phase of acetonitrile and formic acid (0.1%) in water was selected as the best solvent mixture. Various compounds (oxybutynin, tolterodine, solifenacin, midazolam) were tested to decide on a suitable IS which gave satisfactory validation results of UPLC quantification. Finally, we select tolterodine as IS for its proper retention time and favorable peak shape at the optional condition. Multiple reaction monitoring (MRM) mode was used as MS method for quantification of mirabegron and IS, and the electrospray ionization (ESI) source was operated in both positive and negative modes for ion detection. The ion source parameters were optimized to get a proper response. Solid-phase extraction and Liquid-liquid extraction are effective means to improve the sensitivity and robustness of assays[16,17]. However, both of them are time-consuming and expensive and may result in environmental pollution. In our work, we offered sample preparation with a simple protein precipitation method of plasma protein by acetonitrile, which can reduce the sample preparation time when compared with that of solid-phase extraction or liquid-liquid extraction. The whole separation of the analyte and IS was completed within only 2.5 min per sample, which was much quicker than that of previously proposed methods. Mirabegron and IS were eluted at about 0.39 and 0.76 min, respectively.

Calibration curve and sensitivity

The standard calibration curves for mirabegron in rat plasma exhibited excellent linearity over the concentration range 5-2500 ng/mL (r^2> 0.999) using weighted least square linear regression analysis with a weight factor of $1/x^2$. A typical equation of the calibration curve is: y= 0.000220848 x + 0.00239281, r = 0.999942, r^2 = 0.999884, where y represents the ratios of mirabegron peak area to that of IS and x represents the plasma concentration. Fig.2 showed the representative chromatograms of mirabegron and IS in the rat plasma sample. No endogenous interference at the retention time of analytes and the IS was observed. The LLOQ and LOD for determining mirabegron in plasma was 5 ng/mL and 1 ng/mL, respectively.

Precision, accuracy, recovery, and matrix effect

Assay performance data were presented in Table 2. Intra-day precision was 11.06% or less, and the inter-day precision was 11.43% or less at three levels

of mirabegron. The accuracy of the method ranged from -4.61 to 0.09 at each QC level. The mean recoveries of mirabegron extracted from plasma were 84.95%, 92.81%, 93.26% at concentrations of 25, 500 and 2000 ng/mL (Table 2), respectively. The results demonstrated that the values were within the acceptable range and the method was accurate and precise. The matrix effects determined at concentrations of low, medium, high for mirabegron were 89.32% (RSD %, 9.16), 90.23 % (RSD %, 4.11), and 95.41 % (RSD %, 1.60), respectively. The matrix effect for IS (250 ng/ml) was 91.2% with the RSD of 4.55% (n = 6). As a result, the matrix effect from plasma was negligible in this method. These results indicated that the method was reproducible.

TABLE 3. SUMMARY OF STABILITY OF MIRABEGRON UNDER VARIOUS STORAGE CONDITIONS (N = 5).

Condition	Concentration(ng/mL)		CV(%)	Accuracy(RE%)
	Nominal	Found		
Ambient,12h	25	23.12±2.76	11.97	-7.51
	500	512.05±17.13	3.34	2.41
	2000	2017.22±22.72	1.13	0.86
Autosample, ambient, 12h	25	23.01±2.33	10.11	-7.95
	500	509.67±19.40	3.81	1.93
	2000	2017.48±15.10	0.75	0.87
Three freeze-thaw	25	22.35±2.87	12.86	-10.59
	500	505.74±18.39	3.64	1.15
	2000	2005.19±16.07	0.80	0.26
−40 °C, 50 days	25	22.94±3.14	13.71	-8.26
	500	505.14±24.29	4.81	1.03
	2000	1988.28±17.01	0.86	-0.59

TABLE 4. THE MAIN PHARMACOKINETIC PARAMETERS AFTER ORAL ADMINISTRATION OF 20.0 MG/KG MIRABEGRON AND INTRAVENOUS ADMINISTRATION OF 10.0 MG/KG MIRABEGRON IN THE PLASMA OF TEN RATSPLASMA.

Parameters	Mirabegron (oral)	Mirabegron (intravenous)
$t_{1/2}$ (h)	3.207±0.415	4.471±0.571
T_{max}	3.000±0.000	0.083±0.000
C_{max} (ng/mL)	829.06±38.245	9643.37±3812.36
$AUC_{0\to t}$ (ng/mL h)	4214.66±1068.33	13776.37±4409.77
$AUC_{0\to\infty}$ (ng/mL h)	4243.65±1081.86	13990.66±4465.37
$MRT_{0\to t}$ (h)	5.234±0.341	3.961±0.789
$MRT_{0\to\infty}$ (h)	5.389±0.376	4.378±0.882
CLz/F (L/h/kg)	4.99±1.367	0.774±0.237

STABILITY

All stability studies of mirabegron in rat plasma were conducted at three concentration levels (25, 500 and 2000 ng/mL). The results of stability tests of mirabegron in rat plasma are summarized in Table 3 and are well within the acceptable limit. Mirabegron was demonstrated to be stable after being placed at room temperature for 12 h, in the autosampler at room temperature for 12 h, stored at −40 °C for 30 days, and after three complete freeze-thaw cycles. Moreover, the established method was suitable for the pharmacokinetic study.

Application of the method in a pharmacokinetic study

The pharmacokinetic study dosing of mirabegron (dissolved in normal saline) via oral and intravenous administration was performed successfully to validate UPLC-MS/MS method. The plasma concentration-time curves of mirabegron are shown in Fig.3. A non-compartmental model was applied to calculate the following pharmacokinetic parameters: t1/2, Tmax, Cmax, AUC, CL. All parameters were summarized in Table 4. It was found that oral administration of mirabegron had a much lower Cmax(829.06 ± 38.245ng/mL) , AUC0-t (4214.66 ± 1068.33 ng/mL h) , AUC0→∞ (4243.65 ± 1081.86 ng/mL h) than that of intravenous administration (9643.37 ± 3812.36 ng/mL , 13776.37 ± 4409.77 ng/mL h and 13990.66 ± 4465.37) in rat plasma. According to the formula to calculate F (%) = AUC oral/AUC intravenous × 100%. The F Value of Oral administration absolute bioavailability is 15.17%. The Tmax, the clearance CL/F of oral mirabegron was 36-folds and 6.45-folds than that of intravenous administration, respectively.

CONCLUSION

A rapid, sensitive and accurate UPLC–MS/MS method for quantifying mirabegron in rats was established for the first time and validated for linearity, accuracy, precision, recovery, and stability.

The validation has proved this method is reproducible, sensitive, and robust. To the best of

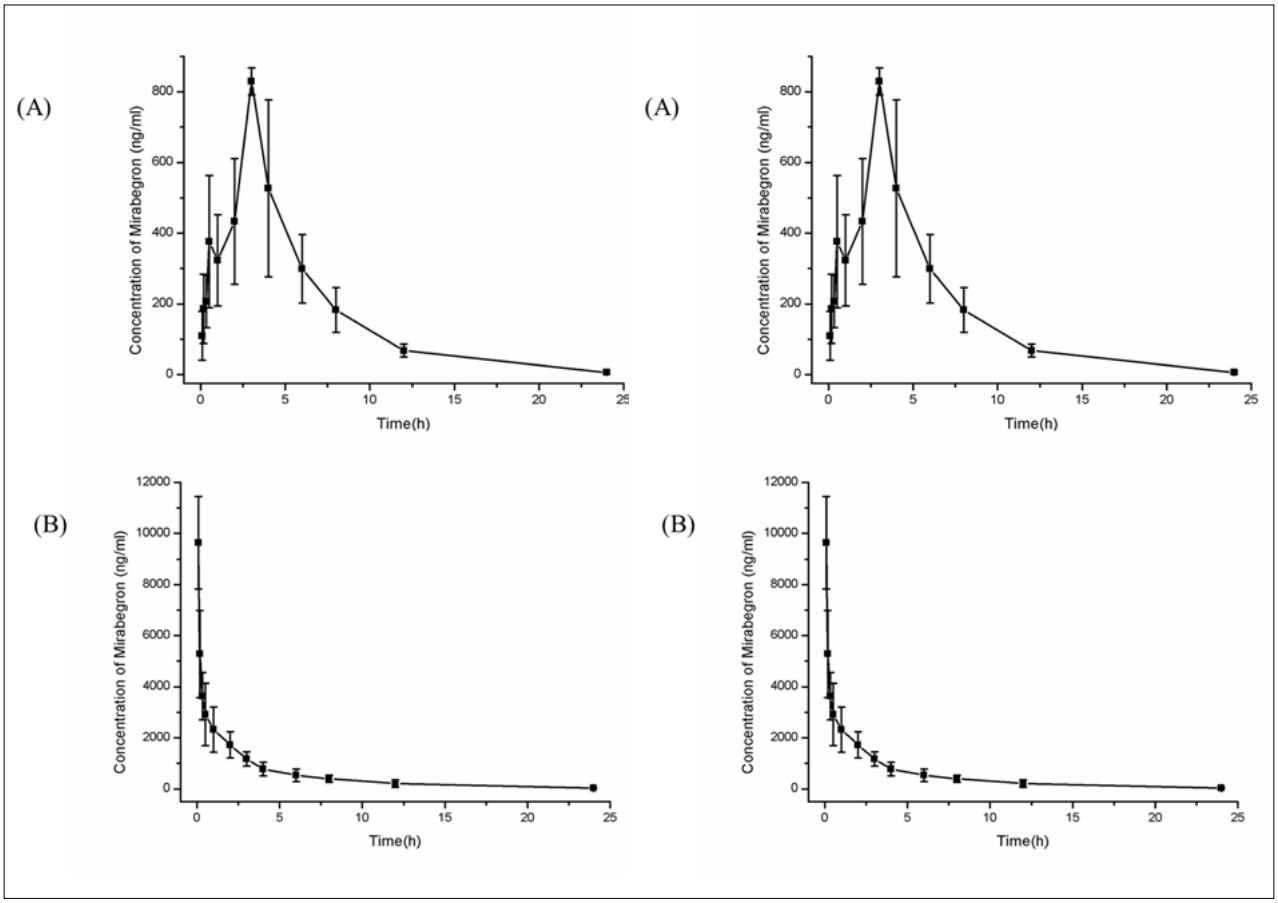

FIGURE 3. Mean plasma concentration time profile after oral administration of 20.0 mg/kg Mirabegron and intravenous administration of 10.0 mg/kg Mirabegron in ten rats.

our knowledge, this is the first report of determining the mirabegron level in rat plasma using a UPLC-MS/MS method. The LLOQ of 5 ng/mL for mirabegron in plasma was achieved, and a simple protein precipitation procedure was developed with average extraction recoveries over 84.95% for each analyte. Compared to other studies, one of the main advantages offered by the method developed is the shorter running time which meets the requirement of high throughput in bioanalysis. To sum up, the validated method has been successfully applied to the pharmacokinetic study of mirabegron in rats, and we suggest it could be applied to human pharmacokinetic studies in the near future.

Conflict of Interest

There is no conflict of interest.

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RESUMO

Mirabegron é um tipo de agonista do receptor adrenérgico beta 3 que demonstra eficácia no tratamento de bexiga hiperativa. Nesta pesquisa, o método UPLC-MS/MS é desenvolvido e validado para o estudo da farmacocinética mirabegron em ratos. Um método de precipitação de proteínas é aplicado para a preparação de amostras com acetonitrilo. 397.3 → 379.6 M / Z, M / Z 326.4 → 121.0 para mirabegron, tolterodina (IS), respectivamente, para o íon positivo foi realizado para quantificação. O método é fiável e reprodutível em nosso estudo (precisão intradia ≤ 11,06%; precisão entredia ≤ 11.43%), com curvas de concentração linear de 5 a 2 ng/ml (R2 > 0,999). Estudos de estabilidade demonstraram que mirabegron permanece estável sob uma variedade de condições de armazenamento. Este método foi aplicado com sucesso para a determinação de mirabegron em ratos após administração oral e intravenosa.

PALAVRAS-CHAVE: *Bexiga urinária hiperativa. Agonistas de receptores adrenérgicos beta 3. Plasma. Administração intravenosa. Administração oral. Ratos.*

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ORIGINAL ARTICLE

Association between sex differences on foot health related to the quality of life in a sample of sedentary people

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SUMMARY

Sedentary (sitting) time may produce many anatomical and physiological consequences which are supposedly associated with a decreased quality of life (QoL) related to foot health. Accordingly, this study aimed to analyze the QoL impact on the overall health and the foot health among male and female sedentary people. A total of 312 participants with an age mean of 39.81 ± 15.40 years completed all phases of the study. In addition, self-reported data were registered. The participants' sedentary lifestyle was determined using the European Prospective Investigation into Cancer and Nutrition (EPIC) physical activity questionnaire.

Furthermore, the scores obtained from the Portuguese version of the Foot Health Status Questionnaire were registered. Sedentary people in the equivalent metabolic energy had 301.09 ± 72.22 (min/week). In the first section, values were higher for foot pain and foot function and lower for general foot health and footwear. In the second section, values were higher for general health and vigor and lower for physical activity and social capacity. The differences between the sex groups of the study were statistically significant for footwear (P = 0.008), physical activity (P= 0.002), social capacity (P = 0.001) and vigor (P = 0.001) showing a worst QoL related to foot health in favor of male subjects in comparison with females. The rest of the domains did not show any statistically significant difference (P ≥ .01). The sedentary population evidenced a negative impact on the QoL related to foot health. This problem may be associated with this lifestyle, especially for males.

KEYWORDS: *Foot. Foot Deformities. Foot Injuries. Quality of life. Sedentary lifestyle.*

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INTRODUCTION

Sedentary (sitting) time may be characterized by a lifestyle with reduced walking or activity, which consequently leads to a sitting, reclining or lying posture and decreased energy expenditure (≤1.5 metabolic equivalent of task)[1]. This condition can have many anatomical and physiological consequences related to impaired health outcomes, leading to the development of anxiety[2], certain types of cancer[3,4] cardiovascular diseases[4,5] cholesterol[6], depression[7], diabetes[8], high blood pressure[9], metabolic syndrome[10], musculoskeletal pain[11], overweight[12], obesity[13], osteoporosis[14], and other mortality causes[15].

Furthermore, the prevalence and severity of sedentary lifestyles in females and males worldwide are high, estimated between 60-85%, with considerable harmful impacts that involve significant public health burdens and an enormous cost for individuals, society, and health care systems[16,17]. However, no prior research has been conducted on the negative health effects on the quality of life (QoL) related to foot health.

Also, there is a high prevalence rate of foot pathologies comprised between 71% and 93% [18] (i.e., cavus foot, flat foot, hallux rigidus, hallux valgus, hyperkeratosis, lesser toe deformities, metatarsalgia, Morton's neuroma, nails disorders, plantar fasciitis and tailor's bunions)[18-2], which could be associated to a sedentary lifestyle and a potential factor in predicting loss of overall health.

Based on the foregoing and on the need for assessment, evaluation, and examination of the foot health of sedentary people may be very important. Indeed, disorders and alterations of the foot, painful or postural conditions, gait disturbance, the risk of falls and other basic diseases may be considered key factors when planning assistance and preventive health activities to improve QoL, wellbeing, and autonomy for the sedentary population.

Consequently, the goal of this research was to analyze the impact of the QoL related to foot health among male and female sedentary people. We hypothesized that sedentary people might present a negative impact on the QoL related to foot health.

MATERIALS AND METHODS

Design and Sample

This research consisted of a cross-sectional observational study carried out in a private podiatric medical and surgical center that provides treatment for foot problems in the city of Porto (Portugal) during the period between October 2016 and October 2017. Participants were selected using a non-randomized consecutive sampling method, and their mean age was 39.81 ± 15.40 years. A sample of 312 volunteers was recruited. The exclusion criteria included injury and a history of foot surgery, psychiatric or neurological disorders, diabetes, rheumatoid arthritis, chronic joint pathology, immunocompromised or lack of partial independence in daily activities, refusal to sign a consent form, the inability to follow the instructions necessary to carry out the present study, as well as patients of other nationalities (non-Portuguese) who did not master Portuguese.

Procedure

Initially, participants were enrolled and interviewed about overall health, sociodemographic characteristics (age and sex) and presence of relevant factors such as arthritis, diabetes, obesity, vascular disease, osteoarticular disorders or participation in sports.

A single trainer researcher conducted a standardized clinical exam. Then, we measured the subject's demographic data, such as height and weight as well as determined the body mass index (BMI), from the height (m), and weight (kg^2), applying Quetelet's equation ($BMI=weight / height^2$). [21]

Next, the voluntary completed the Portuguese version of the European Prospective Investigation into Cancer and Nutrition (EPIC) Physical Activity Questionnaire[22]. This tool presented a higher construct of the degree, content and validity (Spearman correlation coefficient, *r*s) for male (*r*s = 0.47 - 0.89= and female (*r*s = 0.49 - 0.81). Also, this tool showed a high retest reliability (*r*s = 0.32 – 0.81 in male and *r*s = 0.28-072 in female)[23]. This self-administered instrument determines the duration of physical activity intensities average (day, week and month) in the three different tasks (professional, domestic and free time) and for five types of activities. The first task assesses rest (sleeping or sitting/lying awake). The second task assesses transport to or from work (walking, motorized vehicle, or other). The third task assesses professional activity (very light, light, moderate and heavy). The fourth task assesses household activities (very light, light and moderate). The final task assesses very light activities (watching TV, playing cards, reading) and exercise (light, moderate and heavy).

Also, each participant was measured using the energy cost of expenditure in the daily activities ap-

plying standard scores of the metabolic energy equivalent multiplied by the time spent in every task (min/day) for seven days (min/week) [24,25].

Subsequently, participants completed the Portuguese version of the Foot Health and Quality of Life (PFHQL) tool that contains two sections[26]. The first section presents thirteen points related to foot pain (four questions), foot function (four questions), footwear (three questions) and general foot health (two questions), differing from other tools that only measure recorded foot pain, function of the foot and disability[27,28].

Also, PFHQL presented appropriate psychometric properties, a higher construct of the degree, content, and validity (Cronbach α= 0.89 – 0.95). In addition, a high retest reliability (intraclass correlation coefficient, ICC= 0.74 – 0.92) was shown[29,30]. The second section of the tool was adapted from the Medical Outcomes Study 36-Item Short-Form Health[29], and presented items related to overall health (general health, physical activity, social capacity, and vigor), which demonstrated to be a valid measurement questionnaire[31]. The FHSQ software (Version 1.03) showed points from zero (poor health status) to one hundred (better health status).

Sample size calculation

The sample size was calculated using the Clinical Epidemiology and Biostatistics Research Group software, Universidade da Coruña (http://www.fisterra.com/mbe/investiga/9muestras/9muestras2.asp)[32]. The calculation was based on the population living in Portugal with a total of 6.979.785 adults on October 05, 2017 (https://www.pordata.pt/DB/Municipios/Ambiente+de+Consulta/Tabela). Considering a 2-tailed test, an α level of 0.05, a desired power analysis of 95 % with a β level of 20%, a precision of 50% (P=0.5) and assuming a loss of participants of 15%, at least 203 participants needed to be studied. Finally, a sample of 312 participants was included in the study.

Ethical considerations

This study was approved by the local Bioethics Committee of the Hospital-Escola da Fundação Fernando Pessoa in Porto, Portugal (registry number: 30/2017). All voluntary participants gave written informed consent before beginning the investigation protocol. Additionally, the research followed international principles for medical research in human experimentation set forth by the World Medical Association in the Declaration of Helsinki.

Statistical analysis

The descriptive analysis related to the variables, which appear in this research, was carried out. The qualitative variable (sex) was described as an absolute value. The quantitative variables were described as mean ± standard deviation (SD), median ± interquartile range (IR) as well as maximum and minimum values (range) for the male, female and total sedentary sample.

A Kolmogorov-Smirnov test was used to examine the normality of the distribution for all variables, and data were considered normally distributed if $P > 0.01$. The Kruskal Wallis test for independent samples was performed to analyze sociodemographic and PFHSQ differences between men and women in the sedentary sample. In all the analyses, $P < .01$ (with a 99% confidence interval) was considered statistically significant.

The IBM SPSS Statistics 19.0 package for windows was to analyze the results. FHSQ (Version 1.03) was used to obtain the QoL scores related to foot health.

RESULTS

The results for Kolmogorov Smirnov showed a normal distribution (P<0.05) for sociodemographic data and PFHSQ scores. Therefore, the non-parametric Kruskal Wallis test was performed.

A total sample of 312 subjects, between 18 and 87 years old with a mean ± SD of 39.81 ± 15.40 years, concluded the investigation pathway. Most participants were of average weight (BMI, 24.75 ± 3.942 kg/m^2) and their metabolic equivalent of tasks showed a sedentary activity (MET, 301.09 ± 72.22 min/week). Descriptive socio-demographic characteristics of the people, stratified by sex, are presented in Table 1.

The findings of the comparison between QoL related to the foot values of sex groups are shown in Table 2. The first specific section for the feet analyzed four domains: 1) pain, 2) function, 3) overall foot health and 4) footwear. The values were higher for foot pain and foot function and lower for general foot health and footwear. The second general section assessed four health domains: 1) overall health, 2) physical function, 3) social capacity, and 4) vitality. The values were higher for general health and vigor and lower for physical activity and social capacity.

The differences between the groups of the study were statistically significant for footwear (P = 0.008), physical activity (P= 0.002), social capacity (P = 0.001), and vigor (P = 0.001) showing a worst QoL

TABLE 1: SOCIO-DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF THE SAMPLE POPULATION

	Total Group Mean ± SD Range Median ±IR N = 312	Male Mean ± SD Range Median ±IR N= 219	Female Mean ± SD Range Median ±IR N= 93	P-Value Male vs. Female*
Age, years	39.81 ± 15.40 (18-87) 39 ± 24.25	38.15 ± 14.72 (18-87) 37 ± 23	43.72 ± 16.31 (18-86) 41 ± 24	0.005
Weight (kg)	68.57 ± 13.11 (40-115) 67.00 ± 19.00	63.88 ± 10.83 (42-98) 62 ±14	79.62 ± 11.31 (56-115) 80 ± 12.50	0.001
Height (m)	1.66 ± 0.85 (1.45-1.92) 1.65 ±0.11	1.62 ± 0.06 (1.45-1.80) 1.63 ± 0.10	1.74 ± 0.07 (1.50-1.92) 1.75 ± 0.10	0.001
BMI (kg/m2)	24.75 ± 3.942 (16.78-39.82) 24.21 ± 4.92	24.15 ± 4.17 (16.78- 39.82) 23.42 ± 4.97	26.17 ± 2.877 (20.61 – 36.43) 25.82 ± 3.59	0.001
METS (min/day)	43.82 ± 10.60 (24.00-80.24) 40.14 ± 15.80	43.20 ± 9.04 (27.99- 80.24) 40.42 ± 10.50	43.20 ± 9.04 (24.00- 69.83) 40.42 ± 10.50	0.583
METS (min/week)	306.76 ± 74.21 (168.00 – 561.73) 281.00 ± 110.66	302.44 ± 63.34 (168.00 – 488.83) 282.98 ± 73.55	302.44 ± 63.34 (168.00 – 488.83) 282.98 ± 73.55	0.582

Abbreviations: BMI, body mass index; METS, metabolic energy equivalent; IR, interquartile range; SD, standard deviation. In all the analyses, P < .01 (with a 99% confidence interval) was considered statistically significant. * Kruskal Wallis test for independent samples was performed.

TABLE 2: COMPARISONS OF FHSQ SCORES TOTAL GROUP AND SEX GROUP

	Total Group Mean ± SD Range Median ±IR N = 312	Male Mean ± SD Range Median ±IR N= 219	Female Mean ± SD Range Median ±IR N= 93	P-Value Male vs. Female*
Foot Pain	78.84 ± 22.80 (0-100) 84.37 ±18.12	77.31 ± 22.82 (0-100) 84.37 ±18.75)	82.46 ± 22.47 (0-100) 90 ± 21.87	0.013
Foot Function	85.92 ± 20.55 (0-100) 93.75 ±18.75	84.73 ± 21.28 (0-100) 93.75 ±18.75	88.71 ± 18.53 (6.25-100) 100 ±18.75	0.104
Footwear	57.16 ± 28.67 (0-100) 58.33 ±41.66)	54.30 ± 28.74 (0-100) 58.33 ±45.83	63.89 ± 27.51 (0-100) 66.66 ±33.33	0.008
General Foot Health	63.68 ± 23.89 (12.50-100) 60.00 ±42.50	62.09 ± 23.92 (12.50-100) 60 ±42.5	67.42 ± 23.51 (12.50-100) 72.5 ±42.5	0.057
General Health	68.46 ± 22.98 (0-100) 70.00 ±40.00	67.26 ± 23.12 (0-100) 70 ±30	71.29 ± 22.52 (10-100) 80 ±30	0.147
Physical Activity	83.51 ± 20.87 (0-100) 91.66 ±22.22	81.61 ± 21.15 (11.11-100) 88.88 ±27.77	87.99 ± 19.58 (0-100) 94.44 ±11.11	0.002
Social Capacity	78.33 ± 21.13 (12.50-100) 81.25 ±37.50	75.34 ± 21.58 (12.50-100) 75 ±37.50	85.35 ± 18.30† (12.50-100) 87.5 ±25	0.001
Vigor	51.66 ± 19.34 (0-100) 50.00 ±31.25	49.32 ± 18.83 (0-100) 50 ±25	57.19 ± 19.50 (12.50-93.75) 56.25 ±31.25	0.001

Abbreviations: FHSQ, Foot Health Status Questionnaire; IR, interquartile range; SD, standard deviation. In all the analyses, P < .01 (with a 99% confidence interval) was considered statistically significant. *Kruskal Wallis test for independent samples was performed.

related to foot health in male subjects. The rest of the domains did not show any statistically significant difference ($P \geq .01$).

DISCUSSION

This study aimed to analyze the QoL impact on the overall and foot health among male and female sedentary people. Foot health may be very important for the overall health, independence, and QoL in the sedentary population.

Regarding a prior case-control study, a sample of 140 active and sedentary participants with mean age of 40 years in Iran were randomly selected and showed that active people presented better QoL than sedentary people[33]. Nevertheless, the QoL associated with foot health was not analyzed in this context in any investigation.

This is the first study to reveal that sedentary individuals present poor QoL values on the dimensions related to the foot. The outcomes of this research highlighted the need for medical and podiatric care in sedentary people. Sedentary people should be advised about the changes that a sedentary lifestyle will bring to their feet. Therefore, podiatric care would help to improve the lifestyle and overall health in this population.

These findings are consistent with a randomized controlled clinical intervention conducted by Campbell et al.[34], which indicated that leg, ankle, and foot are habitually sites of the most pathologies in sedentary individuals.

Furthermore, this investigation revealed a significant increase in PFHSQ scores for foot pain, foot function, general health, vigor and lower scores for general foot health, footwear physical activity, and social capacity in the levels of QoL related with foot health in sedentary people. This is consistent with research that has demonstrated that lifestyle affects the risk of illnesses, symptoms, poorer response to treatment, and a significant increase in the use of medical services[11,35,36]

However, this study presented several limitations that should be acknowledged. Firstly, a new classification related to different lifestyles may help identify if there are other mechanisms involved. Secondly, sub-categories of metabolic energy equivalent would be beneficial to improve the strength of the study. Finally, expanding data collection to other countries would be beneficial to strengthen this study. This highlights the need for further studies that should focus on foot health, lifestyle and QoL of the population.

CONCLUSIONS

The sedentary population evidenced a negative impact on the QoL related to foot health. This problem may be associated with this lifestyle, especially in the males.

DECLARATIONS

Ethics approval and consent to participate

This study was approved by the local Bioethics Committee of the Hospital-Escola da Fundação Fernando Pessoa in Porto, Portugal (registry number: 30/2017). All voluntary participants gave written informed consent before beginning the investigation protocol.

Consent for publication

The authors declare that they consent for publication.

Availability of data and material

The data set supporting the conclusions of this article is available in the daniellopez@udc.es in the Research, Health and Podiatry Unit. Department of Health Sciences. Faculty of Nursing and Podiatry. Universidade da Coruña, Spain.

Competing interests

The authors report no conflicts of interest in this work.

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The authors did not receive any funding for the design, methods, subject recruitment, data collections, analysis and preparation of the paper or have any personal relationships with other people or organizations that could inappropriately influence (bias) their work.

Authors' contributions

All authors: concept, design, analyses, interpretation of data, drafting of the manuscript and revising it critically for important intellectual content.

Authors' information (optional)

None

RESUMO

O tempo sedentário (sentado) pode produzir muitas consequências anatômicas e fisiológicas que supostamente estão associadas a uma redução de qualidade de vida (QoL) relacionada à saúde do pé. Por conseguinte, o objetivo deste estudo foi analisar o impacto da QV sobre a saúde geral e a saúde do pé entre pessoas sedentárias masculinas e femininas. Uma amostra de 312 participantes com idade média de 39,81 ± 15,40 anos completou todas as fases do processo de estudo. Além disso, os dados autorrelatados foram registrados. O comportamento sedentário dos informantes foi determinado usando o questionário de prospecção prospectiva de câncer e nutrição (Epic). Além disso, os resultados obtidos com a versão em português do Questionário de Status de Saúde do Pé (PFHSQ) foram registrados. As pessoas sedentárias no equivalente de energia metabólica apresentaram 301,09 ± 72,22 (min/semana). Na primeira seção, os valores foram maiores para a dor no pé e função do pé e diminuíram a saúde e o calçado do pé geral. Na segunda seção, os valores foram maiores para saúde geral e vigor e menores para atividade física e capacidade social. As diferenças entre os grupos sexuais do estudo foram estatisticamente significativas para o calçado (P = 0,008), atividade física (P = 0,002), capacidade social (P = 0,001) e vigor (P = 0,001), mostrando uma pior QV relacionada à saúde do pé a favor dos sujeitos do sexo masculino em relação aos participantes sedentários femininos. O restante dos domínios não apresentou diferença estatisticamente significante (P ≥ 0,01). A população sedentária evidenciou um impacto negativo na QoL relacionada à saúde dos pés. Esse problema pode estar associado a este comportamento, especialmente no sexo masculino.

PALAVRAS-CHAVE: *Pé. Deformidades do pé. Traumatismos do pé. Qualidade de vida. Estilo de vida sedentário.*

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ORIGINAL ARTICLE

Analgesic effects of a capacitive-resistive monopolar radiofrequency in patients with myofascial chronic neck pain: a pilot randomized controlled trial

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SUMMARY

BACKGROUND: *To date, there is a lack of prior studies on the use of capacitive resistive monopolar radiofrequency (RF) to treat neck pain. The objective of this study was to investigate the immediate effect of capacitive resistive monopolar radiofrequency (RF=448 kHz), in comparison with a placebo, on (1) reducing neck pain intensity at myofascial trigger points (MTrP), (2) decreasing neck disability and (3) improving cervical range of motion (CROM).*

METHODS: *A randomized, double-blind, placebo-controlled trial (NCT02353195) was carried out. Patients with myofascial chronic neck pain (N=24) with active MTrP in one upper trapezius muscle were randomly divided into two groups: a radio-frequency group, which received eight sessions of a monopolar capacitive resistive radio-frequency application over the upper trapezius muscle, and a placebo group (PG), which received eight sessions of placebo radio-frequency over the same muscle. Visual analog scale (VAS), CROM and Neck Disability Index (NDI) were evaluated after the first session and after the eight sessions.*

RESULTS: *The Wilcoxon test for VAS showed statistically significant differences between baseline, immediately after the first session and after eight sessions (p<.001). No significant differences for PG were found. No differences were observed between groups. NDI improved in both groups after eight sessions, but no differences were found between groups (p<.05). ANOVA for time factor showed statistically significant changes in the right cervical rotation in both groups (F=4.112; p=.026) after eight sessions.*

CONCLUSIONS: *Even though there were no differences between both groups, the monopolar capacitive, resistive RF could have a potential effect on pain intensity.*

KEYWORDS: *Myofascial pain syndromes. Neck pain. Range of motion, articular. Catheter ablation/methods.*

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LIST OF ABBREVIATIONS

- Cervical range of motion (CROM)
- Myofascial pain (MP)
- Myofascial trigger points (MTrP)
- Monopolar capacitive resistive radio-frequency (MCRRF)
- Neck disability Index (NDI)
- Neck pain intensity (NPI)
- Placebo group (PG)
- Radio-frequency (RF)
- Radio-frequency group (RFG)
- Visual analog scale (VAS)

INTRODUCTION

Neck pain is currently a major health problem in developed countries[1] and is the leading cause of disability worldwide[2]. Neck pain can lead to negative coping strategies, disability and reduced quality of life, which may, in turn, lead to episodes of depression or anxiety[3]. It affects approximately 50% of people at some point in their lifetimes[4], and its social and economic impact is considerable[5]. Neck pain tends to be chronic, and its etiology is rarely known; therefore, it is typically classified as nonspecific neck pain or mechanical neck pain[6]. In contrast to this disease entity, myofascial pain (MP) is considered a common cause of neck pain. MP is characterized, upon examination, by the presence of myofascial trigger points (MTrP) in skeletal muscles, which consists of hyperirritable areas located in a palpable, taut band of muscle fibers[7,8].

The effectiveness and safety of combined treatments including both conventional and complementary alternative medical treatment modalities have not been fully established. Various treatment modalities are available for myofascial chronic neck pain including electrotherapy, education, injection therapy, acupuncture, and dry needling interventions[9,10].

Patients with neck pain tend to concurrently utilize both conventional treatment and complementary alternative treatment in the hope of achieving more positive effects with a better safety profile. These alternative therapeutic modalities include radio-frequency (RF). RF energy is currently the most commonly used energy source to generate therapeutic levels of heat. Supraphysiologic temperature has been used medically to produce structural and biologic responses in tissue. Although its most common use is as an electrocautery tool, it is possible to use RF in a nonabrasive form by controlling the heating of target tissues to achieve structural and biologic therapeutic effects[11-13]. This constitutes a noninvasive Monopolar Capacitive Resistive Radio-frequency (448 kHz) (MCRRF).

It has been described that the effect of RF on muscle is mediated by the impact on myogenic precursor cells. RF also induces an inflammatory and antinociceptive response[14]. This is possible due to the production of a reverse thermal gradient and capacitive coupling of the energy into a volume of tissue[12,13]. Molecular and cellular mechanisms other than thermal ones have recently been proposed for tissue repair through the local promotion of mesenchymal cells[15].

A pilot study could evaluate the feasibility of recruitment, randomization, retention, assessment procedures, new methods, and implementation of a novel intervention. This pilot study aimed to evaluate the feasibility of MCRRF for the treatment of myofascial chronic neck pain immediately after one session and after eight sessions. To our knowledge, this is the first study that has investigated the MCRRF in myofascial chronic neck pain.

METHODS

Participants

The participants were recruited from the Rehabilitation Area of the Rey Juan Carlos University between October 2013 and June 2014. The inclusion criteria defined patients aged between 18 and 60 years with myofascial chronic neck pain. The area of neck pain was defined as the cervical region, possibly with referred or radiating pain into the occiput, nuchal muscles, shoulders, and upper limbs. Symptoms had to have been present for at least six months. At least one active MTrP in one upper trapezius muscle had to be present. For bilateral neck pain, the most painful side was designated for treatment[9,10]. All of the procedures used in this study were planned according to the ethical principles of the Declaration of Helsinki and were approved by the Ethics Committee of the Rey Juan Carlos University (15/2013), and registered in Clinical Trials.gov (NCT02353195).

Exclusion criteria included severe disorders of the cervical spine, such as spinal stenosis, disk prolapse, postoperative conditions of the neck and shoulder areas, history of severe trauma, whiplash, spasmod-

ic torticollis, instability, migraine, peripheral nerve entrapment, fibromyalgia, shoulder diseases, inflammatory rheumatic diseases, severe psychiatric illness, and pregnancy[9,10].

Twenty-six patients with chronic nonspecific neck pain were initially recruited.

Active MTrP diagnosis criteria

An active MTrP was selected in one upper trapezius muscle following the Simons et al.[16] criteria. The presence of a nodule in a taut band of skeletal muscle, plus the patient's pain recognition under stimulation and the range of motion limitation at full stretch were necessary to diagnose an active MTrP. In such case, there were more than 1 active MTrP in the upper trapezius muscle; the most hyperalgesic MTrP (which produced more pain intensity under the same pressure of stimulation) was selected in order to be treated[17,18]. In addition, the examiners presented the experience and specialization necessary to achieve a good inter-examiner agreement (Kappa coefficient; $\kappa = 0.63$) in order to diagnose an active MTrP in the upper trapezius muscle[19].

Study design

This pilot study was a prospective, randomized, double-blind, and placebo-controlled trial to determine the feasibility of MCRRF (448 kHz) in patients with myofascial chronic neck pain. The Consolidated Standards of Reporting Trials (CONSORT)[20] guidelines were implemented in this paper.

The findings of this pilot study could guide in the design and implementation of a larger scale efficacy study. Those components that are deemed infeasible or unsatisfactory will be modified in the subsequent trial or removed altogether, i.e., side effects, number of dropouts, electrical dose and changes in outcome measures.

Each subject recruited in the study was randomly assigned to the placebo (PG) or radio-frequency group (RFG) using computer-generated random-sequence numbers, with Graph Pad software (Graphpad Software, Inc., La Jolla, CA 92037 USA), and a table was created beforehand to perform the study with a concealed assignment. The randomization process was blocked. The participants were blinded to the treatment. The allocation was concealed, the patients received the assigned intervention in opaque envelopes, the therapist who applied the interventions was blinded to the assessment outcomes until the end of the entire data collection, and drop-out rates were recorded after randomization.

A questionnaire was compiled to obtain baseline information about the participants' demographics and ages, and patients were given a body chart to mark the location of their pain and answered a questionnaire regarding their pain.

Evaluation of pain intensity and cervical range of movement (CROM) were carried out before the start of treatment and after the first and eighth treatment sessions. The neck disability was evaluated before treatment and immediately after eight sessions, and the evaluation sessions were always scheduled at the same time of day. This was a pilot study.

Intervention

A INDIBA® activ 902 equipment was used for MCRRF (448 kHz). A 35-mm diameter CAP and 30-mm diameter RES movable electrode were used on the affected side, and a planar electrode was used as a return electrode on the abdomen. The application was administered in the following manner: cream was applied to the site with the severest pain and its adjacent area (near to the most hyperalgesic MTrP in the upper trapezius muscle), and the electrical dose was increased by moving the movable electrode within the patient´s tolerance level, while monitoring the skin temperature tolerable to the patient.

The PG was treated with the same device in a nonfunctional application (no energy source). The participants were informed that the system did not generate heat.

Therapy was conducted for 12 minutes, two times per week over four weeks (eight sessions in total). Therapeutic efficacy was evaluated regarding improvement in pretreatment symptoms compared to post-treatment symptoms and between-group comparisons. No other therapies that might affect the judgment of therapeutic efficacy were conducted during the treatment. No medication was taken during this study. An experienced therapist administered the treatments.

A blind-to-the-treatment-allocation evaluator recorded the visual analog scale (VAS), CROM and neck disability Index (NDI) measurements pre- and post-treatment.

Outcome measures

Pain intensity was measured using a 100-mm VAS. This scale consists of a 100-mm horizontal

line with pain descriptors ranging from "no pain" marked on the left side to "the worst pain imaginable" on the right side. The perceived pain level of the patients was measured at rest, by marking the VAS with a perpendicular line. This is a valid method to measure pain level[21], and psychometric properties of the VAS have been reported widely[22]. The minimal important difference of the VAS is based on detecting an 8.6-mm difference (based on a previous study for a score < 40 mm with a pain onset of greater than 12 weeks) immediately after treatment[23]. The VAS has been documented in previous studies as having good reliability and validity[24,25].

The NDI was used. NDI is a self-reporting questionnaire used to determine how neck pain affects a patient's daily life. It consists of ten questions in the following domains: Pain Intensity, Personal Care, Lifting, Reading, Headaches, Concentration, Work, Driving, Sleeping, and Recreation. Each question contains six response options, scored from 0 (no disability) to 5 (complete disability). All section scores are then totaled. Scoring is reported on a 0–50 scale (0 being the best possible score and 50 the worst). The NDI has good construct validity. The NDI is seen as a valid tool to measure neck pain and disabilities in patients with neck pain due to acute or chronic conditions, as well as in patients suffering from musculoskeletal dysfunctions, according to its adequate psychometric properties for test-retest reliability (intraclass correlation coefficient of 0.50) and minimum clinically important difference (19-percentage points)[26-28].

CROM was measured with a CROM device (Performance Attainment Associates, Roseville, MN)[29]. The CROM device was placed on the subject's head, and a magnetic collar, also part of the CROM device, was placed on the shoulders to take into account any rotation of the trunk. The collar was always placed in the same position to the magnetic pole; the chair on which the subjects were sitting was kept in the same position for all data collection. The initial position of the head was set to neutral at the zero mark of the inclinometer for flexion, extension and both side flexion. For rotation, the dial was set to zero with the head in the neutral position. When the motion was performed in one direction, the final position was read and recorded for each trial. Subjects moved their heads back to the initial position once the reading was finished. Three trials were executed consecutively in each direction, and the average of the three trials was computed for the analysis. This is a reliable method of measurement[29], providing intra-meter reliability ranging from 0.70 to 0.90 and inter-meter reliability from 0.80 to 0.87.

Data analysis

The statistical analysis was carried out using the SPSS statistical software system (SPSS Inc., Chicago, IL; version 20.0). Normal distribution for the CROM variable was found using the Shapiro-Wilk test, except for VAS and NDI.

The independent t-test and one-way ANOVA were used for analysis of CROM, as well as the subjects' socio-demographic data (age, and pain duration), comparing the baseline data for the two groups. Analyses were performed according to the intention-to-treat principle.

For the CROM variable, two-way repeated measures within-between ANOVA interaction factors were performed; the factors analyzed were group (RFG vs. PG), time (Pre-, Post- and Post-eight sessions), and group x time interaction. Post hoc analysis with Bonferroni corrections was performed in the case of significant ANOVA findings for multiple comparisons between variables.

We used nonparametric statistics for VAS and NDI variables, which were abnormally distributed. Descriptive statistics were used to summarize data, including means and SDs, medians and interquartile ranges (IQR) for continuous data. For comparisons across and between-groups, we used the U Mann-Whitney. The Friedman test was used to analyze the change from the intra-group results, and the Wilcoxon signed-rank test was used for post-hoc intra-group comparisons. Statistical tests were interpreted at the 5% significance level.

RESULTS

Twenty-six patients with myofascial chronic neck pain in the trapezius muscle were screened for possible eligibility criteria, and twenty-four patients successfully completed the study protocol, of which 14 were randomly assigned to the RFG and completed the study protocol (four male, 10 female; mean age ± SD, 31.8 ± 12.4 ys) and 10 were assigned to the PG (six male, four female; mean age ± SD, 42.1 ± 12.6 ys). Figure 1 shows the diagram of recruitment and dropouts. Demographic and clinical characteristics at the beginning of the study are summarized in Ta-

ble 1. There were no significant differences between the two groups regarding demographic and clinical characteristics at baseline. No adverse events were reported in this study.

VAS score

RFG presented statistically significant differences between pain intensity in baseline versus all measurement periods ($p < .001$) but not PG. In comparisons between groups, no differences were found using the U Mann-Whitney test. The results for VAS are presented in Table 2.

Neck disability outcomes

NDI improved in both groups with statistically significant differences after receiving eight sessions of intervention ($p < .05$). In comparisons between groups, no differences at eight sessions were found using the Mann-Whitney U test. The results for neck disability are presented in Table 2.

CROM outcomes

Repeated ANOVA measures showed no effect for time in all cervical range of motions (Flexion F = 2.47; $p = .101$; Extension F = .044; $p = .957$; hp^2=.003; Right lateroflexion F = 2.812; $p = .076$; hp^2 = .158 ; Left lateroflexion F = 1.472; $p = .246$; hp^2= .089; Left Rotation F = .713; $p = .498$; hp^2 = .045), except right rotation (right rotation F = 4.112; $p = .026$; hp^2= .215); time X group interaction was not found (Flexion F = 1.356; $p = .273$; hp^2 = .083; Extension F = .173; $p = .842$; hp^2 = .011; Right lateroflexion F = .198; $p = .821$; hp^2 = .013 ; Left lateroflexion F = 1.406; $p = .261$; hp^2 = .086; Right Rotation F = .066; $p = .936$; hp^2 = .004; Left Rotation F = .425; $p = .658$; hp^2 = .028). The comparison of the results for CROM within groups is presented in Table 3.

DISCUSSION

The purpose of this study was to evaluate MCRRF (448 kHz) for the treatment of neck pain compared to a placebo intervention. Most studies that analyze the effects of RF in pain intensity have not included a placebo group. Therefore, the placebo effect of RF remains unexplored. The results obtained from this study suggest that RF could potentially be used to treat myofascial chronic neck pain in the trapezius muscle.

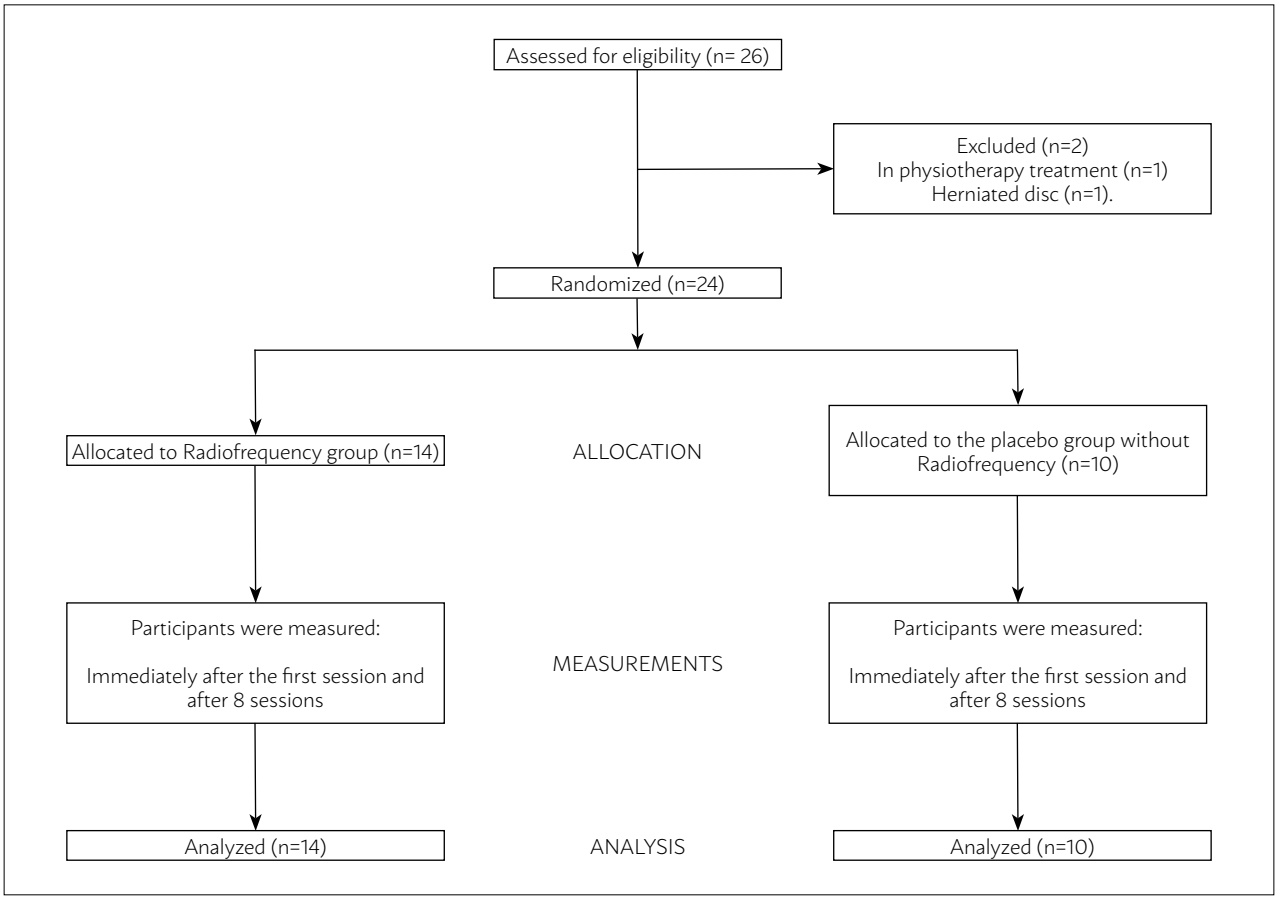

FIGURE 1: FLOW DIAGRAM OF THE STUDY

Neck pain intensity

A significant improvement in pain intensity was observed in the RF group immediately after the first session and after receiving eight sessions.

The magnitude of change was clinically important in the RF group. It has been established that a reduction of 0.85 cm is a minimally detectable change[23], and we initially found 2.9 cm and 4.4 cm after eight sessions on the visual analog scale. The short-term effects showed in this study are slightly less significant than the outcomes found by Llamas-Ramos et al.[30] with a variation of between 4.3 and 5.3 cm immediately and at two weeks during follow-up post-treatment in which dry needling was applied over the trapezius muscle. Nevertheless, Mejuto-Vázquez et al.[31] reported a change between 1.9 cm and 3.7 immediately and one-week post-treatment. However, our results are superior to Seo et al.[32], which treated patients with mean electoral nerve stimulation and botulinum toxin A, although they followed up with the patients for 16 weeks.

Therefore, according to early clinical studies with RF, this technology could have the potential to become an effective therapy for neck pain. However, these differences were not significant between groups. Therefore, further trials should include other types of measures for outcomes in order to assess in-depth the RF effects on pain. In this sense, it could be interesting to include outcome measures such as clinical pressure-pain algometric measurements.

TABLE 1: BASELINE CHARACTERISTICS OF PATIENTS IN BOTH GROUPS

	RFG (n = 14)	PG (n = 10)	Total (n=24)	P value
Age in years	31.8 ± 12.4	42.1 ± 12.6	36.3±13.3	.06
Gender (female/male)	10/4	4/6	14 (58.3)	.12
Neck pain duration (months)	13.35 ± 11.39	12.00 ± 8.64	12.79 ± 10.15	.75
VAS_NPI (0-10 cm)	4.7 ± 1.8	4.9 ± 2.6	4.5 ± 2.03	.34
NDI	10.5 ± 4.6	13.8 ± 6.7	12.45 ± 5.59	.06
CROM FLEX	45.57 ±13.63	43.66 ±2.03	44.90 ±12.74	.80
CROM EXT	63.84 ±10.31	48.77 ±12.50	58.52 ±13.05	.03*
CROM LAT FLEX RIGHT	37.27 ± 8.26	34.00 ± 4.71	36.11 ± 7.22	.76
CROM LAT FLEX LEFT	40.51 ± 6.82	33.11 ± 6.23	37.90 ± 7.38	.69
CROM ROT RIGHT	59.75 ± 13.39	56.66 ± 13.59	58.66 ±13.12	.19
CROM ROT LEFT	62.48 ± 14.69	55.33 ± 18.10	59.96 ±15.80	.10

NOTE. Values are mean ± SD. Abbreviations: RFG= Radio-frequency group; PG=Placebo group; VAS =Visual Analogue Scale; NPI= neck pain intensity; NDI=Neck Disability Index; CROM= Cervical Range of Motion; FLEX=flexion; EXT=Extension; LAT=Lateral.

*The data for botgroups wereas not normally distributed: the Mann-Whitney U test was used.

TABLE 2: NON-PARAMETRIC TESTS OF OUTCOME DATA COMPARISONS WITHIN GROUPS AND BETWEEN GROUNS

		Median (first and third quartiles)			Friedman ANOVA	Wilcox onvalueue) a) Pre vs. First session b) Pre vs. Eight sessions c) First session vs. Eight sessions
Parameter	Group	Basele in	Immediatel after the first session	Immediately after eight sessions		
VAS	RFG	4.9 (2.8 and 6.4)	2.0 (1.0 and 3.0)	0.5 (0 and 2.0)	P=.001*	a) P<.001† b) P<.001† c) P>.05
	PG	5.7 (2.4 and 7.0)	6.0 (1.9 and 7.1)	2.5 (1.1 and 5.5)	P=>.05	a) P>.05 b) P>.05 c) P>.05
Mann-Whitney U test		P=.318	P=.168	P=.104		
NDI	RFG	10.0 (8.0 and 12.0)	__	3.0 (2.0 and 9.0)	P<.05*	b) P<.05
	PG	14.0 (10.3 and 20.5)	__	7.0 (6.0 and 14.5)	P<.05*	b) P<.05
Mann-Whitney U test		P=.060	__	P=.254		

Abbreviations: RFG= Radio-frequency group; PG=Placebo group; VAS=Visual Analogue Scale; NDI=Neck Disability Index.
Within groups analysis: * P<.05 using Friedman ANOVA for k repeated measures; †P<.05 using Wilcoxon test for two related samples. Between groups comparisons: ** P<.05 using Mann-Whitney U test

TABLE 3: CERVICAL RANGE OF MOVEMENT, EXPRESSED IN DEGREES, OVETIME-COMPARISONSNW WITN HITHGROUPSNS

		Baseline	Immediately after the first session	Immediately after eight sessions	ANOVA p-value
Flexion	RFG PG	45.5 ± 13.6 43.6 ± 12.0	52.4 ± 10.8 42.6 ± 11.4	58.0 ± 10.6 45.7 ± 28.0	>.05 >.05
Extension	RFG PG	63.8 ± 10.3 48.7 ± 12.5	64.6 ± 11.8 45.7 ± 14.8	62.9 ± 16.1 48.0 ± 14.0	>.05 >.05
Right Lateroflexion	RFG PG	37.2 ± 8.2 34.0 ± 4.7	40.0 ± 7.5 35.2 ± 6.4	44.4 ± 12.2 38.1 ± 9.8	>.05 >.05
Left Lateroflexion	RFG PG	40.5 ± 26.8 33.1 ± 6.2	43.2 ± 8.2 28.8 ± 9.7	44.1 ± 9.0 34.9 ± 15.5	>.05 >.05
Right Rotation	RFG PG	59.7 ± 13.3 56.6 ± 13.5	60.2 ± 10.9 54.8 ± 12.2	67.8 ± 9.8*† 63.4 ± 12.6*†	.03* .03*
Left Rotation	RFG PG	62.4 ± 14.6 55.3 ± 18.1	62.9 ± 14.3 57.6 ± 11.3	63.5 ± 13.3 62.9 ± 15.0	>.05 >.05

NOTE. Values are mean ± SD. Abbreviations: RFG= Radio-frequency group; PG=Placebo group

*Significantly lower than baseline (P<.05) using two-way repeated-measures ANOVA (Bonferroni adjustment)

†Reached minimal detectable change from pre-intervention (P<0.05)

Neck disability

Both groups decreased from the baseline after eight sessions, but no significant differences were found between groups. As far we know, there are no studies in which radio-frequency has been administered for this condition.

In our study, the RFG achieved a 40% improvement. These changes in neck disability did not exceed the minimal detectable change (7.5 points on the NDI) (28). We also observed a 30% improvement in PG. This placebo effect could be a psychobiological phenomenon that may be attributable to different mechanisms, including the expectation of clinical improvement. Overall, the placebo effect appears to be an excellent model to understand how a complex mental activity, such as expectancy, interacts with different neuronal systems[29].

Cervical range of motion

The administration of MCRRF and placebo did not show a significant improvement in CROM, except for right rotation, but no differences were found between groups. Cervical right rotation showed significant improvement of 8.1º after the eight sessions in the RF group and 6.8º in the placebo group. Cervical right rotation seems to improve 2º more with RFG. The minimal detectable change of 6.1º for right rotation was achieved in both groups, as stated previously [29].

To the best of the authors' knowledge, this is the first time that MCRRF has been investigated on CROM as a treatment for myofascial neck pain, however our results contradict those of previous studies, in which CROM improved after the administration of other therapies, such as trigger point dry needling[30,31] lidocaine injection[30] and transcutaneous electrical nerve stimulation[32-34].

