RAMB

JOURNAL OF THE BRAZILIAN MEDICAL ASSOCIATION

AMB Associação Médica Brasileira

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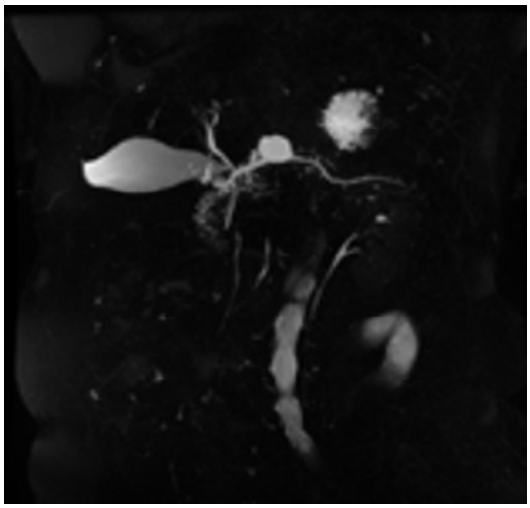
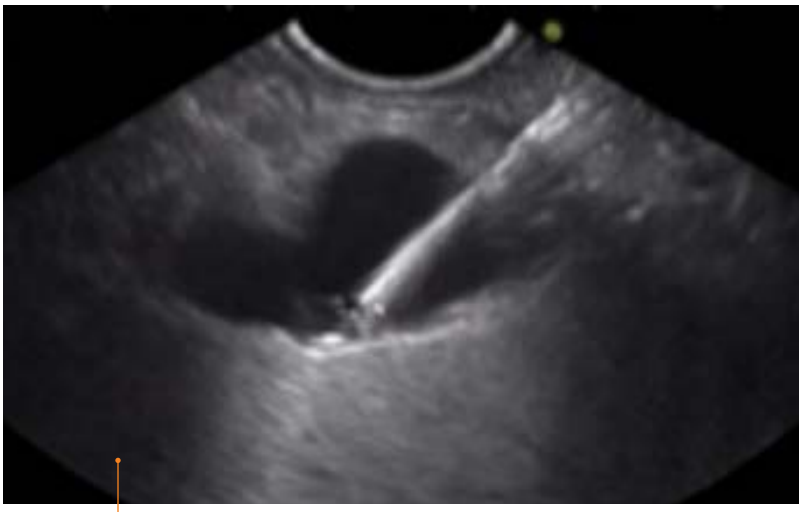
Volume **64** Number **10** **October 2018**
ISSN **0104-4230**
ISSN **1806-9282** (On-line)





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RAMB REVISTA DA ASSOCIAÇÃO MÉDICA BRASILEIRA
JOURNAL OF THE BRAZILIAN MEDICAL ASSOCIATION

RAMB - REVISTA DA ASSOCIAÇÃO MÉDICA BRASILEIRA (JOURNAL OF THE BRAZILIAN MEDICAL ASSOCIATION)

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The RAMB, Journal of The Brazilian Medical Association, *is an official publication of the Associação Médica Brasileira (AMB – Brazilian Medical Association), indexed in Medline, Science Citation Index Expanded, Journal Citation Reports, Index Copernicus, Lilacs, and Qualis B2 Capes databases, and licensed by Creative Commons®. Registered in the 1st Office of Registration of Deeds and Documents of São Paulo under n. 1.083, Book B, n. 2.*

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ORIGINAL ARTICLES

Over-the-counter medications potentially inappropriate for the elderly

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http://dx.doi.org/10.1590/1806-9282.64.10.869

The use of over-the-counter medication – with no need for prescriptions – is part of human behaviour[1]. The Brazilian Health Regulatory Agency (Anvisa) does not consider that to be a relevant factor for public health since they periodically publish a list of over-the-counter medications that are deemed of low-risk for patient health and/or adequate for treating less severe conditions for short periods of time[2].

It is noteworthy that self-medicating poses a potential for adverse reactions and drug interactions just like prescription drugs. That is even more palpable for patients, such as the elderly, who make long-term concurrent use of several medications[3].