The etiology of MTrP is unknown, but the cascade of mechanisms postulated by Simons describes three features involved in MTrP mechanisms, which are as follows: increased acetylcholine release in the motor endplate, a release of sensitizing substances and increase of the muscle fiber tension[35,36]. Considering the previous hypothesis, one plausible explanation for our results is that MCRRF could produce vasodilation due to the increased temperature, reverting local ischemia and hypoxia and decreasing the release of sensitizing substances and acetylcholine. RF induces the inflammatory cascade necessary to remove hemorrhage remnants and produce an antinociceptive response[11]. In our study, if there were more than 1 active MTrP, the most hyperalgesic active MTrP was selected to receive the intervention[17,18]. However, MCRRF would not reduce sarcomere shortening because the patients in our study did not improve in range of motion; however, they did improve in pain intensity.

Study limitations

The primary limitation of the study is the small sample size. Consequently, the results need to be interpreted with due caution. The statistical analysis has shown no differences between groups after the intervention. However, the differences were ob-

served in the RFG. Further studies have to increase the sample size with the aim to increase statistical power. Second, in cases of bilateral neck pain, we only treated the more painful side, which may bias the results. In addition, the localization of the myofascial trigger points may change along the different sessions of treatment. Third, only one muscle was treated, the treatment of more muscles could modify the outcomes. Fourth, the lack of follow-up is another bias that must be taken into account in future studies. Fifth, we did not take into account psychological variables, and the emotional state may influence outcomes. Finally, the placebo procedure used in this study has not been previously validated, and the results should be interpreted with caution.

CONCLUSION

The Monopolar Capacitive Resistive Radio-frequency could have a potential effect on pain intensity. Neck pain intensity improved immediately after one and eight sessions of Monopolar Capacitive Resistive Radio-frequency (448 kHz) in RF group. Additionally, neck disability and right cervical rotation improved after eight sessions of RF and placebo. However, there were no statistical differences between RF and PG in the outcome measures.

The findings of this pilot study show that RF has no side effects and the electrical dose described in this manuscript could be taken into account in a large study. Further trials should include a follow-up assessment, additional outcome measures, and larger sample sizes.

RESUMO

ANTECEDENTES: *Até a data, há uma falta de estudos prévios para tratar a dor no pescoço por radiofrequência (RF) monopolar capacitiva resistiva. O objetivo deste estudo foi investigar o efeito imediato da radiofrequência monopolar capacitiva resistiva (RF = 448 kHz) versus placebo em (1) redução da intensidade da dor no pescoço em pontos de gatilho miofascial (MTrP), (2) diminuição da incapacidade do pescoço e (3) melhorando a amplitude de movimento cervical (Crom).*

MÉTODOS: *Foi realizado um ensaio randomizado, duplo-cego, controlado por placebo (NCT02353195). Os pacientes com dor no pescoço crônica miofascial (N = 24) com MTrP ativo em um músculo trapézio superior foram divididos aleatoriamente em dois grupos: um grupo de radiofrequência, que recebeu oito sessões com uma aplicação de radiofrequência resistiva capacitiva monopolar sobre o músculo trapézio superior, e um grupo de placebo (PG), que recebeu oito sessões de radiofrequência de placebo no mesmo músculo. A escala analógica visual (VAS), Crom e Índice de incapacidade do pescoço (NDI) foram avaliadas após a primeira sessão e após as oito sessões.*

RESULTADOS: *O teste de Wilcoxon para VAS mostrou diferenças estatisticamente significativas entre a linha de base e imediatamente após a primeira sessão e após oito sessões ($p < 0,001$). Não foram encontradas diferenças significativas para PG. Não foram observadas diferenças entre os grupos. O NDI melhorou em ambos os grupos após oito sessões, mas não foram encontradas diferenças entre os grupos ($p < 0,05$). A Anova para o fator de tempo mostrou mudanças estatisticamente significativas na rotação direita cervical em ambos os grupos ($F = 4,12$; $p = 0,26$) após oito sessões.*

CONCLUSÕES: *Apesar de não haver diferenças entre os dois grupos, o RF resistivo capacitivo monopolar pode ter um efeito potencial sobre a intensidade da dor.*

PALAVRAS-CHAVE: *Síndromes da dor miofascial. Cervicalgia. Amplitude de movimento articular. Ablação por cateter/métodos.*

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ORIGINAL ARTICLE

The primacy of ultrasound in the assessment of muscle architecture: precision, accuracy, reliability of ultrasonography. Physiatrist, radiologist, general internist, and family practitioner's experiences

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SUMMARY

OBJECTIVE: *With high-resolution real-time ultrasonography we investigated the muscle architectural parameters of vastus lateralis in healthy volunteers.*

PURPOSES: *We determined the reproducibility and validity of ultrasonography and the role of the ultrasonographer in assessing muscle architecture. We proposed the most appropriate clinical parameters for objective measurements and an ultrasound protocol of muscle architecture.*

METHODS: *We conducted an intraobserver and interobserver study. We investigated 21 healthy male volunteers. The subjects were independently evaluated by four different operators using high-resolution real-time ultrasonography. To assess the reproducibility of ultrasound examinations, four operators repeated measurements using the same ultrasound device. Muscle thickness, muscle volume, muscle fiber pennation angle, and subcutaneous adiposity of the vastus lateralis muscle were measured.*

RESULTS: *Intra-observer (ICC 0.92-0.97), interobserver (ICC 0.78-0.92) reproducibility was good to excellent for all measurements.*

CONCLUSION: *Simple, reproducible, non-invasive ultrasound measurements of muscle structure easily demonstrated differences in muscle morphology. With a protocol and with objective and repeatable measurements, sonographers from different backgrounds could obtain an objective measurement of ultrasound images with little differences and low variability in results, thanks to the upgrading of diagnostic ultrasound imaging and their clinical skills.*

KEYWORDS: *Ultrasonography. Quadriceps muscle. Muscle fibers, skeletal.*

INTRODUCTION

With high-resolution real-time ultrasonography (US) we investigated the muscle architectural parameters of vastus lateralis (VL) in healthy volunteers. US permits to evaluate echo intensity (ECHO), ultrasound imaging of muscle perfusion, transverse and longitudinal sections of the muscle and its thickness at rest

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and during maximal voluntary contractions (MVC), overlying subcutaneous fat, cross-sectional area (CSA), and angled fibers of pennate muscles. Physicians with different specialties could use ultrasound for various applications. Therefore, physiatrist must be able to interpret ultrasound imaging performed by other specialists.

The internal fibers of the vastus muscles are not arranged in any particular manner. They are characterized by the presence of connective-tissue septa. The fascia that surrounds each of the vastus muscles is visualized as a thin hyperechoic layer. Their presence allows distinguishing the vastus intermedius from the VL and rectus femoris. Distinguishing the VL from the VI can be difficult because these two muscles merge laterally[1]. However, US overcomes this difficulty.

The purpose of this study was to examine the reliability and validity of US measures of the architecture of the VL and interrelationships among the architectural parameters. The validity of measurements was investigated by comparing values obtained after three ultrasound measurements from the same operator and other measurements obtained from 4 different specialists: a physiatrist, a radiologist, a general internist, and a family practitioner.

We designed a protocol to detect the most repeatable and objective ultrasound parameters: muscle thickness at rest (MT), CSA, muscle fiber pennation angle (θp) and subcutaneous fat thickness (FT) of pennate muscles. A common language facilitated the interpretation of the ultrasound measurements, even if performed by other specialists. Our ultrasound protocol allowed for a complete evaluation and more effective follow-up.

METHODS

All procedures performed in our study involving human participants followed the ethical standards of the institutional and national research committee, the 1964 Helsinki declaration and its later amendments, or comparable ethical standards. Informed consent was obtained from the participants included in the study.

We conducted an intraobserver and interobserver study. We investigated 21 healthy male volunteers (mean age 32 years, range 26 to 38 years, height 170.2 ± 5.5 cm, mass 68 ± 5 kg), at rest.

The inclusion criteria were as follows: healthy subjects, without previous lesions of the lower limbs, neither fractures, luxations, distortions, nor injuries to the bones, joints, tendons, and muscles. Exclusion criteria were as follows: immobilization, lesions or trauma or musculoskeletal deformities of the lower limbs, peripheral neuropathy, major medical conditions, and anatomical variations of the lower limb muscles.

The subjects were independently evaluated by four different operators using high-resolution real-time ultrasonography (Acuson S3000 Ultrasound System). After using a warm gel, we performed the ultrasound. Time taken for examinations was recorded (each visit lasted approximately 10–15 minutes). To assess the reproducibility of ultrasound examinations, four operators repeated measurements of the same image of the muscle three times, using the same ultrasound device. For each subject, we applied the same machine setting (gain, focal depth, transducer aperture size, beam steering, depth) in order to reproduce the same technical errors and obtain comparable data.

The subjects were examined in the prone position with hip and knee in neutral position. We examined the VL of the dominant leg, which was scanned axially and then longitudinally. The landmarks, defining the sites of measurement of the VL, were the femur, particularly the greater trochanter, and the kneecap. We measured the MT at rest (Fig. 1), CSA (Fig. 2), θp (Fig. 3) and subcutaneous adiposity (Fig. 4). The MT was the vertical distance between the superficial and deep aponeurosis of the VL (muscle belly), measured at the thickest part of the muscle. The CSA was the perimeter of the VL, with a manual tracing of the muscle borders using digital software of our ultrasound system. Pennation angle of the VL was the angle between the muscle fibers and the deep aponeurosis besides the vastus intermedius. We used a 6-18 MHz linear ultrasound probe. When it was possible, we took perforating vessels arising from the muscle, as landmarks.

The Statistical Package for Social Sciences (SPSS, Version 18.0 for Windows; SPSS Inc., Chicago, IL) was used for data analysis. Data were obtained using intra- and interobserver correlation (ICC).

RESULTS

Results were analyzed using intra- and interobserver correlation (ICC) of values obtained after three

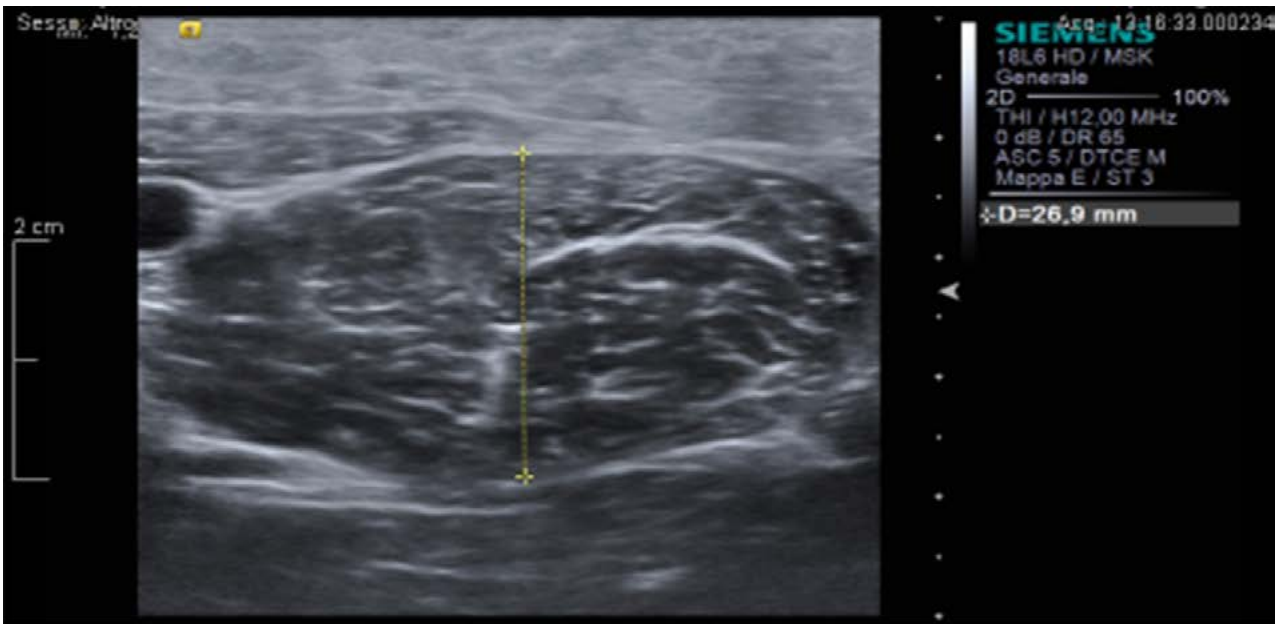

FIGURE 1: MUSCLE THICKNESS AT REST

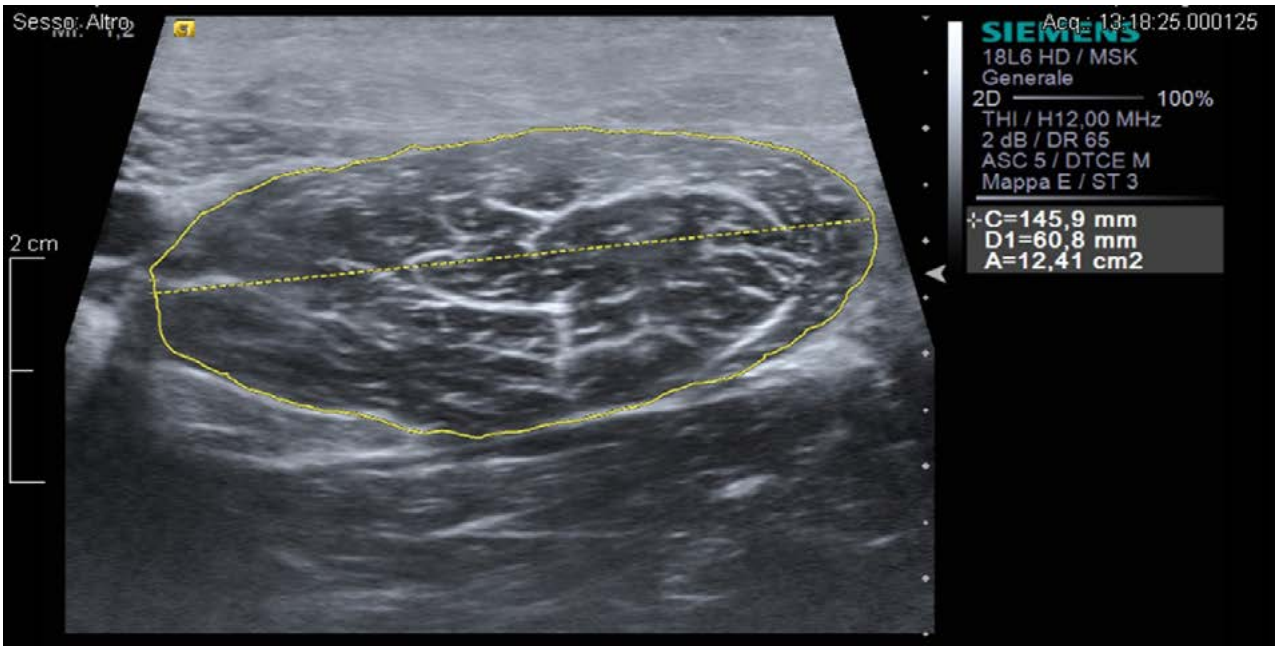

FIGURE 2: CSA

ultrasound measurements measured by the same operator and the other measurements obtained from 4 different specialists: a physiatrist, a radiologist, a general internist, and a family practitioner.

Intra-observer (ICC 0.92-0.97), interobserver (ICC 0.78-0.92) reproducibility was good to excellent for each measurement (Table 1). There were no statistical differences between measurements obtained from the same operator and different operators.

We confirmed the measurement accuracy, after having performed the same measurement three times.

DISCUSSION

Ultrasound is accurate, reproducible and fast; it offers a regional and easy evaluation. It should be taken into consideration when routine clinical examinations are performed or to evaluate patients with specific diseases[2]. The measurement of muscle size is essential to assess the effects of training, disuse, and ageing[3]. Different radiological techniques are used to examine muscle characteristics. The 'gold standard' for CSA measurements is magnetic resonance imaging (MRI). However, MRI is costly and

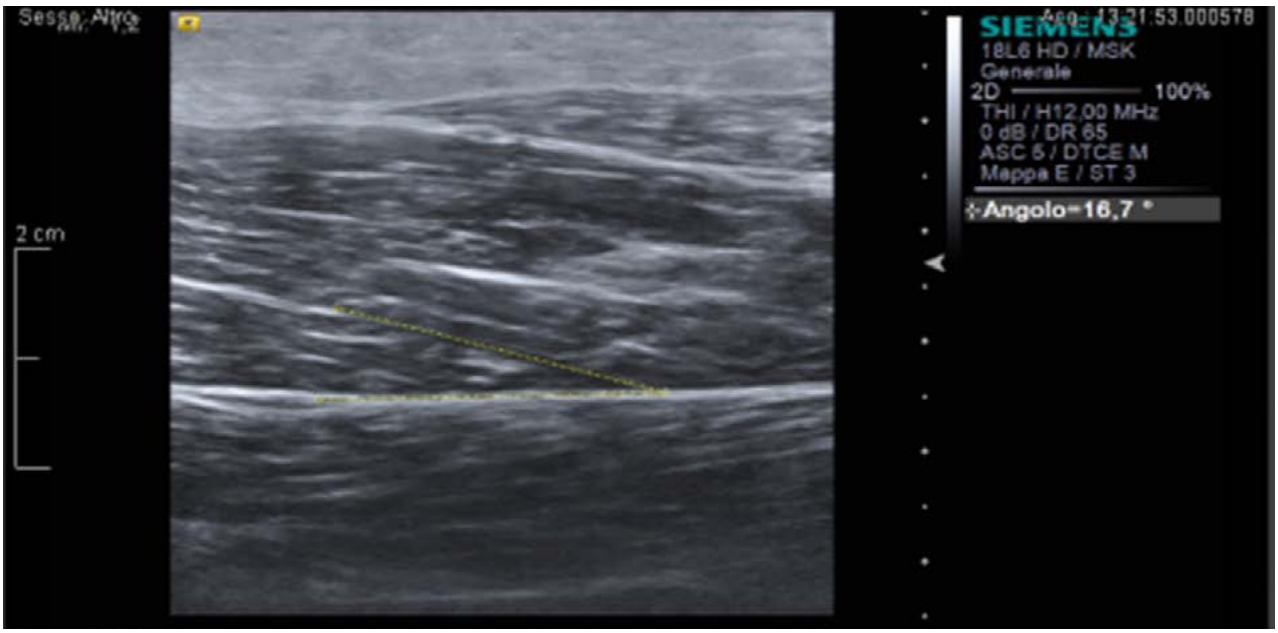

FIGURE 3: MUSCLE FIBER PENNATION ANGLE

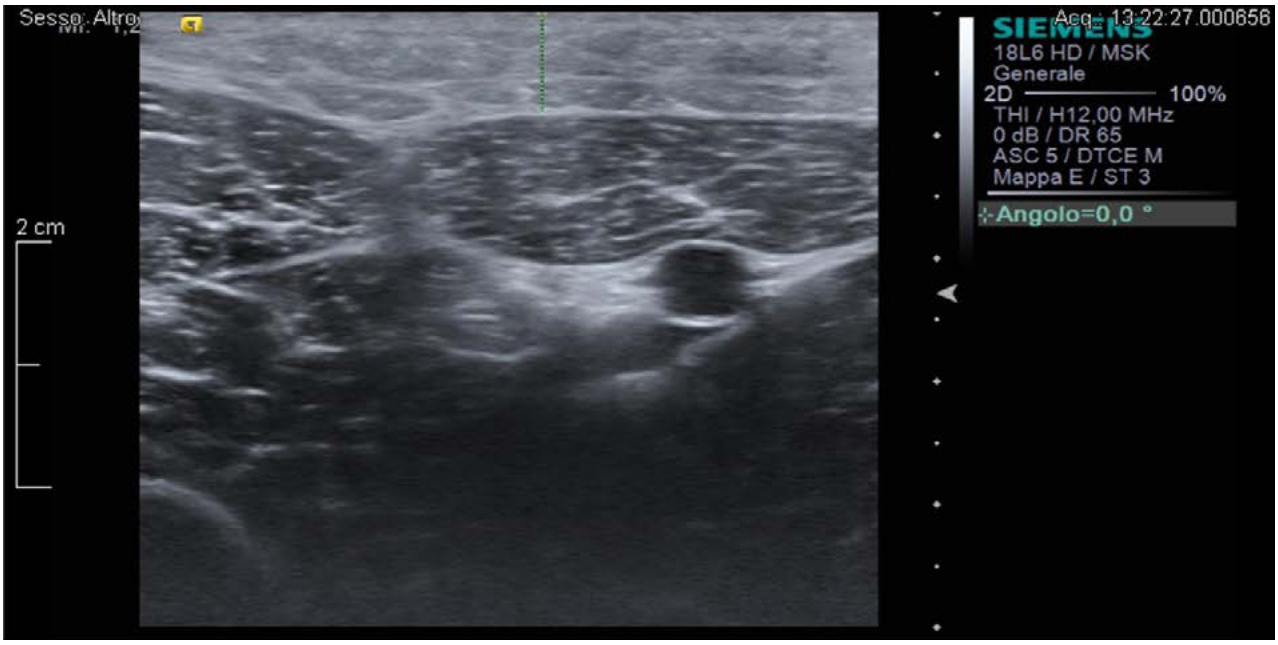

FIGURE 4: SUBCUTANEOUS FAT THICKNESS

often inaccessible[4]. So, US is the method of choice to examine muscle characteristics. Musculoskeletal ultrasound imaging is used to investigate muscle structure regarding architecture (MT, fascicle length, θp) and texture. The gray-scale analysis is commonly used to distinguish between normal and pathologic muscles; it depends on the image acquisition system and its settings[5-13]. Hypertrophy of the pennate muscles is associated with a proportional increase in pennation angle but not necessarily in fascicle length, and with training-induced changes in muscle size[5]. Many pieces of research evaluated the capacity of ultrasound to measure the parameters of the muscles in healthy and unhealthy people. Also, age or gender could influence muscle architecture. The fascicle, muscle, and tendon lengthen proportionally during the maturation; thus the muscle-tendon stiffness and excursion range are likely to be similar in children and adults, but the relatively higher increase in CSA than fascicle length indicates that adult muscles are better designed for force production than muscles of children[10,12]. Another study described a lower ECHO, bigger muscle size in men, who were faster and more potent than women[7]. A study suggested that there

TABLE 1: INTRA-OBSERVER AND INTER-OBSERVER REPRODUCIBILITY

Measurements	Sample size n.	Average	Inter-observer (95% CI)	Intra-observer (95% CI)
MT	21	27.2	0.97	0.92
CSA		12.9	0.92	0.78
θp		16.9	0.95	0.86
Subcutaneous adiposity		4.5	0.97	0.89

MT thickness at rest, CSA cross-sectional area, θp muscle fiber pennation angle

was no statistically significant difference between ultrasound estimation of muscle volume in vivo and in cadavers and there was no statistical difference between operators who determined muscle volume in vivo[6]. Other researchers showed that US is a valid and reliable method to measure quadriceps muscle size. They correlated computed tomography (CT) and US[9] or magnetic resonance imaging (MRI) and US[1]. Our and other studies showed a positive correlation between the MT and the θp[4].

Compared to other researchers, our sample was more homogeneous for relevant sociodemographic variables, general clinical features, and clinical characteristics. Our purpose was to obtain more objective parameters than before and to show our protocol of muscle ultrasound. Few studies paid attention to a specific diagnostic sequence[2,10]. So, the physiatrist could clinically monitor the subjects and their structure of muscle fiber, even if the ultrasound were performed by other health care practitioners. Our ultrasound protocol permitted physiatrists to be more accurate in the evaluation of clinical pictures and in the follow-up. We aimed to standardize the US imaging, to reduce confusion in imaging interpretations, to provide a common language among clinicians regarding the significance of the findings and management recommendations, to facilitate outcomes monitoring and to design an individual exercise programme.

CONCLUSIONS

We observed accuracy, precision, and repeatability of ultrasonography in the evaluation of muscle architecture. Repeated measurements showed that the measurement techniques of this study were highly reproducible, so we concluded that US was a valid method for the evaluation of muscle structure. Simple, reproducible, non-invasive ultrasound measurements of muscle structure easily demonstrated differences in muscle morphology. So, we can evaluate and monitor changes in muscle architecture, due to training in sportsman or due to disease.

The discriminative ability of sonographers attests their levels of training, but expert operators can obtain objective data of the muscles structure. Even if sonographers are competent, guidelines and landmarks are necessary to obtain a better performance of the US study. With a protocol and with objective and repeatable measurements, sonographers from different backgrounds could obtain an objective measurement of ultrasound images with little differences and low variability in results, thanks to the upgrading of diagnostic ultrasound imaging and their clinical skills.

RESUMO

OBJETIVO: *Nós utilizamos ultrassonografia de alta resolução em tempo real para investigar os parâmetros da arquitetura muscular do vasto lateral em voluntários saudáveis.*

PROPÓSITO: *Nós determinamos da reproduzibilidade e validade da ultrassonografia e o papel do ultrassonografista na avaliação da arquitetura muscular. Nós propomos os parâmetros clínicos mais apropriados para uma medição objetiva e um protocolo de ultrassonografia para arquitetura muscular.*

MÉTODOS: *Nós conduzimos um estudo intra-observador e inter-observador. Investigamos 21 voluntários saudáveis do sexo masculino. Os participantes foram avaliados de forma independente por quatro operadores diferentes usando ultrassonografia de alta-resolução em tempo real. Para avaliar a reprodutibilidade dos exames de ultrassonografia, quatro operadores repetiram as medições usando o mesmo equipamento de ultrassonografia. A espessura e o volume do músculo, o ângulo de penação da fibra muscular, adiposidade subcutânea do músculo vasto lateral foram medidos.*

RESULTADOS: *A reprodutibilidade intra-observador (ICC 0,92-0,97) e inter-observador (ICC 0,78-0,92) foi boa a excelente para todas as medições.*

CONCLUSÃO: *Medições de ultrassonografia simples, reprodutíveis e não-invasivas da estrutura muscular demostraram facilmente as diferenças na morfologia muscular. Seguindo um protocolo e com medições objetivas e reproduzíveis, ultrassonografistas com dif-*

erentes formações e experiências podem conseguir uma medição objetiva de imagens de ultrassonografia com poucas diferenças e pouca variação nos resultados graças à melhoria da qualidade nos exames de diagnóstico por imagem de ultrassonografia e das habilidades clínicas.

PALAVRAS-CHAVE: *Ultrasonografia. Músculo quadríceps. Fibras musculares esqueléticas.*

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ORIGINAL ARTICLE

Daily genital cares of female gynecologists: a descriptive study

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SUMMARY

INTRODUCTION: *Genital hygiene can play an essential role in avoiding vulvovaginal discomfort and preventing infections. The scientific evidence on best practices on genital hygiene is scarce, and without doubt, gynecologists should be the best person to discuss and guide the subject.*

OBJECTIVE: *Evaluate the general genital female gynecologist hygiene.*

METHODS: *This descriptive analytic study identified genital hygiene and sexual practices of 220 female gynecologists, through a questionnaire with 60 self-answered questions. The data were analyzed and presented using frequency, percentage, mean and standard deviation.*

RESULTS: *The studied population was constituted by middle age (37.3 years) and white (71.3%) female gynecologists. More than a half (53.6%) declared spending over 10 hours a day away from home and complained of vaginal discharge in 48.1% of the cases. Regular vulvovaginal hygiene: 17.8% reported washing genitals once a day and 52% twice a day. The use of dry paper alone was reported in 66.4% post urination and 78.5% post-evacuation. Using running water and soap was practiced by 25.9% and 21.5% respectively. Vulvovaginal hygiene related to sex: More than half of them had intercourse 1-3 times a week, and 37.4% and 24.1% had frequent oral sex and eventually anal sexof the participants, respectively. Genital hygiene before sex was positive in 52.7% of the subjects and, post-sex hygiene in 78.5% of them. Conclusion: Genital hygiene habits of female gynecologists can be improved, despite the high grade of scientific knowledge they hold.*

KEYWORDS: *Gynecology. Feminine Hygiene Products/adverse effects. Vaginal Creams, Foams, and Jellies. Sexual Behavior. Physicians, Women.*

INTRODUCTION

The modern woman has changed her lifestyle and social behavior, aningd her genital care changed too[1]. According to the Brazilian Institute of Geography and Statistics (IBGE), 49.5% of them work 40 to 44 hours weekly [2]. This new lifestyle imposes situations that may interfere in the vaginal ecosystem, providing or even preventing infections. The use of toilet tissue, soaps with different pH, intimate deodorants, sanitary pads, panty liners, the frequency of sexual intercourse, use of adornments, hair removal, clothing, and physical activity are important factors that should be taken into consideration, but haven't been thoroughly investigated[1].

The anatomy of the female genitalia presents nu-

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merous skin creases, hair and is located in a region that hinders the aeration and increases the friction, making it difficult to remove debris [3]. There is, in this area, sweat and sebaceous glands that cause organic waste, facilitating infection or modifications, promoting odor, vaginal discharge, and vulvar pruritus. Others external factors as ingestion of drugs, sexual activity, and genital hygiene can also affect the genital well-being [1].

Genital hygiene is the set of actions aimed at removing excess residues (dead cells, secretions, oiliness, menstrual blood, lubricants, sperm, and remains of urine, feces, and paper) in the female genital area in order to promote wellness and comfort, besides preventing genital infections [1]. Genital hygiene includes, besides the local cleaning, removal of pubic hair, piercings and tattoos, use of sanitary pads and wearing appropriate clothing.

The ideal feminine genital hygiene has not been established yet and can vary in different countries. Unfortunately, the side effects of unappropriated hygiene and its consequences are not reported regularly. It is known, that total hair removal is linked to a younger age with active sexual life [4]. Sexual activity is an important factor in changing the vaginal environment, either by the presence of semen (due to the alkalinity) or introducing new bacteria or promoting micro fissures [5].

Furthermore, feminine hygiene products such as soaps, lotions, wipes, and sanitary pads are used by women around the world daily without knowledge of its implications [6,7].

Pediatricians, infectious disease doctors, and dentists have already realized the importance of disease prevention practices by promoting hygiene measures and changes in lifestyle habits. Research shows that washing hands with soap reduces by 44% the morbidity from diarrhea and decreases the transmission of various infectious agents, such as the H1N1 virus [8,9].

Gynecologists, due to their professional knowledge, are the professionals who should know better how to guide patients on genital hygiene practices. However, few scientific publications have endorsed the guidelines which are usually presented. In practice, these professionals guide their patients without knowing the real consequences of poor hygiene, based only on common sense.

This research aimed to study the habits of hygiene in female gynecologists (products and approaches for genital hygiene, clothing habits, menstrual products, hair removal, sexual practices), since female gynecologists are excellent representatives of the modern woman, with the addition of having scientific knowledge about vulvar and vaginal conditions. Thus, they should know what is better or not regarding the proper care of female genitals. Therefore, we should know how the female gynecologists take care of their own genitals as they will be health providers for other women. Gynecologists are responsible for guiding other women in their daily care and must be qualified to do so.

METHODS

This is a descriptive, analytical study that enrolled 220 female gynecologists interviewed at scientific meetings of the specialty. The research involved a self-administered questionnaire with 60 questions related to the topic: genital hygiene habits, including cleaning (frequency, time, the specific type of soap, use of wipes), hair removal, usual clothing, and use of sanitary pads or panty liners. The questionnaire was designed by the researchers themselves and is currently undergoing validation. Inclusion criteria: to be a gynecologist, complete the TCLE, and answer more than 75% of the questions. Exclusion criteria: be under antibiotics treatment in the last 15 days, be pregnant, a chronic and/or degenerative patient (cancer, diabetes, immunosuppression), be a syphilis, HIV or hepatitis patient. Some female gynecologists were approached during a specialized congress, between June and September 2013, during the coffee break. To ensure confidentiality and anonymity of the interviewees, the questionnaire was inserted by the participant in a sealed box.

During the approach, there was an acceptance rate of 84.6%. The main reason for denial was the lack of time to answer the questionnaires. After collecting the data, we developed the database in Microsoft Excel 2007. The results were analyzed using descriptive statistics, such as frequency, percentage, mean and standard deviation, where applicable.

All participants signed an informed consent form before filling out the questionnaire. The study was approved by the Research Ethics Committee of a university hospital in São Paulo, Brazil.

RESULTS

The average age of interviewees was 37.3 years (±12.9); 71.4% of them were white, and 46, 8% had been a graduate for between one and ten years-graduated. More than half (53.6%) of the women

are away from home for more than 10 hours a day. Table 1 shows that more than half (55.9%) take two baths per day, 52% wash genitals twice a day, 66.4% of them do not wash genitals after urination, using toilet paper to dry the vulvar area. Only 21.5% wash the anal area after evacuations with soap and water, and 48.6% of them frequently use intimate deodorants. The genital hygiene with liquid soap is made by only 39%, and antibacterial soaps were used by 6.8% of the interviewees; 52.7% and 78.5% sanitize them before and after intercourse respectively. Vaginal douching was reported by 21.4% of the female gynecologists. Table 2 shows that panty liners are used by 41% of them, changing it four to five times a day (34.1%). Pruritus was referred by 83.2% of the panty liners users. Over 83% shave, at least once a month, meanwhile, 31.6% twice or more. After shaving, 48.6% do not use moisturizing products to prevent complications in the region. Table 3 shows that over 85% use cotton underwear or cotton lining during the day, but only 3.63% sleep completely naked. The majority (62.7%) reported wearing tight pants. Sexual profile showed that 50.9% female gynecologists have sexual intercourse one to three times per week, 47.2% and 22.2% practiced oral and anal sex respectively. Over 29% reported pain in relations in varying intensities.

TABLE 1. GENITAL HYGIENE HABITS CHARACTERISTICS OF FEMALE GYNECOLOGISTS

Characteristics		N	%
Daily genital hygiene (freq.)	1	39	17.8
	2	114	52.1
	3 or more	63	28.7
	Don't do	3	1.4
Time of genital hygiene	<1m	63	28.6
	1 - 2m	123	55.9
	Up to 5m	30	13.6
	Over 5m	4	1.8
Vaginal discharge	Often	30	13.7
	Eventually	75	34.4
	Rarely	82	37.6
	Never	31	14.3
Hygiene product used	Only water	10	4.5
	Water and solid soap	84	38.2
	Water and liquid soap	86	39.1
	Liquid and solid soap	21	9.5
	Bactericidal soap	15	6.8
	Other	4	1.8
Vaginal douching	1x day	17	7.7
	1x week	10	4.5
	1x month	20	9.1
	Never	173	78.6
Urination after hygiene	Water shower	57	25.9
	Toilet paper	146	66.4
	Wet towels	17	7.7
Hygiene after evacuation	Water and soap	47	21.5
	Toilet paper	171	78.5
		Missing	2
Hygiene after SR	Yes	116	52.7
	No	104	47.3
Hygiene before SR	No	7	3.2
	Wash	173	78.6
	Toilet paper	21	9.5
	Wet towels	3	1.4
	Others	16	7.3

SR= sexual relationship; m= minutes

DISCUSSION

This is, perhaps, the first study on genital hygiene on gynecologists. Surprisingly, we found that even people with high scientific training could significantly improve hygiene habits and consequently, decrease not only the discomforts of the genital area but mainly prevent gynecological infections. Our attention is drawn to the fact that only 25.9% and 21.5% of the interviewees wash the genital area with running water after urination and evacuation, respectively. We know that the accumulation of residues, whether urine or feces, promote irritation and itching so that they may predispose to the formation of cracks and the increase of bacteria. Removing urine or feces using only toilet paper requires the use of strength and can scrape a region that is delicate and at the same time has many folds of skin. Gynecologists, from a practical point of view, are very similar to any other category of professionals who have to work many hours a day without adequate time and availability to do proper genital hygiene.

The data collected annually by the Federal Council of Medicine provides an overview on distribution, compensation and working hours of Brazilian medical professionals, however, are lacking information about the quality of this medical health [10]. Despite their medical profile, gynecologists are similar to other Brazilian women with a hectic routine, with

TABLE 2. PANTY LINERS AND HAIR REMOVAL PRACTICES OF FEMALE GYNECOLOGISTS

Characteristics		N	%
Panty liners use	No	130	59.9
	Daily	9	4.1
	>3x week	17	7.7
	Sometimes	37	16.8
	Rarely	27	12.3
Tipe of panty liners	Dont use	130	59.9
	With plastic film	11	5
	Without plastic film	72	32.7
	Don't know	7	3.2
Genital hair removal motivation	Do not do it	23	10.4
	More hygienic	95	43.2
	Aesthetic	29	13.2
	Hair disconfort	60	27.3
	Non specified	13	5.9
Type of hair removal	No hair removal	23	10.4
	Shave	65	29.5
	Hot wax	90	40.9
	Cold wax	14	6.4
	Depilation cream	1	0.5
	Fotodepilation	20	9.1
	Others	7	3.2
Use of substances after hair removal	Don't use	107	48.6
	Ointment	20	9.1
	Moisturizing	59	26.8
	Others	34	15.5

TABLE 3. CLOTHING AND SEXUAL HABITS OF FEMALE GYNECOLOGISTS

Habits	Type	N	%
Underwear material			
	Synthetic	22	10
	Cotton	96	43.6
	Synthetic with cotton lining	93	42.3
	Elastane	5	2.3
	Others	4	1.8
Tight jeans			
	Yes	138	62.7
	No	82	37.3
Clothing at sleeping time			
	Panties	140	63.6
	Pijama	54	24.5
	Camisole	17	7.7
	Day clothes	1	0.5
	Naked	8	3.6
SR per week			
	No relationship	49	22.3
	<1x	44	20
	1-3x	112	50.9
	4-6x	14	6.4
	>6	1	0.5
Dyspaurenia			
	Never	79	35.9
	Rarely	76	34.5
	Sometimes	34	15.5
	Frequently	3	1.4
	Always	19	8.6
	Other	9	0.4
Anal SR			
	Never	157	75.8
	Sometimes	46	22.2
	Frequently	1	0.5
	Always	3	1.4
	Missing	13	
Oral SR			
	Never	33	15
	Sometimes	104	47.2
	Frequently	60	27.3
	Always	23	10.4
Intimate lubricants			
	Yes	58	26.4
	No	162	73.6

SR= sexual realtionship

many working hours and unhealthy habits (smoking and physical inactivity), adding to this the constant stress of the complex universe in which they live [10].

Hygiene practices are effective methods and recommended in various specialties to prevent diseases. Take the case of *H Pylori*; research currently proposes that if there was any transmission in childhood, it could be interrupted if new hygiene habits such as washing hands, not sharing oral-oral food or utensils are initiated [11]. Another example, much more palpable and well-studied in the literature, is the control of caries by proper hygiene of the teeth and oral cavity. It is known that in countries where the rate of caries is smaller there is an investment in guidance and education of dental hygiene. [12].

Similarly, there are some premises about female genital hygiene as a prevention method for genital infections. Amiri et al. [13] showed that pregnant women with Urinary Tract Infection presented as main characteristic the habit of not washing hands after going to the bathroom and genitals after coitus. A 2011 Brazilian study found that using acidified liquid soap as an adjunct to metronidazole decreases the time between recurrences of bacterial vaginosis [14]. Among

the interviewees, 52% 7 sanitize themselves before sexual intercourse, and 66.3% say just use toilet paper after micturitions, leading to prolonged accumulation of sperm, urine, and scraps of paper in contact with vulvovaginal mucosa may cause irritation and facilitating any vulvovaginitis.

Approximately 50% of North American and European women use sanitary pads and 10% to 30% daily during the intermenstrual period with the idea of staying clean and dry [15]. A recent survey of Brazilian estimated using sanitary pads in 28.3% [16]. They used it for different reasons and associated improvement in self-esteem and security with their genital hygiene. Our study showed a high prevalence in the use of sanitary pads in the intermenstrual period (41%). It is double the use compared with European standards. This may be explained by the difficulty of work periods or the practicality of the product.

Only 9.5% of the doctors in this study used liquid soaps with acid pH, products that are recommended for the genital area because they have acidic pH similar to that of the skin of the vulva and because they have low detergency (do not excessively remove the fat from the skin and do not resect the genital area). On the other hand, 6.8% of them had the (inadequate) practice of using bactericidal soaps, since the genitals are colonized by bacteria that protect the vulvovaginal ecosystem.

Among those interviewed at least 62,7% wear tight jeans, considering the extended time away from home (more than 10 hours in half of the cases: 53%) the lack of habit of washing the vulva (33.7%) and anus (21.5%) after urination and evacuation inevitably worsens local conditions. Although 83.18% of gynecologists declared wearing cotton underwear, 62.7% of them wear tight jeans but do not have vaginal discharge.

Most of the subjects shave at least once a month (42.7%), while others (31.6%) shave two or more times a month. This implies 74.3% concerned with this process of hygiene. Twenty percent of them declare that they do vaginal douches at varying frequencies, although they are gynecologists and know that this practice is condemned by most studies, due to association with Pelvic Inflammatory Disease, bacterial vaginosis [17]. The sexual profile of interviewees is very similar to the profile of the general population. More than half reported to having sex 1-3 a week with a high prevalence of dyspareunia of 29% [18]. The discomfort to talk about sexuality was evident by the number of lost cases in issues related to sexual activities (13 lost cases).

There is a massive number of hygiene products currently available for women. The need, erroneously propagated to feel fresh, leads many women to live a dilemma between using or not using such products [19]. The reality is that women, regardless of the profession, are making increasing use of hygiene products in search of vulvar health.

Because of the vocational training and science they have, the interviewed gynecologists should know the ideal way to carry out genital hygiene; however, they practice hygiene inappropriately. A survey of 341 university students (biological area, social area, and exact area) of a large Brazilian university shows patterns of genital hygiene and sexual practices very similar to those found in medical gynecologists. In this study, it was found that after sex, 69.3% of women bathe and 14.2% do not bathe but do genital hygiene, and 13.9% do vaginal douching [20].

This study is the first to methodologically investigate the group of medical gynecologists on the subject, extending the line of research in women's clinical care. However, being a descriptive study, we believe that further research and clinical trials need to be conducted to evaluate the effects of the practice of female genital hygiene and thus offer subsidies for proper orientation on how to promote genital hygiene.

CONCLUSION

Female genital hygiene is a vital topic which should be more widespread between gynecologists and society. Gynecologists behave, regarding genital hygiene, like most women with an intense professional and social life, committing the same inadequacies as most women. Surprisingly, despite female gynecologists having previous knowledge about the vaginal environment and how to perform proper genital hygiene, some of their hygiene habits are questionable. More studies (clinical trials) need to be performed in order to provide directions to proper female genital hygiene, as well as guidelines and education for the gynecologists, collaborating with women health.

Conflict of interest

The authors have no conflict of interest to declare.

RESUMO

INTRODUÇÃO: *A higiene genital pode desempenhar um papel importante na prevenção de desconfortos vulvovaginais e infecções. Evidências científicas sobre as melhores práticas em higiene genital são escassas, e o ginecologista, sem dúvida, é a melhor pessoa para discutir e orientar o assunto.*

OBJETIVO: *Avaliar a higiene genital feminina usual de médicas ginecologistas.*

MÉTODOS: *Estudo analítico descritivo que identificou higiene genital e práticas sexuais de 220 ginecologistas por meio de um questionário com 60 perguntas autorrespondidas. Os dados foram analisados e apresentados por frequência, porcentagem, média e desvio padrão. Resultados: A população estudada consistiu de médicas ginecologistas femininas brancas (71,3%) com idade média de 37,3 anos. Mais da metade (53,6%) relatou ficar fora de suas casas por períodos superiores a 10 horas por dia e queixaram-se de descarga vaginal em 48,1% dos casos. Higiene vulvovaginal regular: 17,8% relataram lavar os genitais uma vez por dia e 52%, duas vezes por dia. O uso apenas de papel (seco) foi relatado em 66,4% dos casos após micção e em 78,5% após a evacuação. A higiene ideal com água corrente e sabão foi praticada apenas em 25,9% e 21,5%, respectivamente. Higiene vulvovaginal relacionada ao sexo: mais da metade delas relatou relações sexuais 1-3 vezes por semana, sexo oral frequente e anal eventual em 37,4% e 24,1%, respectivamente. A higiene genital pré-sexo foi relatada por 52,7% das pessoas e em 78,5% após o coito. Conclusão: Os hábitos de higiene genital dos ginecologistas femininos estão sujeitos a melhorias, mesmo considerando o alto grau de conhecimento científico que possuem.*

PALAVRAS-CHAVE: *Ginecologia. Produtos de higiene feminina/efeitos adversos. Cremes, espumas e géis vaginais. Comportamento sexual. Médicas.*

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ORIGINAL ARTICLE

Effect of FOLFOX6 chemotherapy on serum VEGF expression in advanced colorectal cancer patients

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SUMMARY

OBJECTIVE: *To explore the effect of FOLFOX6 chemotherapy on serum vascular endothelial growth factor (VEGF) expression in advanced colorectal cancer patients.*

METHODS: *A retrospective analysis of 81 patients with advanced colorectal cancer who visited our hospital from March 2014 to February 2016 was performed. All the patients were treated with FOLFOX6 chemotherapy. On day 1, patients received oxaliplatin 100 mg/m2 ivgtt (2h), calcium folinate 200 mg/m2 ivgtt (2h), 5fluorouracil 400 mg/m2 iv bolus and 5fluorouracil 2500 mg/m2 ivgtt (5h). The treatment course was 2 weeks, and 4 treatment courses were required. The changes in the levels of VEGF and CRP and quality of life before and after 4 courses of chemotherapy were observed and therapeutic effects and adverse reactions after chemotherapy were evaluated.*

RESULTS: *After treatment, the total efficiency of chemotherapy was 82.72% (67/81) with 24 cases in complete remission, 25 cases in partial response, 18 cases in stable disease and 14 cases in progressive disease. The levels of CRP and VEGF after the treatment were significantly lower than those before treatment (5.69±0.77) mg/L vs. (7.99±1.36) mg/L; (443.26±21.55) pg/mL vs. (542.83±20.44) pg/mL] (P<0.05). The KPS grade after treatment was significantly higher than that before treatment (57.84±4.6) point vs. (50.99±3.73) point] (P<0.05). Among them, 3 cases developed a rash, 5 cases experienced hair loss, and 9 cases developed nausea and vomiting.*

CONCLUSION: *FOLFOX6 chemotherapy can decrease serum VEGF expression in patients with advanced colorectal cancer and enhance the curative effect with high safety, which is good for the improvement of patients' survival.*

KEYWORDS: *Colorectal neoplasms. Vascular endothelial growth factor A. Drug therapy.*

INTRODUCTION

Among various malignant tumors, colorectal cancer is a common one whose morbidity and mortality are at the forefront with a rising trend each year[1]. In China, approximately 400,000 people are diagnosed with colorectal cancer every year[2]. Typical symptoms of early-stage colorectal cancers are absent and only appear as indigestion, discomfort, fecal occult blood, etc. With the further development of cancer, more clinical symptoms appear, mainly as an abdominal mass, blood in the stool,

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abdominal pain, change in bowel habits, bowel obstruction with and without weight loss, fever, anemia, and other systemic symptoms. The affected organs can change because of infiltration and metastasis of the tumor[3-5].

In the treatment of colorectal cancer, excision is the most effective method. However, in many cases, when patients are diagnosed as colorectal cancer, it has already developed to middle and advanced-stage and chemotherapy becomes a major treatment method at this time[6]. The key is how to predict the effect of chemotherapy and take timely and effective treatment measures. In the research for colorectal cancer treatment, the relationship between expression of vascular endothelial growth factor (VEGF), C-reactive protein (CRP), clinical cancer behavior, and the prognosis is deemed as a critical research category[7,8]. To explore the effectiveness of FOLFOX6 chemotherapy, this study analyzes its effects on serum vascular endothelial growth factor (VEGF) expression in advanced colorectal cancer patients. These cases are reported as below.

MATERIAL, SUBJECT, AND METHOD

Study design

This study was mainly designed following the retrospective analysis protocol.

Case selection

A retrospective analysis of 81 patients with advanced colorectal cancer who visited our hospital from March 2014 to February 2016 was performed. Although there is no clear definition of advanced colorectal cancer, it is usually distinguished from early-stage cancer. Generally, both locally advanced cancer and metastatic cancer are included. Here, we enrolled all the clinical stages T3 or T4 or node-positive colorectal cancer, with or without metastasis, as advanced colorectal cancer.

Inclusion criteria[4]:

(1) Patients whose diseases were confirmed by pathological examination. (2) Patients who were conscious and had the ability of healthy communication and exchange and had no history of mental illness. (3) Patients who were in good compliance and could cooperate with the medical staff to complete the study.

Exclusion criteria:

(1) Patients who had received prior radiotherapy and chemotherapy, surgery and other particular treatment methods before this study. (2) Patients whose survival time was less than 3 months. (3) Patients with contraindication to chemotherapy. (4) Previous history of cancer

Drug and instrument

Oxaliplatin Injection, specification: 100ml: 100mg, manufacturer: Jiangsu Hengrui Medicine Co., Ltd, batch No.:20131103; Calcium Folinate, specification: 10ml: 100mg, manufacturer: Jiangsu Hengrui Medicine Co., Ltd, batch No.:20131221; 5fluorouracil, manufacturer: Tianjin Kingyork Co. Ltd, specification: 10ml: 250mg * 5vials, batch No.:20140103.

Room temperature centrifuge: SICMA 1-13, Sigma, Germany; Refrigerator: LC-128, Haier, Qingdao; Automatic biochemical analyzer: ADVID 1800, Siemens, Germany; ELISA Kit for Vascular Endothelial Growth Factor (VEGF), Shenzhen Jingmei Biotech company; CRP ELISA Kit, Roche, the United States.

Grouping and treatment

All the patients were treated with FOLFOX6 chemotherapy. On day 1, patients received oxaliplatin 100 mg/m2 iv gtt (2h), Calcium Folinate 200 mg/m2 iv gtt (2h), 5fluorouracil 400 mg/m2 iv bolus and 5fluorouracil 2500 mg/m2 iv gtt (5h). The treatment course was 2 weeks, and 4 treatment courses were required.

Observation parameters and efficacy evaluation criteria

The changes in the levels of VEGF and CRP before and after 4 courses of chemotherapy were observed. A 5mL sample of the fasting venous blood respectively before and after 4 courses of treatment was collected and mixed. The blood was centrifuged for 10 min at 3000r/min, and the serum was collected and preserved in the refrigerator at -50°C. ELISA was adopted to detect VEGF levels, and immune turbidimetry was used to measure CRP levels. The survival quality points before and after treatment were analyzed using Karnofsky (KPS) with a total point of 100. The evaluation criteria are as follows: death represents 0 points; impending death represents 10 scores; seriously ill represents 20 scores; could not care for self

seriously represents 30 scores; could not care for self and needs special help and care represents 40 scores; often needs someone's help represents 50 scores; mostly cares for self but occasionally needs help represents 60 scores; lives independently but has difficulty maintaining a normal life and work represents 70 points; barely able to carry out normal activities with some signs or symptoms represents 80 scores; carries out normal activities with some mild signs and symptoms represents 90 points; normal without signs and symptoms represents 100 points. The lower the score, the worse the quality of life. The adverse reactions after treatment were observed.

The baseline characteristics were observed before the therapy. Moreover, the responses to chemotherapy were evaluated after 4 courses. A therapeutic evaluation was conducted according to RECIST criteria[9], including complete remission(CP), partial response(PR), stable disease (SD) and progressive disease(PD). The disease control rates (DCR) = CR + PR + SD, the objective response rate (ORR) = CR + PR.

Statistical analyses

All the data were analyzed using software package SPSS 18.0. GRP, VEGF and other quantitative data were expressed as the average±standard deviation (x±s), and t-test was used. The difference was regarded as statistically significant when $P<0.05$.

RESULTS

General information

Among 81 patients with advanced colorectal cancer, there were 48 males and 33 females aged from 41-78 years with an average of (61.32±2.32) years. Forty-six cases had colon cancer, and 35 cases had rectal cancer. Thirty-one cases were poorly differentiated adenocarcinoma, 20 cases were moderately differentiated adenocarcinoma, and 30 cases were highly differentiated adenocarcinoma. Twenty-one cases had hepatic metastases, 12 cases had pulmonary metastasis, 17 cases had lymphatic metastasis, 15 cases had bone metastases, and 16 cases were within the pelvic recurrence. See Table 1.

Recent clinical efficacy analysis

After treatment, 24 cases were in complete remission, 25 were in partial response, 18 were in stable disease, and 14 were in progressive disease; the total efficacy was 82.72% (67/81). See Table 2.

CRP and VEGF level comparison before and after treatment

CRP and VEGF levels were significantly lower after treatment than before. The difference was statistically significant ($P<0.05$). See Table 3.

KPS grade comparison before and after treatment

KPS grade after treatment (57.84±4.6) was significantly higher than before (50.99±3.73). The difference was statistically significant ($P<0.05$). See Table 4.

Safety assessment

After treatment, all the patients had different degrees of adverse reactions, which mainly appeared as vomiting, hair loss, and rashes. Among the adverse reactions, 3 cases developed a rash, 5 experienced hair loss, and 9 developed nausea and vomiting. See Table 5.

DISCUSSION

Colorectal cancer is a common malignant tumor, and 5-year survival rate after surgery is generally about 50%. The factor that leads to failure is largely related to metastasis and recurrence. The main metastasis is in the lymph, and VEGF plays a critical role in the lymphatic metastasis and blood stream[10-12]. A study by Xin et al.[13], among others, have shown that angiogenesis can promote tumor growth and metastasis. VEGF, a substantial factor in stimulating blood vessel formation, was inseparable from tumor growth, metastasis, invasion and angiogenesis[14]. VEGF was mainly for promoting lymphatic endothelial cell growth and strengthening vascular permeability which was conducive to the formation of new blood vessels and proliferation of endothelial cells. It not only supplied the nutrients needed for tumor growth and removed metabolic products but also supplied the route for the corresponding spread of tumor cells. A high level of VEGF in serum reminds that the tumor is widely infiltrated, at an advanced stage, or associated with distant lesion metastasis. Observing the level of serum VEGF can help predict tumor growth, metastasis, and invasion[15,16]. In addition, a high ex-

TABLE 1: GENERAL INFORMATION

Item	
Sex(male/female)	48/33
Age(year)	61.32±2.32
Pathologic types	
Colon cancer	46(56.79)
Colorectal cancer	35(43.21)
Degree of differentiation	
Poorly differentiated adenocarcinoma	31(38.27)
Moderately differentiated adenocarcinoma	20(24.69)
Highly differentiated adenocarcinoma	30(37.04)
Transfer case	
Hepatic metastases	21(25.93)
Pulmonary metastasis	12(14.81)
Lymphatic metastasis	17(20.99)
Bone metastasis	15(18.52)
Within the pelvic recurrence	16(19.75)

TABLE 2: RECENT CLINICAL EFFICACY ANALYSIS (N, %)

Item	CR	PR	SD	PD	ORR	DCR
	24 (29.63)	25 (30.86)	18 (22.22)	14 (17.28)	49 (60.49)	67 (82.72)

CR: Complete Remission; PR: Partial Response; SD: Stable Disease; PD: Progressive Disease;

TABLE 3: CRP AND VEGF LEVEL COMPARISON BEFORE AND AFTER TREATMENT ($\bar{\chi}$±S)

Time	CRP (mg/L)	VEGF (pg/mL)
Before treatment	7.99±1.36	542.83±20.44
After treatment	5.69±0.77*	443.26±21.55*

Compared with before treatment,*P<0.05

TABLE 4: KPS GRADE COMPARISON BEFORE AND AFTER TREATMENT ($\bar{\chi}$±S)

Time	KPS grade(point)
Before treatment	50.99±3.73
After treatment	57.84±4.62*

Compared with before treatment,*P<0.05

TABLE 5: ADVERSE REACTIONS ANALYSIS

Item	Rash	Hair loss	Nausea and vomiting	Overall adverse reactions
	3(3.70)	5(6.17)	9(11.11)	17(20.99)

pression of VEGF is closely correlated with tumor recurrence and prognosis.

CRP is a typical acute phase protein which appears initially in acute inflammation patients and increases in cancer patients. Wenbo et al.[17] and related scholars propose that persistent inflammation caused by tissue damage or chronic inflammation transfers the tumor cells or pro-inflammatory cytokines caused by DNA damage, causing cell transformations that can lead to tumor growth and induce chronic inflammation[18,19]. Relevant research shows that CRP levels in serum of patients with malignant tumors were significantly increased, and was closely correlated with the progress and prognosis of cancer. Studies on patients with colon cancer showed that tumor recurrence and staging were significantly correlated with CRP levels[20-22].

The incidence of colorectal cancer is rising each year, and more than half of patients have metastases. The primary treatment for advanced colorectal cancer is chemotherapy, which can significantly improve patients' quality of life regarding better supportive care. For a long time, 5-fluorouracil has been a core drug in the treatment of gastrointestinal tumors. Its mechanism of action is similar to other platinum compounds. It forms DNA binding of platinum to ultimately inhibit DNA synthesis and DNA repair [23-25]. Compared to cisplatin, the rate of oxaliplatin binding with DNA in vivo is faster than over 10 times and their binding can be firmly combined, which has stronger cytotoxicity[26,27]. A study by Jie et al.[28] and other studies[29,30] showed that oxaliplatin had an excellent therapeutic effect in the treatment of advanced gastrointestinal tumors and its cytotoxic effect was stronger without renal toxicity. In comparison with cisplatin, nausea and vomiting incidence of oxaliplatin was much lower, and hydration was not required. In addition, no severe hearing impairment and cardiac toxicity were found.

The baseline characteristics were observed before the therapy. Also, the responses to chemotherapy were evaluated after 4 courses. Therapeutic evaluation was conducted according to RECIST criteria [9], including complete remission(CP), partial response(PR), stable disease (SD) and progressive disease(PD). The disease control rates (DCR)= CR + PR + SD, the objective response rate (ORR) = CR + PR.

In summary, FOLFOX6 chemotherapy can decrease serum VEGF expression and the CRP level in patients with advanced colorectal cancer and enhance the curative effect with high safety which is good for the improvement of patients' quality of life.

RESUMO

OBJETIVO: *Explorar o efeito da quimioterapia Folfox6 na expressão do fator de crescimento endotelial vascular sérico (VEGF) em pacientes com câncer colorretal avançado.*

MÉTODOS: *Uma análise retrospectiva de 81 pacientes com câncer colorretal avançado que visitaram nosso hospital de março de 2014 a fevereiro de 2016 foi realizada. Todos os pacientes foram tratados com quimioterapia Folfox6. No dia 1, os doentes receberam oxaliplatina 100 mg / m2 ivgtt (2h), folinato de cálcio 200 mg/m2 ivgtt (2h), 5fluorouracil 400 mg/m2 iv bolus e 5fluorouracil 2.500 mg/m2 ivgtt (5h). O curso de tratamento foi de duas semanas e foram necessários quatro cursos de tratamento. Foram observadas as alterações nos níveis de VEGF e CRP e qualidade de vida antes e após quatro cursos de quimioterapia e avaliados os efeitos terapêuticos e reações adversas após a quimioterapia.*

RESULTADOS: *Após o tratamento, a eficácia total da quimioterapia foi de 82,72% (67/81), com 24 casos em remissão completa, 25 casos em resposta parcial, 18 casos em doença estável e 14 casos em doença progressiva. Os níveis de CRP e VEGF após o tratamento foram significativamente inferiores aos do tratamento (5,69 ± 0,77) mg / L vs. (7,99 ± 1,36) mg / L; (443,26 ± 21,55) pg / mL* ***vs.*** *(542,83 ± 20,44) pg / mL] (P < 0,05). O grau de KPS após o tratamento foi significativamente maior do que antes do tratamento (57,84 ± 4,6 pontos)* ***vs.*** *(50,99 ± 3,73 pontos)] (P < 0,05). Entre eles, três casos desenvolveram erupção cutânea, cinco casos sofreram perda de cabelo e nove casos desenvolveram náuseas e vômitos.*

CONCLUSÃO: *A quimioterapia Folfox6 pode, obviamente, diminuir a expressão de VEGF no soro em pacientes com câncer colorretal avançado e melhorar o efeito curativo com alta segurança, o que é bom para a melhoria da sobrevivência dos pacientes.*

PALAVRAS-CHAVE: *Neoplasias colorretais. Fator A de crescimento do endotélio vascular. Tratamento farmacológico.*

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ORIGINAL ARTICLE

An immunohistochemistry and histopathological study of ankaferd blood stopper in a rat model of cervical inflammation

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SUMMARY

OBJECTIVE: *Ankaferd Blood Stopper (ABS) is a medicinal plant extract used topically as a hemostatic, anti-inflammatory, and anti-oxidant agent. Its cytoprotective effect mainly depends on its pleiotropic properties by modulating inflammatory mediators such as IL-1β, IL-6, and TNF-α. This study aims to test the possible therapeutic effect of ABS in the treatment of erosive and inflammatory conditions occurring in the uterine cervix.*

METHODS: *Twenty-four female Wistar Albino rats were used in the present study. Trichloracetic acid was applied intravaginally to establish an experimental rat model of cervicitis. The rats were randomly divided into three groups: group I (injury), group II (injury+isotoinc saline), and group III (injury+ABS). After 3 estrous cycles of ABS and isotonic saline treatment, the amount of inflammation, vascular congestion and erosion were evaluated in the cervical tissues by using a modified semi-quantitative scale of 0-3. Immunohistochemical staining with monoclonal antibodies against IL-1β was also performed.*

RESULTS: *Compared with group I and II, the ABS group showed the least inflammatory cell infiltration, vascular congestion and cervical erosion, compared with the ABS group prominent IL-1β staining observed in group I and group II.*

CONCLUSION: *Our data suggest that ABS is a highly effective alternative to induce normal cervical epithelium and can be used safely in the treatment of cervical inflammation with or without cervical erosion.*

KEYWORDS: *Uterine cervicitis. Uterine cervical diseases. Inflammation. Interleukin-1beta. Plant extracts/*pharmacology.*

INTRODUCTION

Cervicitis, which is characterized by the inflammation of the cervical mucosa, is commonly provoked by distinct etiologic factors including sexual infections, hormonal alterations, irrational use of contraceptive devices and trauma to the cervix.[1] Clinical significance of the condition concerning evaluation, monitoring, and treatment usually depends on the stage of disease as either being acute or chronic rather than histologic appearance. Although rarely diagnosed during pregnancy, it can cause infection of the membranes and gross malformations of the fetus.[2] Moreover, in the postpartum period, cervical inflammation can also provoke the development of fetal abnormalities, intrauterine growth restriction, premature birth and perineal pain with infectious complications to the mother.[3] In this context, the

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appropriate treatment of cervical inflammation in either pregnant or non-pregnant women is paramount for the prevention of further complications associated with an inflammatory reaction within the lower genitourinary tract[4]. Apart from surgical interventions, which are usually preferred in chronic cases, anti-inflammatory and antimicrobial drugs, physiotherapy, immunomodulating agents and drugs that restore the normal biocenosis of the vagina are standard treatment options with variable results.[5]

Ankaferd Blood Stopper (ABS) is a herbal extract that has been reported to have hemostatic, anti-inflammatory, anti-oxidant, anti-infective and wound healing properties. It consists of five distinct plants namely: *Thymus vulgaris, Glycyrrhiza glabra, Vitis vinifera, Alpinia officinarum*, and *Urtica dioica*. Although the exact pathophysiology underlying the unique effect of ABS remains an area of active investigation, ABS induces the formation of an encapsulated complex protein web with vital erythroid aggregation which covers the whole physiological hemostatic process (Figure 1).[6,7] Besides from these hemostatic properties, the anti-inflammatory effect of ABS are reported to be based on the effect of proinflammatory cytokines including tumor necrosis factor-α (TNF-α), interleukin- 6 (IL-6) and its antioxidant potential.[8-10]

Difficult to cure in nature, inflammation of the cervix can be treated with topical application of ABS based on its anti-inflammatory effect, which was proved in several studies.[10,11] Unfortunately, to our knowledge, no study in the literature evaluates the beneficial effect of ABS explicitly on inflammatory response during cervicitis. Therefore, in this study, we attempted to define and characterize the possible role of ABS in the elimination of a chronic inflammatory process in a rat model of cervicitis.

MATERIALS AND METHODS

Materials and experimental design

The study was approved by the Institutional Animal Use and Care Committee of Canakkale Onsekiz Mart University (COMU) and performed following the Helsinki Declaration of World Medical Association recommendations on animal studies. Wistar albino rats were obtained from COMU Experimental Research Application and Research Center. Twenty-four female Wistar Albino rats were used in the study, with a mean age of four months and a mean weight of 240–300 g. The rats were housed in stainless steel cages in an animal room maintained at a standard humidity (45%-50%) and temperature 22±2°C with 12 hour light periods (12 hours of daylight/12 hours of dark). All animals were fed standard food and water and, twelve hours before the study procedure, feeding was stopped, and the rats were only allowed to drink water. The entire experiment was conducted under half-sterile conditions.

Experimental procedure

Before starting the study, on the basis of experimental models of intrauterine synechia induced by trichloroacetic acid[12], we tested trichloroacetic acid intravaginally to induce cervical lesion on 2 Wistar Albino rats. To establish the experimental model, 0.2 ml of trichloroacetic acid was injected intravaginally. After 3 estrous cycles, the animals were sacrificed and confirmed that 1 dose of 0.2 ml intravaginal trichloroacetic acid is sufficient to induce inflammatory cervicitis.

Groups

After establishing and confirming the experimental model, the study was started with 24 Wistar albino rats. The stage of the estrous cycle for each female rat was determined by histological examination of cells in vaginal smears taken daily at 09 a.m. to 10:00 am. It is known that the rat estrous cycle consists of four stages with a typical cycle every 4 to 5 day. The cycle of the rats was synchronized according to their vaginal smear analysis and divided into three groups.

Group I (injury, n=8): after trichloroacetic acid application, rats did not receive any treatment. Rats were further sacrificed after 3 estrous cycles and cervix of the rats were removed for evaluation.

Group II (Isotonic saline group, n=8): after 1 day from the trichloroacetic acid application, rats were received 2 ml/day intravaginal isotonic saline for 3 estrous cycles. Rats were further sacrificed after 3 estrous cycles and cervix of the rats were removed for evaluation.

Group III (ABS group, n=8): after 1 day from the trichloroacetic acid application, rats received 2 ml/day intravaginal ABS (ABS; İmmun İlaç Kozmetik Ltd, Istanbul, Turkey) for 3 estrous cycles. The dosage of ABS was determined from previous studies.[13] Rats were further sacrificed after 3 estrous cycles and cervix of the rats were removed for evaluation.

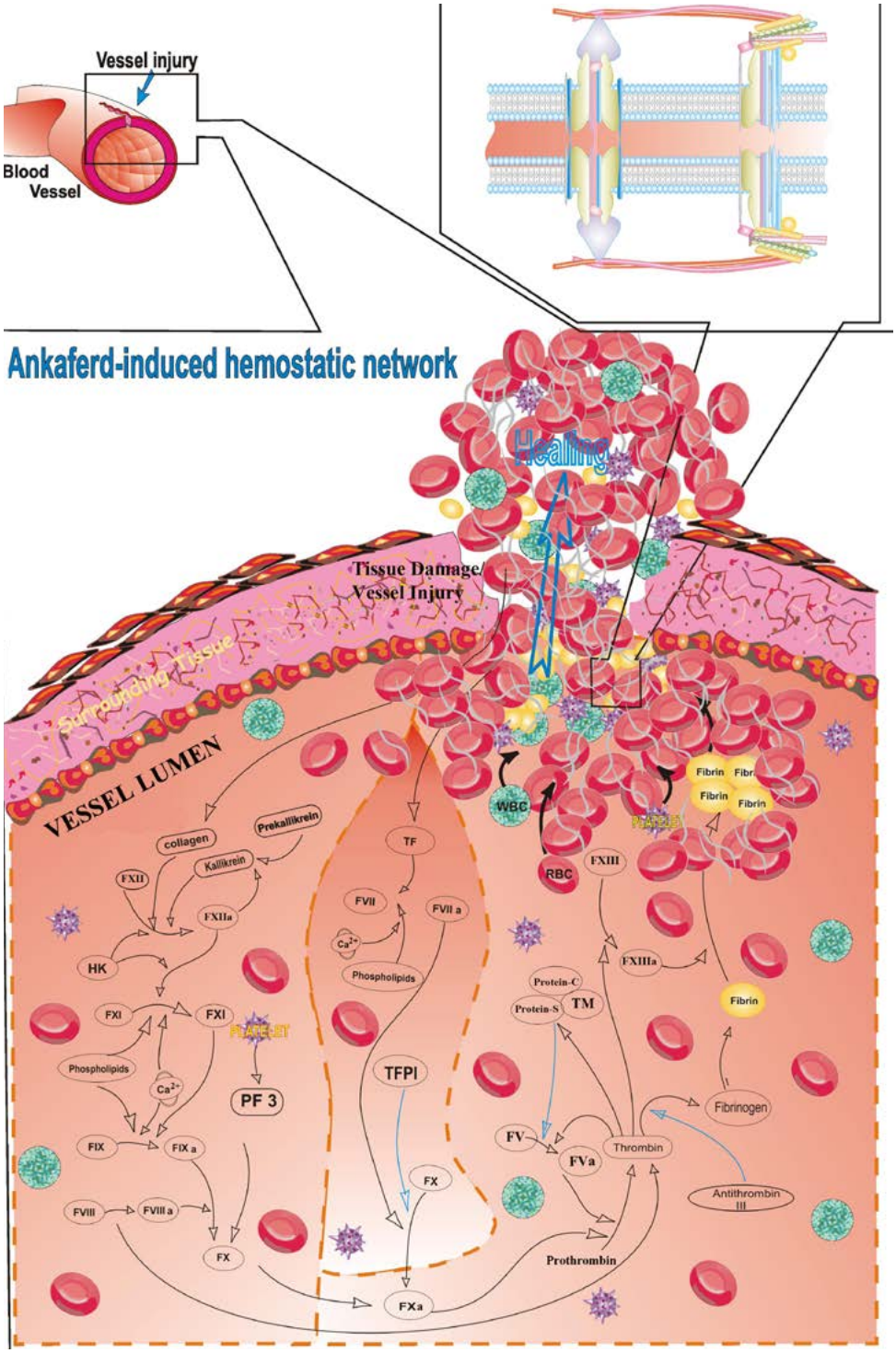

FIGURE 1 The basic mechanism of action for Ankaferd Blood Stopper (ABS) is the formation of an encapsulated protein network that provides focal points for erythrocyte aggregation. ABS-induced formation of the unique protein network within the vital erythroid aggregation covers the entire physiological haemostatic process. Red blood cell (RBC) elements (such as spectrin and ankrin surface receptors, and internal ferrochelatase enzyme), related transcription factors (such as GATA-1) and RBC-related proteins (such as urotensin II) are the main targets of ABS. Source: Modified from Ref. [6] with permission.

Macroscopic and histopathological examinations

Macroscopic findings were recorded by one observer who participated in the injection experiments. Groups were compared macroscopically regarding the presence of erythema, edema and small ulcers.

To investigate histopathologic changes, cervical tissue samples were consecutively numbered and placed in 10% formaldehyde and sent to the histology department of COMU. Evaluation of the pathology specimens was done by a histology specialist who was blind to the three study groups.

After fixation with formaldehyde, cervical tissues were embedded in paraffin. The paraffin blocks were cut in 5 mm thickness on Rotary Microtome (Leica RM2125 RTS), and the sections were stained with hematoxylin and eosin (H&E) and Masson's trichrome method. The histopathologic sections were examined under a light microscope (Zeiss AxioScope A1) for the presence of inflammation, congestion (vascular dilation), and erosion and rated on a modified semi-quantitative scale of 0-3 as also stated by Kilic et al.[12] The amount of inflammation was scored as follows: 0, no inflammation; 1, presence of occasional plasma cells and lymphocytes; 2, significant existence of plasma cells, eosinophils and neutrophils; and 3, existence of many inflammatory cells and microabscesses. Vascular congestion was scored as: 0, no vascular congestion; 1, mild vascular congestion; 2, moderate vascular congestion; and 3; intense vascular congestion. Erosion was scored as: 0, intact epithelium, no erosion; 1, mild epithelial damage; 2, full layer epithelial damage, intact lamina proprium; and 3; damage beyond lamina propria, deep layer involvement. The total tissue damage score was calculated by adding up these scores.

Immunohistochemical examination

Immunohistochemistry for IL-1 β was carried out with a commercial kit (Cell Signaling Technology, Danvers, MA) following the manufacturer's protocol. In brief, approximately 4-micron tissue slices were fixed in 10% neutral-buffered formalin and before proceeding with the staining protocol, slides deparaffinized and rehydrated. Antigen retrieval method was applied to conduct immunohistochemistry on the paraffin sections. Sections were heated in a microwave at 200 watts for 20 minutes in 10mM EDTA and left to cool down at room temperature for 20 min. A PAP pen was used to delineate the tissue sections. Hydrogen peroxide (Thermo Scientific, Erembodegem, Belgium) was dropped to the sections and remained there for 15 minutes. The sections were then washed with phosphate-buffered saline (PBS). Sections that were incubated with primary antibodies were marked with AEC chromogen (ThermoScientific™) and counterstained with Mayer's hematoxylin. Finally, each section was dehydrated and cover-slipped. Immunohistochemical evaluation and scoring were done according to Jiang et al.[14] According to this scoring, IL-1 β immunostaining scores were calculated by initial defining of both the staining intensity (0, no staining; 1, weak but detectable staining; 2, moderate; and 3, strong staining) and the percentage of positively stained cells (0, no staining; 1, <25% staining; 2, >25% and <50% staining; 3, >50% staining). The final score was measured by multiplying scoring intensity by the percentage of positive stained score.

Statistical analyses

Statistical analysis was performed using SPSS for Windows 19.0 (Chicago Inc., Chicago, IL). All continuous variables were expressed as the mean ±standard deviation (SD) and median (minimum-maximum). Because of the small sample size and non-normal distribution of the data non-parametric tests were used to evaluate the results. The mean values were compared by Kruskal–Wallis, and Mann–Whitney U tests. A p-value lower than 0.05 was considered as statistically significant.

RESULTS

All of the rats in group I, II and III survived during the study period. Macroscopic erythema and edema were observed more prominently in group I and II compared to group III. Small ulcers were only seen in one rat in group I and in two rats in group II. No macroscopic ulcers were found in the cervical mucosa of ABS group.

According to total tissue injury scores, mean injury scores were found to be statistically elevated in group I and group II compared to ABS group. Subgroup analysis revealed that group I and II have increased inflammation (p=0.018), vascular congestion (p=0.001) and erosion scores (p>0.001)

TABLE 1: TISSUE INJURY SCORES OF STUDY GROUPS

	Inflammation	Congestion	Erosion	Total
Group I (n=8)				
Mean (±SD)	2.75±0.46	2.37±0.51	2.37±0.74	7.50±0.92
Median (min-max)	3 (2-3)	2 (2-3)	2 (1-3)	7 (6-9)
Group II (n=8)				
Mean (±SD)	2.75±0.46	2.25±0.71	2.28±0.71	7.37±0.91
Median (min-max)	3 (2-3)	2 (1-3)	2 (1-3)	7 (6-9)
Group III (n=8)				
Mean (±SD)	1.75±0.70	1.01±0.75	0.12±0.35	2.88±1.25
Median (min-max)	2 (0-3)	1 (0-2)	0 (0-1)	3 (1-5)
p	0.018a	0.001b	<0.001c	<0.001d

a,b,c,d Group III vs Group I and II

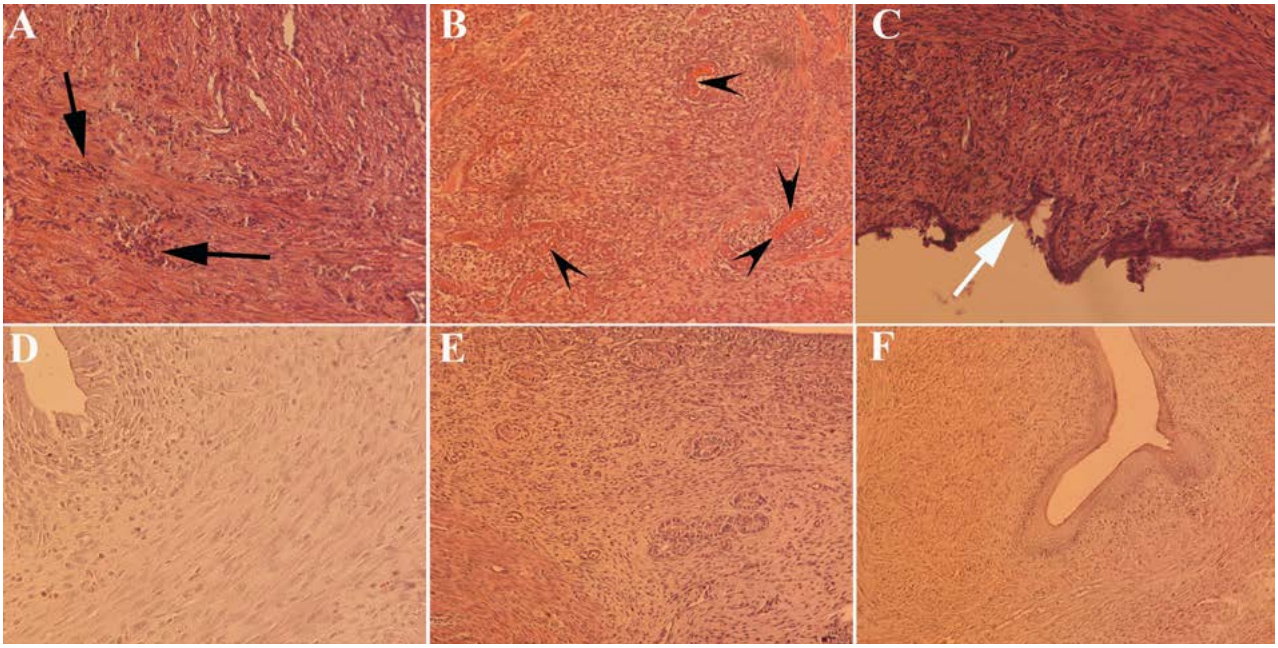

FIGURE 2. Microscopic comparison of inflammation (A,D), vascular congestion (B,E) and erosion (C,F) in group II (injury+isotonic saline) and in group III (injury+Ankaferd BloodStopper [ABS]). Histology of isotonica saline treated group is shown in panels A, B, C and histology of ABS treated group is shown in panels D, E, F. Isotonic saline treated group showed significantly greater histological damage including increased cellular inflammation (A: black arrow), vascular congestion (B: arrowhead) and erosion (C: white arrow) compared to ABS treated group.

compared to ABS group (Table 1). Mean total injury scores for groups I, II and III were found to be 7.50±0.92, 7.37±0.91 and 2.88±1.25 respectively. Group III had significantly lower total scores compared with group I and II ($p<0.001$). Figure 2 shows the microscopic comparison of inflammation, vascular congestion, and erosion in group II and III.

Immunohistochemical analysis revealed that median IL-1 β expression scores in group I, II and III were 4 (2-9), 4 (2-9) and 2 (1-4) respectively. IL-1 β expression was found to be higher in group I and II compared to group III ($p=0.018$).

DISCUSSION

The purpose of the present study was to investigate the macroscopic and microscopic changes in the cervical mucosa as well as immunohistochemical staining for IL-1 β in response to ABS treatment in a chemically induced cervicitis model of rats. We observed that intravaginal ABS injection compared with isotonic saline results in a significant reduction in the number of inflammatory cells in cervical mucosa. Furthermore, ABS seemed to have significant protective effects on vascular congestion and cervical erosion.

ABS is a novel hemostatic agent that has pleiotropic and profound effects on the hematologic and immune system including anti-microbial, anti-neoplastic, anti-mutagenic, and antioxidant as well as tissue-healing properties.[6,15,16] The mechanistic basis of this pleiotropism remains a mystery despite extensive research during the past decade. It has been suggested that the unique action of the ABS mechanism mainly depends on the protein agglutination and polymerization that modulates the erythroid aggregation and vascular endothelium.[7] In a recent study by Simsek et al.[15] a detailed proteomic and transcriptomic analyses of ABS was performed in order to explain the pleiotropic effects of this hemostatic agent. Authors demonstrated that hepatocyte nuclear factor-4a, malic enzyme-1, midkine and protein inhibitor of activated signal transducer and activator of transcription (PIAS)-2 are the main components that are responsible from the pleiotropic effects of ABS. Although less studied, ABS has also shown promising results in inflammatory conditions related to cartilage tissue, gastric mucosa, pericardial tissue, and liver.[9,17-19] Dynactin, Egr-1, Midkine, NF-1, Twinfilin, V-myc, and Yin Yang 1 can contribute both the anti-inflammatory and tissue-healing effects of ABS with various mechanisms.[15]

This study demonstrated that IL-1β expressions are significantly down-regulated in the ABS group compared with the other two groups. Although our study is the first to investigate the expression of IL-1β in ABS treated cervical mucosa, a study by Amanvermez et al.[20] IL-1β and Interleukin-10 (IL-10) investigated expressions during the fracture healing process with or without ABS application to the bone fracture. IL-1β levels were found to be decreased in the ABS-treated experimental rat group. Being a potent inflammatory cytokine, which is up-regulated during inflammation, IL-1β has a vital role in the host defense and inflammatory response.[21] However, overproduction or prolonged expression of IL-1β in the inflammatory course may result in enhanced tissue destruction due to the immune cells overactivation and the production of proteases, along with decreased collagen matrix formation and decreased granulation tissue formation.[22,23] For this reason, ABS could exert its beneficial effects by limiting IL-1β induced vascular permeability, neutrophil recruitment and maturation in the early phases of inflammation.

In addition to the decreased expressions of IL-1β in the ABS group, we found that cervical erosion was less prominent in the ABS group compared to the other two groups. Cervical mucosal erosion, which is one of the unique features of cervicitis, can be caused by trauma, deep lacerations, infections, and contraceptive devices.[24] The eroded cervical surface could enlarge, and complaints like excessive discharge can be more prevalent. Although treatment options may vary depending on the acute or chronic nature of the process, there is still a limited approach to cure cervical erosions related to chronic inflammation. In this context, ABS can effectively promote the healing of cervical erosion with no side effects.

After the injection, all the animals survived after 3 estrous cycles indicating that local application of ABS is safe and has no severe side effects. This is important because ABS is still considered a novel antihemostatic and anti-inflammatory agent since the extensive characterization of the pharmacological properties of ABS needs to be carried out. Although no other study in the literature compares the safety and optimal dosage of ABS in cervical inflammation, data from other trials demonstrated that ABS is safe and efficient in distinct disease conditions including dental surgery, wound healing, epistaxis, gastrointestinal bleeding, and peritoneal adhesion.[13,16,25-28] Unfortunately, because of unknown optimal dosage schedules which can cause premature safety and efficacy studies to fail and may limit further research, it is crucial to conduct preliminary analysis to determine ABS's optimal dosage in cervical inflammation. Therefore, this preliminary study is of paramount importance because it provides important insights into the understanding of the histopathologic and immunohistochemical basis of ABS induced cervical mucosa protection.

One of the limitations of the present study is the lack of immunohistochemical analysis apart from IL-1 β. Immunohistochemical staining with monoclonal antibodies against pro-inflammatory cytokines and growth factors such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), transforming growth factor-alpha (TGF-alpha) and epidermal growth factor receptor (EGFR) would be noteworthy to understand the enigmatic role of ABS in the cervical inflammatory process. Another limitation of our study is the relatively short follow-up period of rats which cannot encompass the natural recovery period.

In conclusion, ABS may be used safely and effectively in cervical inflammatory conditions as a natural remedy.

ABS effectively inhibits the inflammatory process and has a unique role as an effective wound-healing and anti-inflammatory agent. Despite the existence of several pharmacological and herbal options for cervicitis, present work demonstrates that ABS effectively decreases the expression of IL-1β resulting in a decrease of inflammation, vascular congestion, and mucosal erosion. Further studies are required to evaluate the clinical benefits and any possible adverse effects of ABS application on cervical mucosa.

RESUMO

OBJETIVO: *Ankaferd Blood Stopper (ABS) é um extrato de plantas medicinais utilizado topicamente como um agente hemostático, anti-inflamatório e antioxidante. O seu efeito citoproteico depende principalmente das suas propriedades pleiotrópicas por meio da modulação de mediadores inflamatórios tais como IL-1β, IL-6 e TNF-a. O objetivo deste estudo é testar o possível efeito terapêutico do ABS no tratamento de condições erosivas e inflamatórias que ocorrem no colo uterino.*

MÉTODOS: *Vinte e quatro ratas Wistar Albino foram utilizadas no presente estudo. O ácido tricloroacético foi aplicado intravaginalmente para estabelecer um modelo experimental de cervicite em ratos. Os ratos foram divididos aleatoriamente em três grupos: grupo I (lesão), grupo II (lesão + fisiológico sérico) e grupo III (lesão + ABS). Após três ciclos estrais de ABS e tratamento fisiológico sérico, as quantidades de inflamação, congestionamento vascular e erosão foram avaliadas nos tecidos cervicais usando uma escala semiquantitativa modificada de 0-3. Coloração imuno-histoquímica com anticorpos monoclonais contra IL-1β também foi realizada.*

RESULTADOS: *Em comparação com os grupos I e II, o grupo ABS mostrou menos infiltração de células inflamatórias, congestionamento vascular e erosão cervical. Além disso, em comparação com o grupo ABS, observou-se uma coloração proeminente de IL-1β no grupo I e no grupo II.*

CONCLUSÃO: *Nossos dados sugerem que o ABS é uma alternativa altamente eficaz para induzir o epitélio cervical normal e pode ser utilizado com segurança no tratamento da inflamação cervical com ou sem erosão cervical.*

PALAVRAS-CHAVE: *Cervicite uterina. Doenças do colo do útero. Inflamação. Interleucina-1beta. Extratos vegetais/*farmacologia.*

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ORIGINAL ARTICLE

Differentiation between stercoral perforation and colorectal cancer perforation

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SUMMARY

OBJECTIVE: *To determine the computed tomography (CT) signs associated with stercoral perforation and colorectal cancer perforation.*

MATERIALS AND METHODS: *From May 2003 to Feb. 2015, all surgically and pathologically confirmed patients with stercoral perforation (n=8, mean age 68.3 years) or colon cancer perforation (n=11, mean age 66.3 years) were retrospectively reviewed by two board-certified radiologists blinded to the proven diagnosis. The following CT findings were evaluated and recorded for each patient: wall thickness of the distal colon adjacent to perforation site, pattern of the colon wall thickening and enhancement, length of the thickened bowel wall, presence of fecaloma, degree of proximal colon dilatation, and pericolonic inflammation or presence of pericolonic abscess, and number of enlarged pericolonic lymph nodes. These findings were correlated with the pathologic diagnosis.*

RESULTS: *The mean thickness of the distal colonic wall adjacent to the perforation site was 13.6 mm in patients with colorectal cancer perforation and 5.1 mm with stercoral perforation, which was statistically different. There was a significant correlation between colorectal cancer perforation and eccentric wall thickening ($p<0.01$). CT findings of layered enhancing wall thickening ($p<0.01$) and the presence of fecaloma in the proximal colon ($p<0.01$) were significant findings for stercoral perforation. Patients with colorectal cancer displayed more pericolonic lymph nodes (mean 2.27, $p<0.05$).*

CONCLUSION: *Fecaloma in the proximal colon and layered enhancing wall thickening adjacent to perforation site are likely due to stercoral perforation. Eccentric bowel wall thickening at the distal portion of the perforation site with many enlarged pericolonic lymph nodes is most likely due to colorectal cancer perforation.*

KEYWORDS: *Fecal Impaction. Colorectal Neoplasms. Colitis, Ischemic. Intestinal Perforation.*

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INTRODUCTION

Stercoral colitis is caused by pressure necrosis from a fecal mass [1,2] and it can produce stercoral perforation if not promptly treated. It is a very rare cause of colon perforation but can be life-threatening with mortality rates of 23-57% [3]. In stercoral perforation, the diameter of the perforation site can be large, and the degree of contamination by feces can be severe.

Colorectal cancer, the most common cause of colonic perforation [4], can also lead to fecal impaction by progressive luminal obstruction [5]. Imaging findings can be similar to those of stercoral perforation [5-7]. However, treatments for these two conditions are entirely different. The resection of the diseased segment of the colon and its exteriorization is sufficient for stercoral perforation [2], while extensive bowel resection with lymph node dissection and adjuvant chemotherapy is required for the treatment of colorectal cancer perforation [5]. Thus, distinguishing these two conditions with an accurate preoperative diagnosis can facilitate early therapeutic management and improve survival [4,8,9].

Useful imaging features for the differentiation between stercoral perforation and colorectal perforation from statistical evidence is unclearly reported. The purpose of this study was to evaluate the useful preoperative computed tomography (CT) findings to differentiate stercoral perforation and colorectal cancer perforation.

MATERIALS AND METHODS

The study was approved by the institutional review board. Patient consent was not required for the retrospective review of records and images because patient anonymity was preserved.

Patients

One radiologist retrospectively searched electronic medical records and the radiology information system of our hospital for individuals with stercoral perforation identified from May 2003 until February 2015. First, all enhanced abdomen CT studies for which the reports included the words “stercoral colitis” or “stercoral perforation” were selected. Four patients were included in the stercoral perforation group, with both enhanced CT images and histologic results available. We also included another four patients from two same hospital branches identified by the same method during the same period. Finally, eight patients were included in the stercoral perforation group.

Next, the radiologist searched for reports including the word “bowel perforation” with histologic results, using electronic medical records during the designated period. A total of 95 patients were identified. Patients with small bowel perforation (n=33), ischemic colitis (n=5), diverticulitis or appendicitis (n=7), Crohn’s disease (n=3), other malignant tumor except adenocarcinoma (n=6), iatrogenic cause (n=4), and sclerosing peritonitis (n=1) were excluded. The 17 patients who had not undergone enhanced abdomen CT at our hospital were also excluded. Moreover, patients with ascending colon cancer (n=3) or hepatic metastasis (n=1) were excluded. Because of the large diameter of the ascending colon and a relatively large amount of water in the feces of the ascending colon, stercoral perforation rarely does not occur in the ascending colon. Furthermore, the presence of hepatic metastasis is more suggestive of colon cancer than stercoral perforation.

Finally, 11 patients with perforated colon cancer at the rectum, sigmoid colon or descending colon who showed no evidence of other solid organ metastasis were included in the colorectal cancer perforation group.

CT Examinations

CT examinations in our hospital were performed using a 4-, or 16-detector row CT scanner (Sensation 4 or 64; Siemens Medical Systems, Erlangen, Germany). Scanning parameters were reconstruction thickness, 3 mm; reconstruction interval, 1.5 mm; beam pitch, 1.5; tube voltage, 120 kVp; and tube current, 84 mAs.

For the patients in the second hospital branch, a 16-detector row CT scanner (Sensation 16; Siemens Medical Systems) and a 64-detector row CT scanner (Lightspeed VCT XTe; GE Medical Systems, Milwaukee, WI, USA) were used with the administration of 140 mL of same intravenous (IV) contrast medium. Scanning parameters were reconstruction thickness, 5 mm; reconstruction interval, 5 mm; beam pitch, 1.5; tube voltage, 120 kVp; and tube current, 100 mAs.

In third hospital branch, a Lightspeed VCT XTe 16-detector row CT scanner was also used with the same scanning parameters. About 140-150 mL of ionic or nonionic iodinated IV contrast medium (Omnipaque; Nycomed, Princeton, NJ and Optiray 320,

Mallinckrodt, Dublin, Ireland) was routinely injected in all patients following departmental protocols. In all cases, axial and coronal images at the portal phase were analyzed.

Imaging Analysis

Two abdominal radiologists (10 and 1 years of abdominal imaging experience, respectively) retrospectively reviewed the CT images of the patients. The reviewers were blinded to the pathologic results. Any discrepancy during the review was resolved by consensus.

CT findings that were analyzed for each patient included the following. The colon was divided into proximal and distal portions based on suspected perforation site. The thickness (mm) of the distal colon wall was measured by drawing a right angle to the lumen on the utilized PACS system and grouped into eccentric and concentric wall thickening. Wall thickening was considered to be eccentric when there was asymmetry in the thickening of the two walls of the colon. Patients were also divided into three groups according to the length of the thickened wall (<5 cm, 5~10 cm, >10 cm). The pattern of wall enhancement at the suspected perforation site was recorded as a layered or homogenous enhancement. The presence of the fecaloma in the proximal colon and the degree of proximal colonic dilatation (<3 cm, 3~5 cm, 5~7 cm, >7 cm) were also investigated. Fecaloma was considered to be present when hyperdense mass-like feces filled the entire colonic lumen and caused luminal dilatation. The degree of pericolic fat infiltration (mild, moderate, severe) or presence of abscess, and the number of pericolic lymph nodes were recorded. Lymph nodes were defined as measuring greater than 1 cm in the short-axis diameter.

Statistical Analysis

The CT findings were correlated with the pathologic diagnosis of stercoral perforation and colorectal cancer perforation. All statistical analyses were conducted using SPSS 14.0 for Windows (SPSS, Chicago, Illinois, USA). P<.05 indicated statistical significance. The thickness of the colon wall and the mean number of lymph nodes of each group were compared using the Mann-Whitney U test. Statistical differences for other categorical data (i.e., length of the thickened wall) were analyzed using the Fisher exact test.

RESULTS

The results of analyses of the preoperative CT imaging findings in the stercoral perforation and colorectal cancer perforation groups are shown in Ta-

TABLE 1: PREVALENCE OF CT FINDINGS IN STERCORAL PERFORATION AND COLORECTAL CANCER PERFORATION

Morphologic Criteria	Patient Group		P-value
	Stercoral perforation (n=8)	Colorectal cancer perforation (n=11)	
Wall thickness (mm)	5.1	13.6	.001
Eccentric wall thickening	0 (0%)	11 (100%)	.000
Length of bowel wall thickening			
< 5 cm	3	6	.254
5-10 cm	3	5	
> 10 cm	2		
Homogeneous enhancement	1 (12.5%)	11 (100%)	.000
Fecaloma in proximal bowel	7 (87.5%)	2 (18.2%)	.005
Dilatation of proximal bowel			
< 3 cm	0	3	.177
3-5 cm	3	4	
5-7 cm	4	3	
> 7 cm	1	1	
Pericolonic inflammation			
Mild	2	5	.790
Moderate	4	0	
Severe	2	6	
Pericolonic abscess	1 (12.5%)	3 (37.5%)	.603
Number of pericolonic LNs	0.25	2.27	.011

ble 1. The patients in the stercoral perforation group had a significantly higher prevalence of the following findings (P<.05): thinner thick distal colonic wall adjacent to perforation site, concentric wall thickening, layered enhancement of the colonic wall, and the presence of fecaloma in the proximal colon.

The wall thickness of the just distal bowel on suspected perforation sites was about 5.1 mm in the stercoral perforation group and 13.6 mm in the colorectal cancer perforation group. The difference was statistically significant (p=0.001). All patients in the colon cancer perforation group showed eccentric wall thickening at the distal portion, and no patients in the stercoral perforation group showed eccentric wall thickening (p=0.000). The length of the thickened wall displayed a tendency of a longer length in the stercoral perforation group. However, no statistically significant correlation was seen between the two groups (p=0.254).

Significant differences in the pattern of wall enhancement around the suspected perforation site (p=0.000) and the presence of fecaloma in the proximal colon (p=0.005) were noted between the two groups. All patients with stercoral perforation showed a layered enhancement of the colonic wall at the suspected perforation site, and the other patients with colon cancer perforation showed a homogenous enhancement of the colonic wall. In patients with stercoral perforation, 7 of 8 patients (87.5 %) showed fecaloma in the proximal colon (Fig. 1), whereas in patients with colorectal cancer perforation, 2 of 11 patients (18.2 %) showed fecaloma (Fig. 2).

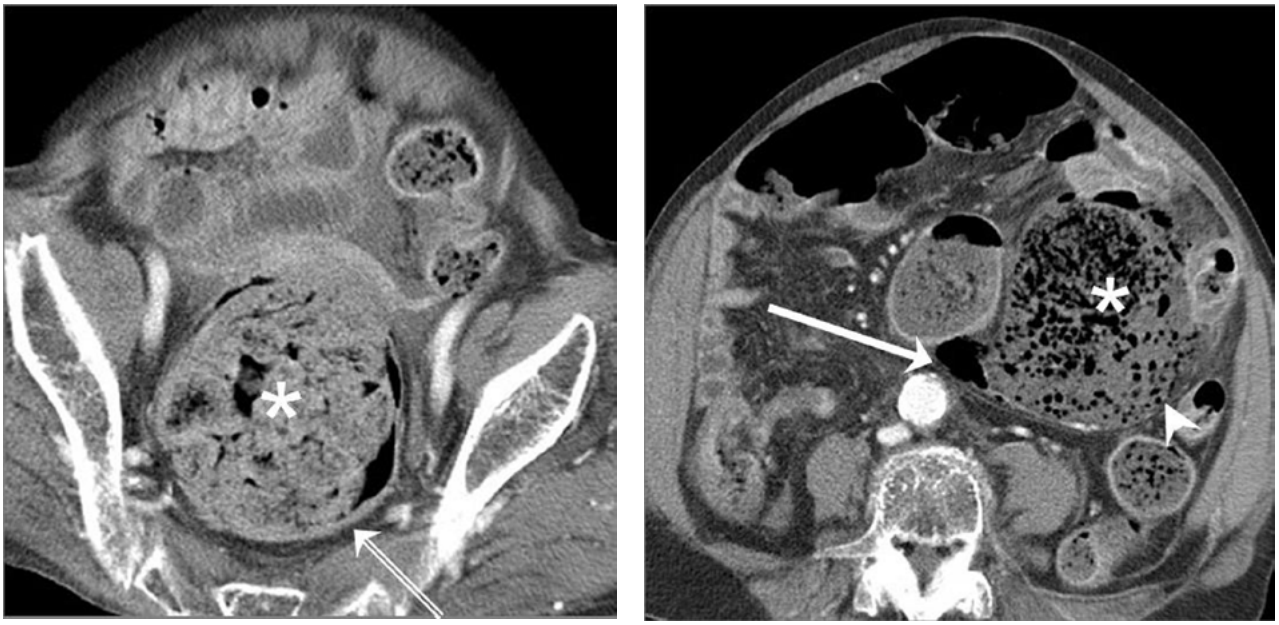
The degree of proximal colonic dilatation was classified into four groups. There was no significant difference (p=0.177). There were also no evident significant differences between the groups in the degree of pericolic fat infiltration (p=0.790) and the presence of pericolic abscess (p=0.603).

The mean number of pericolic lymph nodes was 0.25 for stercoral perforation and 2.27 for colorectal cancer perforation. Significant differences in the number of pericolonic lymph nodes (p=0.011) were noted between the two groups.

DISCUSSION

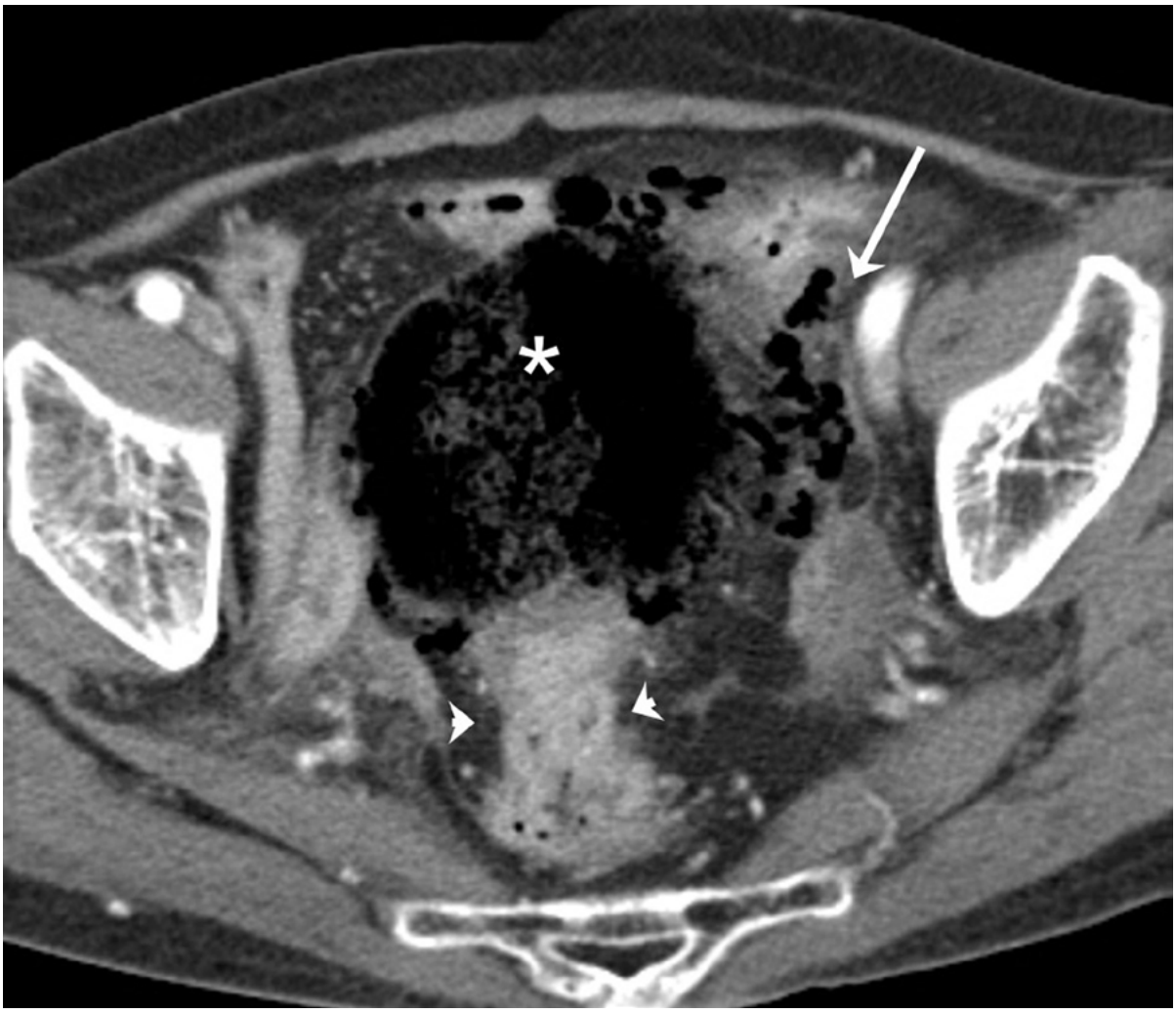
Recently, increasing numbers of patients with chronic constipation have been documented because of a global aging trend with long-term hospitalization of the elderly, as well as radiation therapy for gastrointestinal tract cancer, steroid use in organ transplant patients [1], frequent use of antidepressants and painkillers, and a low fiber diet in younger people. Chronic constipation increases the incidence of stercoral colitis and stercoral perforation. Thus the interest on these diseases is increasingly growing. In these patients, differentiation between stercoral perforation and colorectal cancer perforation is critical

FIGURE 1: A 78-YEAR-OLD WOMAN WITH ABDOMINAL PAIN AND DIAGNOSED WITH STERCORAL PERFORATION.



A and B. Axial contrast-enhanced CT scans show a focal wall defect (arrowhead) in the sigmoid colon with extra luminal feces, free air (arrow), and fecaloma (*) with luminal dilatation and posterior wall thickening (double arrow) in the rectum, which is suggestive of stercoral colitis.

FIGURE 2: A 77-YEAR-OLD WOMAN WITH CONSTIPATION AND DIAGNOSED WITH RECTAL CANCER PERFORATION.



Axial contrast-enhanced CT scan shows fecaloma (*) with luminal dilatation in the rectum, extraluminal free air (arrow) in the pelvic cavity and abrupt luminal narrowing with homogenously enhancing wall thickening (arrowheads) in the distal rectum. Hartmann's operation was performed, and adenocarcinoma was confirmed by the pathologic examination.

in determining the appropriate treatment plan and predicting the prognosis of patients. Therefore, clear differentiation criteria on imaging will be beneficial in clinical medicine.

Fecaloma that can cause stercoral perforation occurs because of the accumulation of feces over the years after absorption of water contents by the colon, with calcification rarely occurring [10]. These fecaloma gradually expand the lumen of the large bowel and increase the intraluminal pressure, which results with a reduction of blood supply to the bowel wall. If it is not treated appropriately and continued, ulceration or perforation of the bowel wall can occur due to ischemia [2]. However, the fecaloma may also occur by partial obstruction due to colon cancer. Thus, when stercoral colitis or perforation is suspected, the possibility of combined colon cancer causing distal bowel obstruction should be considered.

CT is the most useful examination to detect extraluminal gas. The presence of air in the colon wall, mesocolon, or pneumoperitoneum limited to the pelvic cavity will help to predict colon perforation as the cause of pneumoperitoneum [11]. If fecaloma with luminal dilatation of the colon accompanies extraluminal air, the possibility of stercoral perforation should be considered.

In the present study, the sigmoid colon was the most common site of stercoral perforation, which is consistent with the previous report that sigmoid or rectosigmoid colon is the most frequent site of stercoral perforation [1]. Feces in the more distal colon get harder due to water resorption, so it can increase the chance of colon perforation in the distal colon which has a narrowing lumen [2,8]. On the other hand, the cecum, the largest lumen of the colon, is the most common site for colon cancer perforation [12,13]. In our

study, in the stercoral perforation group, the suspected perforation area was the rectosigmoid colon in 6 patients and the transverse colon in 2 patients. Because we excluded patients with ascending colon cancer perforation, meaningful statistical comparisons between both groups were difficult.

In cases of obstructive colitis associated with colon cancer, normal mucosa without inflammation or ulcer can be found in just the proximal or distal portion of the cancer [5]. In this study, while the stercoral perforation group displayed circumferential-enhancing wall thickening at the site of contact with the fecaloma, the colon cancer perforation group feature eccentric-enhancing wall thickening at the adjacent distal bowel, even with the presence of fecaloma. A thicker distal bowel wall and the presence of several enlarged pericolonic lymph nodes around the perforation site are important findings for the differential diagnosis of colon cancer perforation.

In our study, more severe luminal dilatation of the proximal bowel favored stercoral perforation but did not present statistical significance. Stercoral perforation results from luminal expansion by the fecaloma and secondary pressure necrosis of the bowel wall, so most patients show moderate to severe luminal dilatation. However, colon cancer perforation can occur due to tumor necrosis itself [4], as well as secondary perforation due to luminal distension of the proximal colon due to distal bowel obstruction, so it may not show proximal bowel distension. In this study, we might have subdivided the degree of luminal dilatation too excessively, accounting for the lack of statistical significance, or colon perforation may have occurred before the CT scanning and luminal expansion could have been improved by decreased intraluminal pressure. This result is consistent with the prior description that the degree of luminal expansion is not associated with the prognosis of patients[14].

Layered-enhancing wall thickening was more frequently seen in the stercoral perforation group than the colon cancer perforation group. This finding is also evident in ischemic colitis, which shows circumferential-enhancing wall thickening and hyperattenuating mucosa by mucosal bleeding [3]. However, it can be discriminative because ischemic colitis rarely shows fecaloma [11] and involves a longer segment than stercoral perforation with a narrow lumen.

If bowel perforation occurs gradually, it forms a localized pericolonic abscess at the perforation site rather than spreading rapidly into the abdominal cavity due to the viscosity of feces [2]. There were no statistically significant differences between the two groups in the presence of pericolonic abscess formation or the degree of inflammation. These findings are just secondary findings of colon perforation, not specific CT signs for determining the cause of perforation.

Stercoral perforation shows transmural necrosis, and a well-defined ulcer on the histopathologic exam, the shape of ulcer is irregular according to the shape of contacting fecaloma, and the size is often larger than 1 cm [10]. Chronic inflammatory change and atrophy of the colon wall can be seen around the perforation site. Also, stercoral colitis can occur in several places simultaneously, because the fecaloma could have been located anywhere on the colon [15]. This type of perforation more often occurs in the anti-mesenteric border of the bowel, because the blood perfusion at this portion is low and intraluminal pressure exceeds the capillary perfusion pressure at first [10].

The treatment of choice for stercoral perforation is surgery, and Hartman's operation is usually performed to resect the whole involved bowel segment and make the colostomy [10]. To prevent intraabdominal infection, intraabdominal washing with a large amount of saline and extensive antibiotic use are needed. To prevent the stercoral perforation, active treatment for constipation and rapid removal of the fecaloma are required [15].

The limitation of this study is the small number of patients. Because of the low incidence of the disease, reportedly 0.04%-2.3% [14] and the difficulty in histopathologic assessment with nonspecific pressure necrosis and ischemia, it is hard to recruit many patients. Most of the patients with stercoral colitis receive conservative treatment and are only treated surgically in cases of suspected bowel perforation. Further prospective studies with a larger population are likely necessary to prove our results in those populations. Second, we did not evaluate the reproducibility of CT features of stercoral perforation with various observers as we had resolved any discrepancies between the observers by consensus. Third, we retrospectively searched for individuals with stercoral perforation identified at CT, using electronic medical records and the radiology information system of our hospital. Thus, there is the possibility that we missed some patients with this search method. Fourth, our patients were se-

lected from three different hospitals, so they used different scanners and different parameters. However, it could not help because stercoral perforation has a very low incidence.

CONCLUSION

In conclusion, stercoral perforation is a very rare disease, but the conditions that cause chronic constipation or fecalomas are widespread. If perforation occurs, the prognosis is critical. So, accurate preoperative diagnosis of stercoral perforation on CT examination has important clinical implications. In summary, patients with stercoral perforation exhibited fecaloma in the proximal bowel and layered-enhancing wall thickening around the perforation site. In contrast, patients with colon cancer perforation showed thicker eccentric wall thickening in the distal bowel and more frequently presented with enlarged pericolonic lymph nodes.

RESUMO

OBJETIVO: *Determinar os sinais de CT associados à perfuração estercoral e perfuração do câncer colorretal.*

MÉTODOS: *De maio de 2003 a fevereiro de 2015, todos os pacientes cirurgicamente e patologicamente confirmados com perfuração estercoral (n = 8, idade média de 68,3 anos) ou perfuração de câncer de cólon (n = 11, idade média de 66,3 anos) foram revisados retrospectivamente por dois radiologistas certificados por placa cegados ao diagnóstico comprovado. Os seguintes achados CT foram avaliados e gravados para cada paciente: espessura da parede do cólon distal adjacente ao local da perfuração, padrão de espessamento e realce da parede do cólon, comprimento da parede intestinal espessada, presença de fecaloma, grau de dilatação do cólon proximal e inflamação pericolônica ou presença de abscesso pericolônico e número de linfonodos pericolônicos aumentados. Esses achados foram correlacionados com o diagnóstico patológico.*

RESULTADOS: *A espessura média da parede colônica distal adjacente ao local de perfuração foi de 13,6 mm em pacientes com perfuração de câncer colorretal e 5,1 mm com perfuração estercoral, que foi estatisticamente diferente. Houve uma correlação significativa entre a perfuração do câncer colorretal e o espessamento da parede excêntrica ($p < 0,01$). Os achados de CT de espessamento de parede aprimorada em camadas ($p < 0,01$) e presença de fecaloma no cólon proximal ($p < 0,01$) foram achados significativos para perfuração estercoral. Os pacientes com câncer colorretal apresentaram mais linfonodos pericolônicos (média 2,27, $p < 0,05$).*

CONCLUSÃO: *O fecaloma no cólon proximal e o espessamento da parede que aumenta a camada adjacente ao local da perfuração são provavelmente devidos à perfuração estereocálica. O espessamento da parede intestinal excêntrica na porção distal do local da perfuração com muitos gânglios linfáticos pericolônicos aumentados é provavelmente a perfuração do câncer colorretal.*

PALAVRAS-CHAVE: *Impacção fecal. Neoplasias colorretais. Colite isquêmica. Perfuração intestinal.*

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ORIGINAL ARTICLE

Axis screws: results and complications of a large case series

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SUMMARY

OBJECTIVE: *To present the surgical results of patients who underwent axis screw instrumentation, discussing surgical nuances and complications of the techniques used.*

METHODS: *Retrospective case-series evaluation of patients who underwent spinal surgery with axis instrumentation using screws.*

RESULTS: *Sixty-five patients were included in this study. The most common cause of mechanical instability was spinal cord trauma involving the axis (36 patients – 55.4%), followed by congenital craniocervical malformation (12 patients – 18.5%). Thirty-seven (57%) patients required concomitant C1 fusion. Bilateral axis fixation was performed in almost all cases. Twenty-three patients (35.4%) underwent bilateral laminar screws fixation; pars screws were used in twenty-two patients (33.8%), and pedicular screws were used isolated in only three patients (4.6%). In fourteen patients (21.5%), we performed a hybrid construction. There was no neurological worsening nor vertebral artery injury in this series.*

CONCLUSION: *Axis screw instrumentation proved to be a safe and efficient method for cervical stabilization. Laminar and pars screws were the most commonly used*

KEYWORDS: *Axis, cervical vertebra. Cervical vertebrae. Bone screws. Internal fixators.*

INTRODUCTION

The atlantoaxial complex has a unique bone, ligamentous, and vascular anatomy that particularly distinguishes it from the subaxial spine, making it challenging to achieve mechanical stability in this very mobile spine segment[1-3]. Due to the high range of motion of the C1-2 segment, fusion rates at this level have been lower than those at the subaxial spine[4-6].