The common knowledge that the elderly often face serious drug interaction problems and adverse reactions led Beers et al[4-8] (1991, 1997, 2003, 2012, and 2015) to develop a set of criteria for potentially inappropriate medications for 65-year-old adults or older. Potentially inappropriate medications (PIM) for the elderly are defined as those that present higher risks of causing adverse effects than of generating benefits for patients in this age group.

There are several other lists of PIM for the elderly, and they are all useful in clinical practice to assist in the prevention of iatrogenesis[9-12]. However, since Beers' criteria are the most reviewed, updated and most often cited in the literature, they have become the essential reference for PIM for the elderly.

Thus, that is the question that inspired this editorial: Are there, among over-the-counter medications in Brazil, any potentially inappropriate medications for the elderly?

We analyzed a list of drugs exempt from prescriptions that was published on the *Diário Oficial da União* on October 30 2016, according to the Beers' criteria (2015)[2,8]. We found that five therapeutic groups included in the criteria – 14.3% of the groups total – were present on the list of drugs analyzed (antacids/antiemetics, antispasmodics, antihistamines, anti-inflammatory, and muscle relaxants)[2,8].

The version of the Beer's criteria used (2015) does not include topical medications and eye drops, which were present on the list of drugs exempt of prescription[2,8].

Considering that the elderly make long-term concurrent use of several medications, inappropriate use has become a common situation among this age group[1,13]. Self-medicating contributes to the development of drug interactions and adverse effects – often attributed to pre-existing conditions and/or the "age"

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factor –, which in turn leads to the prescription of other drugs by the health professional, to minimize the symptoms[1,13-15]. Before that decision – referred to as a prescription cascade – it is also worth to ask the patient about the use of over-the-counter medications[15,16].

Even if we consider the self-medicating process greater than that of the use of over-the-counter medications, we must acknowledge and measure the inherent health risk caused by the frequent use of this type of drug. That is the justification for the objective of this editorial and the use of Beers' criteria (2015) as a quantitative and qualitative assessment scale for over-the-counter medications[8].

Halila et al[17] (2015) analyzed systematic reviews published on over-the-counter medications and their effectiveness and safety. They found that most of the studies included in the review were favorable to the use of some of these medications but considered that some therapeutic groups require greater evidence to be considered safe for free consumption. The categories of drugs that are exempt from prescription considered PIM for the elderly according to the Beers' criteria (2015) – antacids/antiemetics, antispasmodics, antihistamines, anti-inflammatory, and muscle relaxants – were included amongst those in need of concrete evidence regarding their safety[2,8,17]. Even though this was not the point of this editorial, the systematic review by Reis e Figueiras[18] (2010) on the week evidence of effectiveness and safety of cough medication registered in Brazil is noteworthy. The same is said about the variations in quality of seven different brands of dipyrone (oral solution) sold in drugstores in cities in the interior of the state of Paraná, as observed by Knappmann e Melo[19] (2010).

Seeking to identify the instruments that assess the quality of prescriptions written for the elderly and which drugs could be considered PIM for this age group, Varallo et al.[20] (2014) conducted a review of publications on the subject and identified 15 instruments, including a version of the Beers' criteria before 2015[7]. They considered 15.2% of the over-the-counter drugs sold in Brazil to be PIM for the elderly, a number close to that found by this editorial (14.3%).

These numbers are incredibly relevant, for 26% of the elderly who attended the Elderly Healthcare Clinic of the Hospital of the Catholic University of Brasilia declared they self-medicated, and 40% of these medications were over-the-counter. The types of medication most often used with no medical supervision were nonsteroidal anti-inflammatories, analgesics, antipyretics, phytotherapics, and those of cardiovascular action[1].

Clinical practice often finds situations similar to those described above. Some elderly patients self-medicate with nonsteroidal anti-inflammatories, antipyretics or muscle relaxants to make the journey from home to the clinic and the wait there more comfortable, or due to insomnia caused by night pains. This behavior is often kept from health professionals and has the potential to alter, for example, arterial pressure, thus triggering the previously mentioned prescription cascade[13-15].

Therefore, even if considered of low iatrogenesis risk, when asking elderly patients about the use of medication, one should always ask specifically about over-the-counter drugs due to their potential for drug interactions and adverse effects in patients of this age group. Equally important in the verification of PIM for the elderly, for it reduces the risk of severe adverse reactions to the pharmacotheraphy[21].

In conclusion, there is a significant percentage of potentially inappropriate medications for the elderly, according to Beers' criteria (2015), that are sold in Brazil exempt from a prescription. These criteria are useful to prevent the use of potentially inappropriate medications for the elderly that are sold over-the-counter, even though they do not include all types of medication that are exempt from prescription in Brazil.

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Challenges and perspectives in the treatment of patients with haemophilia in Brasil

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http://dx.doi.org/10.1590/1806-9282.64.10.872

Dear Editor,

Haemophilia affects more than 184,000 people worldwide.[1] Despite increasing advances, it is still associated with a high prevalence of disabilities, posing an economic burden to individuals and health systems.[2] Over the last decades, significant therapeutic improvements have been achieved, including advances in blood safety, prophylaxis with coagulation factor concentrates (CFC), treatment with inhibitors of coagulation factors, orthopedic surgeries to correct limbs deformities and long-acting CFC.[2,3] It is anticipated that new compounds which could be applied subcutaneously and gene therapy will be of benefit to patients with hemophilia (PWH) in the future.[4]

Without prophylactic treatment, approximately 80% of all bleedings in PWH occur inside joints (haemarthroses), mainly ankles, knees and elbows.[5] Although prophylaxis with CFC for children and adults with severe hemophilia is recognized to prevent hemophilic arthropathy,[6] it is not available to all PWH. Accordingly, it is estimated that approximately 75% of PWH worldwide (~350,000) are not regularly treated, if at all.[4]

It is known that CFC accounts for up to 80% of the overall cost of hemophilia treatment[2] presenting a barrier to patient care in developing countries. Indeed, hemophilia health care costs in the United States are approximately $140,000 USD/year;[7] in patients with inhibitors to factor VIII, costs of treatment can be 4.8 times higher.[7]

According to the last Annual Global Survey (Table 1), Brazil has the fourth highest number of registered PWH (n=12,119).[1] The Brazilian federal government provides 100% of CFC to these patients; up to 77% of PWH under 18 years of age and 31% for those over 18 benefit from prophylaxis, although this was only available from late 2011.[1] The Brazilian Unified Health System (SUS), which is part of the 1988 National Constitution, acknowledges that health is a fundamental right of the citizen, which must be guaranteed by the State through a set of

DATE OF SUBMISSION: 22-Jan-2018
DATE OF ACCEPTANCE: 27-Jan-2018
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governmental policies and actions, with community participation (social control).

According to the SUS principles of hierarchy and decentralization, in this context, the roles of the Ministry of Health (MoH) are purchasing the CFCs and establishing national policies and guidelines, while the states and municipalities are responsible for the multidisciplinary care of PWH.

The PWH society encompasses non-governmental organizations (NOG), as well as individuals with inherited bleeding disorders and their relatives. The Brazilian Federation of Haemophilia (FBH), with its regional chapters, represents Brazil in the World Federation of Hemophilia (WFH); they play a key role in advocating for PWH.

The WFH (through FBH) has been providing the MoH with technical, accessible and credible information on products for the treatment of bleeding disorders and their costs, as practiced in other countries, thus supporting better decision-making by the country. It also conducts national and international meetings as well as scientific congresses, which are important to the development of public policies. Moreover, FBH is represented on the *Coagulopathy Committee*, the technical arm of the MoH in the Bleeding Disorders area. This committee, among

TABLE 1. POPULATION DATA FROM THE FOUR COUNTRIES WITH THE LARGEST NUMBER OF PEOPLE WITH HEMOPHILIA WORLDWIDE, ACCORDING TO THE REPORT ON THE ANNUAL GLOBAL SURVEY 2016 FROM THE WORLD FEDERATION OF HEMOPHILIA, 2017.