Spinal diseases such as trauma, congenital malformations, tumors, and inflammatory disorders may lead to cervical instability involving the axis,

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requiring surgical fixation[7,8]. Although the posterior wiring procedure is easy to accomplish, this technique has a high rate of nonunion, leading to incomplete immobilization and pseudoarthrosis[9-12].

Since the first description of the sublaminar wiring technique by Gallie[6] in 1939, many derived procedures for internal fixation have been proposed to treat atlantoaxial instability[4,13,14]. Although the transarticular screw fixation provides superior stability and higher bone fusion rates compared to wiring[12,15], it is a technically demanding procedure with a risk of injury to the vertebral artery and spinal cord[16,17].

Pedicle screws fixation has emerged as an effective form of axis instrumentation and is probably the technique that offers the strongest possible fixation[18,19]. Another screw fixation technique, very similar to the transarticular C1-2 technique, is the insertion of pars interarticularis screws, using shorter length screws, with the same trajectory through the isthmus[20]. In patients who do not require a laminectomy and have a prohibitive pedicle or pars anatomy, the use of laminar screws for immobilization of the axis is possible and without risk of vertebral artery injury[20-22].

We have previously published our preliminary experience with axis screws instrumentation, proposing an algorithm on the decision for axis fixation[23]. In the present study, we present our experience with a more extensive series of cases of axis screw instrumentation, discussing surgical nuances and complications.

METHODS

A retrospective case series of patients who underwent axis stabilization using pars, pedicle, or laminar screws from 2009 to 2017 was performed by a single surgeon (AFJ). Forty-seven more patients were added to our previously published initial case series of 17 patients[23]. All patients that underwent surgery that involved axis fixation with screws were included. The only exclusion criteria were lack of clinical or radiological data. The study was part of a database of spinal patients, performed retrospectively (17337313.7.0000.5404)

The collected clinical data included: age, sex, surgical indication, surgical complications and pre- and post-op neurological status. Radiological images were obtained preoperatively in all patients. 3D CT scans and CT angiogram (whenever assessment of the vertebral artery involvement was necessary) were analyzed by the chief surgeon (AFJ) in order to evaluate axis morphology and decide about the fixation technique to be used.

After the surgical procedure, all patients underwent at least one CT scan for checking screws position and plain radiographs with a dynamic image during follow-up.

RESULTS

Sixty-five patients with complete data were found in our database. Forty patients were male (61.5%), and twenty-five were female (38.5%). The average age was 42.2 years (ranging from 5 to 77 years old). The demographic data are presented in Table 1, and case examples are shown in figures 1 to 2.

The majority of our cases were due to spinal trauma involving the axis (36 patients – 55.4%), followed by congenital craniocervical malformations (twelve patients – 18.5%), degenerative spine conditions (nine patients – 13.8%), spinal tumors (two spinal metastases and one primary giant cell tumor – 4.6%), post-laminectomy kyphosis (three patients – 4.6%) and inflammatory disorders (two cases of rheumatoid arthritis leading to C1-2 instability – 3.1%)

Thirty-seven patients (57%) required concomitant C1 fusion and fourteen cases (21.5%) underwent occipitocervical fusion.

Bilateral axis fixation was possible in the majority of cases (60 patients – 92.3%). Twenty-three patients

TABLE 1. DEMOGRAPHIC DATA

Variable	Median (range)	n (%)
Demographics		
Age	42.2 (5-77)	35.4
Male		40 (61.5)
Female		25 (38.5)
Etiology		
Trauma		36 (55.4)
Congenital malformation		12 (18.5)
Degenerative		9 (13.8)
Tumors		3 (4.6)
Post-laminectomy		3 (4.6)
Inflammatory disorders		2 (3.1)
Concomitant fusion		
C1-2		37 (57.0)
Occipitocervical		14 (21.5)
Subaxial		14 (21.5)

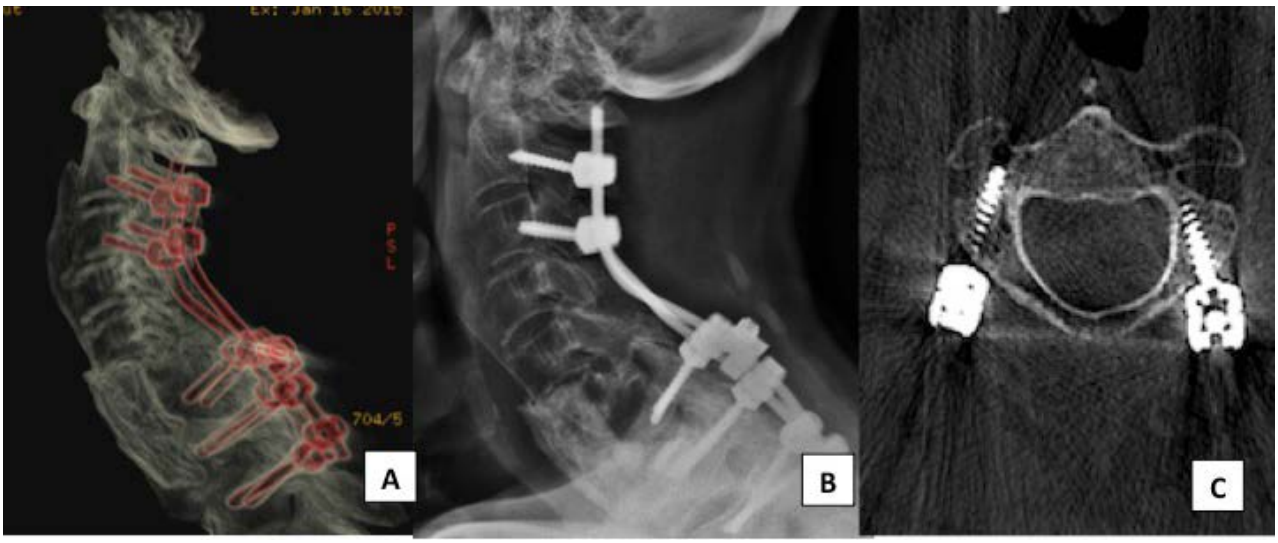

FIGURE 1. A cervico-thoracic fusion for treatment of a post-laminectomy defect. (A) Sagittal CT scan reconstruction, (B) postoperative lateral X-ray and (C) axial CT scan showing a right C2 pars screw and a left C2 pedicular screw.

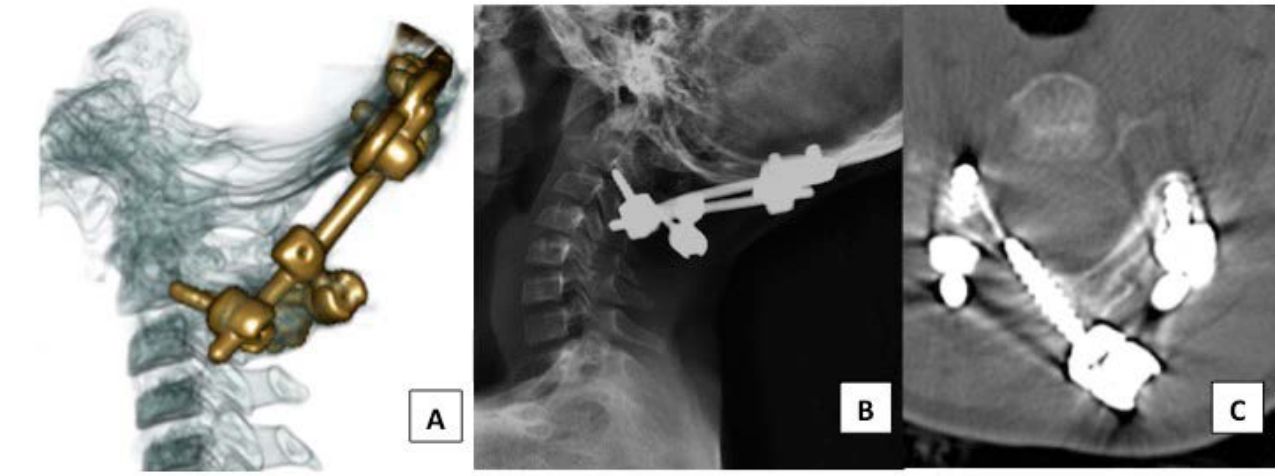

FIGURE 2. An occipitocervical fusion for treatment of a bone cyst of the atlas. (A) Sagittal CT scan reconstruction, (B) postoperative lateral extension cervical spine X-ray and (C) axial CT scan showing a right C2 laminar screw and bilateral C2 pars screws.

(35.4%) underwent bilateral laminar screws fixation; pars screws were used in twenty-two cases (33.8%), and pedicular screws were used in only three cases (4.6%). In fourteen cases (21.5%), we performed a screw hybrid construction: lamina and pars (eight cases), pedicle and pars (three cases), pedicle and lamina (one case), bilateral pars and lamina (one case) and bilateral lamina and pars (one case). In three cases, we had to use unilateral screw fixation: two with one laminar screw and one with pars screw.

Considering hybrid construction, two cases required three axis screws. In one of these patients, with complex axis fracture and C1-2 luxation, we used concomitant C1 lateral mass screw and C2 bilateral laminar and unilateral pars screws. In another patient, a child with a C1 bone cyst, we performed a hybrid construction with bilateral pars and unilateral laminar screws and a concomitant occipitocervical fusion. A total of 132 axis screws were used considering all the patients.

In one case, we had a superficial infection at the site where the graft was harvested. There was no neurological worsening or complications directly related to the use of axis screws, such as vertebral artery injury or neurologic injury. One patient (with a giant cell tumor and a C2-T2 fusion) had a pars screw pulled out during follow-up, but was not reoperated once she had only mild cervical pain. One patient with a C2 pedicle screw had a canal violation but was not reoperated either, since the patient was asymptomatic.

There were no surgical related deaths in this series. One patient, with traumatic brain injury and Type II odontoid fracture, died from pulmonary sepsis one month after the procedure. The most common complication was superficial wound infection in three cases (4.6%), all of them in occipitocervical fusions.

DISCUSSION

Although there are many techniques for screw fixation of C2 available, the unique anatomy of this vertebra and its variations can significantly impact all these procedures[24]. Therefore, solid anatomical knowledge and the surgeon's familiarity with C2 screw fixation techniques are of paramount importance for the efficacy of the procedure.

Transarticular screws are widely used and described in the literature for their superiority in biomechanical immobilization of C1-2[5]. However, this procedure is technically demanding and sometimes even prohibitive because of the potential risk for vertebral artery injury.

Pars screws are an alternative method for axis fixation with less risk of vertebral artery injury[24]. The insertion of a C2 pars screw is similar to the posterior transarticular screw placement, using shorter length screws, with the starting point selected in the medial half of the inferior facet of C2 (2 mm up and 2 mm lateral to the junction of the medial portion of the C2–C3 facet joint)[20]. Hoh et al.[24] retrospectively reviewed a random selection of 50 CT scans and evaluated various starting points and trajectories for C2 pars screws. They concluded that 99% of pars interarticularis are at least 14 mm in length when using a conventional transarticular screw entry point with a trajectory directed toward the superior facet-pars junction. In our series, we used pars screws lengths of 14-16 mm for maximal safety in order to avoid breaching the transverse foramen.

Considering pedicle screws, the entry point is located lateral to the superior margin of the lamina, and the trajectory for drilling the pedicle is about 20 degrees up and 15-25 degrees medially[20]. Preoperative CT scans are fundamental to demonstrate any rotational variations that influence the final direction. Patients with a hypoplastic pedicle do not tolerate a C2 pedicle screw[24]. Igarashi et al.[25] selected 98 dry axis vertebrae of adult skeletons for measurement and showed that 20% of the specimens had a pedicle diameter smaller than 3.5 mm, making the screw placement difficult. Because of this potential risk, pedicle screws were less used in our series.

Frequently, many studies fail to anatomically define the C2 pedicle and the pars and often combine these structures[26]. Elliott et al.[27] reviewed a series of published papers describing C2 pars and pedicle screw implantation and demonstrated that both types can be placed accurately with low morbidity. The risk of vertebral artery injury with C2 pedicle screws was 0.3%, and no injury occurred with shorter pars screws, while longer pars screws (> 16 mm) could increase the risk of artery injury[27]. There was a low rate of clinically significant screw inappropriate positions for both techniques[27]. They also showed that C1 lateral mass screws combined with C2 pars or pedicle screws can provide excellent rates of stabilization without the use of halo-vest immobilization (95-99%)[27].

In the case of laminar screws, the entry point angle and direction of the drilling should match the slope of the lamina[20]. In this technique, the entire length of the screw is within the surgical field, making placement safer and minimizing any risk to the vertebral artery injury[20]. Cassinelli et al.[22] studied the axis vertebrae of 420 human adult cadavers and showed that the lamina can safely accommodate screw placement in the majority of specimens. They also demonstrated gender-related differences in laminar measurements[22]. Laminar thickness was 8.3% smaller in women compared to men[22]. Interesting, there were no statistically significant differences in laminar measurements considering race, height, and weight[22].

A comparison of operative morbidity, accuracy, and durability of laminar versus pedicle screw fixation of C2 was published by Parker et al.[28]. The authors divided the patients into two groups: axial and subaxial fixation[28]. After one year postoperatively, pseudoarthrosis or screw pullout requiring operative revision occurred in 4 (6.1%) patients with laminar screws versus 0 patients with pedicle screws in subaxial constructions[28]. However, no cases of laminar or pedicle screws for axial cervical fusions required reoperation[28]. These findings indicate that both techniques are equally effective and safe for axial cervical fusions, while the durability of laminar screws might be inferior to pedicle screws when extended to the subaxial spine[28].

Biomechanical testing using fourteen cadaveric specimens suggested that C2 pedicle screws offer the strongest fixation[29]. Their results also demonstrate that laminar fixation is superior to pars instrumentation[29]. In our series, we used pedicular screws only in situations that large constructions were required, such as C2-thoracic fusions.

CONCLUSION

In conclusion, axis screw instrumentation was a safe and efficient method used for cervical stabilization, regardless of the technique used. The choice of the suitable fixation technique should be determined by the local anatomy, posterior element fractures or necessity to remove the posterior elements. The choice of the technique also depends on the surgeon's experience and preference.

RESUMO

OBJETIVO: *Apresentar os resultados cirúrgicos de pacientes submetidos à instrumentação com parafusos do áxis, discutindo nuances cirúrgicas e complicações das técnicas utilizadas.*

MÉTODOS: *Série retrospectiva de casos de pacientes submetidos à instrumentação do áxis utilizando parafusos.*

RESULTADOS: *Sessenta e cinco pacientes foram incluídos neste estudo. A causa mais comum de instabilidade foi trauma raquimedular envolvendo o áxis (36 pacientes – 55,4%), seguida por malformação craniocervical congênita (12 pacientes – 18,5%). Trinta e sete (57%) pacientes necessitaram concomitante fusão de C1. Fixação bilateral foi realizada em quase todos os casos. Vinte e três pacientes (35,4%) foram submetidos à fixação com parafusos de lâmina; parafusos de pars foram utilizados em 22 pacientes (33,8%) e de pedículo, isoladamente, em três (4,6%). Em 14 casos (21,5%), realizamos técnicas combinadas. Não houve piora neurológica ou lesão de artéria vertebral nesta série de casos.*

CONCLUSÃO: *A instrumentação com parafusos do áxis foi um método seguro e eficaz para estabilização cervical. A fixação da lâmina e a da pars foram as técnicas mais utilizadas.*

PALAVRAS-CHAVE: *Vértebra cervical áxis. Vértebras cervicais. Parafusos ósseos. Fixadores internos.*

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ORIGINAL ARTICLE

Ameliorative effect of certolizumab on experimentally induced acute necrotic pancreatitis in rats

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SUMMARY

OBJECTIVE: *The effects of Certolizumab, a pegylated monoclonal antibody to tumor necrosis factor α, on experimentally induced acute pancreatitis (AP) were examined.*

METHODS: *Thirty-six Wistar Albino male rats were randomly divided into four groups. Group I was the control group and no medication administered to this group. Group II was the Certolizumab group, and 100 ml/kg serum physiologic administered into the biliopancreatic duct and a single dose of 10 µg Certolizumab was simultaneously administered intraperitoneally. Acute pancreatitis was induced with a retrograde injection of 3% Na taurocholate into the common biliopancreatic duct in the study (Group III) and treatment (Groups IV) groups. Rats were sacrificed 72 hours later. Serum amylase, lipase, lactate dehydrogenase activities, along with pancreatic histopathology, were examined.*

RESULTS: *Certolizumab treatment significantly decreased serum amylase, lipase, and LDH levels; histopathologically edema, hemorrhage, parenchymal necrosis, fat necrosis, and infiltration scores; immunohistochemically MDA, MPO, TNF-α and Caspase-3 activity.*

CONCLUSION: *The results support the idea that certolizumab might be beneficial for the severity of AP.*

KEYWORDS: *Pancreatitis, acute necrotizing. Certolizumab Pegol. Rats.*

INTRODUCTION

Acute pancreatitis (AP) is one of the most common diseases and a huge threat to human health with limited specific therapy. With many complications, its mortality rate ranges from 11.8% to 25%.[1] Clinically, it is characterized by edematous and necrotic features. From all of the gastrointestinal system diseases, AP is one of the conditions with most frequent hospitalization. The incidence of the disease is reported to be 4-41/100.000[2] and is increasing alongside with obesity and bile stone incidence; however, mortality rates did not decrease in the same ratio. [3]

To understand the pathogenesis of acute pancreatitis, experimental models are essential. At the pathogenesis of AP, early trypsinogen activation and

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the nuclear factor- Kappa B (NF-kB) activation are the most common pathways. Then, inflammatory mediators and cytokines, such as TNF, play a role in local and systemic damage. TNF, which secretes immune and non-immune cells such as macrophages, T cells, mast cells, has been shown to have a role in acute pancreatitis. Blocking the TNF can minimize pancreatic damage.[4,5] Given the importance of TNF-α in the pathogenesis of acute pancreatitis, investigators have regarded blocking the action of this mediator as an attractive treatment option.

Certolizumab is a polyethylene glycolic covalent linked to a monovalent Fab1 antibody fragment. Certolizumab, an anti-TNF monoclonal antibody, binds to the soluble and transmembrane forms of TNF-α not including the Fc fragment. It is an approved treatment for rheumatoid arthritis, psoriasis and Crohn disease.[6,7]

This study aims to examine the effect of the new Certolizumab and if it has a different action than anti-TNF-α in experimentally Na-taurocholate induced acute necrotic pancreatitis model in rats.

METHOD

The experiment was approved by the Committee of Institutional Animal Use and Care of the Pamukkale University and was performed in accordance with the National Institutes of Health Guidelines for the Care and Handling of Animals. Rats were kept in the Pamukkale University Experimental Animals Laboratory, which is internationally accredited for its suitable conditions.

Animals

Wistar Albino male rats weighing 180-220g were used for this study. The rats were fed standard rat chow and tap water ad libitum and housed in a laboratory with automatically controlled temperature and humidity throughout the study. The physical condition of each rat was assessed daily for any obvious clinical symptoms.

Experimental Design

Thirty-six rats were randomly allocated into 4 groups of 9 animals. The rats were kept in groups of 3 in a cage before the study. Then, they were kept individually in a cage during the experiment. All rats underwent laparotomy under 60-100 mg/kg ketamine anesthesia (Ketalar; EczacNbasN Warner-Lambert, Istanbul, Turkey). The abdomen was opened via a midline excision to manipulate the duodenum and the biliopancreatic duct. The duodenal wall was punctured on the antimesenteric side with a 27-gauge catheter (inner diameter, 7 mm; introcan-w; Braun, Melsungen, Germany) 2-3 cm far from the duodenal opening of the biliary canal. The catheter was advanced into the papilla of Vater and fixed to the duodenal wall with 6-0 Prolene. For inducing AP, the pancreatic canal was approached, and 3% Na-taurocholate (Sigma-Aldrich, Steinheim, Germany) or serum physiologic (1 mL/kg) was infused according to the retrograde ductal injection model.[8] Both Na-taurocholate and physiological saline were infused at a pressure of 30 mm Hg controlled by a mercury manometer. Only catheters that were properly placed in the rats were included the study. The abdomen was closed in aseptic conditions, and 1 ml/100gr physiological saline was given for fluid replacement.

Study Protocol:

Group 1 (Control group): Physiological saline alone was administered to the common biliopancreatic duct. No drug was given this group.

Group 2 (Certolizumab group): Physiologic saline (100 ml/kg) was given via the common biliopancreatic duct; a single dose of Certolizumab (Cimzia® -UCB-USA) was simultaneously administered at 10µg intraperitoneally.

Group 3 (Na-Taurocholate group): Acute pancreatitis was induced with an injection of 3% Na-taurocholate (T9034, Sigma- Aldrich, Steinheim, Germany) into the common biliopancreatic duct; physiological saline was simultaneously administered at 100 mL / kg intraperitoneally.

Group 4 (Treatment group): Acute pancreatitis was induced with an injection of 3% Na-taurocholate into the common biliopancreatic duct, and Certolizumab was simultaneously administered at 10µg intraperitoneally.

After 72 hours inducing the pancreatitis, rats were anesthetized using 10mg/kg xylazine hydrochloride (Rhompun®, Bayer, Istanbul) and 60 mg/kg ketamine (Ketalar®, Eczacıbası Warner-Lambert, Istanbul). Intracardiac blood was drawn under anesthesia. Then, the rats euthanatized, and pancreatic tissue samples were collected immediately after their death. Tissue samples were put into 10% formalin solution. Serum amylase, lactate dehydrogenase (LDH), malondial-

dehyde (MDA), and myeloperoxidase (MPO) enzyme activities were assessed.

Histopathologic Evaluation

Pancreatic tissue samples were put into 10% formalin, routinely processed, paraffin embedded, and then stained with hematoxylin and eosin to be interpreted using light microscopy. Histopathologic changes were graded in a blinded manner. The examination and scoring of the pancreatic tissues were evaluated by an experienced pathologist from another university. For this purpose, some parameters were determined by the adoption of those indicated in the references and our previous study.[9-11] The severity of pancreatitis was documented by scoring edema, hemorrhages, leukocyte infiltration, parenchymal necrosis, and fatty tissue necrosis. Each criterion was graded on a scale of 0 to 3. Edema and hemorrhage were assessed according to their location. Leukocyte infiltration and parenchymal necrosis were evaluated according to the number of the lobules involved, and fat necrosis was scored based on its occurrence in the peripancreatic tissue. Data from each animal were statistically analyzed.

Immunohistochemical evaluation

All pancreas samples were immunostained with malondialdehyde (MDA) [anti-malondialdehyde antibody (ab6463), Abcam, Cambridge, UK, 1/100 dilution], myeloperoxidase (MPO) [anti-myeloperoxidase antibody (ab45977), Abcam, Cambridge, UK, 1/100 dilution], TNF-α [anti-TNF alpha antibody [52B83] (ab66579), Abcam, Cambridge, UK, 1/100 dilution] and Caspase-3 antibody [anti-Caspase 3 antibody (ab4051), Abcam, Cambridge, UK, 1/100 dilution] using a routine streptoavidin-biotin peroxidase technique for evaluating MDA, Myeloperoxidase, TNF-α and apoptotic activity of the cells. A Novostain Universal Detection Kit (ready to use) [Abbiotech, San Diego, CA,] was used as a secondary antibody according to the manufacturer' instruction.

For evaluation of immunoreactivity, 10 randomly chosen microscopic fields (X40) near the necrotic areas or pancreatic duct for controls were examined for each slide; 100 cells were counted, a percentage of positive cells was calculated, and statistical analysis was performed.

Biochemical analysis

Blood samples were immediately centrifuged at 3000 g for 10 min at room temperature and stored -80° C until examination for LDH, amylase, and lipase levels. Serum LDH, lipase, and amylase activity was measured using a VetTest commercial kit by IDEXX (Westbrooke, Maine).

Statistical Analysis

An one-way analysis of variance (ANOVA) was applied to evaluate the biochemical and histopathological findings and for statistical comparisons between the experimental groups. A significance assessment was carried out using the Duncan test. The results

TABLE I. MEAN STATISTICAL VALUES OF EACH HISTOPATHOLOGICAL, IMMUNOHISTOCHEMICAL, AND BIOCHEMICAL RESULTS OF THE STUDY GROUPS

Parameter	Groups				P value
	Control	Certolizumab	Na-Taurocholate	Na-Taurocholate + Certolizumab	
Edema	0.00±0.00 [a]	0.42±0.20 [a]	1.85±0.40 [c]	1.00±0. 21 [b]	<0.001***
Hemorrhage	0.00±00 [a]	0.14±0.14 [a]	1.71±0.18 [c]	0.85±0.14 [b]	<0.001***
Parenchymal necrosis	0.00±0.00 [a]	0.28±0.18 [a]	1.71±0.35 [c]	1.00±0.21 [b]	<0.001***
Fat necrosis	0.00±0.00 [a]	0.00±0.00 [a]	1.28±0.42 [b]	0.57±0.29 [a]	<0.01**
Leukocyte infiltration	0.00±0.00 [a]	0.42±0.20 [a]	1.57±0.36 [c]	1.00±0.37 [b]	<0.001***
MDA	0.37±0.26 [a]	0.57±0.20 [a]	2.00±0.30 [b]	0.85±0.26 [a]	<0.001***
TNF-α	0.37±0.74 [a]	0.37±0.51 [a]	2.42±0.78 [c]	0.85±0.69 [b]	<0.001***
MPO	0.37±0.18 [a]	0.42±0.20 [a]	1.42±0.20 [b]	1.14±0.26 [b]	<0.01**
Caspase-3	0.62±0.32 [a]	0.71±0.18 [a]	2.00±0.30 [b]	0.85±0.26 [a]	<0.01**
LDH(U/L)	746.33±38.27 [a]	984.28±88.69 [a]	3915.28±183.37 [c]	1984.57±278.67 [b]	<0.001***
Amylase (U/L)	1044.75±52.45 [a]	1126.28±188.13 [a]	2807.28±147.17 [b]	1430.85±135.12 [a]	<0.001***
Lipase (U/L)	41.00±8.05 [a]	68.00±15.68 [a]	342.42±47.69 [c]	166.28±19.28 [b]	<0.001***

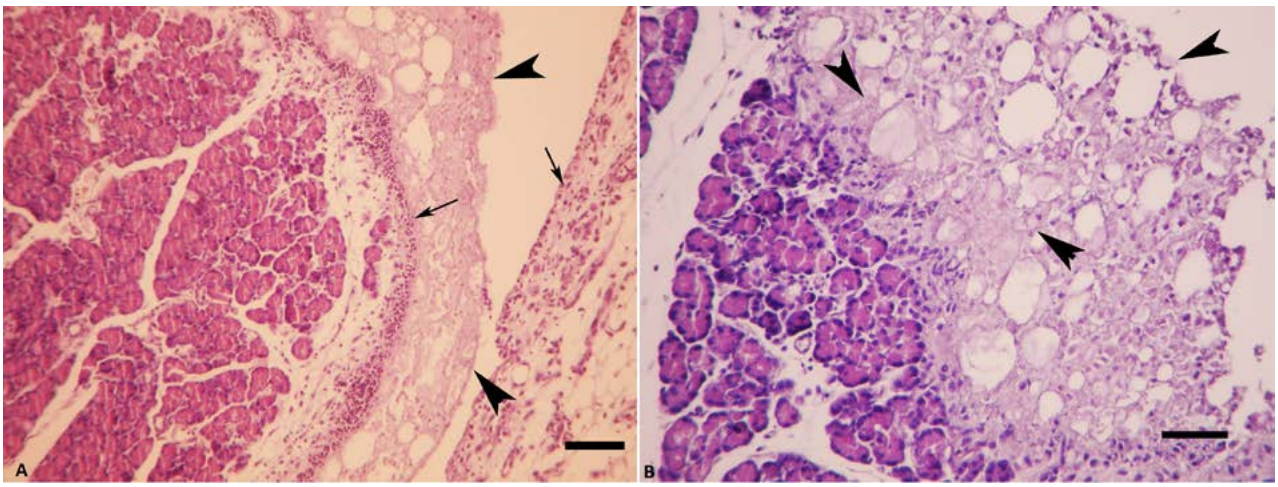

FIG. I: A. Acute necrotic pancreatitis, severe necrosis (arrowheads) and numerous neutrophil leukocytes (arrows) in the pancreas in a rat from the Na-taurocholate group, HE, Bar=100μm. B. Acute necrotic pancreatitis, severe necrosis (arrowheads) in the pancreas in a rat from the Na-taurocholate group, Bar=200μm.

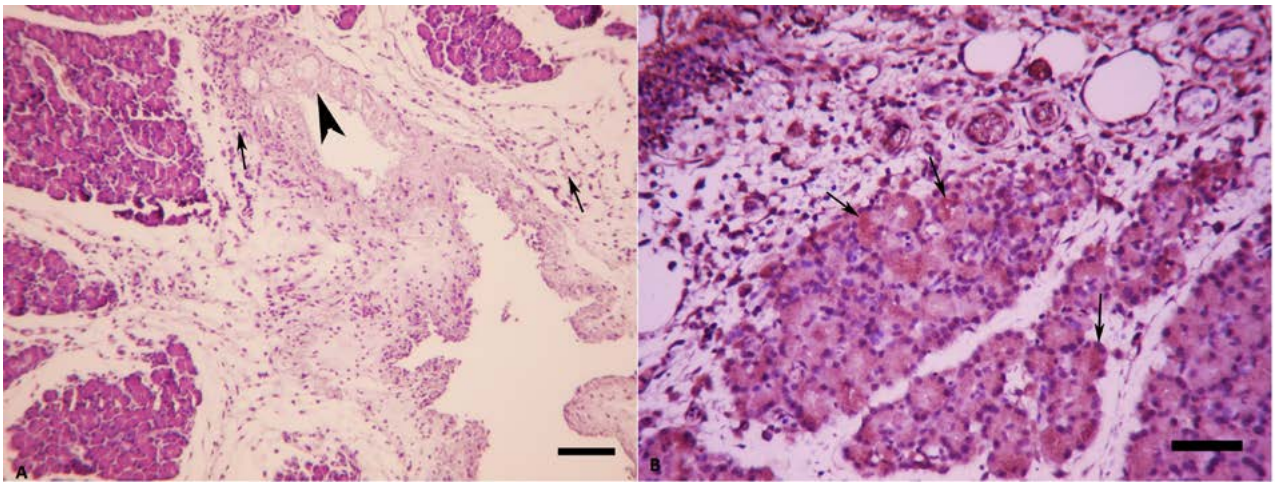

FIG. II: A. Slight necrosis (arrowhead) and neutrophil leukocyte infiltrations in a rat from the Na-taurocholate + certolizumab group, HE, Bar=100μm. B. Marked caspase-3 immunoreaction in acinar cells (arrows) of the pancreas in a rat from the Na-taurocholate group, streptoavidin-biotin peroxidase technique with hematoxylin counterstain, Bar=100μm.

were expressed as mean ± SD. The values were considered to be statistically significant when $P < 0.05$. The statistical analysis was done with SPSS 13.0 software (SPSS Inc, Chicago, Ill).

RESULTS

The most severe clinical and pathological symptoms were observed in the Na-taurocholate group and characterized by severe necrotizing pancreatitis, marked retroperitoneal edema, and hemorrhagic ascites.

In the control group and certolizumab group, the normal glandular architecture was preserved. Na-taurocholate induced pancreatic damage was evident by increased edema, neutrophil leukocyte infiltration, hemorrhage and necrosis in both parenchymal and fat tissues (Fig.1). These lesions were observed in Na-taurocholate and Na-taurocholate + certolizumab groups but were more severe in the Na-taurocholate group. However, treatment with certolizumab reduced histopathological lesions (Fig.2). The statistical analysis results of serum LDH, amylase and lipase levels in study groups are shown in Table 1.

Immunohistochemically-marked increases were observed in MDA, MPO, TNF-α and Caspase-3 expression in the Na-taurocholate group (Fig.2). Certolizumab treatment showed an ameliorative effect in the Na-taurocholate + certolizumab groups.

Serum amylase, lipase, and LDH levels were higher in the Na-taurocholate group and Na-taurocholate + certolizumab groups but levels were markedly higher in the Na-taurocholate group. Amylase, lipase and LDH activities were according to the refer-

ence values in the control group. However, a slight increase was observed in the Na-taurocholate + certolizumab group.

DISCUSSION

AP is a systemic inflammatory disease, characterized by acute abdominal pain associated with an increase in serum amylase and lipase levels.[12,13] It is a common condition of which clinical manifestations may range from a mild, self-limiting disease to an inflammatory process with life-threatening complications. The development of pancreatic necrosis results in a dramatic increase in mortality in AP patients.[14] There are no specific therapies for acute pancreatitis, and the treatment remains largely supportive. A better comprehension of the inflammatory effects, which play a major role in the pathogenesis of the disease, appears necessary for the development of new therapeutic strategies. In the present study, the pathological changes and biochemical results in the treated group improved. In this study, we examined the effect of the anti-TNF-α agent certolizumab for the treatment of acute necrotic pancreatitis.

TNF-α, a cytokine, mediates a broad spectrum of host responses to stress and injury and it is strongly involved in many deleterious events in the course of AP.[15] Our results directly evidence that TNF-α plays a pivotal role in Na-taurocholate induced pancreatitis and that it may be a novel target of therapeutic applications for treating pancreatic inflammation. This study showed that TNF-α markedly increased in pancreatic tissue in AP, and anti-TNF-α treatment caused marked amelioration of both pathological and biochemical findings.

Pancreatic exocrine secretion disruption is an important feature of AP because there has been reported an evident alteration in pancreatic secretory response in pancreatic acini after taurocholate infusion. A marked increase in amylase release during experimental pancreatitis has previously been demonstrated commonly,[11,16-18] suggesting that this increase is not due to a physiological secretory response but to enzyme release from damaged cells.[16] In this study, we also observed a marked increase in pancreatic lipase and amylase levels in serum in 72 hours after inducing pancreatitis. At the same time, pancreatic tissue destruction and oxidative tissue stress and cytokine level increase were observed immunohistochemically.

Certolizumab neutralized both transmembrane and soluble TNF by binding to p55 and p75 TNF-a receptors in vitro.[6] Since Fc fraction was not included, Certolizumab mineralized the Fc mediated complement dependent cytotoxicity (CDC) and antibody dependent cell-mediated cytotoxicity (ADCC). While adalimumab, infliximab, and etanercept induced CDC and ADCC in vitro, certolizumab did not have this effect.[7] Certulizumab does not transfer from the placenta.[19]

During the inflammatory process of acute pancreatitis, PPAR – gamma activation and the early activation of trypsinogen the acinus are the main pathways. Via the inflammatory process triggered by various factors, many mediators appeared. TNF is one of these mediators. Due to the TNF release in the early stages, neutrophils are activated and released oxygen free radicals that caused lipid peroxidation, cellular swelling, and cell death.[20,21] Experimental studies have shown that by blocking TNF, inflammation can be minimized and pancreatic damage limited.[4,5] Pancreatic tissue damage in the treatment group given certolizumab was significantly lower than the group that received treatment. Because the TNF-α is responsible for both local and systemic effects in pancreatitis, the inhibition of TNF-α is thought to diminish local and systemic complications. Indeed, Hughes et al.[5], showed that TNF blockade in acute pancreatitis decreased the complications associated with pancreatitis. Norman et al.15, reported that soluble TNF- α inhibition could decrease the severity of experimental pancreatitis and mortality but not influence the pancreatic vacuolization, necrosis, and inflammatory cell infiltration. Oruc et al.[22] showed that infliximab has anti-inflammatory effects in an edematous and necrotizing pancreatitis model. In the same study, infliximab significantly reduced parenchymal fat necrosis but did not affect the mortality rate, pancreatic edema, and neutrophil activity. In our study, certolizumab showed a positive effect on all of the histopathological parameters. A possible reason for these differences in the results might be due to a difference in efficiency between the different molecules used in the studies. Yilmaz et al.11, reported that a soluble TNF receptor fusion protein etanercept can cause amelioration on histopathological scores and biochemical parameters on Na-taurocholate induced necrotizing pancreatitis model. The study showed that etanercept causes a decrease in pancreatic fibrosis in the long-term. In another simi-

lar study, adalimumab treatment on necrotizing pancreatitis has been described to have improved histological lesion scores, biochemical parameters, and tissue damage indicators.[11,22] In the last 3 studies, infliximab, etanercept, and adalimumab had a positive effect on the serum marker of pancreatic injury. Our findings are also similar to them.

An intracellular calcium concentration increase can cause acinar cell calcium signaling defects, intracellular trypsinogen activation, and at least leads to vacuolization in acinus. The expression of caspase-3 activation is considered an indicator of apoptosis; certolizumab improved Na-taurocholate induced pancreatitis. Free oxygen radicals and neutrophil infiltrations play an important role in the progression of pancreatic injury.[23,24] In this study, in the certolizumab-treated group, tissue MPO and MDA levels were significantly reduced compared to the untreated group.

Pancreatic tissue damage caused increased levels of serum amylase or lipase and LDH levels. Increase started at the beginning of the first day, and enzyme levels returned to normal over the 5 days. There is a parallel condition at the reduction of pancreatic injury and reduced serum levels of biochemical markers. In our study, in the certolizumab-treated group, amylase, lipase, and serum LDH levels were significantly lower than the group not receiving treatment. Our findings are consistent with the literature.

CONCLUSION

As a result, certolizumab treatment in a model of necrotizing pancreatitis reduced damage in pancreatic tissue, and serum levels of indicators were significantly improved. To the best of our knowledge, certolizumab has not been used in other studies.

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RESUMO

OBJETIVO: *Os efeitos de Certolizumab, um anticorpo monoclonal pegilado para o fator de necrose tumoral α, na pancreatite aguda induzida experimentalmente (PA) foram examinados.*

MÉTODO: *Trinta e seis ratos Wistar Albino foram divididos aleatoriamente em quatro grupos. O Grupo I foi considerado o grupo controle e não recebeu medicação; o Grupo II foi o grupo Certolizumab e recebeu 100 ml/kg de soro fisiológico administrado no ducto biliopancreático e dose única de 10 mg Certolizumab administrada por via intraperitoneal simultaneamente. A pancreatite aguda foi induzida com uma injeção retrógrada de uma solução de 3% taurocolato de sódio aplicada no ducto biliopancreático comum nos grupos de estudo (Grupo III) e tratamento (Grupos IV). Os ratos foram sacrificados 72 horas depois. As atividades séricas de amilase, lipase, lactato desidrogenase, juntamente com a histopatologia pancreática, foram examinadas.*

RESULTADOS: *O tratamento com Certolizumab diminuiu significativamente os níveis séricos de amilase, lipase e LDH; edema histopatológico, hemorragia, necrose paranquimatosa, necrose gordurosa e escores de infiltração; atividade imuno-histoquímica de MDA, MPO, TNF-α e Caspase-3.*

CONCLUSÃO: *Estes resultados suportam a ideia de que o Certolizumab pode ser benéfico para a gravidade da PA.*

PALAVRAS-CHAVE: *Pancreatite necrosante aguda. Certolizumab Pegol. Ratos.*

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ORIGINAL ARTICLE

Unison of movements in football players with different nervous systems

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SUMMARY

In this study, we investigated the effect of typological features of nervous system properties on the ability to unite the movements of young football players. A total of 36 young football players aged 11-12 years participated in this experiment. Of them, 18 were engaged in an experimental differentiated method, which is based on using the same exercise and methods for developing the ability to unite movements but with different load components; for players with a strong nervous system (9 children), the load was intensive, but for players with a weak nervous system (9 children) - the load was volumetric. The other 18 athletes made up the control group. After 8 months of the experiment, we observed positive changes in terms of the ability to unite movements in young football players. In the control group, these changes were not significant (P>0.05). In the experimental group studied according to a special method, the indicators changed considerably. The performance of football players with a strong nervous system improved from 6.4±0.2 s to 5.7±0.1 s (P<0.05), and for football players with a weak nervous system from 6.2±0.2 s to 5.6±0.2 s (P<0.05). The study proved the effectiveness of the use of the typological properties of the nervous system as a differentiated method for developing the ability to unite movements in young football players. This approach allows for the improvement of the quality of technical training of young athletes.

KEYWORDS: *Biotypology. Football. Nervous system. Movement.*

INTRODUCTION

To achieve a high level of technical skill, it is necessary to train hard since early childhood. The technical training of the athlete is responsible for the coordination ability [1-3].

There are several classifications of coordination abilities [4-6]. For footballers, the most important are: the ability to adapt and rebuild motor actions, kinesthetic differentiation parameters of the movements, spatial orientation, the ability to unite movements, respond quickly, sense the rhythm of movements and balance. One of the leading coordination abilities for football players is the ability to unite movements, which is manifested in the connection of individual actions into a coherent motor combination [7].

The sensitive period for the development of coordination abilities is during primary school age (8-12 years). The ability to unite movements is better developed at the age of 11-12 years [8-10].

An individual approach is one of the most important principles of general and special pedagogy. The essence of the individual approach is to tailor it to the athletes. An individual approach is aimed at creating favorable conditions for the training and development of athletes, based on their individual capabilities; it is a differentiated approach to coach

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the individual characteristics of a group of athletes during the training process. The implementation of a differentiated approach streamlines the construction of the long-term training of young athletes, understands better the factors limiting the manifestation of motor abilities and implements reserve possibilities of functional systems of the organism of young athlete's [11,12].

Athletes can be dived into groups based on a variety of characteristics, such as physical types, functional indicators, technical training and so on [13-15].

A little-known but effective criteria for grouping athletes are typological peculiarities of nervous system properties of athletes, namely, strength and weakness of the nervous system in the process of excitation [16,17].

Studies that confirm the efficacy of a differentiated approach based on the typology of athletes have been conducted in different sports – athletics, table tennis, basketball [18-20]. However, in football, there have been no such researches.

This study aims to investigate the impact of typological characteristics of the nervous system (nervous system strength) on the ability to unite movements of football players.

Our hypothesis is that the method for developing players' ability to unite movements based on the typological differentiation of athletes will improve their outcome in the sport.

Objectives of the study:

1) To determine the level of development of the ability to unite movements in football players aged 11-12 years;

2) Develop an experimental method to develop the ability to unite football player's movements 11-12, based on the differentiation of loads and the nervous system strength of young athletes;

3) To study the effect of differentiated methods on football sporting activities for players aged 11-12 years.

MATERIALS AND METHODS

The study used methods:

1) Pedagogical experiment

a) Determining the strength of the nervous system of football players;

b) Determining the level of development of the ability to unite movements in young athletes.

2) Methods of mathematical and statistical processing of information.

Subjects

We included in the pedagogical experiment young football players aged 11-12 years who train at sports school nº5, Kirov.

Research procedure

Before the start of the study, 36 football players aged 11-12 years were randomly selected. Taking into account the nervous system strength, they were divided into the experimental group (EG) and control group (CG), each with a total of 18 players divided into two subgroups, one with a strong nervous system and the other with a weak one [21].

During the sports year (8 months), the football players of the EG were engaged in experimental methods, and the ones in the KG followed the standard program of the sports schools [22]. All the athletes trained 3 times a week. Each workout lasted 90 minutes. There was a total of 112 training sessions in each group.

Control tests

1. The strength of the nervous system was initially determined using the "Tapping-test" method.

The test consists of: an A4 sheet of paper divided into six squares (three squares in two rows). The coach gives the signal. Athletes put dots in 1 square for 5 seconds. Every 5 seconds, the athlete needs to move on to the next square. The goal is to put as many dots as possible in each square. When the sixth square is reached, the exercise ends. The results: the number of dots in each square is counted to assess the performance and determine the type of nervous system strength [23].

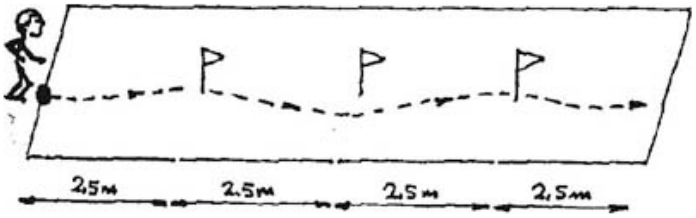
2. The football players' ability to unite movements is determined using the "Slalom with dribbling two balls" test.

The method of the test: three sticks are set over a 10 m straight-line. The first stick is located at a distance of 2.5 meters from the start line. At the coach's signal, the athlete covers the distance between the sticks moving the two balls at the same time using the feet (illustrations 1).

The results: the running time between the sticks, while moving the two balls, with an accuracy of 0.01 seconds [24].

3. Friendly match between the EG and KG teams. The match results are used to objectively estimate the young football players' level of proficiency and ability to unite movements. This is used to validate the effectiveness of the experimental procedure.

ILLUSTRATIONS 1. SLALOM WITH TWO DRIBBLING BALLS



Feature training in the EG:

After the usual warm-up (10-15 minutes), football players of the EG carried out exercises to develop the ability to unite movements in different subgroups, for 20 minutes.

1) Means (physical exercises) – moving hands during dribbling, blowing a ball while jumping, using exercises for general physical training with technical elements, maintaining two balls at the same time and other exercises [7,25].

2) Methods – repeated (the main method), interval, game, and competitive methods [26].

3) Methodical receptions – new exercises, a complication of the previous exercises, change of speed and direction of the movement when performing physical exercises [26].

4) Load components were the main difference in the training of young football players with strong and weak nervous systems. For football players with a strong nervous system, the load was intensive, while for football players with a weak nervous system the load was volume [19].

At the same time, the intensity of the load increased by increasing the number of exercises and reducing rest times. The volume load was increased by increasing the number of repetitions and rest intervals.

The intensity of the exercise was set at 150-170 beats/min for both groups.

The duration of the exercise for football players with a strong nervous system was of 25-30 seconds, and 35-40 seconds for the weak ones.

The resting time for both groups lasted until full recovery, the character of recreation was passive.

The number of repetitions of the same exercise for football players with a strong nervous system was 3-5 times with 6-8 series; for weak ones, it was 4-6 times with 7-10 series.

Statistical analysis

The mathematical and statistical processing of the results of the pedagogical experiment were carried out using a parametrical criterion (t-student) [27]. The correlation analysis was made using BioStat 2009 software. The result was considered significant at a value P<0,05 [21].

STATISTICAL RESULTS

Before the start of the pedagogical experiment, there was a friendly match between the EG and KG teams. The match had a final score of 0-0. During the game, it was objectively visible that football players in both groups moved equally and had equal ability to unite movements.

No significantly relevant indicators of the football players of the EG and KG and also in the subgroups was revealed (P<0,05).

The results of the "Slalom with maintaining two balls" test for all subgroups (from 6.9 to 6.2 c) correspond to the average level of development of the ability to coordinate movements in football players aged 11-12 years [28]. The indicators of the ability to unite movements in football players aged 11-12 years are presented in table 1.

From table 1, it is visible that, after the end of the pedagogical experiment in both groups and subgroups, there were positive changes in the results of the "Slalom with maintaining two balls" test.

TABLE 1: INDICATORS OF ABILITY TO UNITE OF MOVEMENTS OF FOOTBALL PLAYERS OF 11-12 YEARS (M±m)

Indicators	Strength of the nervous system	EG			CG			P (2-5)
		Before 1	After 2	P 3	Before 4	After 5	P 6	
Slalom with maintaining two balls (s)	Strong	6,4±0,2	5,7±0,1	t=2,88 P<0,01	6,9±0,3	6,6±0,2	t=1,11 P>0,05	t=3,74 P<0,05
	Weak	6,2±0,2	5,6±0,2	t=2,18 P<0,05	6,6±0,3	6,3±0,3	t=0,51 P>0,05	t=2,50 P<0,01
		t=0,62 P>0,05	t=0,38 P>0,05		t=0,94 P>0,05	t=0,67 P>0,05		

Young football players from the KG showed an improvement in the indicators, but not a significant one. While the indicators of athletes with a strong nervous system improved from 6.9±0.3 s to 6.6±0.2 s (P>0,05), that of athletes with a weak nervous system improved from 6.6±0.3 s to 6.3±0.3 s (P>0,05).

The indicators of football players aged 11-12 years who trained using differentiated methods considerably improved. The indicators of athletes in the EG with a strong nervous system improved from 6.4±0.2 s to 5.7±0,1 s (P<0,05), and that of athletes with a weak nervous system improved from 6.2±0.2 s to 5.6±0.2 s (P<0,05). Such indicators correspond to a high level of development of the ability to unite movements in football players at the age of 11-12 years [28].

After the pedagogical experiment, the EG team and the KG team played a friendly match again. The match ended with the victory of the EG team, with a score of 6-1. During the game, football players from the EG team looked much quicker, with more technical skills, better ability to unite movements and their actions were more effective than that of the football players from the KG team. The result of a match allows judging efficiency of the experimental method.

DISCUSSION

There is no doubt that coordination abilities are the basis of an athlete's technical skills, especially in sports such as football [1-3].

One of the primary coordination abilities for football players is the ability to unite movements [4-6].

However, research directed at studying the coordination abilities of football players has not been able to show a complete picture of the training process for athletes. Recommendations made are, as a rule, generic; the means and methods for developing coordination abilities are defined, as well as load parameters, for all football players, regardless of their type of nervous system or other criteria [7].

In our research, we detail specific means, methods and load components that should be used in the training process for young football players with different nervous systems.

Our research confirms opinions on the effectiveness of using a differentiated approach during the training process of young athletes. Such an approach takes advantage of the athletes' specific organism, thus having a more positive influence and more effective results in developing their abilities [11,12].

dDuring the experiment, based on our results we defined concrete recommendations for working with young football players aged 11-12 years with different typological features of the manifestation of nervous system properties.

Our hypothesis that the method to develop the ability to unite movements in football players based on differentiating athletes based on typological signs could improve their results in a game was completely proved.

CONCLUSION

The experimental method of using different approaches to develop the ability to unite movements in football players aged 11-12 years based on typological features of the manifestation of athletes' nervous system properties had a positive and progressive impact on football players in the EG (P<0,05).

The indicators of football players from the KG who were trained according to the usual program also improved, but not considerably (P>0,05).

The results of a friendly match at the end of the pedagogical experiment confirmed the effectiveness of the differentiated method. The EG team victory with a score of 6-1 shows that football players from the KG team were technically weaker, with a slower ability to unite movements during the game, unlike football players from the EG team.

This research attracts great interest from trainers who work in sports like football. The research described in detail means, methods, and loads for football players with different nervous system strength. It also determined effective tests to determine nervous system strength during the process of excitation and the level of development of the ability to unite movements in players aged 11-12 years. Such tests can be used for working with young athletes.

RESUMO

Neste estudo, investigamos o efeito das características tipológicas das propriedades do sistema nervoso sobre a capacidade de união dos movimentos de jovens jogadores de futebol. Trinta e seis jovens jogadores de futebol de 11 e 12 anos participaram na experiência pedagógica. Dezoito jogadores de futebol estavam envolvidos na metodologia experimental diferenciada, que é baseada no uso de um mesmo exercício e métodos de desenvolvimento da capacidade de unir os movimentos, mas diferentes componentes da carga; para os jogadores com um sistema nervoso forte (nove garotos), a carga foi intensa, mas, para jogadores com um sistema nervoso fraco (nove garotos), a carga foi volumétrica. Os outros 18 atletas compõem o grupo de controle. Em oito meses de experiência pedagógica houve mudanças positivas em termos da capacidade de unir o movimento de jovens jogadores de futebol. No grupo controle, essas alterações não foram significativas (P > 0,05). No grupo experimental estudado de acordo com uma metodologia especial, os indicadores mudaram consideravelmente. Os jogadores de futebol com um sistema nervoso forte melhoraram a performance de 6,4±0,2 s para 5,7±0,1 s (P < 0,05), e os jogadores de futebol com um sistema nervoso fraco, de 6,2±0,2 s para 5,6±0,2 s (P < 0,05). O novo estudo comprovou a eficácia da utilização das propriedades tipológicas do sistema nervoso como um método diferenciado de desenvolver a capacidade de unir os movimentos de jovens jogadores de futebol. Esta abordagem permite a melhoria da qualidade da formação técnica dos jovens atletas.

PALAVRAS-CHAVE: *Biotipologia. Futebol americano. Sistema nervoso. Movimento.*

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ORIGINAL ARTICLE

Treatment of aortic dissecting aneurysm involving visceral arteries with multi-layer bare stents

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SUMMARY

OBJECTIVE: *Aortic dissecting aneurysms involving visceral arteries are difficult for clinical treatment. This study aimed to explore the clinical efficacy and safety of multi-layer bare stents technique in the treatment of aortic dissecting aneurysms involving visceral arteries.*

METHOD: *The clinical data of 16 patients of aortic dissecting aneurysm involving visceral artery treated with multi-layer bare stents technique from March 2013 to March 2017 in Tianjin Medical University General Hospital were retrospectively analyzed. To explore the clinical efficacy, the number of stents applied, postoperative aortic dissecting thrombosis and postoperative cumulative branch arterial patency of the16 patients.*

RESULTS: *The operations of the 16 patients were successfully completed without peri-operative death cases. The 16 patients were implanted with 39 bare stents with an average of 2.44 per person. There were 2 cases with 1 stent, 8 cases with 2 stents and 7 cases with 3 stents. One month after the operation, CTA showed complete thrombosis in the arterial dissection in 4 cases (25.0%), partial thrombosis in 12 cases (75.0%); CTA showed that celiac artery, left and right renal arteries, and superior mesenteric artery were all unobstructed. There were 4 cases (25.0%) of dissecting artery with reduced diameter, 12 patients (75.0%) without changes in the diameter, and no diameter expanding cases.*

CONCLUSION: *The treatment for aortic dissecting aneurysm involving the visceral arteries using multi-layer bare stents technique is safe and reliable with a higher patency rate of postoperative accumulated branch arteries.*

KEYWORDS: *Aneurysm, dissecting. Aortic aneurysm. Stents. Endovascular procedures.*

INTRODUCTION

Aortic dissection (AD) is a disease with a rapid onset and a dangerous prognosis. Aortic dissecting aneurysms involving visceral arteries are difficult for clinical treatment[1,2]. Conservative treatments of this disease have high risks, and there is high mortality after open surgery[3]. Traditional endovascular graft exclusion is a safe and effective method for aortic dissections that do not involve the visceral artery;

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however, dealing with aortic dissections involving important branches is very difficult[4]. How to reconstruct a vital branch artery is the key to solving this problem. After blocking the proximal crevasse, thoracic endovascular aortic repair (TEVAR) dramatically reduces mortality and the rate of complications. However, distal dissection cannot be cured. What is more, some cases develop into dissecting aneurysms involving abdominal visceral branch arteries. In this regard, the application of endovascular technology still faces significant challenges. Fenestration and the branch stents need space to expand the stent, but the true lumen of the dissection patient is often narrow, which makes the application of these two technologies limited. Also, part of the visceral arteries are from the false lumen of the dissecting aneurysm, so that the fenestration, branches, and parallel stent technology are more difficult to use. In recent years, the emergence of multi-layer bare stent technology for the treatment of dissecting aneurysms involving visceral branch arteries has brought hope[5]. Multi-layer bare stent technology has subverted the traditional concepts of traditional endovascular graft exclusion by regulating the blood flow and making dissection false lumen thrombosis while maintaining visceral artery patency[6]. Moreover, the technical difficulty has been dramatically reduced, so this is expected to become a safe, minimally invasive and effective method for the treatment of aortic dissecting aneurysms involving visceral arteries.

In this study, 16 patients with aortic dissecting aneurysm involving visceral arteries treated with multi-layer bare stent technique from March 2013 to March 2017 were retrospectively analyzed to study its short and mid-term efficacy.

METHODS

General information of the included patients

In this group, there were 16 patients aged 20-82 years old, including 13 males and 3 females. They were associated with symptoms of varying degrees of chest and back, waist or abdominal pain with the onset time 8h-10d. Computed tomography angiography (CTA) examination before the surgery showed aortic dissecting aneurysm involving visceral arteries (Figure 1). Among the 16 patients, there were 15 with hypertension, 3 with coronary heart disease, 1 with diabetes, 1 with nephrotic syndrome, 1 with Marfan syndrome, and 1 with Behcet's disease. Also, this was the first operation for 11 of the patients and reoperation for 5 of them.

Surgical Procedure

Under general anesthesia, the right (or left) common femoral artery was punctured using the Seldinger method; the puncture site was located below the midpoint of the line between the ipsilateral anterior superior iliac spine and the upper edge of the pubic symphysis. The 8Fr sheath was

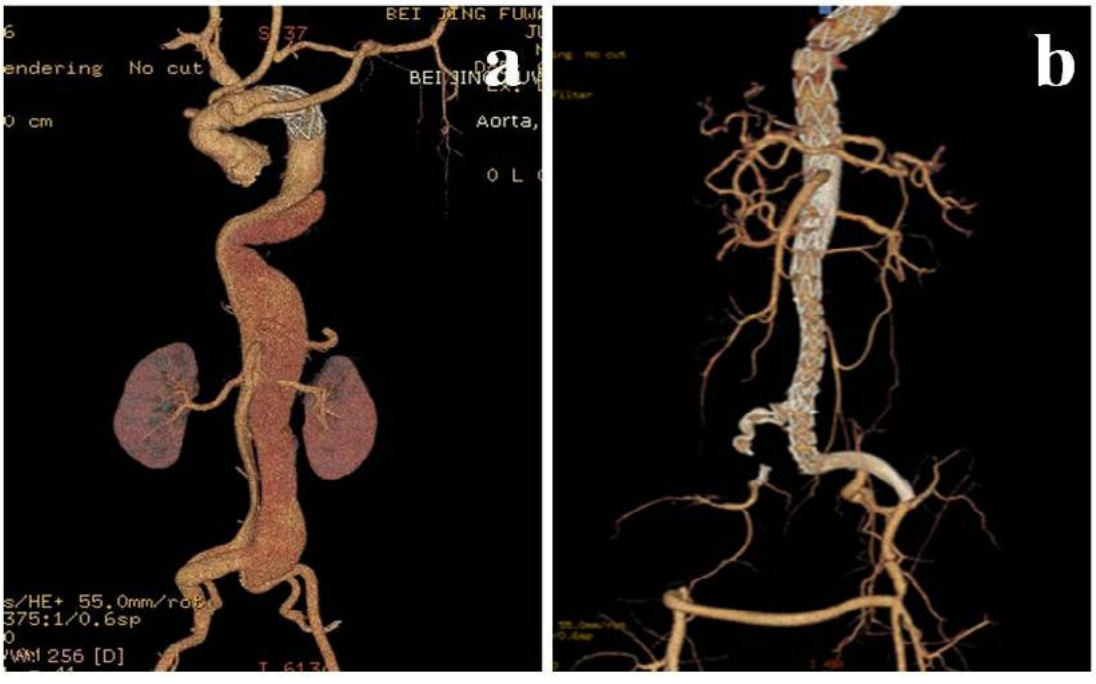

FIGURE 1
Computed tomography angiography (CTA) examination before and after the surgery showed aortic dissecting aneurysm involving visceral arteries(a: CT three-dimensional vascular reconstruction of the thoracoabdominal aorta before surgery; b: Post-surgery thoracoabdominal aorta CT three-dimensional vascular reconstruction)

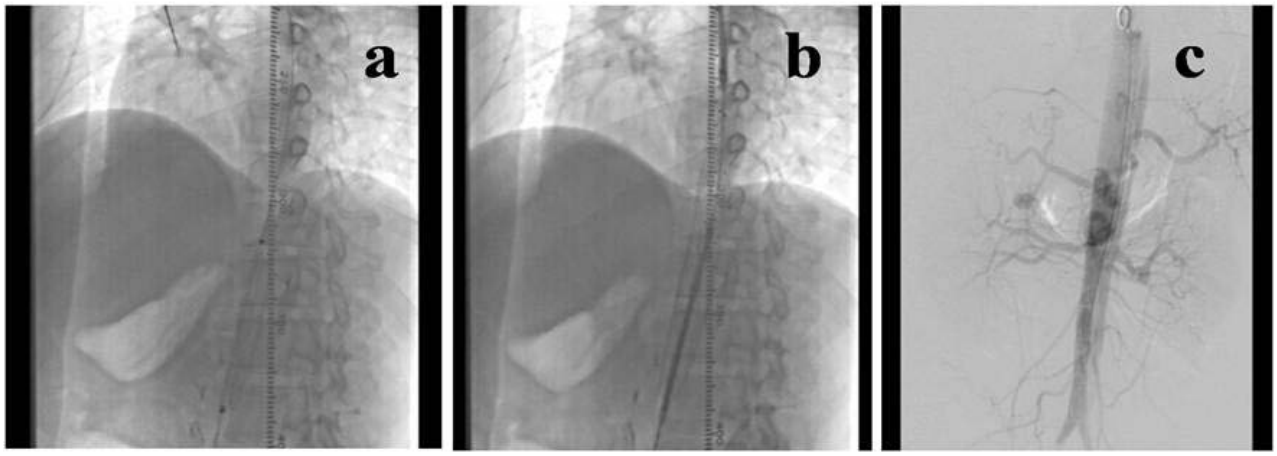

FIGURE 2. Endovascular treatment for aortic dissecting aneurysms (a: Bare stent covering the main visceral artery; b: BareStent covering non-visceral area dissection; c: Postoperative angiography of main visceral arteries patency.)

placed and heparin (80 IU / kg) administered in the sheath. The 0.035-inch ultra-smooth guide wire was led to reach the proximal end of the aortic lesion segment under the surveillance of digital subtraction angiography (DSA). 6Fr Pigtail catheter was exchanged with an injection rate of 20ml/s and a total volume of 40ml, aortic angiography was performed with the upper limit of 1000 psi injection pressure and the lesion location and size was re-determined. Puncture site sheath was changed for the 10Fr sheath, and the 0.035inch super-hard guide wire was guided to go across the aortic lesion segment. The guide wire was used to guide into the bare stents (Sinus-XL stent, Germany Optimed Company) one by one and overlap them over the lesion. The size of the stent was chosen to be about 10-20% of the diameter of the proximal neck of the aneurysm as described in the instructions. The proximal or distal end of the bare stent was extended to continuously place the covered stent in order to cover all the crevasses. The final number of bare stents depended on the results of intra-operative angiography, which is based on a slowdown in the flow rate of contrast media in the tumor cavity. The branches of the aorta, including CA, SMA, RA, and SA, were directly covered without revascularization or spinal cord protection. After the release of the stent, the guide wire catheter was removed, the puncture point was sutured using a vascular suture device, was the area was binded up with local pressurization (Figure 2).

Statistical analysis

The statistical analysis was performed by SPSS17.0 statistical software (http://www-01.ibm.com/software/analytics/spss/), the measurement data were expressed with $\bar{x} \pm s$, and the enumeration data were expressed with a relative number. $P<0.05$ meant a statistical difference.

RESULTS

Stent application

Sixteen patients successfully completed the operation with a total of 39 bare stents (average of 2.44 per person). Also, there were 2 cases with 1 stent, 8 cases with 2 stents and 7 cases with 3 stents.

Arterial dissection thrombosis

All the patients received CT angiography (CTA) examination during 1-4 weeks after the operation to assess the arterial dissection thrombosis. CTA showed complete thrombosis in the arterial dissection in 4 cases (25.0%) and partial thrombosis in 12 cases (75.0%), (Figure 3)

The main branch arterial patency

One to 4 weeks post-operation, the CTA showed that the celiac artery, left and right renal arteries and superior mesenteric artery were unobstructed.

Changes in the size of the dissection arterial diameter

CTA examination was performed one month after the surgery to assess the changes in the size of the dissection arterial diameter of patients, and the results showed that 4 cases had diameter reduction (25.0), 12 cases had no changes in diameter in (75.0%), and there were no diameter expanding cases.

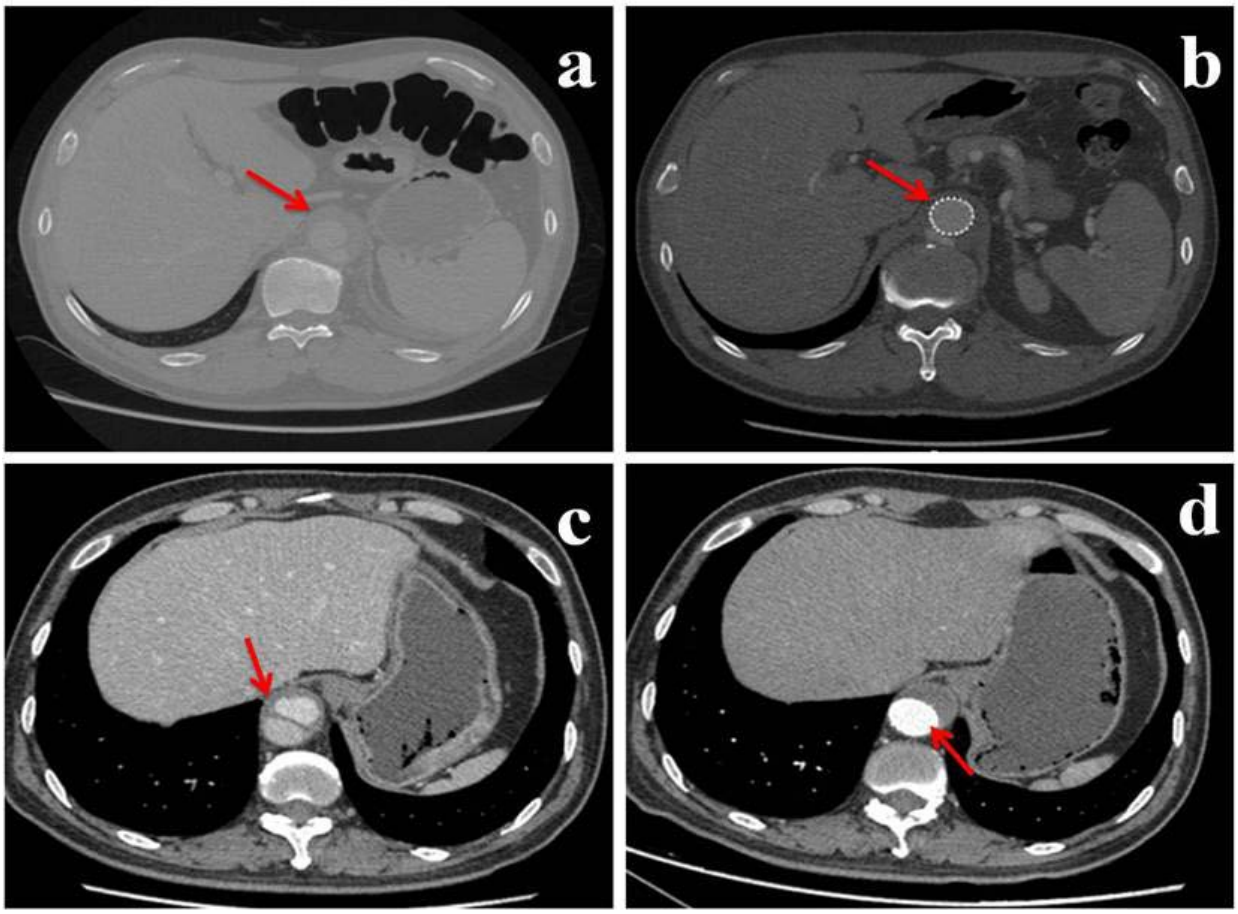

FIGURE 3. CT scan showed the arterial dissection thrombosis 1 to 4 weeks after operation (a: Dissecting aneurysm of abdominal aorta before surgery; b: Post-surgery, CT scan showed partial thrombosis; c: Dissecting aneurysm of abdominal aorta before surgery of another case; d:Post-surgery, CT scan showed complete thrombosis)

DISCUSSION

Aortic dissecting aneurysm involving visceral branch arteries has been a challenge for endovascular graft exclusion[7]. The traditional covered stent also covers the adjacent important branch arteries together while isolating aneurysms, resulting in target organ ischemia, necrosis, and dysfunction[6,8]. In order to solve this problem, the branch/fenestrated stents (Zenith fenestrated endovascular graft) came into being. According to the important branch open position and angle shown by the patient's preoperative CTA image, fenestration must be performed in the corresponding position of the straight-type covered stent; the fenestration must be made toward each branch artery while releasing the stent. Then the branch stent must be placed through the fenestration position to both isolate the aneurysm and protect the patency of important branch arteries[8]. However, such complex surgical procedures and the individualization of the stent make this technology challenging to be performed in smaller medical institutions[9]. The fenestration and branch stents need space to expand, but the true lumen of the dissection patient is often narrow, which limits the application of these two techniques. Moreover, because part of the visceral arteries are from the false lumen of the dissecting aneurysm, the fenestration, as well as branch and parallel stent technologies, are more difficult to apply. The near or mid-term treatment effect of flat renal or suprarenal abdominal aortic dissecting aneurysm is good, but because its indications are strict, the requirements to the surgeon's experience and techniques are high, and stent customization costs a long time and much money too, so it cannot be popularized widely at present. There are no regular products of branch stents, but only some case reports about it. Also, chimney stent complications, such as leakage and stent compression occlusion and other problems, still plague clinical practice. The emergence of multi-layer bare stent technology to solve the problem of branch artery patency provides a new

way of thinking. The objective of the covered stent is to build a mechanical barrier between the normal arterial lumen and the false lumen of the dissecting aneurysm cavity, and the multi-layer bare stent is designed to reduce the blood flow velocity and shear stress in the dissection, thereby inducing the formation of thrombosis in the dissection and achieving the "physiological" isolation, thus achieving the purpose of the treatment[10]. With the formation of thrombosis in the dissection, the shear stress of the arterial dissection wall is gradually reduced. At the same time, thrombosis increased the effective thickness of the wall and reduced the radius of the arterial lumen to achieve the effect of reducing the wall tension. However, bare stent-induced thrombosis is a relatively slow process. It may take several months to reach an effective level. During this period, the patient is still at risk of dissection fracture. In this study, CTA follow-ups 1 week and 1 month after the operation have found basic thrombosis in the dissection. There were no cases of dissection fracture. However, patients with hypertension should strictly control blood pressure to minimize the risk of dissection fracture while the thrombosis in the dissection has not yet fully formed.

In this present study, the maximal diameter of arterial dissection was reduced by 3.5mm on average, and there were no complications such as dissection expanding and fracture. A previously published study[4] reported using optimed bare stent multi-layer overlapping method for the treatment of 13 patients with dissecting aneurysms with an average follow-up of 20.9 months. The mean arterial diameter was reduced from 52.7 mm to 51.5 mm, but this was not statistically significant. The false lumen of the dissecting aneurysm completed thrombosis in 12 months in only 16.7% of the patients. Analysis of the reasons for that showed that most of the aortic dissections have distal crevasses, the distal end of the false lumen has outflow tracts, and the blood forms a stable circulation method. Therefore, the simple implantation of multi-layer bare stents has poor efficacy. However, it has good efficacy for dissection patients without obvious outflow tracts in the distal thrombosis. In this study, all the patients received covered stents placed in the dissecting non-visceral blood vessels area to cover the dissection crevasses and destroy the stable laminar flows, and they were placed into the bare stents sequentially, to achieve a satisfactory therapeutic effect.

In our present study, the average number of bare stents used was 2.44, with a maximum of 3 and a minimum of 1. Mainly based on the results of intra-operative angiography results, the number of bare stents stacked was flexibly controlled until the observed dissection blood flow velocity significantly decreased and the contrast agent density was significantly reduced. Thus the risk of incomplete isolation was greatly reduced. On the retention of the branch arteries, the short and middle term results of this study were satisfactory. No clinical symptoms of target organ ischemia were observed during the follow-up. There are also disagreements about postoperative anticoagulation therapy. In the literature[11], postoperative routine combined with antiplatelet therapy (aspirin 100mg / d, clopidogrel 75mg / d) is recommended to maintain the patency of the branch arteries. However, antiplatelet therapy can delay the formation of thrombosis in the dissection, which is not conducive for reducing the risk of dissection fracture. In this study, routine antiplatelet therapy was not performed. For clinical effects, even if not given conventional antiplatelet therapy, branch arteries can still maintain good patency. This may be related to the "circulatory pathway" hypothesis that the blood flows go through the branch arteries into the target organ, and the corresponding venous flows go back to the heart, forming a closed circulatory system, which drives the blood flows to the branch arteries[12].

CONCLUSION

In summary, the initial results of this study are satisfactory. However, some problems need to be further investigated. (1) How to determine the dissection prognosis during the surgery by the quantitative determination of DSA results; (2) how to deal with long-term arterial stenosis or occlusion in the stent coverage area is a matter of concern; (3) the current lack of multi-layer bare stent treatment norms and guidelines. Besides, the study was designed as a single center retrospective clinical study, and the number of cases included is relatively small. It is limited by the relatively short follow-up time. Moreover, long-term safety and effectiveness of multi-layer bare stent technology cannot be determined so far.

Declaration of conflict of interest: None

RESUMO

Objetivo: Aneurismas dissecantes da aorta envolvendo artérias viscerais são de difícil tratamento clínico. O objetivo deste estudo foi explorar a eficácia e segurança clínica da técnica de stents multicamadas não farmacológicos para o tratamento de aneurismas dissecantes da aorta envolvendo artérias viscerais.

Métodos: Foi feito um estudo retrospectivo usando os dados de 16 pacientes com aneurisma dissecante da aorta envolvendo artérias viscerais e tratados com stents multicamadas não farmacológicos de março de 2013 a março de 2017, do Hospital da Escola de Medicina da Universidade de Tianjin. Foram analisados nos 167 pacientes: a eficácia clínica, o número de stents aplicados, trombose dissecante da aorta no pós-operatório e patência cumulativa pós-operatória do ramo arterial.

Resultados: As operações dos 16 pacientes foram concluídas com sucesso sem nenhum óbito perioperatório. Os 16 pacientes receberam 39 stents não farmacológicos, com uma média de 2,44 por indivíduo. Houve 2 casos com 1 stent, 8 com 2 stents, e 7 com 3. Um mês após a operação, a ATC mostrou trombose completa da dissecção arterial em 4 casos (25,0%) e trombose parcial em 12 casos (75%). Também mostrou que a artéria celíaca, as artérias renais direita e esquerda e a artéria mesentérica superior estavam todas desobstruídas. Houve 4 casos (25,0%) de artéria dissecante com diâmetro reduzido, 12 (75,0%) pacientes sem alteração no diâmetro, e nenhum caso de aumento de diâmetro.

Conclusão: O tratamento para aneurisma dissecante da aorta envolvendo artérias viscerais com a técnica de stents não farmacológicos multicamadas é seguro e confiável, com uma taxa mais alta de patência de ramos arteriais acumulados no pós-operatório.

Palavras-chave: Aneurisma dissecante. Aneurisma da aorta. Stents. Procedimentos endovasculares.

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ORIGINAL ARTICLE

Nutritional and hematological factors associated with the progression of Alzheimer's disease: a cohort study

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SUMMARY

OBJECTIVE: *We studied the users of the Specialized Drug Distribution Program of the public health network.*

METHODS: *A prospective cohort examined the elderly at two intervals of three years and included 30 patients in phase I and 16 in phase II. The methodology was composed of home visits, anthropometric, nutritional and hematological evaluation. For the progression of AD, the Clinical Dementia Rating (CDR) scale was used.*

RESULTS: *According to the CDR, the disease evolved, since in 2014 most of the patients were in CDR 3. In the analysis of the micronutrients, only the B vitamins (B1, B2, B3, B5, B6) presented a significant reduction in 2014. The consumption of carbohydrates and lipids increased in the 2014 evaluation, and protein consumption decreased. As for the average weight of the elderly, there was an increase in 2014, 65.9 (± 15.6) Kg, with a BMI of 26.75 (± 4, 5), in 2011 the average weight was 62.44 kg (± 14, 36), BMI 24.64 (± 4.97).*

CONCLUSION: *The hypothesis that patients are likely to be overweight or obese before the development of AD and that this may be associated with an increased risk of dementia is suggested.*

KEYWORDS: *Alzheimer disease. Obesity. Macronutrients. Dementia.*

INTRODUCTION

Alzheimer disease (AD) is a degenerative neurological disease, progressive and irreversible that affects about 35.5 million people around the world, being characterized as the most common dementia. It manifests itself insidiously as a result of neuronal lesions and consequent degeneration of nervous tissue [1].

Patients with AD present deterioration of the superior cortical functions that lead to loss of memo-

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ry, disorientation, difficulty of comprehension, calculation, learning ability, language, and judgment, making it difficult to perform activities of daily living [2,3]. The first symptom of the perceived disease is often the decline of memory, especially of recent events (episodic memory), and spatial disorientation, cognitive aspects most often subordinate to hippocampal formation [1].

One of the main factors associated with the disease progression is malnutrition due to agnosia (difficulty in distinguishing an object even with tactile stimulus), and apraxia (loss of ability to perform characteristic movements and gestures), symptoms of AD which decrease energy intake by accelerating the process of weight loss, making the patient more and more dependent on their caregivers [4]. Changes in swallowing, such as dysphagia, also affect individuals with AD, impairing food intake, along with anorexia caused by atrophy of the mesial temporal cortex (MTC), the area of the brain responsible for eating behavior. The difficulty in self-feeding is a determining factor for the diagnosis of dementia according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) [5].

Although weight loss is frequently observed in elderlies with AD, weight gain due to hyperphagia has also been documented in these individuals [4,6]. Thus, during the progression of dementia, it is possible to observe a functional loss of the body weight regulation process [4], since the deferred elderly can present weight gain, periods of acute weight loss and, thus, great body mass instability.

Epidemiological and observational studies suggest that nutritional status, lifestyle factors, and some associated pathology (e.g., hypertension, cardiovascular disease, diabetes mellitus, and metabolic syndrome) are directly related to cognitive impairment and dementia [7,8].

Studies have reported that patients with AD present nutritional deficiencies of many vitamins and minerals [7-9]. Micronutrient deficiencies may result not only in loss of lean mass, decline in immune functions and increased risk of fractures but also in oxidative damage in the brain and deficiencies in neurotransmitters, impairing cognitive function [10].

Therefore, the knowledge of the nutritional and cognitive reality of patients with AD is important to establish a means of possible improvement of the general health of patients and even avoid the progression of AD.

In this context, our study aimed to follow and monitor the nutritional, biochemical and cognitive status of a cohort of patients with Alzheimer's disease, users of the Specialized Medicines Distribution Program of the public health network in the city of Guarapuava-Paraná, Brazil, from 2011 to 2014.

METHOD

Study type

The methodological approach used was a descriptive quantitative research, with a longitudinal and prospective design. The elderly were examined on two occasions, with three years interval: evaluation I (August to October 2011) and evaluation II (January 2014).

Study population

The AD elderly cohort was performed in Guarapuava city, on the Middle East region of Paraná state, Brazil. The identified participants were citizens of the community registered on the public health system (SUS), provided by the Health Ministry, which received specific AD medicines at no charge. These patients confirmed the AD diagnosis according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's disease and Related Disorders Association (NINCDS-ADRDA) [5].

From 66 patients registered in SUS, 30 (45%) received anthropometric measurements and hematological examinations. The remaining 36 patients were excluded for the following reasons: 07 had died, 11 moved from Guarapuava, 02 were not found, and the caregivers of the remaining 16 did not agree to participate in the study because of their weakness. Thus, the 2011 cohort was completed with data from 30 patients.

In January 2014, after a 27-month interval, a new cohort data collection was performed. From the 30 elderly individuals in stage I (2011), 09 died, and there were 05 follow-up losses (3 change of municipality and 2 refusals in participating in the study), so in the evaluation 2 (2014) the sample was composed of 16 patients. Only the participants with the initial and segmental evaluations participated in the analysis, totaling 16 patients for this study in 2014.

Data collection

Considering the studied population, it was idealized a set of applied instruments on the following order: Interview (personal data, socioeconomic condition, clinical history, used medication); anthropometrical evaluations; dietary evaluation using Mini nutritional evaluation (MNA) [11], and 24 hours reminder (R24H). Besides, a hematological evaluation (hemogram) was performed [12]. For the disease staging, a Clinical Dementia Rating (CDR) was used [13,14].

Data collection proceed

The interview for data collection was conducted in the patients' residences using a semi-structured questionnaire. The elderly's medications were analyzed, separated and counted, according to the pre-established pathologies: systemic hypertension, diabetes mellitus, hypercholesterolemia, and AD. The weight was determined using a portable digital scale (Plenna®, Brazil) with 150 kg capacity and 100 g precision. Anthropometrical measurements of weight, height, arm, wrist, waist, hip, circumferences, triceps, skin folds, and demi-span were also performed using standard equipment (CMS Pesando Equipment Ltd., London, UK). When the weight and height measurements were not possible, the estimative was done through theoretical formulas, using arm, calf circumferences, knee height, and subscapular skinfold thickness, according to Chumlea et al. [15]. All the anthropometrical measurements were performed by a direct method.

Based on the parameters of base and height, the body mass index (BMI) of each patient was calculated, which corresponds to the ratio of body weight (kg) to height (m) squared. The cut-off points recommended by the Pan American Health Organization for body mass index (PAHO, 2002) were used as reference, and the PAHO classification recommends the following cut-off points (kg / m2) [16]: appropriate BMI (> 23 and <28), BMI indicative of low weight (≤ 23), BMI of overweight (≥ 28 and <30) and BMI indicative of obesity (≥ 30).

The patients nutritional status were evaluated through MNA, composed of anthropometrical measurements of CB, CP, BMI and weight loss percent, general evaluation associated to life quality, subjective evaluation and dietary survey, which was developed with the intention of elderly malnutrition detection [17].

The patient's food consumption was evaluated through the 24 hours reminder, which consists on questions to the respondent (or responsible) to describe the ingestion of food and beverages consumed on the past day [17]. The caregiver provided information about the time, food, type of preparations and quantities of each food consumed during the 24 hours before the interview. All the food and beverages reported were submitted to a dietary analysis composed of energy values (kcal), macronutrients (carbohydrates, lipids, and proteins) and micronutrients. The data were analyzed with computer program Avanutri® 4.0 version [18].

Blood analysis

For the blood composition verification, a blood sample was collected. The blood analysis for the hematological parameters evaluation was carried on the hematology cell counter Hema-Screen 18. The following rates of blood cell were evaluated: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), hemoglobin, total leukocytes, and platelets, using reference values advocated by Matos et al. [12]. All the exams were carried on in the Midwest State University (UNICENTRO), in Guarapuava.

Cognitive evaluation

For the evaluation of dementia in Alzheimer's disease, as well as classification of patients in stages of the disease, the clinical dementia rating scale (CDR) was used, as proposed by Montaño et al. [14].

This evaluation classifies patients concerning memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care, with score: healthy (CDR 0), questionable dementia (CDR 0.5), mild dementia (CDR 1), moderate dementia (CDR 2) or severe dementia (CDR 3) [13,14].

Ethical aspects

This study was submitted to the Research Ethics Committee (CEP) of the Midwest State University (UNICENTRO) and approved by the decision No. 611.316/2011.

Statistical analysis

The statistical analysis was performed using package SPSS 20.0 version. The non-parametrical analysis was chosen due to the high number of variables that have not acceded presumption of normal distribution evaluated by the Shapiro-Wilk test. It

consisted of the evaluation of the subjects during 3 years, using the Wilcoxon test. Additionally, possible correlations and associations among the variation (Δ %) on the segment were evaluated.

RESULTS

General characterization of the elderly in 2011 and 2014

In the initial sample, 30 elderly patients with AD were evaluated in 2011, 33% (n = 10) were evaluated in early-stage dementia (CDR-1), 26% (n = 8) 2) and 40% (n = 12) in the advanced stage of dementia (CDRconti3). Among the 30 elderly evaluated, in 2011, 60% (n = 18) were women, and 40% (n = 12) were men. The age of the sample ranged from 54 to 91 years in 2011, with the mean age of all the elderly being 77 (± 9.3 years). After the three years, 8 patients died, and another 6 patients chose not to be part of the study. The mean number of years of AD diagnosis was 3.5 (± 2.51). In the 2014 sample, mean years of diagnosis of AD were 5.6 (± 2.91).

Among the 16 elderly patients with AD, who were followed up in 2011, 25% (n = 4) were diagnosed with dementia at the early stage of the disease (CDR-1), 31% (n = 5) 2) and 44% (n = 7) in the advanced stage of dementia (CDR-3).

In 2014 the sample was reassessed and from the 16 elderly with AD, 25% (n = 4) were evaluated with early-stage dementia (CDR-1), 25% (n = 4) and 50% (n = 8) in the advanced stage (CDR-3). The sample of 2014 had 8 males and 8 females, ranging in age from 57 to 94 years, and the mean age of all the elderly was 79 (± 10.5) year

Comorbidities of the elderly in 2011 and 2014

Among comorbidities present in AD, systemic arterial hypertension (SH) was the most prevalent among the elderly 66%, regardless of cognitive function, followed by the diagnosis of Diabetes mellitus (DM) 33%, hypercholesterolemia 33%.

In 2014, the most prevalent comorbidities among the elderly continued to be hypertension, with 56% of the elderly affected, followed by the diagnosis of Diabetes mellitus 43%, hypercholesterolemia 18.7%.

Anthropometry of the elderly in 2011 and 2014

Regarding the average weight of the elderly in 2011, 62.44 kg (± 14.36), with a mean BMI of 24.64 (± 4.97) for the 2011 sample. Following the classification, for the elderly of PAHO, the (n = 1) were underweight, (n = 0) of the elderly were eutrophic, 31.25% (n = 5) were overweight, and 62.50 % (n = 10) were obese.

In 2014, the mean weight of the elderly was 65.9 (± 15.6) kg, with a mean BMI of 26.75 (± 4.5). The BMI of the 2014 sample, according to the classification, for PAHO the elderly showed that 6.25% (n = 1) were underweight, (n = 0) elderly were eutrophic, 18.75% (n = 3) were overweight, and 75% (n = 12) were obese.

Comparing the two samples in relation to CP and CB data, it indicates a trend in both circumferences in the 2014 cohort. BMI and weight also tended to increase in the 2014 sample.

Profile of the sample according to BMI, calf circumference, arm circumference and total weight in 2011 and 2014. Nonparametric analysis was used because of the high number of variables that did not meet the standard distribution assumption assessed by the Shapiro- Wilk. The analysis consisted of the evaluation of the variation of subjects over 3 years. For this purpose, the Wilcoxon test was used. Comparing the two samples in relation to CP and CB data indicates a trend in both circumferences in the 2014 cohort. BMI and weight also tended to increase in the 2014 sample.

Diet of the elderly in 2011 and 2014

Regarding the consumption of carbohydrates, there was an increase in the evaluation of 2014, as well as in the consumption of lipids. In relation to proteins, its consumption decreased in 2014.

Comparison of the consumption of carbohydrates, proteins, and lipids in the sample in 2011 and 2014. Nonparametric analysis was used because of the high number of variables that did not meet the standard distribution assumption evaluated by the Shapiro-Wilk test. The analysis consisted of the evaluation of the variation of subjects over 3 years. Wilcoxon's test was used for this purpose. Concerning the consumption of carbohydrates, there was an increase in the evaluation of 2014, as well as in the consumption of lipids. In relation to proteins, the consumption of proteins decreased in 2014 (figure 1).

In figure 2 it can be seen that in the analysis of micronutrients, only the B vitamins (B1, B2, B3, B5, B6) showed a significant reduction between 2011 and 2014.

The complex B vitamin intake of the sample in 2011 and 2014 was evaluated using the non-paramet-

ric analysis due to the high number of variables that did not meet the standard distribution assumption assessed by the Shapiro-Wilk test. The analysis consisted of the evaluation of the variation of subjects over 3 years. The Wilcoxon test was used in the analysis of micronutrients, only the B vitamins (B1, B2, B3, B5, B6) presented a significant reduction between 2011 and 2014, P <0.05.

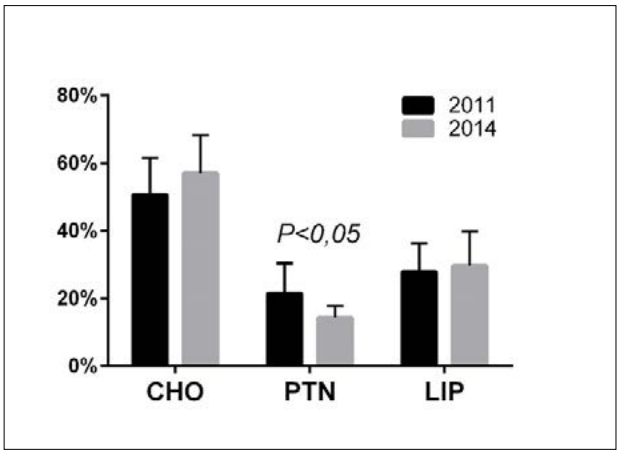
Blood count

According to Table 1, in relation to the blood picture data and hemoglobin in the samples, there was a decrease in erythrocytes, MCHC, and RDW in the 2014 review, but no significant change amounts occurred in the hemoglobin, confirming that none of the samples had anemic patients according to standardized values.

Regarding the medicines used, the average was the same in the two evaluations 3.7 medications per patient. The change occurred in the number of patients in this average, which in 2011 was 80% and in 2014 increased to 87%. The drugs evaluated were those used in the most prevalent pathologies, hypertension, diabetes mellitus, cholesterol, and AD (table 1).

Hematological evaluation of the sample in 2011 and 2014 was expressed as mean ± SD - Student T-test for paired samples; Median and interquartile range (25 - 75%) - Wilcoxon; percent - chi-square. p significant <0.05. Being significant for erythrocytes, RDW and CHCM.

FIGURE 1: COMPARISON OF THE CONSUMPTION OF CARBOHYDRATES, PROTEINS, AND LIPIDS OF THE SAMPLE, IN 2011 AND 2014

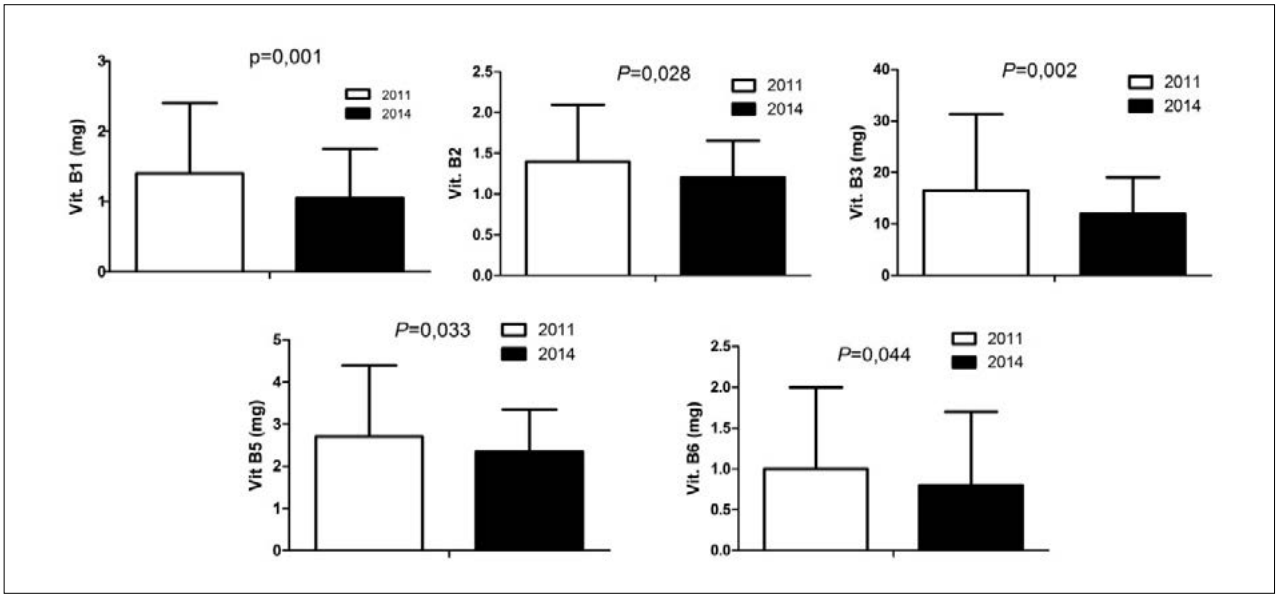


**CHO Carbohydrate, PTN, protein, LIP, lipid

DISCUSSION

Different epidemiological studies have attempted to find associations to explain the progression of disease in Alzheimer patients [6,8,19]. The genetic and biochemical conditions are the main topics discussed [2,3]. The present study aimed to identify the pattern of food consumption, its alteration over 2.7 years, and if these modifications would correspond to cognitive changes the and progression of AD since the alterations in food behavior, especially micronutrient reduction seem to show association with cognitive loss.

FIGURE 2: COMPLEX B VITAMIN CONSUMPTION OF THE SAMPLE IN 2011 AND 2014



The Clinical Dementia Rating Scale (CDR) is a measure to detail the nature and severity of cognitive and functional impairment of Alzheimer's disease (AD) and other dementias. Before this study, it was observed that the majority of patients in 2011 were between the mild and severe stage (CDR 1 and 3). In 2014, it was observed a predominance in the severe stage of AD in (CDR 3). In another study in southern Brazil with patients with diverse types of dementias, differences were also observed between the stage where most, 80% of the patients, were mild to moderate stages (CDRs 1 and 2) [4]. This fact may be associated with the late diagnosis of AD in the analyzed community. In 2011, the mean time of diagnosis of the patients was 3.5 ± 2.51 and in 2014, 5.6 ± 2.91.

Regarding the types of conditions associated with both evaluation years, hypertension was the most prevalent comorbidity among the elderly independently of cognitive function, then the diagnosis of Type 2 diabetes mellitus and hypercholesterolemia. These findings corroborate the studies of the United States Alzheimer's Association [20], where patients with AD older than 65 years had comorbidities associated with systemic hypertension prevalence in (60%) and coronary heart disease (26%).

An epidemiological study demonstrated the relationship between high blood cholesterol levels with increased risk of developing a cognitive disease, vascular alterations and Alzheimer's disease [21], mainly because of the strong relationship between the deterioration of cerebral lipid homeostasis associated with a carrier of cholesterol, the apoE4.

Diabetes mellitus is a disease that has common risk factors for Alzheimer's disease [21]. There are some hypotheses, such as central insulin resistance, along with reduced levels of insulin in the brain, which may be due to DM 2, lead to accumulation of β-amyloid and, consequently, AD.

Concomitant with AD, other comorbidities may affect the elderly, making the practice of polypharmacy common at this stage of life [22].

It is considered light polypharmacy, the use of two to three drugs, moderate from four to five and severe, more than five [23]. This polymerization can influence both the nutrition of the elderly and the pharmacokinetic processes, compromising nutrition and therapy (22). All of these pathologies associated with AD cause the elderly in the sample to be polymedicated. Studies reported that elderly patients who used a high number of drugs were 60% more likely to be at nutritional risk (22). This polymerization can influence both the nutrition of the elderly and the pharmacokinetic processes, compromising nutrition and therapeutics.[23] These interactions are facilitated since most drugs are administered orally [24]. All the patients of the study in 2011 and in 2014 were using at least one cholinergic drug, donepezil, galantamine or rivastigmine acetylcholinesterase inhibitors that are recommended in the treatment of mild to moderate AD.

Concerning body weight, the sample showed an increase. In 2011, the average body weight was 62.44 kg changing to 65.9 kg in 2014. This effect was observed similarly in a study that aimed to identify the relationship between weight loss and temporal

TABLE 1: HEMATOLOGICAL EVALUATION OF THE SAMPLE IN 2011 AND 2014

	2011		2014		
	Medium	P75-P25	Medium	P75-P25	P
Leukocytes	7310.0	2720.00	6100.0	1400.0	0.155
Lymphocytes	2152.5	783.60	1800.0	852.0	0.799
Eosinophils	222.6	352.5	264.5	336.0	0.262
Erythrocytes	4.6	0.50	4.50	0.70	0.022
Hemoglobin	13.7	2.00	13.95	2.30	0.906
Neutrophils	4190.4	1762.10	3472.0	2570.0	0.328
Monocytes	585.0	286.60	601.0	310.0	0.168
RDW*	13.1	0.60	15.45	2.00	0.003
CHCM**	32.9	1.60	31.00	1.20	0.003

* RDW Distribution Range of Red Blood Cells ** CHCM mean corpuscular hemoglobin concentration). Reference values 13.0 to 18.0 g/dL for men and 12.0 to 16.0 g/dL for women, values of (RDW) 11.6 to 14.6% for both sexes.

lobe atrophy in AD patients. This hypothesis, however, has not been confirmed given the weight gain occurred in most study patients [25].

Confirming the hypothesis of the weight gain in the sample, we see that the BMI also increased, in 2011 it was 24.64 kg / m² and in 2014 it was 26.45 kg / m²; the majority of the patients studied presented obese nutritional status. These BMI and total weight data are complemented with the CP and CB measurements of the elderly who also had weight gain in 2014.

There are two possible explanations for these episodes, one involving the patient, the other the caregiver. According to some authors, the finding of weight gain in some people suggests that body weight regulation is dysfunctional in AD [25,26]. This dysfunction may be a consequence of the pathophysiology of AD because the complex regulation of energy intake involves eating disorders, including hyperphagia (overfeeding) [25].

In AD, there is usually a reduction in the levels of 5-hydroxytryptophan (5-HT), which is related to feelings of delayed satiety, a preference for sweet foods, and a reduction in protein intake. Hyperphagia has been reported in scientific studies in 10 to 36% of patients with Alzheimer's disease [27].

Without the intervention of the caregivers, Alzheimer's patients who develop hyperphagia will continue to eat erroneously and may even evolve into clinical disorders resulting from weight gain, which may contribute to a considerable increase in disease morbidity [28].

In another study, with a group of 85 individuals, 35% had hyperphagia at some stage during the disease, and 54% of individuals were in the habit of eating more sweet foods when compared to ingestion before acquiring the disease [28]. Similar results were found in other studies in which researchers studied dietary behavior in a group of 33 individuals with Alzheimer's and found a food preference for sweet foods in 24% of individuals [29]. Another hypothesis for weight gain or the mean eutrophic of the sample is that patients were probably already overweight or obese before the development of AD.

Some studies show that a high BMI in adulthood may be associated with dementia increased risk [29,30]. In the study by Pasinetti and Eberstein [30], obese participants had a 35% higher risk of dementia compared to healthy weight. The study concluded that obesity in adulthood increases the risk of dementia mainly by factors that bind to fat AD as hyperinsulinemia, derived hormones of adipocytes (adipokines and cytokines), and the influence of adiposity on risk vascular and cerebrovascular disease [30].

It is possible that the caregivers are burdened by the disease process, so they invest adequate resources to enable patients with AD to feed appropriately. This explanation can be defended by the results observed in the present study. It was observed an increase in body mass, CP and CB, outcomes, possibly stabilization of macronutrient consumption and a decrease in micronutrient consumption. These combined conditions indicate low nutritional quality in the patients' diet, that is, empty calories. Conversely to this study, other studies have documented a reduction in body weight, and in some cases, this reduction was considered a predictive factor of cognitive changes [6,9,31]. It is known that the decline in total body mass and muscle mass are related to functional loss [6,31]. The reduction in macronutrient intake, especially of protein order, is associated with a reduction of muscle mass and weight loss, which in high degrees characterize sarcopenia [32].

Swallowing disorders such as dysphagia, chewing problems associated with loss of teeth also affect individuals with AD, affecting food intake by limiting the consumption of solid food such as fruits, vegetables, and meats [9].

Compared to 2011, the consumption of protein decreased in the evaluation of 2014. Kalmijn et al. [33] in his study reported that a high intake of total fats increases by 2.4 times the chance of developing dementia. The consumption of lipids increased in the 2014 sample, and the consumption of fat food above 35% is directly related to cognitive decline [31,32] . Other research also found an association between high fat intake and cognitive impairment [33].

Different from what was seen in this study, Kwan et al. [34] in order to identify factors associated with weight changes in patients with Alzheimer's disease, conducted a study with 45 patients and 36 being the control. Among the different tests, the authors found that patients had a higher caloric intake, increased protein consumption compared to the control group.

Individuals with AD tend to prefer carbohydrate-rich foods to protein sources due to a change in neurotransmitters, usually serotonin, which may alter food preference [31].

The diet of the patients should contain higher

levels of protein and lower carbohydrate from the beginning of treatment of disease, particularly to maintain the levels of neurotransmitters such as serotonin, adrenaline, and dopamine, involved in feeding regulation of behavior and some cognitive deficits [35]. Neurotransmitters need some precursors such as tryptophan, choline, and tyrosine, which are present in protein foods.

The study by Forlenza [36] reaffirms that reduced food intake, together with low protein intake, causes a reduction in plasma levels of tryptophan thus altering cortical serotonin levels and contributing to the onset of some disorders such as depression, agitation, and aggressiveness. In the analysis of micronutrients, only B vitamins (B1, B2, B3, B5, B6) showed a significant reduction between the years, decreasing significantly in 2014.

The B complex vitamins in reduction in the study have, as their primary food source, the proteins, which corresponds to the previous data where, in 2014, there was a decrease in protein consumption

The majority of the elderly population present higher rates of nutritional deficiencies than the young population due to physiological changes, such as a decrease in basal metabolism, redistribution of body mass, alterations in digestive functioning. These occur mainly in relation to B vitamins and antioxidant nutrients (vitamin C and E, selenium) [19]. In a study conducted in Greece with 100 elderly patients with AD, 48% were overweight, and the vast majority had inadequate intakes of vitamins A, B6, D, E and K19 [19].

Many thiamine (vitamin B1) dependent processes are decreased in brains of patients with AD. Karuppagounder et al. [37], in their study, demonstrated that thiamine deficiency exacerbated amyloid plaques in transgenic rats, increased the area occupied by plaques in the cortex, hippocampus and thalamus, and induced inflammation in plaque forming areas. The intake of vitamin B3 from the diet is inversely associated with AD, having a protective effect on disease development and cognitive decline [34]. In the present study, there was a decrease in vitamin B3 consumption in 2014, which may increase the risk of cognitive decline, and may be associated with an increase in CDR3 patients when compared to 2011.

Regarding RDW and hemoglobin data, there was an increase in RDW in the 2014 sample. High RDW is common, for example, in iron deficiency, in which the lack of this element prevents the formation of normal hemoglobin, leading to the formation of smaller red cells [12]. There were no changes in hemoglobin, confirming that there were no anemic patients, in practice, the hemoglobin turns out to be the most accurate in the evaluation of anemia, the reference values varied from 13.0 to 18.0 g / dL for hemoglobin in men and from 12.0 to 16.0 g / dL for women [37].

The population of our study, despite their intake of protein, iron sources for the body, reduced in 2014 did not present anemia according to the CBC. In our study, hematocrit was not performed in any of the samples because, according to the Nandigam et al. [38] study with 100 elderly patients in the United States, it is recommended not to evaluate the hematocrit in the elderly, due to the physiological decrease of their plasma volume, which could overestimate the values of this indicator. This study had relevant limitations, which were the small sample size and the significant loss of patients throughout the cohort.

CONCLUSION

The initial sample was 66 patients, but only 30 accepted to be part of the research and at the end of the study, in the year 2014, the analysis counted on only 16 patients. However, even given the reduction of the study population, the results were relevant to the final sample population. In both evaluations that followed, it was observed that the most advanced stage of AD (CDR 3) was prevalent and may indicate a lack of proper and specialized care for early diagnosis.

Regarding nutritional status, there is a hypothesis that patients were already overweight or obese even before AD, which may be associated with an increased risk of dementia. It was observed that BMI and body weight of individuals tended to increase in the 2014 evaluation, although the majority of patients in both evaluations were eutrophic.

The consumption of macronutrients, such as carbohydrates and lipids, increased in the sample of 2014, already in relation to the proteins, it is observed that there was a decrease in the intake. The evaluated micronutrients, B vitamins (B1, B2, B3, B5, B6), showed a reduction in both analyzes, decreasing significantly in 2014.

The present study evidences the need to develop other studies in order to evaluate the serum micronutrient dosage of patients with long-term AD. The importance of frequent nutritional monitoring is also emphasized in order to avoid compromising the nutritional status of patients with Alzheimer's disease.

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Conflict of interest

The authors declare that there is no conflict of interest.

RESUMO

OBJETIVO: *Foram estudados os usuários do programa de distribuição de medicamentos especializados da rede pública de saúde de Guarapuava, Paraná, Brasil.*

MÉTODOS: *Uma coorte prospectiva, em que os idosos foram examinados em dois momentos, com um intervalo de três anos, com 30 pacientes na fase I e 16 na fase II. A metodologia foi composta por visitas domiciliares, avaliação antropométrica; avaliação nutricional e hematológica. Para a progressão da DA, utilizou-se a escala Clinical Demential Rating (CDR). Os testes de Shapiro-Wilk, teste de Wilcoxon e correlações com associações (Δ%), p < 0,05 para as análises estatísticas.*

RESULTADOS: *A progressão da doença, segundo o CDR, evoluiu, pois, em 2014, a maioria dos pacientes encontrava-se em CDR 3. Na análise dos micronutrientes, somente as vitaminas do complexo B (B1, B2, B3, B5, B6) apresentaram redução significativa em 2014. O consumo de carboidratos e lipídeos aumentou na avaliação de 2014, e o consumo de proteínas diminuiu. Quanto ao peso médio dos idosos, houve um aumento em 2014, 65,9 (± 15,6) kg, com IMC 26,75 (± 4, 5); em 2011, o peso médio foi 62,44 kg (± 14,36), IMC 24,64 (± 4,97).*

CONCLUSÃO: *Não foram encontrados pacientes anêmicos ou desnutridos na amostra. A hipótese de que os pacientes provavelmente já apresentavam sobrepeso ou obesidade antes do desenvolvimento da DA, e que isso pode estar associado com um aumento de risco de demência, pode ser sugerida.*

PALAVRAS-CHAVE: *Doença de Alzheimer. Obesidade. Macronutrientes. Demência.*

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REVIEW ARTICLE

Mapping scientific research on the negative aspects of the medical school learning environment

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SUMMARY

OBJECTIVE: *We sought to understand the landscape of published articles regarding medical schools' learning environments (LE) worldwide, with an explicit focus on potentially harmful aspects of the LE as an effort to identify areas specifically in need of remediation or intervention that could prevent future unprofessional behaviors, burnout, violence and mistreatment among students and physicians.*

METHODS: *A bibliometric analysis was conducted in six electronic databases (PubMed/Medline, Web of Science, Cochrane Library, SCOPUS, ERIC-ProQuest, and PsycINFO) up to December 31, 2016, including 12 themes: learning environment – general, hidden curriculum (harmful), unethical behaviors, bullying/hazing, violence, sexual discrimination, homophobia, racism, social discrimination, minorities discrimination, professional misconduct, and other negative aspects.*

RESULTS: *Of the 9,338 articles found, 710 met the inclusion criteria. The most common themes were general LE (233 articles), unprofessional behaviors (91 articles), and sexual discrimination (80 articles). Approximately 80% of articles were published in the 21st century.*

CONCLUSION: *There is a definite increase in scientific articles on negative aspects of the medical school LE in high-quality journals, especially in the 21st century. However, more studies are needed to investigate negative LE aspects with greater attention to experimental, longitudinal, and cross-cultural study designs.*

KEYWORDS: *Learning Environment, Medical Education, Medical Students, Ethics, Professionalism.*

INTRODUCTION

The first study that focused on studying learning environments (LE) in higher education dates back to 1958.[5] Concerning medical schools' LEs, Hutchins (in 1961)[6] developed the first questionnaire and made the first attempt to understand quantitatively how the environment might impact students' attitudes,

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values, and behaviors. Since then, many tools have been developed to assess medical students' perceptions of their LE,[7] showing the growing importance of this subject to medical researchers. According to Cohen,[8] if medical schools intend to deal with the erosion of professionalism during medical training, "purging their own learning environments of unprofessional practices" (p. 610) is a crucial endeavor.

Much research now supports Cohen's[8] idea that this purging of negative aspects of the LE is critical for developing a professional physician. Much of this focuses on the cognitive/curricular or social aspects of the LE and was derived from small populations of students.[9] Reported perceptions of poor LE have already been correlated with high levels of student burnout and a worse perception of quality of life,[10] decreased personal growth,[11] worse academic performance on the United States Medical Licensing Examination Step 1,[12] and less time spent by students on activities involving direct patient contact.[13]

However, many LE general instruments used in research might not encompass all aspects of the LE that influence students' lives. Specifically, it may be especially important to consider the negative aspects of the LE because these are likely to have ensuing negative effects on medical students, as has been demonstrated for unprofessional/unethical behaviors, violence, and harassment, and their impact on students' professionalism and quality of life.[14-18]

Mapping the research on the negative aspects of the LE will help to identify areas that are already well explored, areas where more work needs to be done, how interests have trended over time among researchers and the geographic and cultural areas where interest in these topics is most significant. Thus, the purpose of this study was to build on our current understanding of LEs through a comprehensive bibliometric analysis that develops a broader framework for LEs and their negative aspects, which can guide further investigation about each specific topic (such as original studies and systematic reviews) and medical curricula interventions.

METHODS

From September 2016 to January 2017, we carried out a bibliometric analysis to evaluate all original articles related to medical schools' LE up to December 31st, 2016. This bibliometric approach is defined as "a tool by which the state of science and technology can be observed through the overall production of scientific literature"[19] (p. 6) and is used to map a field of research, providing a statistical description of this (recent or historical) data.[19-21]

Since this is a review of the literature, ethical approval was not required for this project. The sequence of the main phases is described below.

Keywords selection

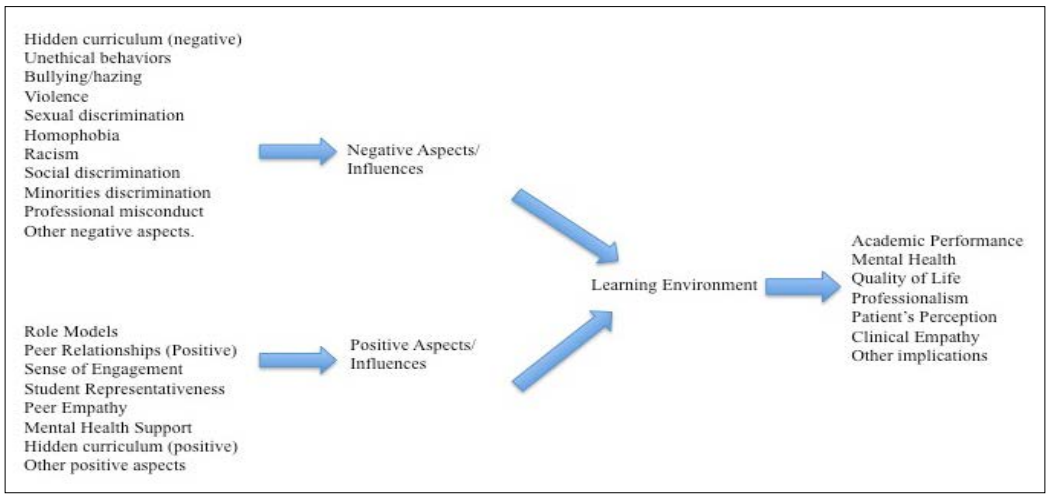
Initially, three authors (R.F.D., A.O.C., J.G.O.) reviewed a sample of LE articles and independently generated a list of keywords focusing on capturing all potentially harmful aspects of medical school LEs. After each list was created, four authors (R.F.D., A.O.C., J.G.O., G.L.) examined the list to eliminate redundancies and add new words to the list. Then, each independent researcher (R.F.D., A.O.C., J.G.O.) created a list of themes (clusters) made up of related keywords and compared them with the Learning Environment literature to check for alignment with the current scientific data. For this initial stage of development of themes, we examined systematic reviews and the most prominent articles published in high tier journals.