Country	Population	People with hemophilia	Haemophilia A		Mean per capita factor VIII use*	Haemophilia B		Mean per capita factor IX use*
India	1,324,171,354	18,353 (756 type unknown)	Age 0 to 4	2%	0,105 (including humanitarian aid)	Age 0 to 4	2%	0,002
			Age 5 to 13	15%		Age 5 to 13	13%	
			Age 14 to 18	11%		Age 14 to 18	13%	
			Age 19 to 44	36%		Age 19 to 44	41%	
			Age ≥45	7%		Age ≥45	9%	
			Age not known	28%		Age not known	22%	
			Total	15,218		Total	2,379	
United States	323,127,513	16,949	Age 0 to 4	9%	9,532	Age 0 to 4	9%	1,656
			Age 5 to 13	25%		Age 5 to 13	24%	
			Age 14 to 18	13%		Age 14 to 18	11%	
			Age 19 to 44	33%		Age 19 to 44	29%	
			Age ≥45	20%		Age ≥45	26%	
			Age not known	0%		Age not known	0%	
			Total	12,996		Total	3,953	
China	1,378,665,000	14,390	Age 0 to 4	3%	Data not available	Age 0 to 4	3%	Data not available
			Age 5 to 13	20%		Age 5 to 13	4%	
			Age 14 to 18	13%		Age 14 to 18	26%	
			Age 19 to 44	49%		Age 19 to 44	50%	
			Age ≥45	15%		Age ≥45	27%	
			Age not known	1%		Age not known	1%	
			Total	12,533		Total	1,857	
Brazil	207,652,865	12,119	Age 0 to 4	5%	3,556	Age 0 to 4	5%	0,578
			Age 5 to 13	16%		Age 5 to 13	15%	
			Age 14 to 18	11%		Age 14 to 18	13%	
			Age 19 to 44	49%		Age 19 to 44	47%	
			Age ≥45	18%		Age ≥45	19%	
			Age not known	0		Age not known	0	
			Total	10,123		Total	1,996	

Adapted from the data available on the Report on the Annual Global Survey 2016 (World Federation of Hemophilia, 2017) . * The number of IU/capita is different in each region. The World Federation of Hemophilia has established that one international unit (IU) of FVIII clotting factor concentrate per capita should be the target minimum for countries wishing to achieve survival for the hemophilia population. Higher levels would be required to preserve joint function or achieve a quality of life equivalent to an individual without hemophilia. Only countries that completed the 2016 questionnaire were included in the report.

FIGURE 1

other responsibilities, helps the MoH to formulate public policies and develop technical guidelines.

Nevertheless, it is recognized that recently other NOGs and individuals have also pledged health rights through judicial institutions, such as the Public Chambers of Attorney and the *Brazilian Government Agency for Law Enforcement (in Brazil, Ministério Público)*.

Frequently, global care to PWH has been jeopardized by oscillations in CFC supply, exposing the patients to risks of bleeding and associated complications. The increased dismantling of the SUS and scrapping of public services, especially at state level, but also affecting the municipal and federal scope, is a challenge which can only be overcome through close cooperation with hemophilia patient societies and those that treat hemophilia. The infographic in Figure 1 presents a brief historical description of the achievements and obstacles of PWH in Brazil.[8,9]

Brazil also has some unmet needs, such as the provision of faster and easier access to the most often necessary orthopedic surgeries and to hemophilia laboratory diagnosis in some centers. A more organized health system should also educate the patients and their families so that they can take advantage of the treatment offered by the Haemophilia Centres (in Brazil, Hemocentros). Indeed, despite an appropriate prescription, 54% of patients do not understand and correctly adhere to the prophylaxis prescribed by healthcare practitioners.[10]

Although the benefits of prophylaxis are well established, this treatment is not available for all Brazilian PWH, and in many cases, it is not able to control the progression to hemophilic arthropathy. Considering the burden that chronic pain adds to the quality of life and daily life activities in PWH, the establishment of evidence-based guidelines for the evaluation and treatment of these complications is vital. When looking forward in healthcare provision, it is essential to prepare for the incorporation and application of new upcoming technologies, as well as to reduce the stigma associated with hemophilia in the general population, further educating patients, family members and non-specialist medical professionals about the disease.

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Benign prostatic hyperplasia

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Final version: *May 7, 2018*

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http://dx.doi.org/10.1590/1806-9282.64.10.876

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.

SUMMARY

The minimally invasive procedures (mips) for the treatment of symptoms of benign prostatic hyperplasia (bph) are presented as attractive techniques due to their ease of accomplishment and the possibility of outpatient treatment. This guideline aims to present recommendations that may assist in decision making in patients with benign prostatic hyperplasia and indication of the different minimally invasive therapies. For this, a systematic review of the literature was performed, with the descriptors according to the pico: patient with benign prostatic hyperplasia, minimally invasive therapy, clinical outcome and adverse events. With no time restriction, in medline, cochrane central and lilacs databases via vhl, 1,007 papers were retrieved, of which 16 were selected to respond to clinical doubt. Details of the methodology and results of this guideline are set out in annex I

INTRODUCTION

The minimally invasive procedures (MIPs) for the treatment of symptoms of Benign Prostatic Hyperplasia (BPH) are presented as attractive techniques due to their ease of accomplishment and the possibility of outpatient treatment. The development of newer minimally invasive procedures seeks new approaches that rival the standard methodology, ideally providing an effective therapy and with fewer adverse effects. From a patient's point of view, a successful MIP would provide: good tolerability, rapid and long-lasting relief of symptoms, short recovery time with rapid return to daily activities, minimal adverse events and accessibility. As many men discontinue drug therapy, but of these, proportionately, few seek surgery, there is a great medical need for an effective treatment that is less invasive than traditional surgery, reducing the risk of imminent bladder dysfunction.

RESULTS

What is the efficiency and safety of different minimally invasive therapies in the treatment of low urinary tract symptoms in benign prostatic hyperplasia?

1. Transurethral Thermotherapy with microwaves (Tumt)

In Tumt method, the emission of microwave radiation through an intraurethral antenna provides heat to the interior of the prostate, which leads to

tissue destruction, apoptosis and denervation of α-receptors, thereby reducing resulting infravesical obstruction[1] **(A)**.

A systematic review (RS) with meta-analysis including 15 randomized controlled trials (RCTs) evaluated Tumt in 1,585 patients with symptomatic BPH with a follow-up of 3-60 months. Comparing Tumt with "sham" thermotherapy, Tumt reduced the severity score of clinical symptoms measured by the International Prostate Symptom Score (IPSS) weighted mean difference [WMD] -5.15, IC 95% -6.04 to -4.26] in the analysis of four studies with 482 patients and increased maximal urinary flow (Qmax) [WMD 2.01 mL/s, IC 95% 0.85-3.16 mL/s] in the analysis of six trials with 643 patients. Tumt also showed a significant improvement of IPSS (WMD -4.20, IC 95% -3.15 to -5.25) and Qmax (WMD 2.30 ml/s, IC 95% 1.47 to 3.13) in comparison with alpha-blockers (in a study of 103 patients). This RS also found that transurethral resection of the prostate (TURP) was better for Qmax (119% vs. 70%) and that these patients (1/100 person/year) were less likely to require retreatment in patients treated with Tumt (8/100 persons/year). In contrast to TURP, Tumt was associated with reduced risks of retrograde ejaculation, stenosis treatment, hematuria, blood transfusions, and transurethral resection syndrome, but increased the risks of dysuria, urinary retention, and retreatment of BPH symptoms. No studies have evaluated the effects of symptom duration, patient characteristics, prostate specific antigen levels or prostate volume in response to treatment 2 **(A)**.

Tumt is an outpatient procedure and an alternative for elderly patients with comorbidities who are at elevated anesthesia and/or surgical risk or are unfit for invasive treatment[1,2] **(A)**.