One author (R.F.D.), in collaboration with the institution librarian, was responsible for comparing each list of themes, merging similar ones and removing duplicates. A discussion among all authors brought a consensus of twelve thematic clusters, including the "general LE" and eleven negative aspects of medical schools' LE: hidden curriculum (harmful), unethical behaviors, bullying/hazing, violence, sexual discrimination, homophobia, racism, social discrimination, minorities discrimination, professional misconduct, and other negative aspects. These clusters were arbitrarily defined by the authors of this paper. The general theme was based on articles with a focus on the Learning Environment, usually containing "Learning Environment" in the title and using LE measurement instruments. However, the negative aspects included research that did not always define the negative aspect as a component of the LE. Figure 1 summarizes the conceptual framework of the LE used by this manuscript.

Eligibility criteria

Inclusion criteria were: original studies (longitudinal studies - cohort and case-control, cross-sectional studies, case reports, experience reports, and experimental studies) carried out with medical stu-

FIGURE 1. CONCEPTUAL FRAMEWORK OF LE USED ON THIS ARTICLE.



dents and related to their LE. Studies considered out of area (not related to LE) and out of population (not with medical students) were excluded. Furthermore, as our focus was on original studies, reviews, replications, theoretical pieces, articles without abstracts (because we needed the abstracts in order to review the paper), and book chapters were not included. No language limit was applied.

Databases search

We searched six electronic databases (PubMed/Medline, Web of Science, Cochrane Library, ERIC-ProQuest, SCOPUS, and PsycINFO) including all studies published up to December 31, 2016. A variety of Boolean expressions based on the twelve thematic clusters were created to guide the search in these databases (see Supplemental Material 1), and then each database outcome was exported to Mendeley Desktop version 1.17.6 (a free reference management program - ELSEVIER®) and sorted alphabetically in order to facilitate the review process. A hand search of references from the oldest articles identified one additional article[6] that was included in our analysis.

Data collection

Three reviewers (R.F.D., A.O.C., J.G.O.) independently screened the title, authors, and abstracts to determine if they met inclusion and exclusion criteria. If excluded, the reason for exclusion as described in the previous section was noted, and if included, the classification into one of the twelve clusters as defined above was noted. Papers that mentioned more than one theme cited above were discussed by all authors, who came to consensus on the most relevant finding of the article. Any discrepancies were resolved by a discussion among the reviewers in a follow-up meeting. The intra-class correlation between reviewers was assessed for the first 100 studies, based on the choice of inclusion/exclusion criteria, and we found an intra-class correlation coefficient of 0.915, showing excellent reliability between the three reviewers.

Bibliometric analysis

All included articles were exported to Excel for Mac version 14.7.2 (Microfoft®), and then each article was classified according to its characteristics: title, authors' name, journal title, journal's impact factor (by Web of Science, 2015), study design, year of publication, article's number of citations (by Web of Science and Google Scholar), and country of origin (of the corresponding author). Then descriptive statistics of all variables were analyzed.

RESULTS

We found 9,337 articles across 6 databases and 1 article via manual search, resulting in a total of 9,338 articles (see Supplemental Material 2). After dropping duplicates (using the automatic Mendeley function), 5,155 articles remained. Based on our eligibility criteria, 4,445 articles were withdrawn due to one of the following reasons: no abstract (416 articles); book chapter (20 articles); duplicate (missed by Mendeley; 376 articles); review (123 articles); theoretical articles (467 articles); out of population (not on medical students; 567 articles); and out of area (not on LE; 2,476 articles).

Finally, 710 articles were included in this bibliometric analysis. Each article was classified into one

of the twelve themes, resulting in a final distribution as follows: learning environment – general (233 articles – 32.8%); unprofessional behaviors (91 articles – 12.8%); sexual discrimination (80 articles – 11.3%); minorities discrimination (76 articles – 10.7%); violence (65 articles – 9.1%); hidden curriculum (52 articles – 7.3%); unethical behaviors (49 articles – 6.9%); racism (16 articles – 2.2%); homophobia (13 articles – 1.8%); bullying/hazing (7 articles – 1.0%); social discrimination (5 articles – 0.7%); and other (23 articles – 3.2%).

Supplemental Material 3 shows the distribution of publications of all articles related to medical schools' LE included in this manuscript. The first such publication dates back to 1961;[6] publications remained relatively stable and infrequent until the 21st century when there was a notable increase in manuscripts related to medical schools' LE. In fact, 80% of all articles related to medical schools' LE were published after 1999. Notably, a significant spike occurs at approximately 2006; almost 70% of all articles were published from 2006 through 2016.

The characteristics of these studies are shown in Table 1. Most of them (77.6%) are cross-sectional and quantitative studies (67.4%). We found only 41 (5.8%) experimental studies and 75 (10.5%) longitudinal studies. Almost two-thirds of the articles were published in journals with an impact factor (IF) greater than 1.00, with one third published in journals with IF greater than 3.00. *Academic Medicine* (IF 4.194), *Medical Education* (IF 3.369), *Medical Teacher* (IF 2.355), *BMC Medical Education* (IF 1.312) and *Teaching and Learning in Medicine* (IF 1.159) represent five leading journals in this area. Most articles on this topic had corresponding authors who resided in the United States (43.5%), followed by the United Kingdom (8.6%) and Canada (5.5%).

Finally, Table 2 presents the 20 most-cited articles in the area of medical schools' LE. The number of citations for these articles is quite high, with 6 articles having more than 100 citations in Web of Science (WoS) and 10 articles having more than 200 citations in Google Scholar.

Supplemental Materials 4-7 present the characteristics of each of the 12 themes defined by this article. The journal *Academic Medicine* has published the most articles on medical students' LE across most of the dimensions we examined. Whereas researchers from the United States published most of the papers in this field, Pakistan published the most in bullying/hazing. Finally, some areas are entirely new in the scientific literature, and until the 1980s there were no published articles on the following areas: unprofessional behaviors, violence, hidden curriculum (harmful), unethical behavior, racism, homophobia, and bullying/hazing.

In relation to the instruments used to measure the LE, we found a high use of the following tools: the Dundee Ready Education Environment Measure (DREEM),[2] the Medical Student Learning Environment Scale (MSLES),[22,23] and the most recent Johns Hopkins Learning Environment Scale (JHLES).[4] Comparing articles using these tools (cluster: learning environment – general), we found an even higher

TABLE 1. CHARACTERISTICS OF STUDIES ON MEDICAL SCHOOLS' LEARNING ENVIRONMENT

All Studies (N = 710)	
Characteristics	No. Studies
Publication year	
Until 1980	15 (2.1%)
1981-1990	32 (4.4%)
1991-2000	97 (13.7%)
2001-2010	236 (33.2%)
2011-2016	330 (46.5%)
Study Design	
Cross-Sectional	551 (77.6%)
Longitudinal	75 (10.6%)
Experimental	41 (5.8%)
Experience Report	39 (5.5%)
Case Report	4 (0.6%)
Measurement Methods (only if cross-sectional or longitudinal)	
Quantitative	422 (67.4%)
Qualitative	160 (25.6%)
Qualitative / Quantitative	44 (7.0%)
Journals	
Academic Medicine	126 (18.4%)
Medical Education	67 (9.8%)
Medical Teacher	48 (7.0%)
BMC Medical Education	29 (4.2%)
Teaching and Learning in Medicine	17 (2.5%)
Impact factor journals (WoS)	
No impact factor	188 (26.5%)
0.000 - 1.00	65 (9.2%)
1.01 - 3.00	219 (30.8%)
> 3.00	237 (33.4%)
Countries (by author's affiliation)	
United States	309 (43.5%)
United Kingdom	61 (8.6%)
Canada	39 (5.5%)
Australia	32 (4.5%)
Netherlands	23 (3.2%)

TABLE 2. MOST CITED ARTICLES ON MEDICAL SCHOOLS' LEARNING ENVIRONMENT

Rank	Article	No. WoS Citations	No. Google Scholar Citations	Average Citations/ Year - WoS
1	Lempp H, Seale C. The hidden curriculum in undergraduate medical education: qualitative study of medical students' perceptions of teaching. BMJ. 2004;329(7469):770-3.	190	471	15.8
2	Sheehan KH, Sheehan DV, White K, Leibowitz A, Baldwin DC Jr. A pilot study of medical student 'abuse'. Student perceptions of mistreatment and misconduct in medical school. JAMA. 1990;263(4):533-7.	190	311	7.3
3	Papadakis MA, Hodgson CS, Teherani A, Kohatsu ND. Unprofessional behavior in medical school is associated with subsequent disciplinary action by a state medical board. Acad Med. 2004;79(3):244-9.	189	358	15.7
4	Christakis DA, Feudtner C. Ethics in a short white coat: the ethical dilemmas that medical students confront. Acad Med. 1993;68(4):249-54.	151	248	6.6
5	Dyrbye LN, Massie FS Jr, Eacker A, Harper W, Power D, Durning SJ, Thomas MR, Moutier C, Satele D, Sloan J, Shanafelt TD. Relationship between burnout and professional conduct and attitudes among US medical students. JAMA. 2010;304(11):1173-80.	149	318	24.8
6	Richman JA, Flaherty JA, Rospenda KM, Christensen ML. Mental health consequences and correlates of reported medical student abuse. JAMA. 1992;267(5):692-4.	122	236	5.1
7	Kassebaum DG, Cutler ER. On the culture of student abuse in medical school. Acad Med. 1998;73(11):1149-58.	97	205	5.4
8	Moffat KJ, McConnachie A, Ross S, Morrison JM. First year medical student stress and coping in a problem-based learning medical curriculum. Med Educ. 2004;38(5):482-91.	95	298	7.9
9	Karnieli-Miller O, Vu TR, Holtman MC, Clyman SG, Inui TS. Medical students' professionalism narratives: a window on the informal and hidden curriculum. Acad Med. 2010;85(1):124-33.	90	154	15
10	Baxter N, Cohen R, McLeod R. The impact of gender on the choice of surgery as a career. Am J Surg. 1996;172(4):373-6.	87	136	4.3
11	Madigosky WS, Headrick LA, Nelson K, Cox KR, Anderson T. Changing and sustaining medical students' knowledge, skills, and attitudes about patient safety and medical fallibility. Acad Med. 2006;81(1):94-101.	85	167	8.5
12	Papadakis MA, Osborn EH, Cooke M, Healy K. A strategy for the detection and evaluation of unprofessional behavior in medical students. University of California, San Francisco School of Medicine Clinical Clerkships Operation Committee. Acad Med. 1999;74(9):980-90.	85	127	5
13	Patenaude J, Niyonsenga T, Fafard D. Changes in students' moral development during medical school: a cohort study. CMAJ. 2003; 168(7): 840–844.	83	223	6.4
14	Hicks LK, Lin Y, Robertson DW, Robinson DL, Woodrow SI. Understanding the clinical dilemmas that shape medical students' ethical development: questionnaire survey and focus group study. BMJ. 2001; 322(7288): 709–710.	76	207	5.1
15	Roberts LW, Warner TD, Lyketsos C, Frank E, Ganzini L, Carter D. Perceptions of academic vulnerability associated with personal illness: a study of 1,027 students at nine medical schools. Collaborative Research Group on Medical Student Health. Compr Psychiatry. 2001;42(1):1-15.	74	135	4.9
16	Dyrbye LN, Thomas MR, Harper W, Massie FS Jr, Power DV, Eacker A, Szydlo DW, Novotny PJ, Sloan JA, Shanafelt TD. The learning environment and medical student burnout: a multicentre study. Med Educ. 2009;43(3):274-82.	70	153	10
17	Edwards MT, Zimet CN. Problems and concerns among medical students--1975. J Med Educ. 1976;51(8):619-25.	68	98	1.7
18	Stern DT, Frohna AZ, Gruppen LD. The prediction of professional behaviour. Med Educ. 2005;39(1):75-82.	66	130	6
19	Frank E, Carrera JS, Stratton T, Bickel J, Nora LM. Experiences of belittlement and harassment and their correlates among medical students in the United States: longitudinal survey. BMJ. 2006;333(7570):682.	64	162	6.4
20	Wolf TM, Randall HM, von Almen K, Tynes LL. Perceived mistreatment and attitude change by graduating medical students: a retrospective study. Med Educ. 1991;25(3):182-90.	63	116	2.5

proportion of manuscripts published between 2011-2016 (53%) that used these popular measurement tools.

DISCUSSION

This study represents the first comprehensive bibliometric description for learning environment (LE) research in medical schools with a focus on negative aspects. We identify here the relatively recent rapid increase in interest in LE globally, demonstrating increased awareness of the importance of this topic, and also the recent attempts to improve LE research study designs. For educators, this is an urgent call to increase our attention to the importance of the medical school LE as well as to explore in greater depth the potentially harmful aspects of medical school LEs.

To facilitate our understanding of the conceptual framework of the LE area, we developed a concept map (Figure 1) based on the authors' own experience and the most prominent studies reviewed by this manuscript. First, our understanding is that both positive and negative aspects might influence students' perception of their LE. In this article, we decided to focus more on the negative ones. We have identified ten main negative areas that might impact students' perception of their LE (in parentheses the most cited on each area): unprofessional behaviors;[17] sexual discrimination;[24] minorities discrimination;[25] violence;[26] hidden curriculum (negative);[27] unethical behaviors;[28] racism;[28] homophobia;[30] bullying/hazing;[31] and social discrimination.[32] These negative themes may be concomitantly the source and the consequence of a "bad" LE; that is, these negative environments may create a poor perception that creates positive feedback on these unacceptable behaviors. Educators should be aware of this, attempting to prevent a state where the "mean" turns inherent and cannot be seen, such as in the famous study of the Stanford prison experiment.[33]

Noteworthy is that while there has been an increasing number of articles published in medical education in general, not all content areas receive the same attention.[34] In our study, we found a recent and rapidly growing interest in medical school LE research, with the vast majority (approx. 70%) of studies having been published from 2006 onward and in journals with an IF greater than 1.00, further indicating interest in this field among medical education researchers. What could be driving this interest in the medical school? First, our data suggest that the availability of LE measurement instruments may facilitate research. Over half of the published articles that utilized the most frequently used measures were published since 2011. Second, interest is likely being driven by a greater concern for the potential negative impact of poor learning environments in medical schools, particularly student mistreatment and overwork, as these impact trainees' empathy and well-being.[10,34-36] For example, in the U.S., the American Medical Association, driven by these concerns, began supporting a longitudinal study of medical students at 28 medical schools in 2010 that has already led to several publications.[37,38] Third, medical school accreditation standards may play a role, as the creation of a new Liaison Committee on Medical Education[39] (LCME) standard related to LE quality coincides with the rapid increase in LE studies.

The United States has by far the most LE research identified in this study, followed by the United Kingdom, Canada, Australia, and the Netherlands. These findings are similar to analyses of all medical education articles[40,41] and global scientific production as a whole. Given that medical education research on the learning environment is dominated by several countries that do not represent most of the world's medical schools, educators and investigators must be cautious to ensure that they focus on local needs and do not seek to overgeneralize their results. For example, in the field of empathy research, a decline in student empathy during medical schools seems to have been primarily driven by several studies from the U.S., and they were not corroborated by the bulk of international studies.[42] At the same time, when reviewing the top countries by topic, we saw that some developing countries had the highest number of publications for certain clusters. For example, Pakistan had the most publications on bullying/hazing and Nigeria had the third most in violence. Future studies should explore whether these findings reflect the initiative of individuals or research teams or a more significant trend in negative LE aspects. Considering that, like most medical education research, cross-sectional and single site studies comprised the majority of LE research, future work should use multiple methods across cultures to better understand the complex interactions between students and their LEs.

Our findings have implications for health managers and medical educators, providing further ev-

idence that the LE could have a positive but also a negative influence on medical students. Educators must promote appropriate infrastructure, active learning strategies, good clinical scenarios, high- and low-fidelity labs, formative feedback, valuable educational content, and consistent theoretical and practical assessments. On the other hand, educators must be aware that unethical behaviors, bullying, violence, sexual discrimination, professional misconduct, and the hidden curriculum could impair medical students' academic performance and mental health. Thus, they must become aware of these possible behaviors occurring in their curricula. The early identification of these negative aspects of the LE and the implementation of educational and preventive interventions should serve to minimize medical training distress and future unprofessional behaviors.

This research has several limitations. First, we focused only on original studies to characterize the current evidence base related to negative aspects of the LE, which meant that we excluded many other highly cited reviews and theoretical studies that may be influential in this field. Second, we may not have identified every relevant original research article, as no database has every paper and no search strategy can find every paper. However, we did not limit by language, so this is indeed an international search. Third, the databases that we searched tended to be more focused on American and European journals, possibly excluding important contributions from the southern hemisphere. Fourth, this is a novel operationalization of the learning environment and has limitations and potential biases; however, it is essential to initiate a discussion about all these possible important aspects of the LE. Fifth, many of the articles included more than one of the negative areas identified in this study. However, we tried to isolate the most critical subject of each manuscript in order to facilitate our understanding. Finally, we focused more on the negative aspects of the learning environment. Positive aspects, such as role models, peer relationships, and a sense of engagement, clearly are essential in understanding medical students' LE. However, we concentrated on negative aspects because the focus of this paper was to identify research that is related to negative outcomes for medical students and that has the potential for remediation. In the future, it will be important to incorporate both negative and positive aspects of the medical school LE that contribute to changes in students' empathy and well-being.

Our analysis identifies the most critical areas that articles, authors, and countries have studied or reported on concerning negative aspects of the learning environment in medical schools. We demonstrated an important growth of scientific production in high-quality journals, especially in the 21st century. However, more studies are needed that investigate the negative aspects of medical students' LE, with particular attention to experimental and cross-cultural/multi-school studies. Heightened awareness of negative aspects of the medical student LE should be useful in empowering medical professionals to make changes in the LE that will, in turn, improve student professionalism.

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RESUMO

OBJETIVO: *Buscou-se entender o panorama dos artigos publicados sobre os ambientes de aprendizagem (AA) das escolas médicas em todo o mundo, com um foco explícito nos aspectos potencialmente negativos do AA como um esforço para identificar áreas especificamente necessitadas de remediação ou intervenção que poderiam evitar futuros comportamentos não profissionais, violência e maus-tratos entre estudantes e médicos.*

MÉTODOS: *Foi realizada uma análise bibliométrica em seis bases de dados eletrônicas (PubMed / Medline, Web of Science, Biblioteca Cochrane, SCOPUS, ERIC-ProQuest e PsycINFO) até 31 de dezembro de 2016, incluindo 12 temas: ambiente de aprendizagem - geral, currículo oculto (negativo), comportamentos antiéticos, bullying/trote, violência, discriminação sexual, homofobia, racismo, discriminação social, discriminação de minorias, má conduta profissional e "outros" aspectos negativos.*

RESULTADOS: *Dos 9.338 artigos encontrados, 710 preencheram os critérios de inclusão. Os temas mais comuns foram LE geral (233 artigos), comportamentos não profissionais (91 artigos) e discriminação sexual (80 artigos). Aproximadamente 80% dos artigos foram publicados no século XXI.*

CONCLUSÃO: *Há um claro aumento em artigos científicos sobre aspectos negativos da escola de medicina LE em periódicos de alta qualidade, especialmente no século XXI. No entanto, mais estudos são necessários para investigar aspectos negativos do LE com maior atenção aos desenhos de estudos experimentais, longitudinais e transculturais.*

PALAVRAS-CHAVE: *Ambiente de Aprendizagem, Educação Médica, Estudantes de Medicina, Ética, Profissionalismo.*

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REVIEW ARTICLE

The importance of the early diagnosis of aorta coarctation

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SUMMARY

INTRODUCTION: *Coarctation of the aorta is a congenital heart disease characterized by a narrowing that occurs in the aortic artery. This constriction can occur anywhere along its entire length; however, it is more common between the origin of the left subclavian artery and the ductus arteriosus. Its incidence corresponds to 3 cases per 10,000 births. Thus, it is a common cardiopathy, but with high mortality and morbidity rates, which are related to a failure in the early diagnosis.*

METHOD: *In the research, articles of the national and international literature in Pubmed, Scielo and Lilacs databases were selected using the following descriptors: coarctation, aorta, diagnosis, heart diseases, congenital abnormalities.*

RESULTS: *The pathophysiology of CoA and its systemic implications in the life of newborn and adults are well elucidated. However, due to the lack of habit to palpate pulses and to check the blood pressure in both upper and lower limbs during the physical examination, it is still a pathology little diagnosed in childhood. There are several techniques used in the repair of coarctation, each with their specifics, although, when not treated, aneurysms, heart failure, coronary diseases, and stroke are the main complications arising from the evolution of this pathology, which explains the low survival rate of these patients.*

CONCLUSION: *Coarctation of the aorta is, therefore, a cardiac malformation of significant importance due to its incidence and its significant mortality risk. In this sense, the early diagnosis stands out as an essential piece for better prognosis of the patient.*

KEYWORDS: *Aortic coarctation. Heart defects, congenital. Diagnosis.*

INTRODUCTION

It is estimated that approximately 60% to 80% of newborns with aortic coarctation are not diagnosed before hospital discharge.[1] Thus, it is essential to understand the physiopathology of aortic coarctation and its systemic implications on the life of the newborn and the adult to contribute to the early diagnosis of these patients.

Aortic coarctation is a congenital heart disease characterized by a narrowing of the aorta. This constriction can occur in any location along its entire length; however, it is more common between the origin of the left subclavian artery and the ductus arteriosus.[2,3]

It has an incidence of 5-10% among all congenital heart diseases and is predominant in males, at a ratio of 2:1.[4-8] It is classified as the fifth most common con-

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genital heart disease.[9] Its incidence in comparison with the number of births corresponds to three cases per 10,000 births.[10] It is a common heart disease, but it has high mortality and morbidity rates, which are associated with failure to diagnose.[11]

Aortic coarctation can be divided into two distinct groups: critical aortic coarctation and asymptomatic aortic coarctation. In the critical coarctation, severe symptoms are observed over the first two months of life and when left untreated can lead to death. Whereas in the asymptomatic coarctation the main characteristic is the late onset of hypertension on upper limbs.[12]

Aneurysms, heart failure, heart diseases, and cerebrovascular accidents are complications the stem from the evolution of the pathology when left untreated, and such manifestations are direct consequences of secondary hypertension.[2]

Aortic coarctation can be classified as simple or complex. The complex form is associated with the presence of heart anomalies. In the simple form, such anomalies are absent. Heart anomalies usually associated with aortic coarctation are: interventricular communication, bicuspid aortic valve, subvalvular aortic stenosis, and alterations in the mitral valve.[13,14]

The most usual symptoms in patients are: arterial hypertension in the upper limb, reduction of pulses in lower limbs, headache, epistaxis, claudication in the legs and cyanosis.[4] Coarctation appears as a consequence of the increase in the arterial pressure of the upper limbs and head.[15] This increased flow can mean a higher risk of saccular aneurysms, increased frequency of headaches, and the development of heart diseases and heart failure[16] (Figure 2).

This work aims to review the literature on the physiopathology of aortic coarctation and the systemic implications of the condition in the life of the newborns and adults to contribute to the early diagnosis of patients, since this pathology is still underdiagnosed in childhood, since it is not customary to measure the pulse and blood pressure in the upper and lower limbs during a physical examination.

METHODS

This is a narrative literature review of the literature. Searches were carried out in the PubMed, SciELO, and Lilacs databases. During the search, we selected articles of narrative and systematic reviews, original articles, clinical trials and case reports from 2009 to 2018, in national and international literature, using the following keywords: coarctation, aorta, diagnosis, heart disease, congenital abnormalities.

DISCUSSION

Pathophysiological aspects

Aortic coarctation is considered a flow-obstruction pathology. One of its consequences is the increased pressure in the left ventricular chamber, which is overloaded since it needs to pump against the resistance caused by the obstruction of the aorta.[17]

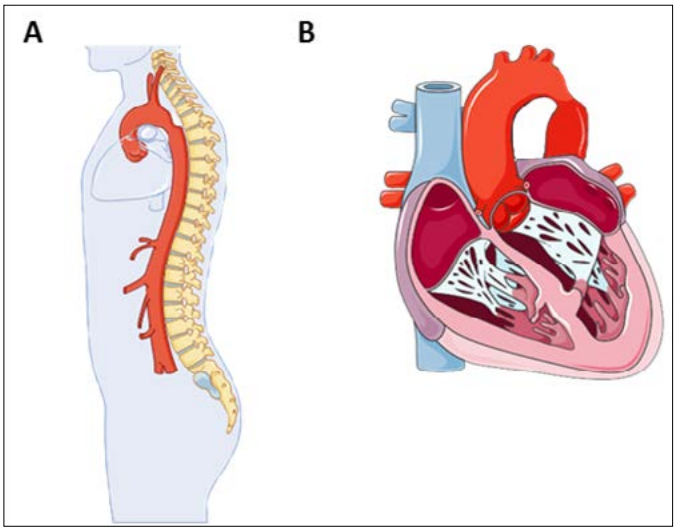

FIGURE 1. Representation of coarctation of the aorta. A) Profile representation of aortic narrowing. Coarctation of the aorta is evidenced by the black arrow. B) Representation in the frontal section of the heart and large vessels; the figure shows the narrowing of the aorta after the origin of the left subclavian artery (evidenced by the black arrow).

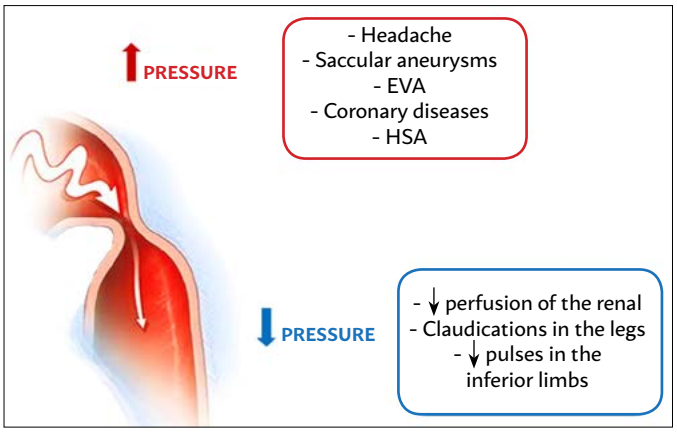

FIGURE 2. Systemic consequences of aortic coarctation. With the increase in pressure (before coarctation), the most common consequences are headache, sacral aneurysms, stroke, coronary heart disease, and systemic arterial hypertension. With the decrease in pressure (after coarctation), there is a decrease in renal perfusion, leg claudication and in the pulses of lower extremities.

From an embryo point of view, coarctation is still not well understood. However, some theories suggest that there is a disorderly migration of smooth muscle cells from the ductus arteriosus to the wall of the aorta artery; others suggest that there is a change of blood flow in the fetal circulation, with a decrease in the amount of blood that exits the left ventricle to the aorta.[13]

Diagnosis

In clinical practice, it is observed that many patients are undiagnosed due to the lack of a detailed clinical examination, since measuring the pulse of upper and lower limbs is not routine.[4] It is estimated that approximately 60% to 80% of newborns with aortic coarctation are not diagnosed before hospital discharge. However, this is a pathology that can be easily recognized in clinical practice.[1]

The signs and symptoms vary according to the type of clinical manifestation. The most frequent are hypertension, heart murmurs, headache, epistaxis, and fatigue in the legs.[18] A general and thorough physical examination with the measurement of arterial pressure can reveal a discrepancy in limb arterial blood pressures (differential SBP popliteal/brachial greater than or equal to 20 mmHg). There is, in these cases, arterial hypertension in the upper limbs, which is related to the obstruction of the coarcted area and the activation of the renin-angiotensin system in response to the decrease in the perfusion of the renal artery below the obstruction. Measuring peripheral pulses (femoral, popliteal, posterior tibial and dorsalis pedis) can reveal weak or missing pulses in the limbs.[18] During cardiovascular auscultation, systolic murmurs can be noticed on the precordium and, in collateral circulation, heart murmurs in axillary and scapular regions.[19,20]

Aortic coarctation presents a varied clinical spectrum depending on the age of the patient. It can manifest as heart failure in newborns after the closure of the ductus arteriosus, or in asymptomatic forms in adolescents and adults.[7,21] During the neonatal period, symptomatic newborns have a higher risk of mortality due to congestive heart failure, metabolic acidosis, and shock; on the other hand, asymptomatic children or adults develop complications at a later stage of life, especially related to systemic arterial hypertension.[22,23]

Clinical manifestations depend not only on the severity and location of the coarctation but also on the existence or not of associated anomalies.[4]

Some complementary examinations, such as x-ray, electrocardiogram, transthoracic and transesophageal echocardiography, angiography, computed tomography, and magnetic resonance imaging may assist in the diagnosis.[24] Prenatal diagnosis using echocardiography has been used and proved to reduce mortality rates and improve survival; nevertheless, it is still challenging to establish a definitive diagnosis using this method.[1] However, if a prenatal ultrasound suggests the presence of coarctation, the mother can be forwarded to a hospital unit that has a pediatric heart surgery center so that post-natal follow-up can be conducted.[22,25]

In chest X-rays, it is possible to see notches bilaterally in the costal margin, resulting from the erosion of the ribs caused by collateral circulation. This happens because there is an increase in pressure before the coarcted area, so the anterior intercostal arteries present high pressure as well. However, the posterior intercostal arteries, which stem from branches of the thoracic aorta, have lower pressure, so they need to dilate in order to accommodate the high pressure coming from the anterior arteries, due to the anastomosis. The increase in pressure causes the arteries to dilate, and they eventually start to brush against the ribs, forming the notches. In newborns' X-rays, it is possible to notice an increase in the cardiac area and pulmonary congestion.[19,26,27]

The electrocardiogram can show normal results or hypertrophy of the left ventricle; neonates, it can show a deviation of the cardiac axis to the right and overload of the right ventricular.[16]

The transthoracic echocardiography is the first clinical routine examination to be conducted when there is a suspected case of aortic coarctation since it is a simple exam that provides hemodynamic variables. Although it does not always offer a direct view of the coarcted area, it is important to rule out other cardiac defects, such as bicuspid aortic valve and interventricular communication. During the exam, it is also possible to observe the turbulence of the blood flow from the aorta before the coarcted area and the pressure gradient caused by the coarctation. The transesophageal echocardiography, although invasive, can also be used in the diagnosis of this pathology.[13,28]

Angiography used to be the most widely used method for diagnosing aortic coarctation. However, nowadays it is more indicated for therapeutic intervention or in cases which there is need of additional hemodynamic data.[10]

A computed tomography (CT) allows the doctor to identify with high resolution both intracardiac and extracardiac structures, with the possibility of reconstructing the vessel anatomy in 2D and 3D. The examination has as a disadvantage of using ionizing radiation, especially in pediatric patients.[19]

Cardiac magnetic resonance is currently the gold standard test for evaluating aortic coarctation, and it can be used diagnosing and monitoring the pathology.[29] With this imaging method it is possible to carry out an anatomical and functional assessment of the congenital cardiopathy.[30]

Magnetic resonance imaging allows identifying a more specific location of the aortic coarctation, which is important for a subsequent surgical approach. Besides, it is a method that generates multiplane images, which allow for better viewing. This imaging technique is essential for the pre-operative evaluation of all patients submitted to percutaneous and surgical interventions.[27] With it, it is possible to also observe other anatomical findings, such as the presence of collateral circulation and abnormalities in heart valves. The advantage of this examination over CT is the non-use of ionizing radiation.[28]

Treatment

Without intervention, the survival of patients with aortic coarctation is approximately 35 years, with a mortality rate of 75% at 46 years of age.[31,32] The reasons that lead to death are diverse and include: cerebrovascular accident, congestive heart failure, rupture of the aorta and bacterial endocarditis.[10]

In 1945, the first successful surgical intervention was described by Crafoord and Nylin. However, during these initial techniques, the recoarctation rates were over 50%, so these strategies were not a permanent solution. Currently, there are several techniques for correcting heart defects, ranging from surgical interventions to the use of a transcatheter.[21,33] Clinical therapy is based on the initial control of blood pressure, mainly through the use of ACE inhibitors, ARB (angiotensin receptor blocker) and beta-blockers.[34]

Systemic arterial hypertension in children and adults, with a difference in the differential systolic arterial pressure (upper limb/lower limb) greater than 20 mmHg is the most widely accepted indication for intervention.[20] Other criteria to perform the intervention are: evidence of significant aortic coarctation and collateral circulation in imaging examinations.[13,34]

There are several techniques used to repair the coarctation: resection of the coarcted area with termino-terminal anastomosis, dilatation with a balloon catheter, a flap of the subclavian artery, intravascular stents, catheterization, and aortoplasty with prosthetic graft are some of them.[35,36]

When surgical repair is used, it can be accomplished technically by resecting the coarcted segment, which is usually the treatment of choice in neonates and infants. In adults, resection with interposition grafting is also an option.[9] In the elderly, calcifications and atherosclerosis may hinder surgical repair.[37] The success rate of the procedure is high, with surgical mortality rates lower than 1%.[9]

Balloon angioplasty can be a palliative option before the definitive correction. Its goal is to stabilize high-risk patients that cannot be submitted to immediate surgical intervention, such as, for example, premature newborns.[13] Moreover, it is an alternative when there are no stents available.[38]

The use of endovascular stents is currently an innovation in the treatment of aortic coarctation. These devices provide structural support to the artery and decrease the rates of vascular trauma in the aorta and aneurysms when compared to balloon angioplasty. The use of stents has contributed mainly in cases of complex coarctation anatomy and in the elderly since they often present arteriosclerosis and calcifications. Currently, with technological advances, efforts are being made to achieve the characteristics of an ideal stent, with a higher potential of dilation, greater flexibility, and lower profile. The four most used types worldwide are Palmaz "10", Genesis XD, Ev3 Maxi and CP.[7,10] These are currently the first option for children (weight > 25 kg) and adults. This procedure has the advantage of being less invasive.[13,34,36]

Some guidelines recommend that the repair is done as early as possible, as in early childhood, to increase the life expectancy of the patient and reduce long-term morbidity.[35,39] It is important to emphasize that, irrespective of the interventional technique used, it can result in complications in the long term, the main ones being hypertension, aortic aneurysm, and recoarctation. In 10% of patients undergoing a surgical intervention to correct aortic coarctation, hypertension remains or relapses; however, the earlier the correction, the higher the chances of the individual maintaining a healthy blood pressure behavior. It is estimated that recoarctation can occur regardless of the technique used, with rates ranging from 5% to 14%,

but it tends to be more common in neonates.[36,40,41]

The ideal treatment for correcting aortic coarctation remains under debate due to the lack of long-term clinical and imaging data to compare the different techniques used for correction.[7,42] Thus, it is noteworthy that the adequate technique should be selected by a multidisciplinary team, taking into consideration the specificities of each patient, such as age, comorbidities and other associated cardiac lesions.[41]

After choosing and performing the appropriate treatment, it is necessary to have a permanent follow-up of the patient with the cardiology team, with regular examinations such as electrocardiogram, echocardiogram and treadmill stress tests, in order to monitor and assess cardiac function, as well have an early detection of changes, so as to intervene with a specific treatment. It is also always necessary to monitor the blood pressure since systemic hypertension is the major risk factor for the onset of other pathologies.[38,40]

CONCLUSION

Aortic coarctation is a common congenital cardiopathy that has high morbidity and mortality rates and is usually associated with failure in diagnosis. This pathology can be divided into two distinct groups: critical aortic coarctation and asymptomatic aortic coarctation. Aneurysms, heart failure, heart diseases, and cerebrovascular accidents are complications the stem from the evolution of the pathology when left untreated, and such manifestations are direct consequences of secondary hypertension.[14]

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Ethical aspects:

The authors declare there are no conflicts of interest.

RESUMO

INTRODUÇÃO: *A coarctação da aorta é uma cardiopatia congênita caracterizada por um estreitamento que ocorre na artéria aorta. Essa constrição pode ocorrer em qualquer local ao longo de toda a sua extensão, entretanto, é mais comum entre a origem da artéria subclávia esquerda e o ducto arterioso. Sua incidência corresponde a três casos a cada 10.000 nascimentos, sendo, desse modo, uma cardiopatia comum, porém com elevada taxa de mortalidade e morbidade, as quais estão relacionadas à falha no diagnóstico precoce.*

MÉTODOS: *Este artigo trata-se de uma revisão bibliográfica narrativa da literatura. Na pesquisa foram selecionados artigos na literatura nacional e internacional nas bases de dados PubMed, SciELO e Lilacs, utilizando-se os seguintes descritores: coarctação, aorta, diagnóstico, cardiopatias, anormalidades congênitas.*

RESULTADOS: *A fisiopatologia da coarctação da aorta e as implicações sistêmicas dessa cardiopatia na vida do recém-nascido e do adulto estão bem elucidadas. Entretanto, devido à falta de costume em palpar pulsos e aferir a pressão arterial nos membros superiores e inferiores durante o exame físico, ainda é uma patologia pouco diagnosticada na infância. Existem diversas técnicas utilizadas no reparo da coarctação, cada uma com suas especificidades, porém, quando não tratada, aneurismas, insuficiência cardíaca, coronariopatias e acidentes vasculares encefálicos são as principais complicações provenientes da evolução dessa patologia, o que explica uma baixa sobrevida desses pacientes.*

CONCLUSÃO: *A coarctação da aorta é, portanto, uma malformação cardíaca de importância relevante devido a sua incidência e ao seu potencial risco de mortalidade. Nesse sentido, o diagnóstico precoce destaca-se como peça fundamental para um melhor prognóstico do paciente.*

PALAVRAS-CHAVE: *Coartação aórtica. Cardiopatias congênitas. Diagnóstico.*

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REVIEW ARTICLE

Use of sodium-glucose cotransporter-2 inhibitors and urinary tract infections in type 2 diabetes patients: a systematic review

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SUMMARY

Sodium-glucose cotransporter-2 inhibitors (SGLT2i) are drugs that act by maintaining glycosuria. Recent studies have shown promising effects of these in the treatment of type 2 diabetes mellitus (DM2). However, there may be an increased risk of developing urinary tract infections (UTIs) in patients treated with these. Our study aims to analyze the association between the risk of UTI in patients treated with SGLT2i. A systematic review of the literature was carried out by randomized clinical trials, totalizing at the end of the selection 23 articles that were statistically evaluated. The incidence of UTI was generally demonstrated in articles and in different subgroups: patients on SGLT2i monotherapy or on combination therapy; according to specific comorbidities of each sample or according to the drug used. They noticed an increase in the chance of UTI in the SGLT2i groups compared to the control groups on placebo or other oral antidiabetic agents. This increased chance was found predominantly with the use of Dapagliflozin, Canagliflozin, and Tofogliflozin, regardless of the dosing. Lastly, stands out that the dimension of UTI chances for DM2 patients who use SGLT2i remains to be more strictly determined.

KEYWORDS: *Sodium-glucose transporter 2 antagonists/inhibitors. Diabetes mellitus, type 2. Urinary tract infections.*

INTRODUCTION

The type 2 diabetes mellitus (DM2) accounts for approximately 90-95% of the diabetes cases worldwide. Its pathophysiology results from a combination of genetic, environmental and metabolic factors that initiate a state of hyperglycemia in the subject from peripheral insulin resistance and progressive failure of beta cells, resulting in decreased secretion of insulin by the pancreas.[1,2]

The pharmacological therapies, although effective in the glycemic control, sometimes present limitations that condition their use by patients. For this reason, new therapeutic agents are frequently researched. The sodium-glucose cotransporter-2 inhibitors (SGLT2i) propose an alternative therapy for DM2 based on an innovative mechanism substantiated on the renal importance of plasma glucose reabsorption.[1,3]

The kidney reabsorbs the filtered glucose through sodium-glucose cotransporters (SGLT 1 and 2) located in the tubular epithelium. In the proximal contort-

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ed tubule, SGLT2 is responsible for the reabsorption of 90% of the filtered glucose. The action of SGLT2i is based on the inhibition of this transport, which reduces renal reabsorption of glucose and induces through the increase of glycosuria a decrease in the plasma glucose of the diabetic patient.[3,4]

Some studies consider SGLT2i a promising therapeutic solution in the treatment of DM2. Given their mechanism of action independent of insulin, these can be used at any stage of the disease progression, with possible advantages such as decreased glycated hemoglobin, decreased fasting and postprandial glucose, weight reduction, blood pressure lowering and prevention of micro and macrovascular complications of diabetes, therefore improving the patient's quality of life. However, in these same studies that prove the efficacy of SGLT2i, cases of genito-urinary infection have also been observed in users; an effect that binds to glycosuria induced by SGLT2-1.[1-4]

The aim of this article was to review the association between the use of SGLT2i in patients with DM2 and the development of urinary tract infections (UTI) to estimate and analyze the risk of this adverse effect on the therapeutic approach.

METHODS

A systematic computerized search was performed using the following databases: PubMed (Public MEDLINE - Medical Literature Analysis and Retrieval System Online - US National Library of Medicine National Institutes of Health), SciELO (Scientific Electronic Library Online) and Cochrane Library. Keywords included "sodium glucose cotransporter-2 inhibitors", "diabetes *mellitus* type 2" and "urinary tract infection". The following filters were also used in the search: "clinical trials", "randomized," and "human". The results of the search process are described below (Figure 1).

Study selection was completed using the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement. Initially, screening was conducted to exclude duplicate, irrelevant titles, and remaining abstracts. Subsequently, remaining full-text articles were independently screened by two authors for eligibility in the review. After, still as part of the eligibility process, some full-text articles were excluded if: they did not have statistical data on cases of UTI in their sample, did not give access to numbers and assessment of UTI cases, had inappropriate size sample (n<100), treatment duration with SGLT2i <12 weeks (0,25 years), or did not mention the method for UTI detection.

At the end of the selection, 23 studies were included for the proposed systematic review. The data for analysis were arranged in tables in Excel (Microsoft, version 15.3) to produce the results. Afterward, the data obtained were transferred to RevMan (Cochrane Collaboration, version 5.3) for statistical evaluation. The measure of association was the odds ratio (OR) and confidence interval (CI) of 95%.

RESULTS

Twenty-three clinical trials, published between 2011 and 2016, were included and analyzed in the review. In these, the incidence of UTI was compared in patients with DM2 using SGLT2i *versus* the control group using a placebo or another antidiabetic of proven efficacy. The duration of SGLT2i treatment ranged from 0.5 to 2.6 years in the studies. In the selected articles, the following drugs used were: Dapagliflozin[5-15], Canagliflozin[16-20], Empagliflozin[21-26], and Tofogliflozin[27]. The characteristics of each study are in Table 1.

The profiles of the patients analyzed in the studies were those with DM2, using or not oral antidiabetic drugs or insulin, with or without associated comorbidities and of both genders. The total sample

FIGURE 1: FLOWCHART OF THE PROCESS FOR SYSTEMATIC REVIEW OF THE LITERATURE USING THE PRISMA (PREFERRED REPORTING ITEMS FOR SYSTEMATIC REVIEWS AND META-ANALYZES) STATEMENT.

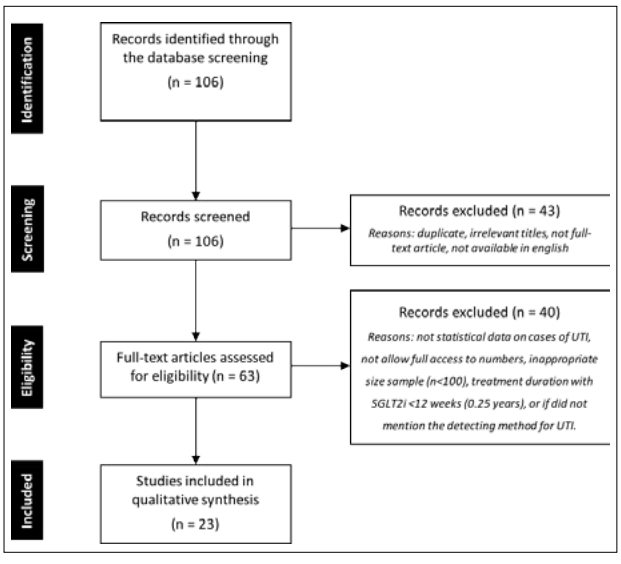


TABLE 1: CHARACTERISTICS OF THE STUDIES INCLUDED IN THE REVIEW.

Author	Year	Sample	SGLT2i analyzed	Sample characteristics	Treatment time
Cefalu et al	2013	1450	Canagliflozin	Metformin use	1 year
Inagaki et al	2014	272	Canagliflozin	With and without medication and inadequate glycemic control	0.5 year
Neal et al	2014	2072	Canagliflozin	Risk of cardiovascular disease and inadequate glycemic control	1 year
Nicolle et all	2012	451	Canagliflozin	Metformin use	0.25 year
Nicolle et al	2014	11752	Canagliflozin	With or without oral antidiabetic	2 years
Bailey et al	2014	167	Dapagliflozin	With and without medication and inadequate glycemic control	2.1 year
Bolinder et al	2012	182	Dapagliflozin	Female postmenopausal and man (30-75 years) on Metformin	0.5 year
Bolinder et al	2011	140	Dapagliflozin	Female on Metformin use	2.1 year
Del Prato et al	2015	814	Dapagliflozin	Metformin use	4.3 year
Jabbour et al	2014	451	Dapagliflozin	With and without medication and inadequate glycemic control	1 year
Ji et al	2014	393	Dapagliflozin	With and without medication and inadequate glycemic control	0.5 year
Leiter et al	2014	965	Dapagliflozin	With established cardiovascular disease	1.08 year
Nauk et al	2013	814	Dapagliflozin	Metformin associated or not to another oral antidiabetic	1 year
Rosenstock et al	2012	420	Dapagliflozin	Pioglitazona associated or not to another oral antidiabetic	1 year
Wilding et al	2012	808	Dapagliflozin	Obese in use of Insulin	1 year
Wilding et al	2014	808	Dapagliflozin	Use of up to 2 antidiabetics	2.1 year
Cherney et al	2016	3215	Empagliflozin	Micro or macroalbuminuria	1 year
Ferrannini et al	2013	659	Empagliflozin	Metformin use or not	0.25 year
Ridderstrale et al	2014	1545	Empagliflozin	Previous use of Metformin	2 years
Rosenstock et al	2014	563	Empagliflozin	Obese in use of Insulin associated or not with Metformin	1 year
Rosenstock et al	2013	495	Empagliflozin	Previous use of antidiabetic	0.25 year
Zinman et al	2015	7020	Empagliflozin	Cardiovascular disease without treatment with antidiabetic	2.6 years
Ikeda et al	2015	394	Tofogliflozin	With and without medication and inadequate glycemic control	0.25 year

for review was of 25,736 patients, and in the studies, the largest sample was 11,572 patients and the lowest 140, with a mean of 1,118 patients per study.

Together, the studies showed an increase in the chance of UTI in the SGLT2i group compared to the placebo (SGLT2i 2,133 of 23,594 [0.09%] *versus* placebo [0.08%], OD 1.18; 1.03–1.34; p=0.02), (Figure 2). The I^2 index of the Higgins Inconsistency Test in this analysis was 32%, indicating moderate heterogeneity of the sample. In addition, individual UTI trends in patients using SGLT2i were noted in the individual analyzes of the studies.[1-13] There was no evidence of publication bias observed by statistically constructed Funnel Plot.

In addition, we analyzed the incidence of UTI in different subgroups: in patients on SGLT2i monotherapy or in combination therapy, according to specific comorbidities of each sample, and according to each drug used.

Six articles analyzed patients on SGLT2i monotherapy[5,9,15,17,21,22] and fourteen used patients on SGLT2i in combination with another oral antidiabetic agent[6,8,9,11,14,16,20-25,27,28]; most of the associations were with Metformin. The odds of developing UTI in both monotherapy and combination therapy were higher in the group using SGLT2i compared to the placebo (monotherapy: OD 1.24; 0.88–1.77; p=0.22; combined therapy: OD 1.24; 0.94–1.64; p=0.13).

Two studies analyzed the adverse effects of SGLT2i in patients on insulin therapy[13,28]. Both used obese patients in the sample. SGLT2i was associated with insulin alone or with Metformin. For the combined use of insulin and Metformin, there was no difference in the chance of UTI in patients compared to the placebo (OD 1.0; 0.62–1.63; p=0.48)[28]. For the association of SGLT2i with insulin, however, there was an increased chance of UTI in the experimental group (OD 1.70; 0.78–3.70; p=0.48)[13]. Statistical analysis of the studies together showed an increase in the chance of UTI with SGLT2i compared to the placebo (OD 1.19; 0.73–1.95; p=0.48).

In patients with an established cardiovascular disease or with risk factors for the development of this pathology, there was also an increased chance of UTI in the group using SGLT2i (OD 1.17; 0.81–1.69; p=0.4). Samples with these characteristics were selected in three studies[10,18,26]. However, these showed marked heterogeneity among them (I^2=72%).

One study observed the risk of UTI in 3215 patients with microalbuminuria or macroalbumin-

uria in use of SGLT2i[21]. In this study, there was no increased chance of UTI in patients on SGLT2i compared to the placebo (OD 0.93; 0.58–1.49; p=0.75).

Separate analysis of the drugs showed an increased chance of UTI in the groups using Dapagliflozin, Canagliflozin, and Tofogliflozin, regardless of the dose taken (OD 1.67; 1.35–2.08; p<0.00001; OD 1.15; 1.01–1.32; p=0.04; OD 1.63; 0.20–13.22; p=0.65, respectively). Only the Empagliflozin did not demonstrate an increased chance of UTI in comparison with the placebo (OD 0.95; 0.85–1.06; p=0.39) in the studies. However, one article included in the review showed that when Empagliflozin is used at a 50mg dose, there was an increased chance of developing UTI (OD 1.57; 0.25–9.69; p=0.63). However, in this same study, there was also an increased chance when the drug was used at lower doses[25]. The characteristics of the drugs analysis on each study are shown in Figure 3.

DISCUSSION

Diabetic patients have an increased incidence of UTI compared to non-diabetic patients and present a increased severity of the disease. Diabetic women present bacterial cystitis, asymptomatic bacteriuria, and symptomatic UTI associated with complications, more frequently than non-diabetic women, with an estimated risk four times higher. A possible explanation for this risk in diabetics is in the physiopathology, in which the high levels of glucose in the urine can promote bacterial growth. In addition, the restriction of the peripheral blood circulation caused by prolonged diabetes can cause abnormalities in the defense system, increase the probability of infections and gravity of this condition. [29,30]

Glycosuria induced by SGLT2i, therefore, has been avidly researched in correlation with the infectious processes of the genito-urinary tract. Revision studies have shown an increase in the occurrence of genital infection in men and women using SGLT2i. [29,30] Regarding UTI's, some recent reviews have shown, based on trial analysis, that patients treated with SGLT2i may have a slightly increased risk of developing UTI. The results demonstrated in our study corroborate such analyzes. We compared our study to the 2016 review conducted by Rizzi and Trevisan[29] that applied a similar methodology to evaluate, among other genito-urinary infections, UTI correlated with the use of SGLT2i in diabetic patients.

FIGURE 2: CHANCES OF URINARY TRACT INFECTION (REPRESENTED BY "EVENTS" IN THE CHART) IN PATIENTS WITH SGLT2I USE *VERSUS* THE CONTROL GROUP IN THE STUDIES INCLUDED. THE CHART ACCOMPANIES THE FORREST PLOT. ABBREVIATIONS: SGLT2I MEANS COTRANSPORTER-2 SODIUM-GLUCOSE INHIBITORS; CI, CONFIDENCE INTERVAL; I^2 CORRESPONDS TO THE INDEX OF THE HIGGINS INCONSISTENCY TEST IN THIS ANALYSIS.

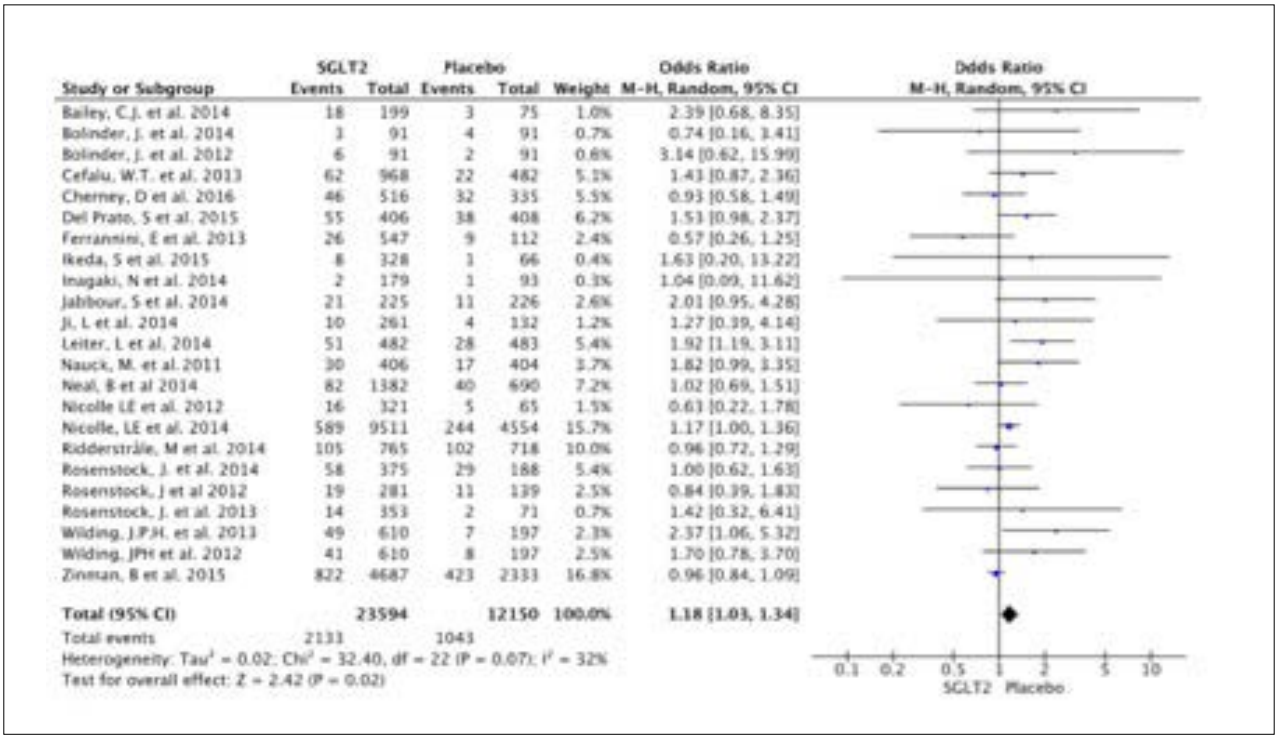
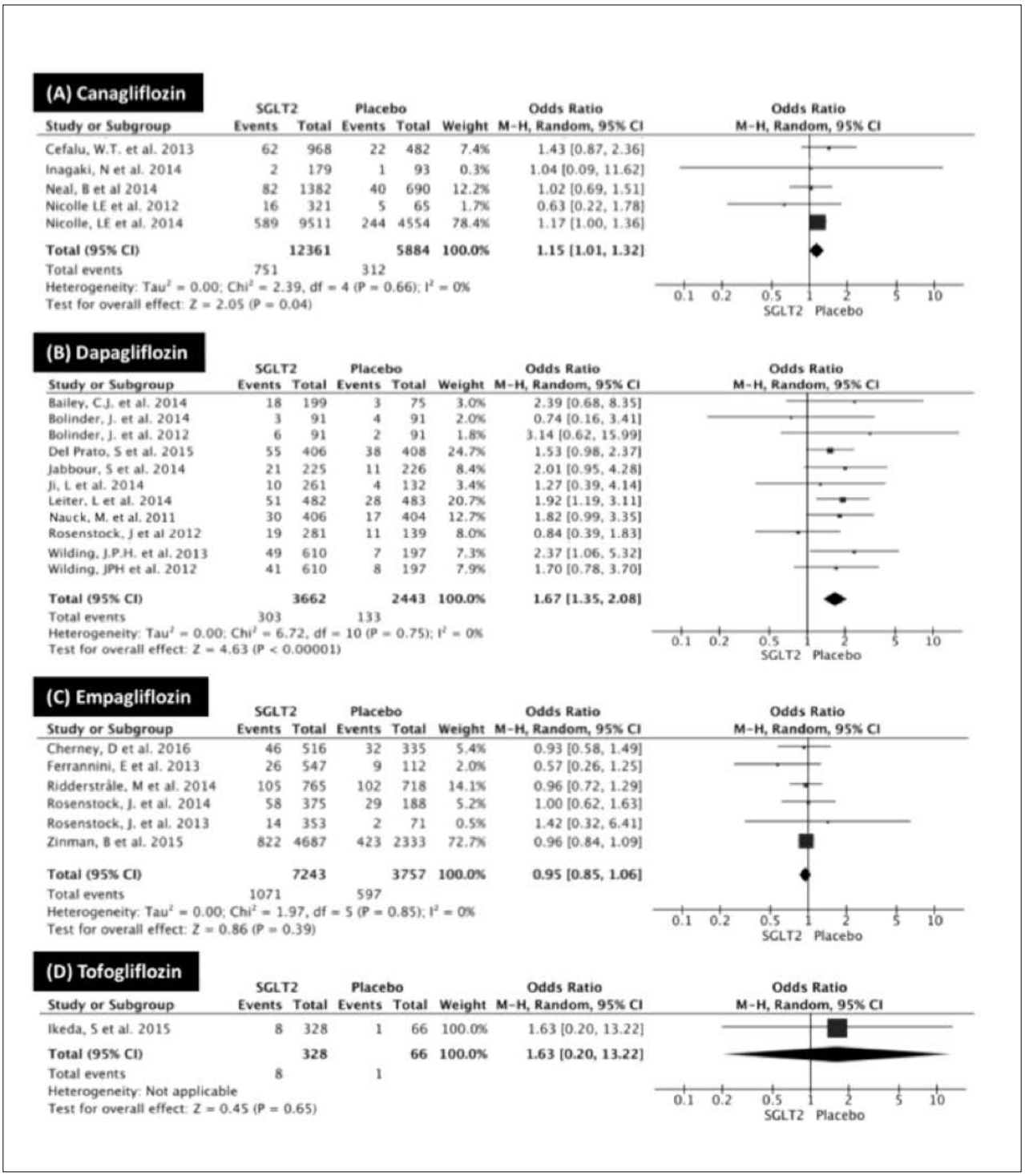


FIGURE 3: CHANCES OF URINARY TRACT INFECTION (REPRESENTED BY "EVENTS" IN THE CHART) IN PATIENTS WITH SGLT2I USE *VERSUS* A CONTROL GROUP, GROUPED ACCORDING TO THE DRUG ANALYZED IN EACH STUDY. THE CHARTS ACCOMPANY THE FORREST PLOTS CORRESPONDENCE IN (A) CANAGLIFLOZIN, (B) DAPAGLIFLOZIN, (C) EMPAGLIFLOZIN, AND (D) TOFOGLIFLOZIN. ABBREVIATIONS: SGLT2I MEANS COTRANSPORTER-2 SODIUM-GLUCOSE INHIBITORS; CI, CONFIDENCE INTERVAL; I^2 CORRESPONDS TO THE INDEX OF THE HIGGINS INCONSISTENCY TEST IN THIS ANALYSIS.



Studies matching Canagliflozin have shown that the chances of developing UTI are greater with the use of this inhibitor than it was with the placebo. There was a relationship with the dose of Canagliflozin, with a greater risk in patients who use 300mg per day than in patients who use 100mg per day.[30] In our study, we observed an increased risk of UTI in patients who used Canagliflozin compared to the placebo, but we found no association with the dosing.

It has been shown that patients treated with Da-

pagliflozin may be at increased risk of UTI compared to the placebo group. The comparative difference was in the dose of the drug. While in the Rizzi and Trevisan[29] study the dose of 2,5mg Dapagliflozin per day was associated to a lower risk of UTI than the placebo, in our study, we found no significant relationship between the risk of UTI and the dose of Dapagliflozin.

As for Empagliflozin, studies have shown an increased probability of developing UTI in comparison with the placebo, except at a dose of 5mg per day, for which the chances of UTI were much lower than the placebo.[30] Our study showed that Empagliflozin does not present a risk of increased UTI when compared to the placebo at doses of 5, 10, or 25 mg per day. A single study in our sample experimented with 50mg of Empagliflozin and found an increase of UTI at all doses tested (10, 25, 50 mg/day).[25]

As for Togogliflozin, no review study evaluated its relationship with UTI. In our study, we included a single article focusing on this drug. It was found an elevated risk of developing UTI with SGLT2i in comparison with the placebo, although we did not see any dose relationship in the analyses.

Regarding the associations of SGLT2i with other oral antidiabetics in treatment, we found there is a superior chance of developing UTI in comparison with the placebo when the patient was in combination therapy.

There were no statistically significant results regarding the incidence of UTI among the different groups of patients analyzed: in monotherapy, in combined therapy, and insulin therapy; or in relation to associated clinical comorbidities, such as cardiovascular diseases and presence of renal injury (micro and macroalbuminuria).

Some limitations found limited the studies analyzed. The main limitation was in the diagnosis of UTI proposed in the methodology of the trials. We excluded the records that did not identify methods of detection of UTI in the methodology. Most studies used pre-specified queries, using lists of preferred terms from the Medical Dictionary for Regulatory Activity (MedDRA) to determine UTI in patients. Looking for evidence on the effectiveness and safety of the drugs, the trials did not focus on the procedures to detect UTI in their samples. Since there was a clinical identification through the symptoms reported by the patients during the period of treatment with SGLT2i, the possibility of these patients already having ITU prior to the start of the treatment has not been clearly excluded. This is an important fact since this comorbidity already has a high prevalence in diabetic patient.

CONCLUSIONS

Diabetic patients treated with SGLT2i had an increased chance of developing UTI compared to those who did not use it. This increased chance was found predominantly with the use of Dapagliflozin, Canagliflozin, and Tofogliflozin, regardless of the dosing. However, the dimension of UTI chances for the DM2 patients in use of these drugs still remains to be more strictly determined. The currently increased use of SGLT2i in clinical practice an requires an analysis of urinary disorders, such as excluding the presence of UTI prior to the treatment, and the elaboration of more specific diagnostic methods for this pathology. These are imperative to determinate the long-term impact of increases in UTI episodes with SGLT2i use.

Conflict of Interest

The authors have no conflict on interest in this review.

RESUMO

Os inibidores do cotransportador de sódio-glicose do tipo 2 (SGLT2i) são medicamentos que atuam mantendo a glicosúria. Estudos recentes têm demonstrado efeitos promissores desses no tratamento de diabetes mellitus tipo 2 (DM2). No entanto, pode haver um risco aumentado de desenvolver infecções do trato urinário (UTI) em pacientes tratados com essa classe de medicação. Nosso estudo tem como objetivo analisar a associação entre o risco de desenvolver UTI em pacientes tratados com SGLT2i. Uma revisão sistemática da literatura foi realizada por ensaios clínicos randomizados, totalizando, ao final da seleção, 23 artigos que foram avaliados estatisticamente. A incidência de UTI foi demonstrada genericamente de acordo com os dados dos artigos e em diferentes subgrupos: pacientes em monoterapia com SGLT2i ou em terapia combinada, de acordo com as comorbidades específicas de cada amostra ou de acordo com a droga utilizada. Verificou-se um aumento na chance de UTI nos grupos SGLT2i em comparação com os grupos de controle em placebo ou outros agentes antidiabéticos orais. Essa chance aumentada foi encontrada predominantemente com uso de Dapagliflozina, Canagliflozina e Tofoglifozina, independentemente da dosagem. Por fim, ressaltou-se que as chances de UTI em pacientes com DM2 em uso de SGLT2i ainda precisam ser mais bem determinadas.

PALAVRAS-CHAVE: *Inibidores/antagonistas do transportador 2 de glucose-sódio. Diabetes mellitus tipo 2. Infecções urinárias.*

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REVIEW ARTICLE

Intraoperative vancomycin powder and post-operative infection after spinal surgery: a systematic review and meta-analysis

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SUMMARY

KEYWORDS: *Vancomycin. Intraoperative care/methods. Spine/surgery. Surgical wound infection/prevention & control.*

INTRODUCTION

Spinal infections after spinal surgeries are important complications that increase morbidity and even mortality, besides their economic and social impact[1-3]. Infections may lead to osteomyelitis, problems with wound healing, instrumentation failure, pain and systemic complications such as sepsis and death[2,4]. Incidence varies tremendously, from 0.5% to 15% in these cases[1,5].

Some studies suggest benefits of adding vancomycin powder into the surgical wound concomitant to conventional parenteral antibiotics prophylaxis to avoid staphylococcal infections[6,7].

The objective of this study is to evaluate the use of intraoperative vancomycin powder delivered into surgical wounds in spinal surgery to decrease post-operative spinal infections.

METHODS

A systematic literature review was performed following the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA)[8].

Search Strategy, selection of studies and data collection

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The PICO acronym was used with the following criteria:

P – Patients – any patient who underwent spinal surgery, of any age, with or without instrumentation.

I – Intervention – patients who receive vancomycin powder into the surgical wound.

C – Control – patients who did not receive vancomycin powder into the surgical wound.

O – Outcome – post-operative infection rates in both groups

The search strategy was based on the following Mesh descriptors terms and word text: "vancomycin"; "spine"; "surgical procedures," "operative." The sources of the articles were PubMed, Embase, Central Cochrane Database and LILACS - on July 09, 2017. Articles in English, Spanish and Portuguese were revised and evaluated.

SELECTION OF STUDIES

Titles and abstracts were reviewed by three authors (AFJ, JWD, RVB). The selected titles had their full papers evaluated. Discrepancies were solved by consensus among all authors using virtual web meetings.

Types of evaluated studies: randomized trials and, if not available, controlled clinical studies evaluating the use of vancomycin powder were deemed to be evaluated.

Data extraction: Data was extracted in a specific spreadsheet according to the number of patients, infection rates, vancomycin doses, spinal procedures, and complications. The process of literature selection is illustrated in the Prisma Flow Chart Diagram (Figure 1). Methodological Quality Evaluation: For randomized trials, the risk of bias was evaluated according to the Cochrane Collaboration guidelines[9], which include random sequence generation (selection bias), allocation concealment (selection bias), blinding of the participants and personnel (performance bias), blinding of the outcomes assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias) and other sources of bias.

For the observation papers, Risks of Bias (ROB) were evaluated following the Newcastle Ottawa Scale (NOS)[10].

Individual selected studies were graded according to their level of evidence following the OXFORD level of evidence-based medicine[11].

GRADE recommendation guidelines were used to evaluate the effect of vancomycin powder in decreasing post-operative spinal infections[12].

Statistical Analysis: The software used for meta-analysis was "R" core Team (R Foundation for Statistical Computing, Vienna, Austria). Statistical heterogeneity was evaluated using the Cochran's Q test and I2. Random effect model was used in case of substantial inconsistencies.

RESULTS

The electronic search identified 64 articles on Medline, 92 on Embase and one in LILACS. After removal of duplicated articles, 151 titles were identified. Abstracts were evaluated, identifying 78 articles for the full-text evaluation. Twenty-two papers were finally analyzed. One article[13] was a randomized trial (Level 2B), and another 21 were case-control studies (Level 3B) (Table 1). Of note, the studies included different spinal levels, surgical approaches and, in the majority of them, instrumented posterior fusions[14,18-21,30,31].

Risk of Bias

Randomized trial

Tubaki et al.[13] published in 2013 the only identified randomized paper in this review.

Selection bias: Randomization was done using a computer-generated sequence. Samples with the use and non-use of vancomycin had no baseline differences in characteristics. Both groups were well comparable.

Performance bias: there was no attempt to conceal the allocation of samples for treatment. There were no Blinding of participants, personnel and outcome assessors. Wound infections were monitored during the follow-up period. All patients were followed up for at least 12 weeks from the date of surgery.

Attrition bias: there were no described losses in the final follow-up. There was no difference in outcome loss and withdrawals from the samples in this study. Patients were followed for a sufficient time to reveal the desired outcome (12 months).In the Vancomycin group infection rate was 1.61% and in the control group, 1.68%. This meager infection rate may have contributed to the lack of vancomycin effect in this trial. Along with the infection rates described above for both samples, estimating the 95% confidence interval, one statistical test with 80% power, the estimated sample size to reveal differences would

TABLE 1 - CHARACTERISTICS OF THE 22 STUDIES USED IN THE META-ANALYSIS

Author/year	Groups appraisals	Surgical site infection rate (N patients/ Infections%); Comparisons between control (non-SSVP) and treatment groups (with SSVP)	Follow–up and general considerations
1. O'Neil et al.[14], 2011	Posterior spine instrumented fusions in traumatic aliments; All spinal segments	Control group: 54/13%; Treatment group: 1 g SSVP, 56/zero (p=0.02)	Median: 25 weeks; No adverse effects
2. Sweet et al.[7], 2011	Posterior spine instrumented fusions in deformity, traumatic, neoplastic aliments; Lumbar and thoracic spinal segments	Control group: 821/2.6%; Treatment group: 2 g SSVP, 911/0.2% (p<0.0001)	Average: 2.5 years; No adverse effects
3. Pahys et al.[15], 2013	Posterior spine instrumented fusions in degenerative, deformity, traumatic, neoplastic, congenital aliments; Cervical spine	1. Control group: IV ATB, 483/1.86%; 2. Control group: IV ATB+Skin alcohol foam + drain, 323/0.3% (p=0.047); 3. Treatment group: IV ATB+ Skin alcohol foam + drain + 500 mg SSVP, 195/zero (p=0.048)	Minimum: 3 months; Risk factors: A BMI* of>30 kg/m^2 and rheumatoid arthritis had the strongest association with acute postoperative infections No adverse effects
4. Strom et al.[16], 2013	Posterior spine instrumented fusions in degenerative, infectious, traumatic, neoplastic aliments; Cervical spine, occipitocervical and cervicothoracic spinal segments	Control group: 92/10.9%; Treatment group: 1 g SSVP, 79/2.5% (p=0.0384)	Control group: Mean 4.5 years; Treatment group: Mean 2.2 years; Absence of complications; Adverse effect: pseudarthrosis: Control group 92/5.4%; Treatment group 79/5.1% (p=1.000)
5. Strom et al.[17], 2013	Posterior spine instrumented and non instrumented fusions in degenerative, infectious, traumatic, neoplastic aliments; Thoracic and lumbar spinal segments	Control group: 97/11% overall rate (non instrumented 20/10%, instrumented 77/12%, p=0.0008); Treatment group: 1 g SSVP, 156/zero overall rate (non-instrumented 68/zero, instrumented 88/zero (p=0.049)	Control group: Mean 4.5 years; Treatment group: Mean 1.9 years; Absence of complications and no adverse effects
6. Caroom et al.[18], 2013	Posterior cervical decompression Instrumented in multilevel cervical spondylotic myelopathy (CSM); Cervical spine	Control group: 72/15%; Treatment group: 1 g SSVP, 40/zero (p=0.007)	Control group: Follow-up NI; Treatment group: Minimum of 6 months, average 18 months; No adverse effects
7. Kim et al.[19], 2013	Posterior, anterior and lateral approaches instrumented in degenerative, traumatic and neoplastic aliments; All spinal segments	Control group: 40/12.5%, all in posterior approaches: Treatment group: 1 g SSVP, 34/zero (p=0.033)	Follow-up: NI; Risk factor: Elderly patients No adverse effects;
8. Godil et al.[20], 2013	Posterior cervical approach instrumented in traumatic aliments; Cervical spine	Control group: 54/13% Treatment group: 1 g SSVP, 56/zero (p=0.02)	Control and treatment groups: median 25 weeks; No adverse effects
9. Tubaki et al.[13], 2013	Open instrumented and non instrumented spine surgery; Aliment types: NI; All spinal segments	Control group: 474/1.68% Treatment group: 1 g SSVP, 433/1.61% (p>0.05)	Control and treatment groups: minimum of 12 weeks; No adverse effects
10. Martin et al.[6], 2014	Open instrumented spine surgery in deformity; Thoracolumbar and lumbar spinal segments	Control group: 150/5.3%; Treatment group: 2 g SSVP, 156/5.1% (p=0.936)	Control and treatment groups: 30 days; No adverse effects
11. Emohare et al.[21], 2014	Open instrumented and non- instrumented spine surgery; Thoracic, thoracolumbar and lumbar spinal segments	Control group: 207/NI, return-to-surgery for infection = 6.71%; Treatment group: 1 g SSVP, 96/NI, return-to-surgery = zero (p=0.0841)	Follow-up: NI; Adverse effects: NI
12.Theologis et al.[22], 2014	Open instrumented spine surgery in deformity; Thoracic, thoracolumbar and lumbar spinal segments	Control group: 64/NI, readmissions within 90 days for SSI = 10.9%; Treatment group: 2 g SSVP, 151/NI, readmissions within 90 days for SSI = 2.6% (p=0.01)	Control group: median 34 months; Treatment group: 18 months; No adverse effects
13. Martin et al.[3], 2015	Open posterior instrumented spine surgery in degenerative, deformity, neoplastic and traumatic aliments; Occipitocervi cal, cervical only, and cervicothoracic spinal segments	Control group: 174/6.9%; Treatment group: 2 g SSVP, 115/5.2% (p=0.053)	Control and treatment groups: 30 days; No adverse effects
14. Scheverin et al.[23], 2015	Open posterior instrumented spine surgery in degenerative aliments; Lumbar spine	Control group: 281/4.98%; Treatment group: 1 g SSVP, 232/1.29% (p=0.0245)	Control and treatment groups: mean 10 months; Risks for SSI: age > 65 years, obesity, prolonged surgery, surgical blood lose; No adverse effects
15. Tomov et al.[24], 2015	Open and percutaneous, anterior and posterior, instrumented and non instrumented spine surgery in deformity, degenerative, traumatic, neoplastic aliments; All spinal segments	Control group: NI; Treatment group: 1 g SSVP, NI; SSI rates were reduced by 50% after the intervention with SSVP (p=0.042)	Follow-up: NI; Risks for SSI: anemia, prior operation, vertebral fracture; Adverse effects: NI

Author/year	Groups appraisals	Surgical site infection rate (N patients/ Infections%); Comparisons between control (non-SSVP) and treatment groups (with SSVP)	Follow–up and general considerations
16. Liu et al.[25], 2015	Open posterior spine surgery in degenerative, deformity, neoplastic aliments; Cervical, thoracic, lumbar spinal segments	Control group: Non-tumor, non-SSVP, 129/7%; Tumor, non-SSVP, 25/8% (p=0.011). Treatment group: Non-tumor, 0.5 mg - 2 g SSVP, 153/0.7%; Tumor, 0.5 mg - 2 g SSVP, 27/14.8% (p=0.442).	Control and treatment groups: 3 months; Preoperative radiotherapy may contribute to the increase of SSI; No adverse effects
17. Heller et al.[26], 2015	Open posterior spine surgery in degenerative, deformity, neoplastic; traumatic aliments; Cervical, thoracic, lumbar spinal segments	Control group: 341/3.89%; Treatment group: 0.5 mg-2 g SSVP, 342/1.1%; (p=0.029)	Control and treatment groups: 90 days. Risk factors for SSI: Discharge to skilled nursing or rehabilitation facilities; No adverse effects
18.Schroeder et al.[27], 2016	Open posterior or anterior, non instrumented and instrumented cervical, thoracic, lumbar spine surgery (anterior cervical excluded); Spinal aliments: NI	Control group: 2253/1.33%; Treatment group: 1-1.5 g SSVP, 1224/0.40% (p=0.04)	Control and treatment groups: 12 months; Adverse effects: NI
19. Lee et al.[28], 2016	Open posterior lumbar spine surgery; Spinal aliments: NI (excluded traumatic)	Control group: 296/10.5%; Treatment group: 1 g SSVP, 275/5.5%	Control group: mean 11 months; Treatment group: mean 8 months; Risk factors: Diabetes mellitus, cardiovascular disease, and longer hospital stay; No adverse effects
20. Hey et al.[29], 2017	Open posterior, lateral spinal surgery; Degenerative, developmental, traumatic, infectious, neoplastic, revision; Non-instrumented and instrumented; Cervical, thoracic, lumbar	Control group: 272/6.3%; Treatment group: 1 g SSVP, 117/0,9%;	Control and treatment groups: 3 months; Adverse effects: NI
21.Van Hal et al.[30], 2017	Spinal surgery (laminectomies and arthrodesis)	Control group: 652/NI; Treatment group: SSVP dose NI, 496/5.6%	Follow-up:NI
22. Chotai et al.[31], 2017	Open posterior and anterior spinal surgery: Degenerative, deformity, neoplastic; With and without instrumentation	Control group: 1587/2.5%; Treatment group: 1 g SSVP, 1.6%	Control and treatment groups: 1 year; No adverse effects

Abbreviations: N: number of included patients; IV: intravenous; SSVP: Surgical site vancomycin powder; g: gram(s); mg: milligram(s); ATB: antibiotic; NI: Not informed; BMI: Body Mass Index; SSI: Surgical site infection

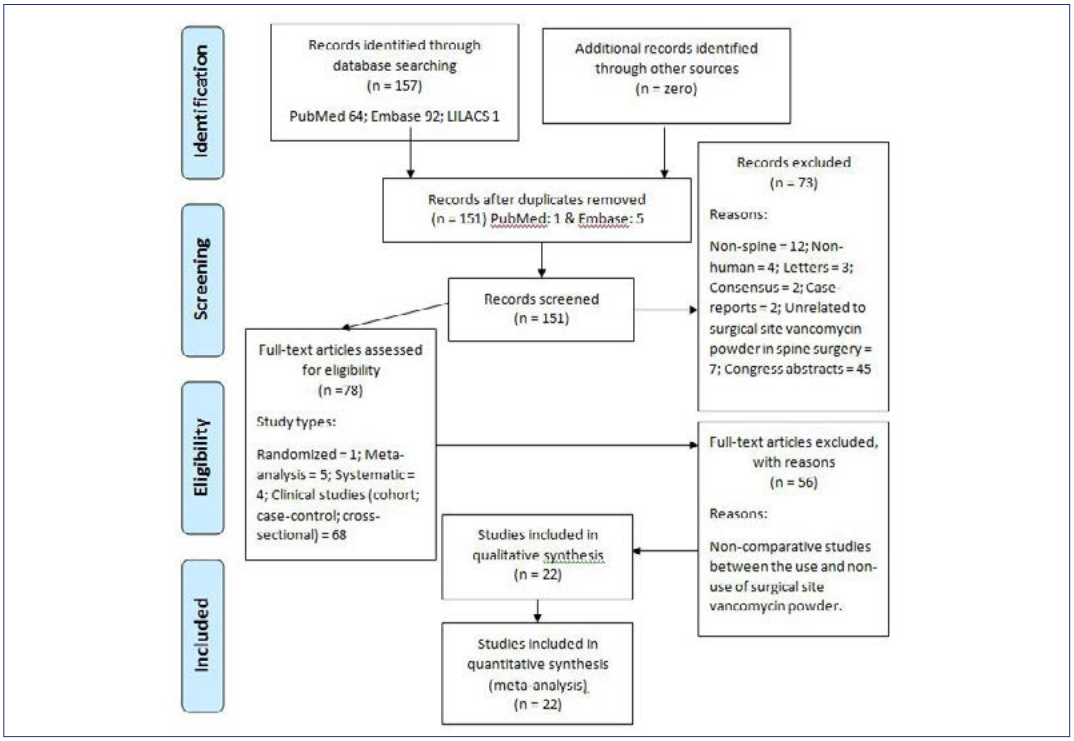

FIGURE 1

TABLE 2 - RISK OF BIAS OF CASE-CONTROL TRIALS: NEWCASTLE OTTAWA SCALE (NOS).

Study	Selection	Comparability	Exposition	
Van Hal et al.[30], 2017	****	*	**	Current vs. previous
Martin et al.[6], 2014	****	*	**	Current vs. previous. Use of propensity score adjustment.
Liu et al.[25], 2015	****	*	**	Current vs. previous. Significant differences between samples.
Tomov et al.[24], 2015	****	*	**	Current vs. previous. Data from the Healthcare Infection Management and Infection.
Martin et al.[3], 2015	****	*	**	Current vs. previous. Significant differences between samples.
Theologis et al.[22], 2014	****	*	**	Current vs. previous. Significant differences between samples.
Kim et al.[19], 2013	****	*	**	Current vs. previous.
Strom et al.[17], 2013	****	*	**	Current vs. previous. Imbalanced for instrumentation.
Heller et al.[26], 2015	****	*	**	Current vs. previous. Only 8%follow-up losses Imbalanced for age, arterial hypertension and use of hair cut.
Schroeder et al.[27], 2016	****	*	**	Current vs. previous. Significant differences between samples.
Lee et al.[28], 2016	****	*	**	Current vs. previous. Uni and multivariate analysis for covariates.
Pahys et al.[15], 2013	****	*	**	Data collected and analyzed by three independent reviewers. Significant differences between samples.
Hey et al.[29], 2017	****	*	**	Significant differences between samples
Scheverin et al.[23], 2015	****	*	**	Vancomycin indicated according to the surgeon's preference. Significant differences between samples
Godil et al.[18], 2013	****	*	**	Samples based on surgeon preferences. Non controlled for confounders but without differences between samples.
Chotai et al.[31], 2017	****	*	**	Not controlled for confounders but without differences between samples.
Carrom et al.[18], 2013	****	*	**	Current vs. previous. The intervention group trended toward slightly more complex procedures.
Sweet et al.[7], 2011	****	*	**	Current vs. previous. No significant differences between samples
Emohare et al.[21], 2014	****	*	**	Patient allocation-specific surgeon or on-call admission. Significant differences between samples.
O'Neil et al.[14], 2011	****	**	**	The treatment and control groups were statistically similar.
Strom et al.[16], 2013	****		**	Current vs. previous. No significant difference between samples.

be well above the studied sample size. Infection rates were meager and raised questions whether a study aiming to decrease infection rates should be done in this low infection rate scenario.

According to the NOS, the topic "selection" is composed of 4 components: adequate case definition, representativeness of cases selection of controls, and definition of controls. Post-operative spine infections are clinically important cases, and the review protocol admitted only papers with sufficient follow-up time, so all articles received four stars in this topic (Table 2).

In the topic "comparability," two stars may be given to each paper. Both cases and controls must be matched in the design or confounders must be adjusted for in the analysis. Although in some of the articles the authors did evaluate the importance of confounding factors, odds ratios for the exposure of interest were not adjusted in any of the articles. Thirteen papers were of current vs. previous sample of cases or non-concurrent case-control trials. Several papers had severe imbalances among cases and controls, most of them imputing greater risk of infection in the vancomycin sample. The biases described occurred more frequently in the experimental vancomycin groups, which in theory would expose the vancomycin groups to higher rates of infection, which did not occur, strengthening the revealed effect. O'Neil's paper was a concomitant case-control study without imbalance between samples and received two stars[14].

In the topic "exposure," there are two items: ascertainment of exposure and non-response rate. Only one paper described a non-response rate of only 8%. As all cases and controls were exposed to infection in surgery, and likewise, the described losses to follow-up were low, all articles received two stars.

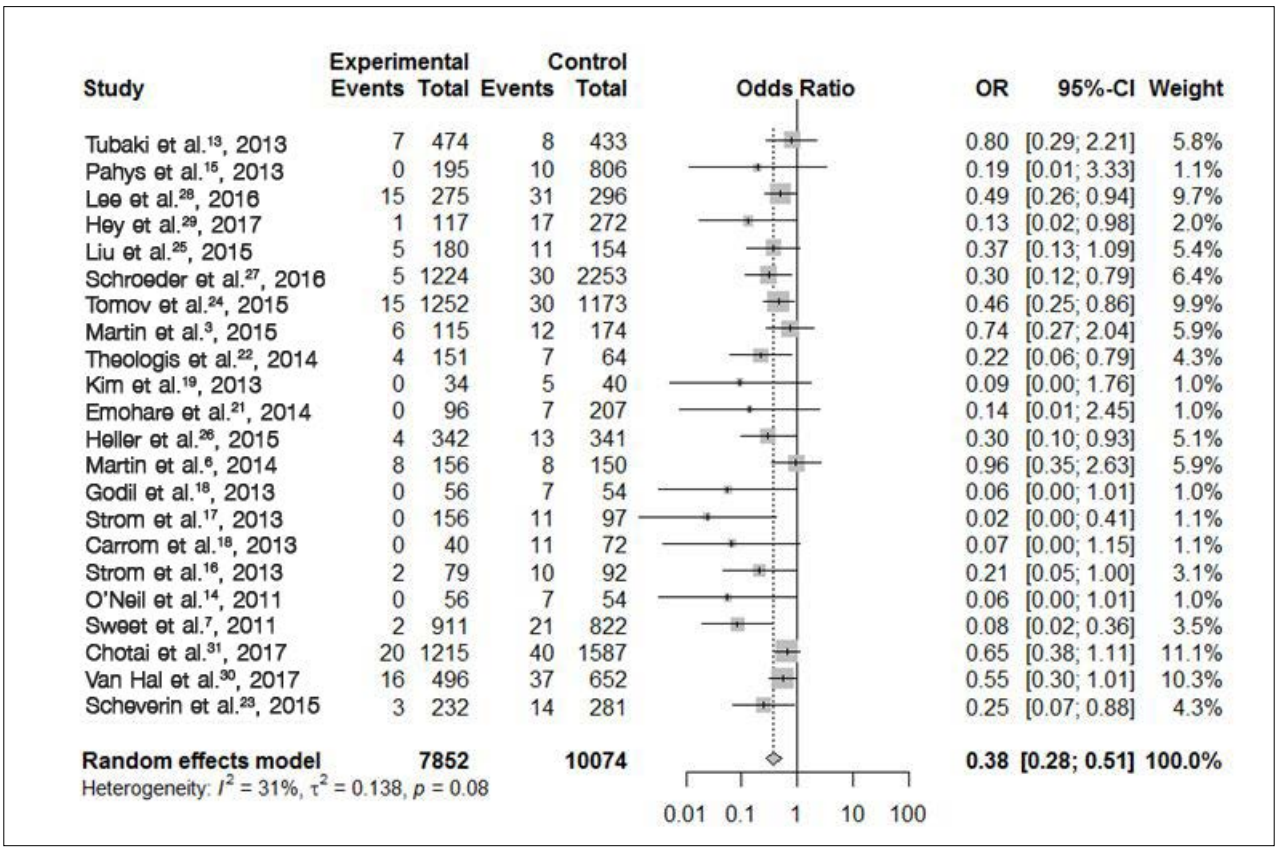

FIGURE 2

TABLE 3 – INTERVENTION EFFECTS ACCORDING TO THE IR (LOW < 2%; MEDIUM 2-4% AND HIGH ≥ 4)

Results for subgroups (random effects model):						
	k	OR	95% - CI	Q	tau^2	I^2
IR = low	3	0.4499	[0.2139; 0.9461]	2.26	0.056	11.5%
IR = medium	5	0.3612	[0.1918; 0.6800]	8.32	0.2367	51.9%
IR = high	14	0.3484	[0.2252; 0.5391]	19.99	0.2039	35.0%

Intervention Effects: Twenty-two papers were included in the pooled analysis. One article was randomized[13]. This article was evaluated alone because of its methodological superiority and level of evidence. However, this article indicated meager infection rates in both groups, even in the group that did not receive vancomycin. Each of the groups had an infection rate of less than two percent. Presented data indicated that the sample sizes needed to reveal significant differences in infection rates and would have to be larger in number. Sample sizes performances were questioned, and for this reason, we considered that this randomized study evaluated the effect of the intervention, but the outcome of interest was infrequently encountered. This way, all of the articles were pooled for analysis.

All other studies were case-control comparing the use and non-use of intraoperative topical vancomycin powder or not. Seven thousand eight hundred and fifty-two (7852) patients received vancomycin, and 10074 did not receive it. The odds ratio to develop post-operative infection was 0.38 (CI 95%: 0.28-0.51), z=-6.26, p< 0.0001, random effects model, favoring vancomycin use (Figure 2).

Subgroup analysis and intervention effects: Due to differences in infection rates (IR) among the articles, the intervention effect of vancomycin powder was tested by distributing the articles according to

the encountered IR into: low (IR <2%), medium (IR 2-4%), high (IR => 5%). Vancomycin remains effective in the 3 subgroups without significant differences (Q=0.34, p-value=0.8421) (Table 3).

To reveal the clinical benefits, results were either described with risk differences to calculate NNT (Number need to be treated to show benefits). The risk difference (random model) was: 0.0286 [-0.0383; -0.0188] (P=0.0002) favoring Vancomycin. The NNT was 35 (34.96) patients. Quantifying heterogeneity: tau^2 = 0.0003; H = 1.95 [1.58; 2.41]; I^2 = 73.7% [60.0%; 82.7%].

The characteristics of the 22 included studies used in the meta-analysis are listed in Table 1.

DISCUSSION

Post-operative spine infections represent about 22% of the costs with infectious diseases, estimated in 1 to 10 billion dollars a year[32]. After spine surgery, the incidence of surgical site infections (SSI) depends on many factors, ranging from 0.5% to 15%, with higher rates in instrumented surgeries and in deformities[33]. Staphylococcal infections (for *S. aureus* and *S. epidermidis*) are the most common agents, with an increased incidence of Methicillin-Resistant *S. aureus* (MRSA)[19,34]. These agents are not affected by commonly used cephalosporin and generally require glycopeptides antibiotics, such as vancomycin or teicoplanin. The rationale for the use of vancomycin powder into the surgical wound is that the endovenous administration has not only more systemic side effects but also an unpredictable concentration into the bone tissues, compared with elevated concentration into the wound after direct application (128 to 1457 ug/ml)[35-38].

In this review, the only prospective study did not show any advantage of the use of vancomycin powder in decreasing infection rate[13]. However, the infection rate in this study was meager (1.8% in the control group). This meager infection rate may influence the reported lack of vancomycin effect. Along with this low infection rate in both samples, considering an 80% power test and 20% type b error, the number needed to be treated to reveal a statistical difference would be much larger than those studied. Then, although this study was a randomized trial, it was evaluated along with the other observational trials.

The remaining 21 studies were case-control studies comparing the use of intraoperative topical vancomycin or its non-use. The OR to develop infection was 0.38 (CI 95%: 0.28-0.51; p< 0.0001) favoring vancomycin use.

The best quality case-control studies have been adjusted to remove the effect of confounding factors. However, ORs adjusted for confounders were not provided.

Evaluating the Vancomycin effect by the NNT, 35 treated patients are necessary to reveal benefits. Although this may be suggestive of a small effect, considering the potential damage of each infected case, potential worsening in clinical results in an infected patient and the hospitalization costs, conflicting with low cost of intraoperative vancomycin powder and almost no side effects, vancomycin effect seems robust. Besides this, unlike most randomized trials, the risk of bias in these studies contributed to a decrease in the effect of vancomycin: in cases where intraoperative antibiotics were used, they were those with the highest potential for infection. Therefore, the effect of vancomycin may even be higher than that demonstrated. According to GRADE recommendations guidelines, observational studies produce low evidence that may have an upgrade in large effects[12]. Also in line with GRADE's recommendations, it is possible to make a strong recommendation based on low-quality evidence if the desirable effects clearly outweigh undesirable effects or vice versa, or if there is evidence for at least one critical outcome from observational studies. The recommendation may change when higher quality evidence becomes available.

LIMITATIONS OF THIS META-ANALYSIS

Although the evidence of this meta-analysis suggested the benefits of adding vancomycin powder into the surgical wound in decreasing infection rates, caution is required when interpreting these results. Different patients' samples were included, as well as different procedures, in many spinal sites, although the majority of the patients were those who had posterior instrumented fusions. Moreover, our results were based on case-control studies, with a low grade of evidence, once the only randomized study had a meager rate infection rate and a relatively small number of cases to demonstrate the effects. Additionally, it is our perception that in surgeries with a very low risk of spinal infection, the benefits of adding powder vancomycin may decrease when compared with high-risk populations.

CONCLUSIONS

Based on our meta-analysis, the use of intraoperative vancomycin powder in spinal surgeries reduces post-operative spine infections with moderate evidence according to GRADE guidelines. However, this recommendation is mainly based on case-control studies with a low level of evidence. Future randomized studies with homogeneous patient populations that undergo spinal surgeries are necessary to improve the grade of recommendation as well as to select patient subgroups that may have a higher benefit with this procedure.

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PALAVRAS CHAVE: *Vancomicina. Cuidados intraoperatórios/métodos. Coluna vertebral/cirurgia. Infecção da ferida cirúrgica/prevenção & controle.*

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REVIEW ARTICLE

Opioids in the immune system: from experimental studies to clinical practice

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SUMMARY

INTRODUCTION: *Opioids interact with both innate and adaptive immune systems and have direct effects on opioid receptors located on immune cells. Research on this topic has provided evidence of the opioid influence on the immune response associated with surgical stress. The immunological effects of opioids are currently being investigated, particularly whether they influence the outcome of surgery or the underlying disease regarding important aspects like infection or cancer progression. This review addresses background research related to the influence of the opioid receptor on the immune system, the immunosuppressive effect associated with major opioids during the perioperative period, and their clinical relevance. The objective of the study was to review the effects of opioids on the immune system. Methods: A search strategy was conducted in PubMed, Embase, and the Cochrane databases using the terms "immunosuppression," "immune system," "surgical procedures," "analgesics," "opioids" and "perioperative care." Results: The immunosuppressive effect of opioids was identified over 30 years ago. They include signaling and acting directly through immune cells, including B and T lymphocytes, NK cells, monocytes, and macrophages, as well as activating the downstream pathways of the hypothalamic-pituitary-adrenal (HPA) axis leading to the production of immunosuppressive glucocorticoids in the peripheral and sympathetic nervous system.*

KEYWORDS: *Immunosuppression. Immune system. Surgical procedures, operative. Analgesics. Analgesics, opioid.*

INTRODUCTION

Millions of people undergo surgical procedures under general anesthesia or sedation annually, many of whom are immunosuppressed patients, such as elderly, oncology, or immunomodulatory therapy patients. According to the distribution of the population pyramid, these groups of individuals are expected to increase.[1]

Opioids are necessary substances used medically for pain management and anesthesia during the perioperative period for any major surgery.[2] The effect of opioids on the immune system has become a research field of great interest, given that opioid use could be related to a poor surgical outcome or a variety of disease processes, such as infection or cancer.[3]

A significant inflammatory response characterizes the perioperative period of major surgery. Immunosuppression accompanies this secondary to the interaction of several factors, such as surgical stress, anesthetics, analgesics, hypothermia, lung ventilation, and the patient's underlying disease.[4]

Several studies have demonstrated that opioids influence the immune system by altering the migration and functional activity of innate immune responders, creating a compromising environment

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that is detrimental to the host's ability to eradicate pathogens (Fig 1).[5] Morphine may decrease the effectiveness of both natural and adaptive immunity and significantly reduce cellular immunity (Table 1).[6] Furthermore, morphine treatment has been associated with increased morbidity and mortality due to the development of infection and accelerated cancer progression in animal models.[6] Additionally, some studies suggest that opioids that are widely used in clinical practice and during the perioperative period, such as morphine, fentanyl, and remifentanil, may worsen the immunosuppressive effect of surgery.[7-9]

The primary objective of this review is to discuss the clinical impact that opioids have on the immune system during the perioperative period considering the current academic literature. In addition, we review the expression of opioid receptors in the immune system, the immunosuppressive effect of surgical stress and the investigations related to this field in humans.

METHODS

A literature search of indexed scientific articles was conducted in MEDLINE and EMBASE using Medical Subject Heading (MeSH) and DeCS keywords in English. The English keywords included "immunosuppression", "immune system", "surgical procedures", "analgesics", "opioids" and "perioperative care". Articles that reported findings of opioids such as fentanyl, morphine, remifentanil, and tramadol were included. Given the paucity of literature including humans, all papers found in the literature were reviewed.

TABLE 1. INNATE VERSUS ADAPTIVE IMMUNITY

	Innate	Adaptive
Features	Primitive and broad	Highly specific (T and B cell reports)
Speed of onset	Immediate	Approx. 3-day lag
Regulation	+/-	++++
Potency	Lower	Higher
Kinetics	Fast (hours-days)	Slow (days-wks.)
Amplification	No (insignificant)	Yes
Duration	Short (days)	Long (months/yrs.)
Memory	No	Yes
Activity	Always present	Normally silent
Specificity	Unspecific	Highly specific

Results

Expression of opioid receptors in immune cells and their immunomodulatory effect

Opioid receptors belong to the family of transmembrane G protein-coupled receptors (GPCR). Four classical opioid receptors have been identified, and ORL-1 (nociceptin/orphanin FQ receptor). Furthermore, there is pharmacological evidence that suggests the presence of subtypes and variants of opioid receptors.[10]

The immunosuppressive effect of opioids was identified over 30 years ago when Wybran et al.[11] first reported the presence of opioid receptors in normal human T lymphocytes. Subsequent studies documented that endogenous and exogenous opioids not only induce analgesic effects by regulating pre- and post-synaptic sensory neurons but also interact with peripheral opioid receptors present in the immune system, resulting in other biological effects including immunomodulation.[5]

Opioid peptides are found in many leucocyte subpopulations including lymphocytes, monocytes, and granulocytes in the peripheral blood and lymph nodes, and also at the site of experimentally induced or clinical inflammation.[12] In peripheral inflamed tissue, opioid peptides including endorphin, (met)-enkephalin, dynorphin A, and endomorphin are produced by leukocytes and released in response to a stimulus, leading to an antinociceptive effect.[13] the blocking of endorphin activity, achieved by either administration of opioid antagonists (naloxone and naltrexone) or immunoglobulins, was reported to increase natural killer (NK) cell activity and lymphoproliferation within minutes. These observations indicate that removing the effect of the opioid by way of an antagonist or an antibody can affect specific immune responses, suggesting that there is an endogenous opioidergic effect on some immune functions.[14] As endorphins play a role in modulation of the immune response, inhibition of endorphins should theoretically lead to Th1-type immune responses, while increases in endorphins should lead to Th2-type immune responses. [3,15]

The interactions between opioid receptors and several molecules involved in the immune response are complex and multifactorial[3]. Endogenous opioid peptides play an immunomodulatory role; however, the effect of exogenous opioids can extend to the physiological control of the immune system, which has an important impact on immune responses.[3] Endog-

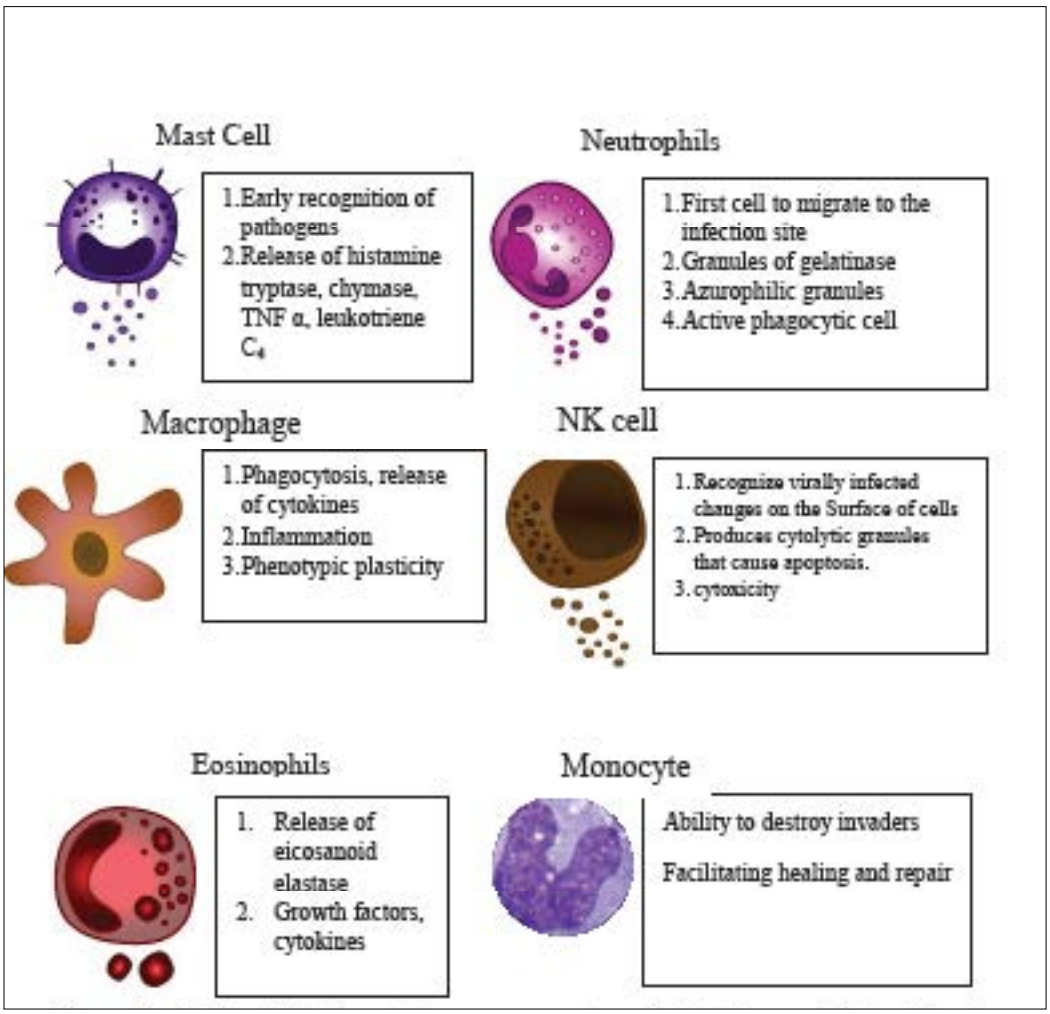

FIGURE 1. CELLS OF THE INNATE IMMUNE SYSTEM AFFECTED BY APIOID ANALGESICS

enous opioids modulate the immune system, while exogenous opioids mediate immunosuppression .[16,17]

The immunosuppressive effects of opioids have been observed in several different models. These effects include signaling and acting directly through immune cells including B and T lymphocytes, NK cells, monocytes, and macrophages, as well as activating the downstream pathways of the hypothalamic-pituitary-adrenal (HPA) axis leading to the production of immunosuppressive glucocorticoids in the peripheral[5,6] and sympathetic nervous system, causing the release of noradrenaline. Both glucocorticoids and noradrenalin negatively modulate the immune system. [6,18]

PERIOPERATIVE PERIOD

Surgical stress and immune suppression

The perioperative period of any major surgery is characterized by an inflammatory response accompanied by immune suppression resulting from the interaction of several factors including surgical stress, anesthetics, analgesics, hypothermia, lung ventilation, and the patient's underlying disease. [4,19] In addition, tissue injury secondary to a surgical lesion can cause changes in metabolic function and defense mechanisms in the patient, leading to an increase in catabolism, immunosuppression, and postoperative morbidity. Thus, the type of anesthetic and surgical technique should be modified to control this response, especially in major surgeries in which it can cause the most harm and increase patient morbidity.[20]

Opioids are essential drugs that provide the analgesic component of general anesthesia as well as controling the neurovegetative response to surgical trauma.[2] Their ability to reduce the stress response is related to the modulation of nociception in different sites of the neuraxis. Also, they can influence the neuroendocrine response in the central nervous system and act as potent inhibitors of the HPA axis.[2]

Opioids administered during the perioperative period

The classic opioid receptors, where the opioids act, are distributed widely throughout the central nervous system and throughout the periphery, occupying sites within the vas deferens, knee joint, gastrointestinal tract, heart, and immune system, amongst others. Opioid receptors have a series of endogenous ligands including endorphins, (met)-enkephalin, leu-enkephalin, dynorphin (A and B) and endomorphins (1 and 2).[21]

Opioids are widely prescribed during the perioperative period to block autonomic responses related to surgical trauma and acute postoperative pain control.[22] Almost all patients undergoing major surgery have uncontrolled postoperative pain even if they receive non-opioid analgesics. Consequently, opioids are a principal component of postoperative analgesia in patients undergoing major surgery.[23]

In this paper, we analyze the research available on opioids that are frequently used during the perioperative period including fentanyl, remifentanil, morphine, and tramadol.

Fentanyl

Fentanyl is a μ-opioid agonist which is widely used in clinical practice for analgesia, sedation, and anesthesia.[24] High doses suppress the associated response to surgery stress. Furthermore, it has a fast mechanism of action because it is highly lipid-soluble. Recovery depends on several factors including dosage, time of infusion and the number of doses administered.[24] It is metabolized to a non-toxic metabolite, and its renal clearance is less than 10%, making fentanyl a drug that is safe to use in patients with renal and hepatic impairment. However, it should be administered with caution in continuous infusions.[25,26]

An *in vitro* study evaluated the effect of fentanyl on natural killer cell cytotoxicity (NKCC) in 40 different patients who were exposed to either a high (75–100 μg/kg) or low (1–5 μg/kg) dose of the drug during the perioperative period.[8] This study found that both doses had the same effect on NKCC on the first postoperative day; however, patients exposed to the low dosage showed faster recovery of NKCC suppression, while patients given the higher doses of fentanyl still showed suppression 48 hours after surgery.[8]

Similar findings were reported in a study by Yardeni et al.[27] who investigated the *ex vivo* effects of high, intermediate, and low doses of fentanyl on immune function during the postoperative period of 60 patients. Secretion of the proinflammatory cytokines IL-1 and IL-6 were significantly diminished in patients treated with the high and intermediate doses of fentanyl when compared with the low dose. However, similar suppression of NKCC and IL-2 secretion was observed among the three groups .[27]

Yeager et al.[28] administered fentanyl to seven healthy volunteers at an initial dose of 3 μg/kg followed by an infusion of 1.2 μg/kg/h for 2 hours to evaluate the effects on innate and acquired immunity in humans and on leukocyte subpopulations in peripheral blood. This short-term exposure to fentanyl produced a significant increase in circulating CD16+ and CD8+ lymphocytes. Likewise, they reported elevated NKCC. This was due to an increase in the population of NK cells, not to an increase in activity. There was no significant effect in any other immunological parameter.[28]

Jacobs et al.[29] treated seven healthy individuals intravenously with fentanyl at a dose commonly applied to induce or balance anesthesia (0.2 μg/kg), with five subjects treated with saline as a placebo. There was an increase in NK cell numbers (CD16+/CD56+); however, pretreatment with naloxone prevented the fentanyl-induced NK cell increase in approximately half of the individuals.

Contradictory results have been reported in studies. However, it is important to note the insufficient number of patients and the low doses used in these studies.

Remifentanil

Remifentanil is an ultra-short-acting μ-opioid agonist. It is used in general anesthesia and has the capacity to rapidly suppress the autonomic, hemodynamic, and somatic responses to a noxious stimulus.[2] This drug has a unique ester structure that allows it to be hydrolyzed by blood esterases, thus resulting in fast-acting metabolism and a rapid reduction in serum concentrations of the drug after interruption of the infusion.[2]

Remifentanil has several clinical advantages when compared with other opioids, one of which is that organ dysfunction does not alter the pharmacokinetics of remifentanil, which has been proven for renal and hepatic impairment. Although its primary metabolite is excreted through the kidney, it has only 1/300 to 1/4600 of the activity of remifentanil[30]. These properties have led to the extensive use of

remifentanil for general anesthesia and sedation in a variety of patients and clinical scenarios. [30-33]

The current literature related to the specific effects of remifentanil on the immune system is limited. Sacerdote et al.[34] evaluated the effects of remifentanil on the immune system of rats and identified early suppression of the immune response caused by a significant reduction in the activity of NK cells and proliferation of lymphocytes without any change in leukocyte number. A study performed by Cronin et al.[35] assessed the effects of a low-dose (0.02–0.04 μg/kg/min) remifentanil infusion on NK cells in healthy volunteers, concluding that there was no alteration in the number nor the cytolytic function of these cells after an 8-hour infusion.

Cytokines such as TNF-α, IL-6, and IL-8 are important mediators of the immune response. The human neutrophil activation induced by lipopolysaccharides occurs because of the activation of mitogen-activated protein kinases (MAPKs) to produce cytokines activated by lipopolysaccharides.[3] Unlike other opioids, remifentanil attenuates the activation of human neutrophils exposed to lipopolysaccharides, reducing intracellular signaling pathways such as p38 and extracellular signal-regulated protein kinases 1 and 2 (ERK1/2). Furthermore, there is decreased expression of proinflammatory cytokines including TNF-α, IL-6, and IL-8 in a dose-dependent fashion.[36] The effect of decreased activation of cytokines and MAPKs has been reported to be reversed with a kappa-opioid receptor antagonist.[36]

The effects of clinically relevant concentrations of remifentanil on polymorphonuclear neutrophil migration through endothelial cell monolayers (ECM) has been assessed, with dose-dependent inhibition reported.[37] Large concentrations of this drug lead to a significant reduction in neutrophil migration through ECM. The main effect of remifentanil on endothelial cell adhesion is reduced expression of endothelial cell adhesion molecules; however, this inhibitory effect is weaker than that of fentanyl.[37] In a study of a prospective cohort of 235 patients, Inagi et al.[9] found that remifentanil-based anesthesia increased the incidence of surgical site infection after colorectal surgery and transiently altered the number of leukocytes and neutrophils.

Morphine

Morphine has been widely studied and is commonly considered to be the archetypal opioid analgesic and the agent to which all other painkillers are compared.[21] It has been well characterized in both clinical and preclinical studies, and decreases in several functions of both natural and adaptive immunity have been reported, with a significant reduction in cellular immunity after acute and chronic administration of morphine.[6]

In general, the effects of *in vivo* administration of morphine are associated with a reduction in innate immunity, mainly macrophages, and monocytes, with inhibition of proliferation and differentiation induced by macrophage colony-stimulating factor.[14]

The literature points to the clear conclusion that *in vivo* administration of morphine suppresses the function of NK cells, T cells, B cells, and polymorphonuclear leukocytes.[17] In 1996, Yeager et al.[38] conducted a clinical study to evaluate the *in vivo* effects of morphine on human immunity. Participants were healthy volunteers who received continuous intravenous exposure to morphine for 24 hours. The study was conducted in two sequential phases. During the first phase, participants received a low dose of morphine (loading dose of 0.025 mg/kg and infusion of 0.015 mg/kg/h) or high dose of morphine (loading dose of 0.05 mg/kg and infusion of 0.03 mg/kg/h). Peripheral blood for analysis was drawn before, during, and after morphine exposure to study the effects on the immune system. Morphine administration resulted in significant suppression of NK cytotoxicity at 2 and 24 hours after beginning intravenous morphine exposure.[38] Additionally, significant suppression of NKCC was observed at 2 and 24 hours after termination of morphine infusion in the high-dose study group. Gamma-interferon-stimulated NKCC and antibody-dependent cell cytotoxicity were also decreased after 24 hours of intravenous morphine exposure. These results suggest that morphine administration, at doses within the range of analgesic use, can cause marked suppression of components of the human cellular immune system.[38]

The effect of intrathecal morphine on NK cell activity was investigated in patients who underwent a hysterectomy.[39] This study divided 40 patients into four groups of 10. Three groups received 0.5 mg intrathecal morphine, 0.1 mg intrathecal morphine or 10 mg intravenous morphine, and the control group received inhalational anesthetics alone. Blood samples were drawn at different times to determine blood NK cell activity.[39] The group that received 0.5 mg intrathecal morphine had lower NK cell activity on a post-

operative day 1 when compared to baseline level and showed recovery on postoperative day 2. The control group and the groups administered 0.1 mg intrathecal morphine, and intravenous morphine showed no significant change in NK cell activities.[39] These results are consistent with the study by Yokota et al.[40] in which NK cell activity was shown to decrease on a postoperative day 1 in groups receiving 0.5 mg intrathecal morphine. Additionally, this study concluded that the combination of morphine and noradrenaline prolonged the suppression of NK cell activity. [40]

Tramadol

Tramadol is a centrally acting analgesic with a double mechanism of action. It binds with low affinity to μ-opioid receptors and inhibits serotonin and noradrenaline uptake with the activation of central monoaminergic pathways. This drug has been studied in animals and humans with consistent results.[3,7]

The effect of tramadol on immune response has been evaluated in rats, evidencing that, unlike morphine, tramadol does not suppress immune cell function. Instead, it has been reported to enhance NK activity, lymphocyte proliferation, and IL-2 production. [41]

The phagocytic capacity of polymorphonuclear cells and monocytes sampled from healthy volunteers was evaluated *in vitro*, in which morphine and tramadol were compared.[42] It was shown that tramadol did not affect the percentage of cells with phagocytic activity nor the phagocytic index.

On the contrary, morphine decreased the phagocytic capacity of polymorphonuclear cells and monocytes in a dose-dependent fashion.[42]

A study conducted by Sacerdote et al.[7] assessed the immune consequences of morphine and tramadol for the management of postoperative pain in 30 patients undergoing abdominal surgery for uterine carcinoma. The authors observed faster and more complete recovery of immune function with tramadol compared to morphine.

Comparable results were reported in a prospective, randomized clinical trial designed to investigate the effect of patient-controlled analgesia administration of morphine, tramadol and the combination of tramadol and lornoxicam on subtypes of T cells, NK cells and activated T cells in patients planned to undergo elective surgery for gastric cancer.[43] In the morphine group, none of the cellular subtypes had returned to their basal values within 48 hours of surgery, which contrasted with what was observed in the tramadol group. After 72 hours, cellular subtypes of NK cells and activated T cells remained at low levels in the morphine-exposed group .[43]

DISCUSSION

Clinical relevance

Studies with human subjects come with ethical considerations that restrict their use, particularly controlled, randomized clinical trials using placebo medications in acute pain and intensive care areas to produce high-quality evidence. Therefore, the conclusions in these settings are limited.[23] Opioids are undoubtedly the fundamental component of analgesia in general anesthesia, as they control the response to surgical stress and alleviate the acute postoperative pain associated with major surgery.[2]

The potential immunosuppressive effect of opioids during the perioperative period has been documented; however, it is known that inadequate pain control and the increased autonomic and metabolic response associated with surgical trauma in major surgery increases perioperative morbidity.[20] This immunosuppressive effect in surgery is associated with both inhaled and intravenous anesthetics. They also have a variable effect on cytokines depending on the agent used for general anesthesia,[44] which further limits interpretation of the results of current studies.

Opioids have a different mechanism of action related to the immune system, and differences in immunosuppressive potential exist between subgroups of opioids.[45] In addition, there is evidence that the immunosuppressive effect of opioids is independent of their antinociceptive effect; therefore, it is essential to individually evaluate the effect of each opioid on the immune system. Opioids with low receptor affinity such as tramadol have been demonstrated to have a lower immunosuppressive potential in animal and human studies. This may be clinically relevant when choosing an analgesic for immunocompromised patients.[42] Experimental studies have shown that opioid treatment or abrupt interruption of treatment leads to immunosuppression or an increased incidence of infections. However, the results of clinical trials are not conclusive. There is not sufficient evidence to conclude that opioids generate a clinically significant increase in the incidence of perioperative infections. [23] Further-

more, the clinical relevance of these trials remains unknown. Currently, it is not possible to provide clear guidelines for how to handle different scenarios in clinical practice.[46,47]

Every type of drug has a different effect on the immune system. Thus, future studies should characterize opioids and their effect on specific subpopulations, including immunocompromised and critically ill patients.[46] However, while there is a need to determine the underlying mechanisms by which opioids modulate the immune system, in addition to improving the methodological design of further studies, the current literature suggests that the potential effects of treatments with opioids should be considered, especially in susceptible patients.[3]

CONCLUSIONS

Opioids are essential drugs to control the response to surgical stress during both anesthesia and acute postoperative pain treatment. Despite their positive effects, they may also enhance the immunosuppression generated by surgery and pain.

The intraoperative use of high doses of fentanyl may prolong NK cell cytotoxicity suppression associated with surgery, in addition to disrupting proinflammatory cytokine production during the postoperative period. However, there is no consensus in the current literature.

The suppressive effect of morphine on NK cell activity during the postoperative period has been documented for both intravenous and intrathecal administration in humans, and there is a dose-effect relationship.

Remifentanil has been associated with an increased incidence of surgical site infection in patients undergoing colorectal surgery. Furthermore, it has been shown to alter the number of leukocytes and neutrophils transiently.

Opioids have different effects on the immune system regardless of their antinociceptive potential. Opioids with lower receptor activity, such as tramadol, have been shown to have a reduced immunosuppressive effect, with consistent results in both animals and humans.

Most *in vitro* and *in vivo* studies that assessed the effect of opioids including fentanyl, remifentanil, and morphine during the perioperative period have shown that these drugs accentuate the immunosuppression associated with surgical stress in a dose-dependent fashion. These results should be interpreted with caution due to the different methods used to measure the functionality of the immune system, the variability of the dose used and the small number of participants in the studies available.

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RESUMO

INTRODUÇÃO: *Os opioides interagem com ambos os sistemas imunes, inato e adaptativo, através de efeitos diretos sobre os receptores dos opioides localizados nas células imunes. As pesquisas neste assunto têm fornecido evidência da influência dos opioides sobre a resposta imune associada ao estresse cirúrgico.*
Os efeitos imunológicos dos opioides estão sendo pesquisados na atualidade, principalmente se eles determinam o resultado da cirurgia ou doença consequente devido a fatos importantes como infecção ou progressão do câncer.
Essa revisão tem como alvo ver antecedentes em pesquisa relativa à influência dos receptores dos opioides no sistema imunológico, o efeito imunossupressor associado com opioides maiores durante o período peri-operatório e sua importância clínica.
O objectivo da pesquisa foi revisar os efeitos dos opioides no sistema imunológico.
MÉTODOS: *Uma estrategia de procura foi dirigida na mídia PubMed, e no cadastro de Embase e The Cochrane, usando os termos "imunosuppressão", "sistema imunológico", "procedimentos cirúrgicos", "analgésicos", "opioides" e "cuidado peri-operatório".*

RESULTADOS: *O efeito imunosuppressor dos opioides foi identificado há mais de 30 anos. Os efeitos imunosupressores incluem sinalização e ação diretamente através das células imunes, mesmo com os linfócitos B e T, células NK, monócitos e macrófagos, também como ativando as vias de corrente do eixo hipotálamo- hipófise- adrenal (HPA) levando à produção de glucocorticoides imunossupresores no sistema nervoso periférico e simpático.*

PALAVRAS CHAVE: *Imunossupressão. Sistema imunitário. Procedimentos cirúrgicos operatórios. Analgésicos. Analgésicos opioides.*

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REVIEW ARTICLE

Lactose intolerance: what is a correct management?

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SUMMARY

Individuals with Lactose Intolerance (LI) tend to exclude milk from their diet since this behavior seems to relieve the symptoms caused by the disease. However, milk is a food of high nutritional value, and complete exclusion of dairy products may favor the development of bone diseases such as osteopenia and osteoporosis. The objective of this review was to evaluate the scientific evidence on the adequate management of patients with LI. This study was carried out from the review of the scientific literature in PubMed and SciELO databases. Complete exclusion of conventional dairy products is not necessary since most individuals with LI can tolerate up to 12 grams of lactose daily in a single dose. Yogurts and cheeses matured for having low amounts of lactose are part of the strategy that allows consumption of dairy products by patients with LI. Currently, there is a diversity of products considered as "milk substitutes" and supplements aimed at individuals with LI. However, these strategies still require better-designed studies.

KEYWORDS: *Lactose intolerance. Lactose. Lactase. Dairy products*

INTRODUCTION

The terms hypolactasia, lactose malabsorption (LMA), lactose intolerance (LI) are usually used as synonyms; however, they have different meanings.[1] Hypolactasia is the reduction in lactase enzyme activity, which can be classified as a primary, secondary or congenital disability. Some individuals with hypolactasia can present difficulty to digest, and consequently absorb, lactose and are considered poor lactose absorbers. When LAM is associated with the presence of symptoms such as diarrhea, flatulence, abdominal pain and/or abdominal distension, that is characterized as LI.[2,3]

Most individuals with LI usually exclude milk and dairy products from their diet, since this approach seems to mitigate the symptoms caused by the disease. However, it is known that the complete exclusion of such products is not necessary since most of these individuals can tolerate up to 12g of lactose in a single dose and have a good tolerance for fermented dairy products.[3] Cow milk has been widely consumed by populations of several locations for thousands of years. It has a high nutritional value and is a source of several nutrients, such as calcium, a protein of high biological value, magnesium, potassium, selenium and complex B and D vitamins.[3-5] Thus, the complete exclusion of such food items from one's diet can cause nutritional deficiencies which, in the long term, can

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help the onset of bone diseases, in addition to modifying the historical and cultural characteristics of a location.[3,4,6] Currently, there are several strategies for treating LI; however, there is controversy concerning their effectiveness.[3] Considering the nutritional and cultural importance of milk and dairy consumption and divergences concerning LI treatment, the objective of this review was to evaluate the scientific evidence on the correct management of patients with this disease.

MANAGING LACTOSE INTOLERANCE

Individuals with hypolactasia or LMA do not necessarily present the characteristic LI symptoms. Thus, treatment is recommended exclusively to those diagnosed with LI (Figure 1).[7]

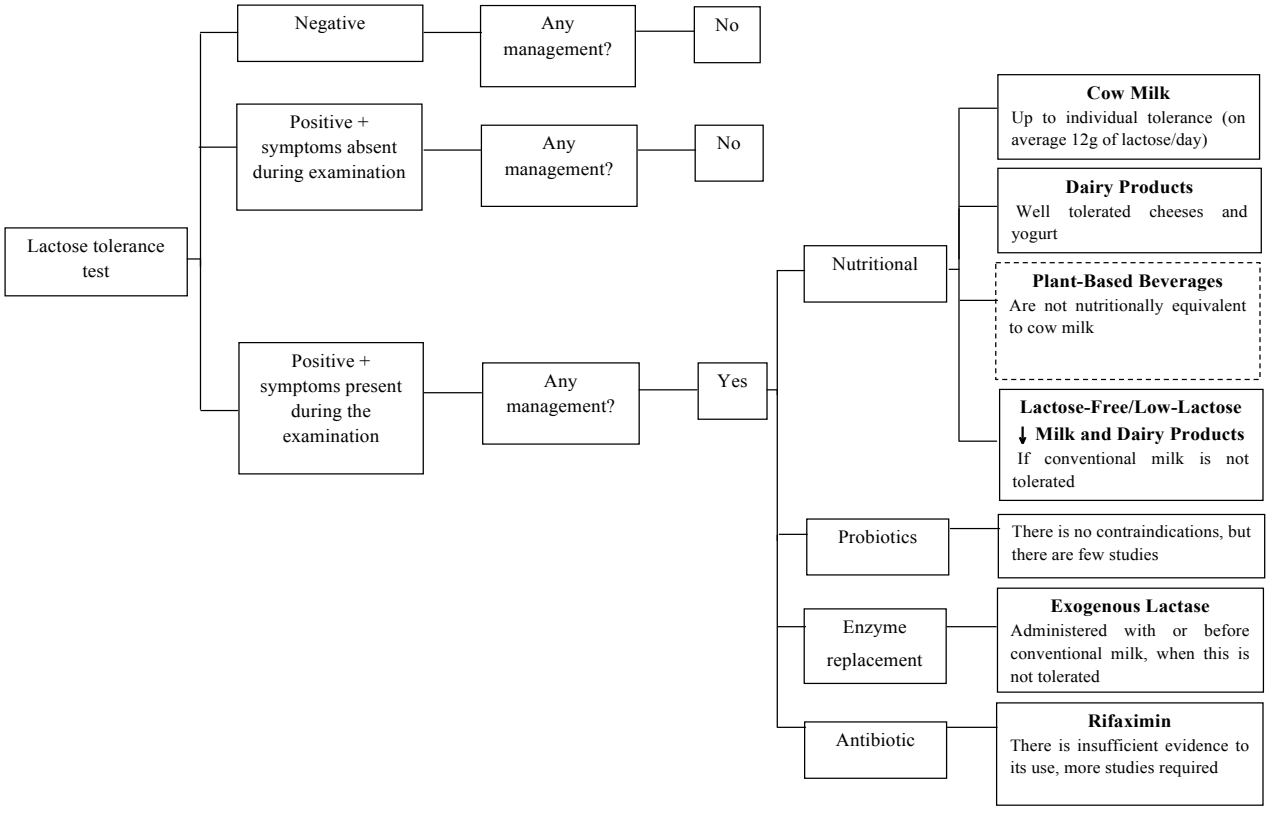
The LI treatment will depend on the etiology and is already well defined in the literature. If the LI is a consequence of primary hypolactasia, the consumption of lactose must be temporarily avoided so that there is a remission of the symptoms.[3,7,8] Most individuals with LI can tolerate up to 12 g of lactose per day (equivalent to 240 ml of milk) in a single dose.[3] Thus, milk and dairy products must be reintroduced respecting individual tolerance.[8] Regular and increasing consumption seems to reduce gastrointestinal symptoms from the bacterial adaptation in the colon, probably due to the increase of beta-galactosidase activity in feces. Nonetheless, results are still conflicting.[9]

If the LI is a consequence of secondary hypolactasia, all food that contains lactose must be temporarily excluded until the underlying disease is controlled.[8] It is noteworthy that individuals with diseases that affect the digestive tract, such as inflammatory bowel disease, will not necessarily develop LI. Thus, the exclusion of such foods must not be generalized.[10]

Some changes in diet can be helpful in improving lactose tolerance, such as portioning the daily amount of milk consumption into smaller doses, ingesting it with other foods, and ingesting fermented and matured dairy products, which naturally have low amounts of lactose.[8]

Individuals with a congenital lactase deficiency must exclude lactose from their diet permanently.[7]

FIGURE 1. DIAGNOSIS ALGORITHM AND MANAGEMENT OF LACTOSE INTOLERANCE



COW MILK

Cow milk is one of the primary sources of lactose. It has several nutrients, especially proteins with high biological value, bioavailable calcium and vitamin D for fortification. Thus, its permanent exclusion from one's diet can result in nutritional deficiencies, with a predisposition to the development of osteopenia and osteoporosis.[3,6,11] Although there are other sources of calcium, milk is one of the foods with a higher concentration and a high absorption rate of the nutrient. The high bioavailability of calcium in cow milk and in its products is attributed to the presence of lactose, caseinate, and citrate.[12]

According to the Food Pyramid adapted to the Brazilian population, in order to reach ideal levels of protein and calcium, it is recommended that healthy individuals consume three daily servings of dairy products.[13,14] Thus, they should be supplemented with calcium and vitamin D.[8,15] However, the effectiveness of ingesting this nutrient from non-dairy products and supplements must be evaluated concerning bone health.[3] Also, the isolated supplementation of calcium seems to be linked with the presence of gastrointestinal symptoms, kidney stone diseases, and other clinical complications.[16]

Nonetheless, these nutritional strategies may not be enough to improve LI symptoms.[15] As a result, the industry has produced supplements, medication, and food products targeted at this audience with the purpose of improving lactose tolerance.

DAIRY PRODUCTS

Dairy products derive from cow milk and usually present lower amounts of lactose.[17] One of the most widely consumed is yogurt, which comes from cow milk and is pasteurized, homogenized and added with two bacteria: *Streptococcus thermophilus* and *Lactobacillus Bulgaricus*.[18] These bacteria are capable of fermenting lactose, transforming it into lactic acid.[19,20] The amount of lactose present in 200 ml of yogurt is lower than what is usually tolerated (12 grams); that is why its consumption must be encouraged.[3,19] Some factor might influence the lactose amount in these products, such as the effectiveness of the bacterial lactase, resistance to gastric acidity, expiration date, and storage temperature.[19]

Like yogurt, cheese is a fermented dairy product that can be classified as fresh or matured. The first is ready for consumption right after production, while the second must go through biochemical and physical exchanges that are characteristics of each type.[21] Most lactose is found in milk whey, and cheese is made of, basically, of the solid portion, which makes it a dairy product with low amounts of lactose. Also, during the process of maturation, lactose is metabolized by lactic acid bacteria, which is why the final amount of lactose in maturated cheese is minimal.[22] Some cheeses, such as mozzarella, have only traits of lactose.[17]

PLANT-BASED BEVERAGES

Some individuals with LI consume plant-based beverages as an alternative,[23] since they are lactose-free. Their production process consists in extracting plant material in water and subsequently homogenizing it, which gives the liquid a milk-like texture and appearance.[24]

Most plant-based beverages have low levels of calcium and other nutrients. This nutritional deficit usually is related to the presence of antinutritional factors and the loss of nutrients during the heating process. Thus, this type of beverage should not be considered a suitable replacement for cow milk.[24] In addition to the low level of calcium,[25] soy-based beverages, one of the most widely consume types, have a poor acceptance due to its unpleasant taste, astringent and bitter, similar to "raw or green beans".[26]

In order to minimize or mitigate these problems, the industry has been using different techniques, such as adding enzymes to break the antinutrients,[27] adding micronutrients[24,25] and other additives, such as juices, fruit extracts, and sucrose to improve palatability.[28] However, the bioavailability of these nutrients and the effects of adding such products are not evident yet.

LACTOSE-FREE/LOW-LACTOSE MILK AND DAIRY PRODUCTS

Over the past years, the production of lactose-free or low-lactose food has been growing due to the desire to service individuals with LI and compensate for market losses due to a decrease in the consumption of the traditional products.[29]

In the industry, there are several methods for making this type of milk, and hydrolysis with soluble enzymes is considered the most simple one since it does not require special equipment. Despite the

TABLE 1 – NUTRITIONAL COMPOSITION OF CONVENTIONAL AND LACTOSE-FREE MILK

Brands	Nutritional composition	Whole milk powder (100 g)		Whole milk (100 ml)		Skim milk powder (100 g)		Skim milk (100 ml)	
		Traditional	Zero lactose	Traditional	Zero lactose	Traditional	Zero lactose	Traditional	Zero lactose
Brand 1	Protein (g)	25.77	25.77	3.30	3.30	34.5	-	3.20	3.20
	Carbohydrates (g)	36.92	36.92	4.40	4.40	50.0	-	5.00	5.00
	Lactose (g)	-	0.00	-	0.00	-	-	-	0.00
	Lipids (g)	27.31	27.31	3.30	3.30	0.00	-	0.00	0.00
	Calcium (mg)	919.23	919.23	115.00	115.00	1300	-	130.00	130.00
Brand 2	Protein (g)	26.15	-	3.30	3.30	34.50	-	3.40	3.40
	Carbohydrates (g)	38.46	-	4.70	4.70	50.00	-	4.85	4.85
	Lactose (g)	-	-	4.70	0.00	-	-	4.85	0.00
	Lipids (g)	26.54	-	3.10	3.10	0.00	-	0.00	0.00
	Calcium (mg)	911.54	-	119.00	119.00	2500	-	150.00	150.00
Brand 3	Protein (g)	26.15	18.08*	3.20	3.20	33.50	26.40*	3.20	3.20
	Carbohydrates (g)	38.08	57.69	4.90	4.90	50.00	60.00	5.00	5.00
	Lactose (g)	-	0.00	-	0.00	-	0.00	-	0.00
	Lipids (g)	26.92	18.85	3.45	3.45	0.00	0.00	0.40	0.40
	Calcium (mg)	946.15	734.62	119.00	119.00	2500.00	2000.00	167.00	167.00

Source: Product label[34].* Milk Composite.

low production costs, skim milk powder produced using this method does not have good acceptance by the consumers, since it has accentuated sweetness, besides being more prone to the Maillard reaction, which causes darkening and reduction of the nutritional value (deterioration of proteins) and changes in smell.[31]

In Brasil, until 2016, there was no legislation determining the maximum lactose allowed in such products and defining the expressions used in labels. That is why there is great variation in the information contained in labels and the actual amount of lactose in the product, which can lead the consumer to purchase products that do not meet their individual needs, especially of those with severe intolerance. Two resolutions concerning the labeling of food containing lactose, RDC135/2017, and RDC136/2017, were published in Brasil. Manufacturers will be required to add the words "*contém lactose*" [contains lactose] on the label of products containing over 0.1% of lactose.[32] The expressions "*Zero Lactose*", "*Isento de Lactose*" [Lactose Free], "*0% Lactose*", "*Sem Lactose*" [No Lactose] ou "*Não Contém Lactose*" [Does Not Contain lactose] can be added to products with a lactose content equal to or lower than 100 mg/100 g or ml. Those with contents over 100 g and lower than or equal to 1 g can have the expressions "*Baixo Teor de Lactose*" [Low Lactose Content] or "*Baixo em Lactose*" [Low Lactose] on the label. These last regulations must be complied with by the end of 2019.[33]

In the Brazilian market, there are several types of *Ultra High Temperature* (UHT) milk that are lactose-free or have low amounts of lactose, with no differences in nutritional content in comparison with conventional milk, according to the information contained on the label. In contrast, there are not many options of milk powder – most are a type of milk composite* – that have lower amounts of macronutrients and calcium (Table 1). That is why it is essential to, when recommending replacing conventional milk for a low-lactose or lactose-free substitute, the nutritionist must now the macro- and micronutrient composition of different brands, choosing the best products with the purpose of avoiding adverse impacts on the diet. These products have a calcium content similar to that of traditional products; however, there are few data about the effects of its consumption on bone mineral density in individuals with LI. Furthermore, there is insufficient evidence concerning their ingestion and the symptoms associated with the disease. They are usually more expensive products, costing up to 71.9% more than the lactose version of the same product,[34-36] so it is not something accessible to everyone.

PROBIOTICS

Probiotics are "living microorganisms capable of improving the intestines microbial balance, produc-

ing beneficial effects on health."[37] These microorganisms contain beta-galactosidase, which can contribute to the digestion of lactose in individuals with LI.[38]

Although recent studies show an improvement in LI symptoms after supplementation with *Lactobacillus Acidophilus*[39] and the combined use of *Lactobacillus casei Shirota* e *Bifidobacterium Breve*,[40] the use of probiotic for managing LI is not well established since the studies available have limitations in sample size, different inclusion criteria, types, sources, and concentrations of different probiotics.[34]

ENZYME REPLACEMENT THERAPY WITH EXOGENOUS LACTASE

The exogenous lactase enzyme, usually obtained from fungi or yeasts, acts as the natural lactase that is missing or with low activity. Such enzymes can be administered with milk or in the form of capsules, pills, or liquids before the intake of dairy products. The hydrolysis ability of the lactase depends on its origin, and enzyme activity is different for every commercial product.[41]

When evaluating the effectiveness of supplementation with this enzyme, some authors found a reduction of expired hydrogen and improvement of LI symptoms.[42,43] However, the supplement is not capable of fully hydrolyzing the lactose, with different results in each individual.[15] There are reports on the association of lactase supplements and allergic reactions and anaphylaxis.[44] Thus, more studies are needed to evaluate lactase and its hydrolysis ability in order to ensure more safety when using this supplement.

RIFAXIMIN

Rifamixin is a broad-spectrum antibiotic capable of destroying anaerobic bacteria that ferment lactose, producing hydrogen, carbon dioxide, and methane.[45] It is believed that LI symptoms are related to the composition of the intestinal microbiota since different bacteria metabolize lactose in different ways.[46] Thus, the use of this antibiotic could contribute to the treatment of LI.[47]

Some authors have shown that the use of this drug reduced the production of hydrogen and gastrointestinal symptoms in individuals with LI.[47] However, the evidence available is insufficient for using this antibiotic in the management of the disease.[34] Since its effectiveness has been proved, it is necessary to perform studies with prolonged administration of rifaximin and monitoring for a more extended period, to meet the need of this population.[47]

CONCLUSION

Excluding milk and dairy products from the diet of LI patients is not recommended in general, considering such products can be tolerated using some diet strategies. This would allow the maintenance of palatability of food preparations, cultural habits, and adequate nutritional supply. In order to assure an improved tolerance in these patients, some therapeutic strategies have emerged; however, its benefits and safety are still unclear. Given this scenario, we need scientific studies to evaluate the effectiveness and safety of these strategies, including an investigation of the nutritional composition and bioavailability of nutrients in plant-based beverages and lactose-free milks.

RESUMO

Os indivíduos com Intolerância à Lactose (IL) tendem a excluir o leite da alimentação, uma vez que essa conduta parece aliviar os sintomas ocasionados pela doença. Entretanto, o leite é um alimento de alto valor nutricional e a exclusão completa dos laticínios pode favorecer o desenvolvimento de doenças ósseas como osteopenia e osteoporose. O objetivo desta revisão foi avaliar as evidências científicas sobre o manejo adequado de pacientes com IL. Este estudo foi realizado a partir da revisão da literatura científica nas bases de dados PubMed e SciELO. A exclusão completa dos produtos lácteos convencionais não é necessária, pois a maior parte dos indivíduos com IL consegue tolerar diariamente até 12 g de lactose em uma única dose. Os iogurtes e queijos maturados, por terem baixa quantidade de lactose, fazem parte da estratégia que garante consumo de produtos lácteos por pacientes com IL. Atualmente existe uma diversidade de produtos considerados "substitutos do leite" e suplementos voltados para os indivíduos com IL, no entanto, essas estratégias ainda requerem estudos mais bem desenhados.

PALAVRAS-CHAVE: *Intolerância à lactose. Lactose. Lactase. Laticínios.*

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REVIEW ARTICLE

Advances in medical technology and new digital educational platforms

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KEYWORDS: *Technology, Telehealth, CardioTrials, CardioLearning, inovation*

INTRODUCTION

Mediums of communication had evolved exponentially over time, and several technology peaks have changed the way we see and understand the world (Figure 1). Over the last decades, we have witnessed a cybernetic revolution motivated by an increase in the density of such technology peaks. Where this revolution will lead humanity remains a mystery.[1,2]

THE IMPACT OF TECHNOLOGY ON MEDICINE

Some areas of professional activity have been deeply affected by these new technology trends. Especially in medicine, there are changes taking place from educational practices – with hybrid systems that mix long-distance learning platforms and in-person activities with realistic simulations – to medical assistance, with the use of apps and devices to assist in the decision making process with the patient.[3]

Following this trend of medical advances, along with the greater diffusion and integration of knowledge that the cybernetic revolution has brought, there has been a spontaneous scientific trend towards medical practices with an increasingly objective academic approach, which marked the beginning of the "evidence-based medicine" era.[4]

It that context, we saw a boom in academic publications over the past two decades, reaching almost 250 published papers in 2016. (Figure 2).[5,6]

However, although technology facilitated the creation, diffusion, and access to digital platforms, it also fostered an environment prone to the dispersion of attention and content, making it difficult to take advantage of all the material produced. Another negative factor of this "technological paradox" is the medical activity itself, which is becoming increasingly intense with very high workloads, making it even

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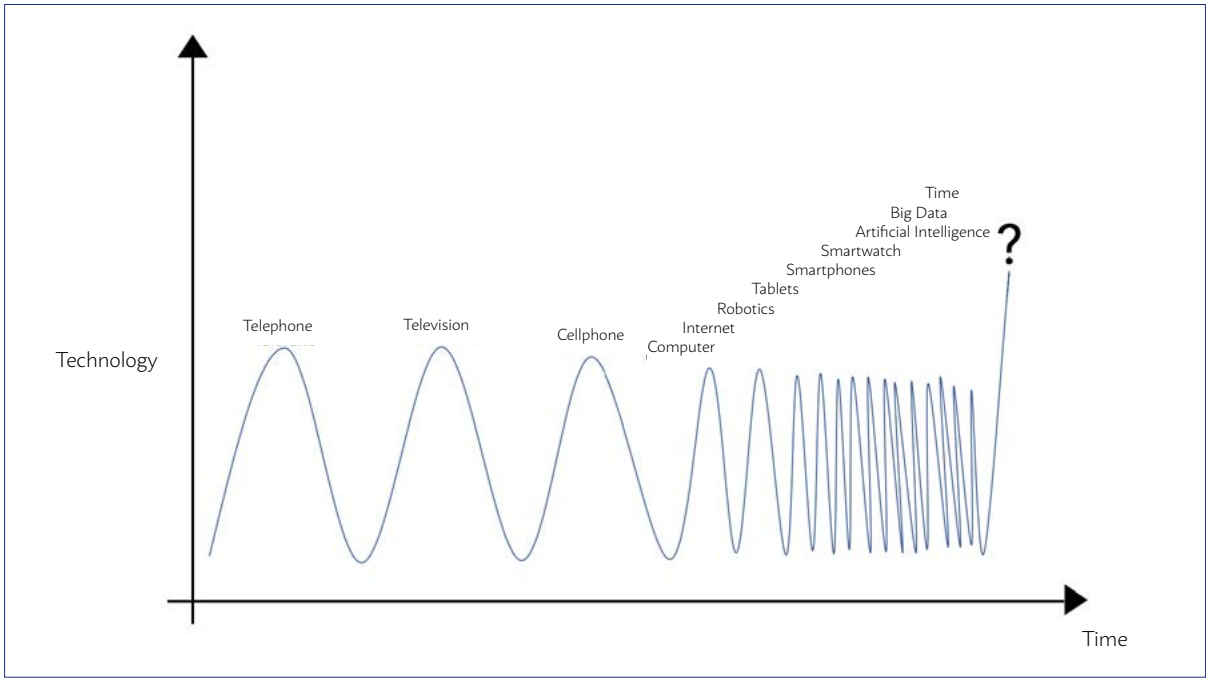

FIGURE 1 - TECHNOLOGICAL GROWTH

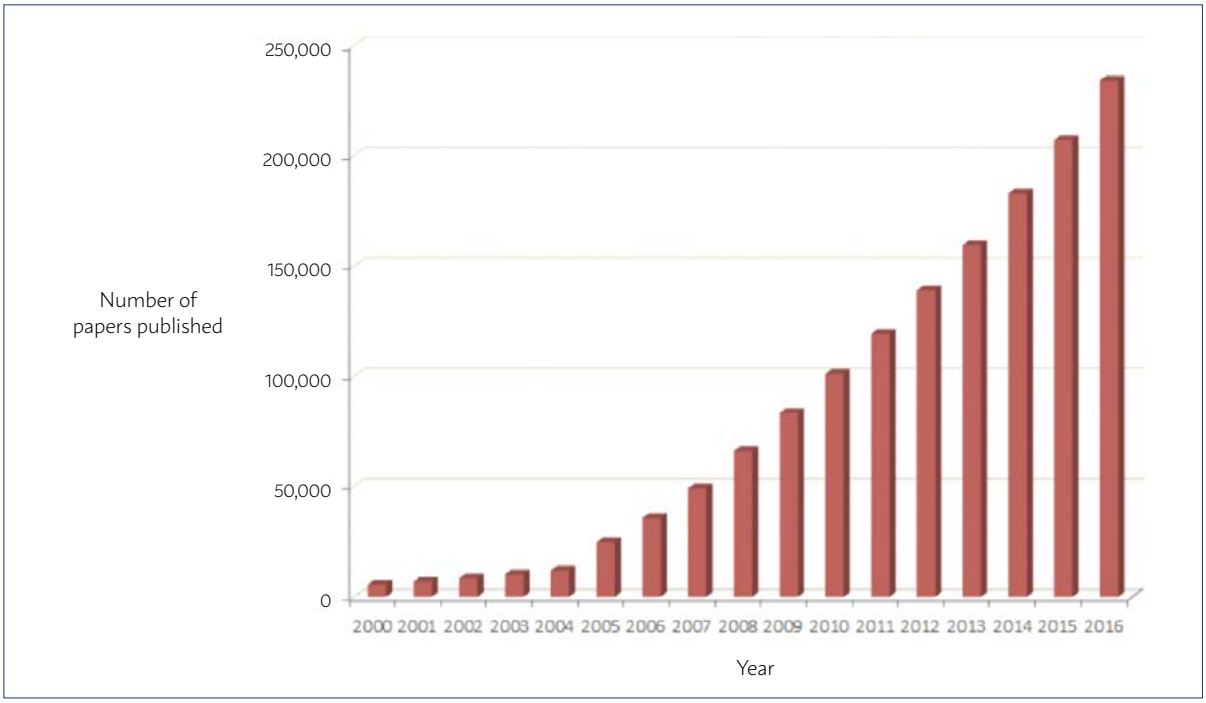

FIGURE 2 - PUBLICATIONS IN RECENT YEARS. SOURCE: ADAPTED FROM CLINICALTRIALS.GOV

more difficult to stay updated with the latest medical findings.[7]

The ever-growing knowledge, the creation of multiple digital platforms, and the enormous amount of simultaneous information that gets dispersed combined with the scarce time to consume it all created a new generation of doctors who need information facilitators, such as electronic devices that ensure quick access to information that is pragmatic and with high technical qualifications.[8,9,10]

CARDIOTRIALS APP

It was the understanding of this scenario that motivated the creation of the CardioTrials app (Figure 3), whose purpose is the critical analysis of scientific papers published in major cardiology journals. The app is an actual database of hundreds of papers updated weekly.

The app was conceived for health professionals, including nurses, physical therapists, and doctors of all specializations but, more specifically cardiol-

FIGURE 3 - CARDIOTRIALS APP

FIGURE 4 - APP SCREENSHOT

FIGURE 5 - APP SCREENSHOT

ogists. In Brazil, 100-thousand professionals are expected to benefit from this free and high-quality scientific product.

The papers are divided into 15 subspecialties of cardiology (categories) to facilitate their access and search (Figure 4).

The app brings the leading medical studies that guide medical conduct, all summarised and translated into Portuguese.

All articles described are subdivided into quick topics to facilitate reading and understanding: acronym, title, magazine, publication date, objective, comparison groups, methods, inclusion/exclusion criteria, baseline, outcomes, perspective, conclusion, reference, link and sponsors (Figure 5).

Thus, the CardioTrials App emerges as an important platform for the dissemination of knowledge in the current scientific scenario. The app brings a dynamic, simple and pragmatic solution for the impairments and difficulties of keeping up to date with the latest medical knowledge, being a extremely useful tool for professional training in this new technological era.

FIGURE 6 - CHANNELCARDIOLEARNING BRINGS TWO WEEKLY VIDEOS, NO LONGER THAN 5 MINUTES EACH

FIGURE 7 - OPENING OF THE "COFFEE AND JOURNAL" VIDEO

FIGURE 8 - OPENING OF THE "COFFEE AND JOURNAL" VIDEO WITH A CONFERENCE COVERAGE

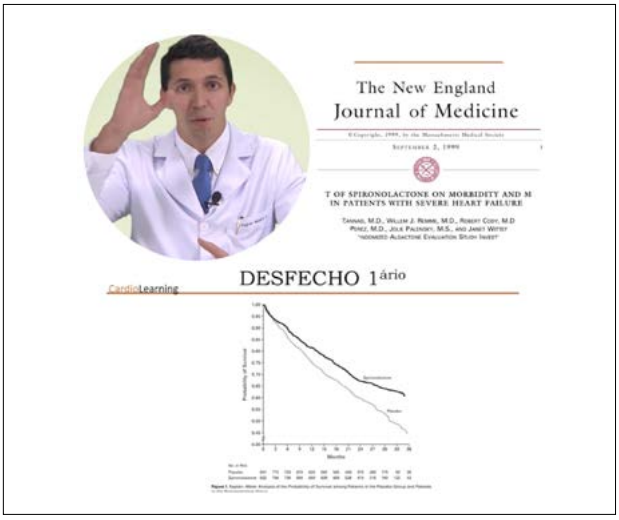

FIGURE 9 - OPENING OF THE "5'TRIALS" VIDEO

CARDIOLEARNING PROJECT

Other cybernetic platforms, in addition to apps, are emerging to add even more to this process of knowledge diffusion and facilitation. The current educational model, which requires a fast track approach to learning, motivated the creation of the CardioLearning YouTube channel (Figure 6).

The first weekly video, called "Coffee and Journal" (Figure 7), posted on the beginning of the week, is an editorial in the form of short videos. In it, editors make a summary of the main articles published on cardiology during the week in a newscast format, bringing headlines of the most important updates. In addition, the channel covers the major cardiology conferences (Figure 8).

The second weekly video, posted during the weekend, is called "5' Trials" and brings in-depth discussions with critical analyses of the greatest trials in cardiology history – all that in a maximum of 5 minutes(Figure 9).

CONCLUSION

The technological revolution has brought several permanent changes in the medical area, which made possible unprecedented scientific growth. With the huge volume of content produced, devices that facilitate the access to knowledge have become essential for keeping up to date with medical findings. In this scenario, apps like CardioTrials and educational platforms like the CardioLearning Project are noteworthy diffusers of high-technical-quality knowledge with an academic commitment.

Statement of contribution

Vagner Madrini Junior, Francisco Akira Malta Cardozo, Brenno Rizerio Gomes, Mozar Suzigan de Almeida contributed in writing this article. Thiago Luis Scudeler was responsible for the scientific review and contributed with the intellectual concept of the study.

PALAVRAS-CHAVE: *tecnologia, telessaúde, CardioTrials, CardioLearning, inovação*

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REVIEW ARTICLE

Uremic neuropathy: an overview of the current literature

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SUMMARY

INTRODUCTION: *Peripheral neuropathy is a disorder that affects the cell body, axon or myelin of motor or peripheral sensory neurons and occurs in 60-100% of patients who are submitted to dialysis due to chronic kidney disease. Uremic neuropathy is attributed to the accumulation of organic waste, evident in patients with reduced glomerular filtration rate. Objectives: This review aims to make clinical characteristics of uremic neuropathy evident enabling early diagnosis and treatment. Methods: This is a literature review of articles published on PubMed over the last 10 years using "Uremic Neuropathy" as "Title/Abstract". Results: A total of nine articles that met the inclusion criteria were included. UN is a distal symmetric sensorimotor polyneuropathy that occurs due to the accumulation of uremic toxins associated with an oxidative stress-related free radical activity. Hyperkalemia is thought to play an important role in its pathophysiology. Diagnosis depends on nerve conduction studies, and treatment includes dialysis or renal transplant. Conclusion: Clinical presentations of UN are broad and non-specific; nonetheless, it is important to detect early changes in order to avoid its progression. The earlier UN is diagnosed and treated, the more successful are the clinical outcomes.*

KEYWORDS: *Neural conduction. Dialysis. Kidney Transplantation. Peripheral Nervous System Diseases. Uremia/complications.*

INTRODUCTION

Peripheral neuropathy (PN) is a disorder that affects the cell body, axon or myelin of motor or peripheral sensory neurons and can respectively be classified as neuropathological, axonal or demyelinating. This condition is either hereditary or acquired and may be further subdivided into sensory, motor or autonomic [1]. PN has a large spectrum of causes (such as nutritional deficiencies and toxic neuropathies [2] as well as clinical presentations [3]; however, constant and recurring pain occurs in almost all types of this disorder [4].

The overall prevalence of peripheral neuropathy is 2.4%. However, this number increases exponentially in certain age groups, and it may even be an underestimate since traumatic causes are not included in this percentage [3].

Peripheral neuropathy occurs in 60-100% of patients who are submitted to dialysis due to chronic

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kidney disease (CKD) [5]. Uremic neuropathy (UN) occurs when renal dysfunction impairs filtration, leading to the accumulation of organic waste. This is evident in patients with reduced glomerular filtration rate (GFR) usually attributed to end-stage renal disease (ESRD) [6].

This review aims to make the clinical characteristics of uremic neuropathy evident enabling early diagnosis and treatment in order to prevent the effects of the advanced stages of this condition. The secondary purpose is to discuss the prognosis of uremic neuropathy based on data hinted in literature.

METHODS

This is a literature review of articles published on PubMed over the last 10 years using "Uremic Neuropathy" as "Title/Abstract". A total of 15 articles were found and 11 of them were available. Then, 9 articles were included as they met the inclusion criteria (Figure 1) – they were clinical studies and discussed uremic neuropathy.

RESULTS

Prevalence

In 1961, Martin and Tyler published the first report on uremic neuropathy in patients with hereditary intestinal nephritis with distal sensory-motor polyneuropathy[7,8]. Asbury et al, in 1963 used the term uremic polyneuropathy to describe distal sensorimotor changes due to uremic toxins. Uremic neuropathy is more frequent in males than in females[8] and is a common condition: studies have shown that it's prevalence varies from 50-100% in patients with chronic kidney disease [7,9-11]. This large range of values is due to the application of different criteria for the diagnosis of UN. The prevalence of UN in the pediatric population is unknown [10].

Pathology and pathophysiology of Uremic Neuropathy

UN is a distal symmetric sensorimotor polyneuropathy that typically affects lower limbs [7,12] and is due to length-dependent axonal degradation and secondary focal loss of myelin sheaths [7,8]. This is considered a demyelinating condition which leads to axonal degeneration and loss [8-10].

The accumulation of uremic toxins (the "middle toxins": guanidine compounds, parathyroid hormone, and myoinositol) [10,11] associated to oxidative stress-related free radical activity causes motor, sensory and autonomic nerve damage which leads to UN [8,13,14]. Although this exact mechanism remains unknown [10], there are hypotheses supporting the role of electrolytes in this process [8,9,12]. Hyperkalemia and hyperphosphatemia cause chronic uremic depolarization of nerves, contributing to the development of UN [8,12]. This occurs because potassium disrupts the normal ionic gradient and therefore activates calcium-mediated processes leading to axonal death [9].

UN is usually asymptomatic until renal function is under 15%, and glomerular filtration is lower than 10–12 ml/min, which usually happens 10-15 years after the onset of the underlying disease, such as di-

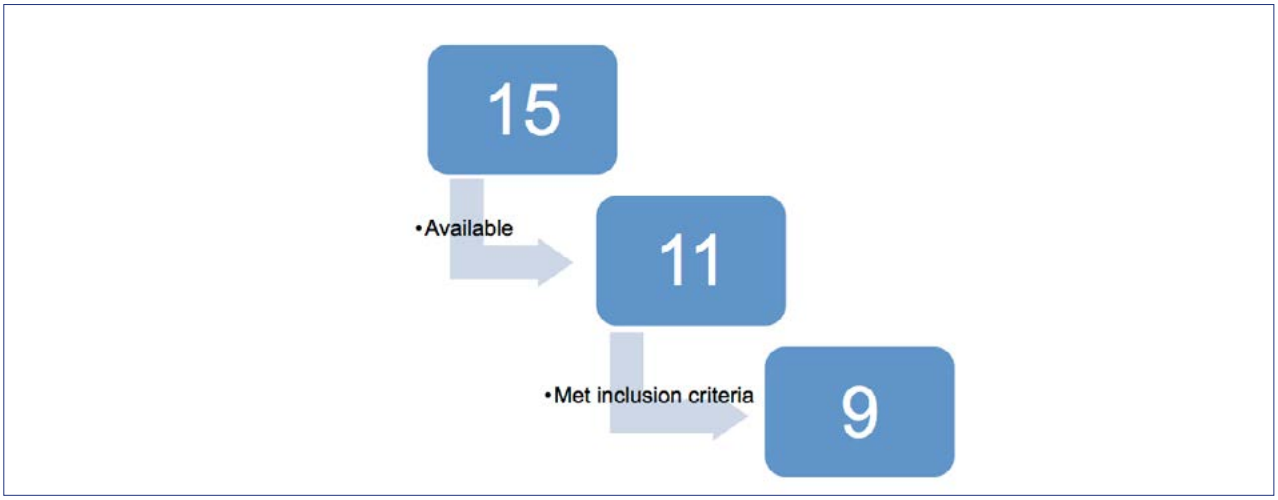

FIGURE 1. SELECTION PROCESS OF ARTICLES INCLUDED IN THIS REVIEW.

FIGURE 2. REPRESENTATIVE SCHEME OF UREMIC NEUROPATHY POSSIBLE CAUSES

abetic neuropathy [7,8,10,11]. This type of neuropathy is one of the most frequent neurological manifestations of end-stage renal disease (ESRD) [11].

Both slowly and rapidly progressing sensorimotor axonal neuropathies are relatively common [10]. However, there have also been reports of fulminant motor neuropathies, which occurred under specific clinical conditions such as sepsis and severe CRF [8].

Hyperkalemia and hyperphosphatemia increase the risk of developing UN [9]. Other risk factors for UN are diabetes, advanced age and low creatinine and clearance [10].

A schematic representation of uremic neuropathy causes can be seen in Figure 2.

Symptoms of Uremic Neuropathy: Sensory and motor changes due to Uremic Neuropathy

Symptoms of UN vary, but it typically presents as a slowly progressing sensorimotor axonal neuropathy [9] which advances proximally, starting from the lower limbs and may spread to upper extremities [7]. Early symptoms are paresthesia, paradoxical heat sensation, restless leg syndrome, increased pain sensation, and cramps [7,9]. Long-term symptoms include weakness, impaired deep tendon reflexes, imbalance, numbness, and atrophy of the lower limbs [7-11].

Nerve conduction and quantitation of sensory loss

Quantitation of sensory loss and nerve conduction is one of the main tools used to evaluate UN, as well as electromyography [11]. In patients suffering from this condition, nerve conduction velocity usually falls to 50-60% of normal values [8]; however, light touch and vibratory perception thresholds are more sensitive to evaluate either progression or recovery of UN than conduction velocity [8].

Some patients suffer thermal sensitivity impairment before they have sensory and motor damage. The number of functional axons in a nerve is evaluated according to changes in the amplitude of muscle response and sensory nerve action potentials [8]. The myelination of nerve fibers and their density is tested by velocity conduction. The most common morphological change in UN is the loss of large myelinated fibers, and positive neuropathic symptoms tend to correlate with quantitative results in conduction and sensory tests [8].

After nerve transplantation, there is an increase in nerve conduction due to remyelination [8]. Studies have shown that motor nerve conduction (MCV) is a significant predictor of mortality [13] in hemodialysis patients and achieves statistically significant values: (HR= 0.92; CI (0.86–0.99); $p < 0.05$) [15]. MCV

correlates significantly with dialysis dose; however, further investigation is needed in order to confirm this hypothesis [13].

Uremic Neuropathy in Children and Teenagers

The prevalence of UN in children is unknown [10], and this population usually does not present clinical evidence of UN; however, nerve conduction is altered, likewise in adults [8]. Authors showed that mean peroneal motor nerve conduction velocity (MNCV) was significantly decreased in children with mild renal failure (serum creatinine concentration, 1.5 to 2.9 mg/dL, normal range: 0.8–1.2 mg/dL), while ulnar MNCV was significantly decreased only when the serum creatinine value was at least 9 mg/dL. Within a year of renal transplantation, ulnar MNCV tends to return to normal values, and it takes 3 years for peroneal MNCV to go back to baseline values.

These parameters could potentially be used to evaluate the development of UN. However, they are only meaningful when renal function is very low or after a long period of time. Therefore, the periodic measurement of nerve conduction velocity is not useful to follow UN in children undergoing chronic hemodialysis [8].

Uremic Optic Neuropathy

Uremic optic neuropathy (UON) is a possible manifestation of UN that causes sudden vision deterioration and involves focal edema of the optic nerve head [14]. Other related ophthalmic disorders include swelling of optic nerve heads, blurred margins of the optic disks seen using an ophthalmoscope. This disease should be taken into consideration as a possible diagnosis when patients with advanced chronic kidney failure present vision deterioration.

Seo et al. [14] described a patient suffering from UON who presented all the manifestations mentioned above, and the visual-evoked potential tests revealed reduced amplitude and increased latency in one of the eyes. This patient was treated with hemodialysis and corticosteroids, and his visual acuity and visual field improved, and the optic disk swelling was resolved.

The pathogenesis of UON is not well known. However, it has been shown to be related to the accumulation ofthat dialyzable toxin metabolites. Hemodialysis in combination to corticoids is the standard treatment for UON [14].

Diagnosis

The gold standard method to diagnose UN is a nerve conduction study [7,9]. Complementary methods include neurological assessment and biopsy of distal axons [12]. Since some UN symptoms are subjective and cannot be quantified using clinical tests, it might also be interesting to include psychological evaluation in order to investigate UN [15]. UN, however, may remain asymptomatic for a long time and only cause symptoms when severe damage has already been done. Therefore, an investigation should take place even in asymptomatic patients who present risk factors for UN [7].

Treatment of Uremic Neuropathy

Treatments that may reverse the effect of UN and improve nerve function are dialysis and renal transplantation [7,9,10]. Studies have shown a more significant reduction in the progression of UN due to an increase in dialysis dose, be it peritoneal or conventional dialysis [9]. It is controversial whether patients submitted to peritoneal dialysis have inferior results in the treatment of UN compared to those submitted to conventional hemodialysis [11]. Hemodiafiltration is another therapeutic option that also benefits motor nerve excitability.

Renal transplantation is the only definite treatment that interrupts the progression of UN and reverts symptoms. It is important to note that results are inversely proportional to the disease duration prior to the transplant: a shorter disease time leads to better post-transplant clinical outcomes [10]. Nonetheless, in patients who may not undergo this procedure, either due to clinical restrictions or personal denial, effective dialysis is definitely a good therapeutic option [11] as it also normalizes most nerve excitability parameters [8].

Comparison of uremic neuropathy with other types of neuropathies:

There are many different types of neuropathies other than Uremic, including Diabetic, Alcoholic, Chronic Inflammatory, and Infectious. Below is a comparative table with the symptoms and treatments of these main types of neuropathies.

TABLE 1. ASSOCIATION OF DIFFERENT TYPES OF NEUROPATHY AND ITS TREATMENTS

NEUROPATHY	MAIN SYMPTOMS	TREATMENT
Uremic Neuropathy	-Pain, numbness, and tingling in feet and legs; -Cramps, muscle twitches, or increased pain sensation in the feet and legs; -Muscle weakness [16]	-Dialysis -Kidney Transplant [16]
Diabetic Neuropathy	-Slowly and progressive primarily sensory deficit following a "stocking-glove distribution" [17]	-Treatment can range from lifestyle modification and strict glucose control to the use of immunosuppressant medications [17,18]
Alcoholic Neuropathy	-Painful paresthesia; -Muscle weakness; -Sensory and motor symptoms extend proximately into the arms and legs; -Gait impairment may be present [19,20]	- Thiamine treatment has been shown not to be effective; - Therapy ought to include cessation of alcohol ingestion, aiming at the toxic target(s) of alcohol [22]
Chronic Inflammatory Neuropathy	-Weakness is typically symmetric and characteristically involves proximal and distal muscles; -Sensory symptoms include numbness, tingling, gait imbalance, and, at times, painful paresthesias [21]	-Steroids, plasma exchange, and intravenous immunoglobulin may be used as 1st line treatment options [21]
Infectious neuropathy (HIV, HCV, CJ, LD[23])	-HIV, HCV, LD: pain, weakness, paresthesia and absent ankle reflexes -CJ: acute, ascending, motor neuropathy associated with Guillan-Barré syndrome (GBS) [23,24]	-HIV: analgesics, anticonvulsants [24] -HCV: steroids, antiviral therapy, rituximab, interferon alfa [25,26] -LD: doxycycline, amoxicillin, cefuroxime,ceftriaxone [27-29] -CJ: azithromycin, erythromycin, fluoroquinolones; treatment for GBS such as plasmapheresis, plasma exchange and intravenous immunoglobulin [30,31]

CONCLUSION

UN is a prevalent condition, affecting 60 to 100% of patients who suffer from chronic kidney disease, depending on the classification criteria used. The exact mechanism of the demyelinating process leading to axonal degeneration and loss is still uncertain. However, electrolytes, such as potassium, have shown to play an important role in the pathophysiology of UN. Clinical presentations of UN are broad and may be non-specific; nonetheless, it is essential to detect early changes in order to diagnose UN and avoid its progression. The earlier the signs and symptoms of UN are detected, the earlier UN is diagnosed using nerve conduction studies and treated with dialysis or renal transplant, leading to greater clinical success.

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RESUMO

INTRODUÇÃO: *A neuropatia periférica (NU) é um distúrbio que afeta o corpo celular, o axônio ou a mielina do motor ou neurônios sensoriais periféricos e ocorre em 60%-100% dos pacientes que são submetidos à diálise por doença renal crônica. A neuropatia urêmica é atribuída à acumulação de resíduos orgânicos, evidente em pacientes com taxa de filtração glomerular reduzida. Objetivo: O objetivo desta revisão é fazer com que as características clínicas da neuropatia urêmica sejam evidenciadas, permitindo o diagnóstico e tratamento precoce. Método: Esta é uma revisão da literatura de artigos publicados no PubMed nos últimos dez anos usando "Neuropatia Urêmica" como "Título/Resumo". Resultados: No total, foram incluídos nove artigos que atendem aos critérios de inclusão. A NU é uma polineuropatia sensório-motora simétrica distal que ocorre devido ao acúmulo de toxinas urêmicas associadas à atividade de radicais livres relacionados ao estresse oxidativo. A hipercalemia tem um papel importante na sua fisiopatologia. O diagnóstico depende de estudos de condução nervosa e o tratamento inclui diálise ou transplante renal. Conclusão: As apresentações clínicas das NU são amplas e não específicas; no entanto, é importante detectar mudanças iniciais para evitar sua progressão. Quanto mais precoce for a detecção e tratamento da NU, melhor será o resultado clínico.*

PALAVRAS-CHAVE: *Condução nervosa. Diálise. Transplante de rim. Doenças do sistema nervoso periférico. Uremia/complicações.*

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Comment: "Mapping the scientific research on the negative aspects of the medical school learning environment"

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The term learning environment encompasses a set of physical, social, and psychological contexts in which a student learns [1]. This is a broad concept that includes the teaching and learning processes, as well as resources and technology, teaching strategies, and connections to society and the global scenario. Medical educators have the responsibility of continuously reassessing their curriculum and the learning environment, rethinking the roles of teachers and students in the construction of a healthy and appropriate learning environment. Most educators agree that the main goals of the education process are to promote students' wellbeing and a healthier environment for the society in which they live. Nevertheless, how should we achieve these goals without a secure and comfortable learning environment?

The study published by Damiano et al. [2] in this edition of RAMB is an attempt to answer this question. Brazilian medical education is facing a serious moment right now, with several reports of suicide attempts and mental health problems in medical students. Some learning environment aspects could be responsible for these outcomes such as the work overload, little time to extra-curricular activities, teacher-centered activities, limited social and psychological support, unethical faculty behaviors, hidden curriculum, and discriminations [3]. Thus, revisiting the scientific articles on the negative aspects of the learning environment at medical schools around the world is a meaningful way to understand which evidence we have now and what should we do to advance this field of research.

In the comprehensive bibliometric analysis proposed by Damiano et al. [2], authors identified the most important areas in this field, the type of articles, the trends of publications and the most prolific authors and countries. It is quite clear in their results that the interest in this issue is growing in the last decades, although more experimental, longitudinal, and cross-cultural study designs are needed. Knowing and understanding the positive and also the harmful practices in medical education can foster a broader discussion in this field of research and empower educators to make appropriate changes in their learning environments in order to achieve a desired inclusive education. In my opinion, this article could serve as excellent reading for those interested in education and in the formation of medical and healthcare students. It also arrives in a very good moment for the Brazilian scenario.

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ERRATUM

Regarding the article "Clinical features of patients with chronic non-specific neck pain per disability level: A novel observational study", with DOI number: http://dx.doi.org/10.1590/1806-9282.64.08.700, published in Journal of the Brazilian Medical Association, 2018;64;08, page 700:

Where was written: "Roy La Touche , PT, PhD[1,2,3,4]"
Now Read: "Roy **LA TOUCHE** , PT, PhD[1,2,3,4]"


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quem faz as perguntas.
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