2. Transurethral prostatic ablation with needle (Tuna)

In Tuna method, a low level of radiofrequency energy is supplied to the prostate through transurethral needles inserted up to the prostatic parenchyma.

A RS with meta-analysis of 35 low quality studies, of which only nine (26%) were comparative, showed that Tuna significantly improved the IPSS and Qmax in relation to the baseline. However, compared to RTU, these improvements were significantly lower at 12 months (mean difference [DM] from 4.7 to IPSS and 5.9 mL/s for Qmax). Tuna was associated with a higher rate of retreatment (analysis of 17 non-comparative studies), with a mean follow-up not reported (odds ratio [OR] 7.44, IC 95% 2.47-22.43) and lower rate of complications (OR 0.14, IC 95% 0.05-0.14)[3] (B).

In comparison to TURP, Tuna is associated with a lower prevalence of adverse events, including mild hematuria, urinary tract infections, urethral stenosis, urinary incontinence, erectile dysfunction, and ejaculatory disorders.

Tuna is not suitable for prostates > 75 mL or isolated obstruction of the bladder neck. In addition, Tuna can not effectively treat the median lobe[5] **(D)**.

3. Prostatic Stent

Prostatic stents were designed primarily as an alternative to delayed bladder catheterization in patients without clinical conditions for the surgical procedure; however, were also evaluated as a primary treatment option in patients without significant comorbidities. The use of stents requires well-functioning detrusor muscles[6] **(A)**. Permanent stents are biocompatible, allowing epithelialization. Temporary stents are non-epithelializable and may be biostable or biodegradable[7] **(D)**.

There are no studies comparing stenting with other treatments or sham, only one RCT compared two versions of a temporary stent in patients with benign prostatic obstruction (OPB)[8] **(B)**.

A RS with 20 series of cases evaluated the placement of the biocompatible and re-epithelializing permanent urethral stent in 990 patients with BPH. Fourteen studies included only patients at high surgical risk. These studies reported significant improvements in symptoms and Qmax. Data combined with catheter-dependent patients showed that 84% of patients (148/176) regained urination capacity after treatment. In 606 patients evaluated, a total of 104 stents (16%) failed in one year and migration was the most common cause of failure (38 stents or 37%). The majority of patients had perineal pain or urinary irritation symptoms after stenting. Therefore, 1 in 6 patients had the stent removed within a year due to complications and the inadequate follow-up prevented conclusions on stent durability beyond one year[9] **(C)**.

Another RS included data from 14 case series and evaluated the efficiency of self-expanding, non-epithelializing metal prostatic stenting in 839 high-risk patients with BPH. Most studies were of poor quality and poor follow-up. Five studies reported reduction in IPSS from 11 to 19 points after stent insertion. All seven studies evaluating Qmax showed an increase in their rate (3-11 mL/s), and the four studies that

described post-urination residual volumes showed a reduction of Qmax[10] **(C)**.

Temporary stents may provide short-term relief of lower urinary tract symptoms secondary to OPB in patients temporarily unsuitable for surgery or after minimally invasive treatment.

4. Prostatic Urethral Lift (PUL)

Prostatic Urethral Lift (PUL) is a minimally invasive treatment performed by cystoscopy under local anesthetic associated with sedation, or general. The PUL consists of a non-absorbable suture wire with metal bundles at each end that act as anchors. It is implanted by transfixing the lateral lobes of the prostate, where one end is externally located in the capsule and the other inside the adenoma. It acts by compressing the lateral lobes and enlarging the lumen of the obstructed urethra. The procedure aims to create a continuous light from the bladder neck to veromontano.

In a RCT, 206 patients with at least 50 years of age with an Auasi Index (American American Association Symptom Index), 13 or greater, Qmax ≤ 12 mL/d and prostate gland from 30 to 80 cc, were randomized 2:1 for prostatic urethral lift (N=140) or simulated procedure (N=66). The primary endpoint evaluated was the comparison of the Auasi reduction at three months. Patients in the PUL group were followed for up to one year and evaluated for symptoms of lower urinary tract, maximum urinary flow, quality of life and sexual function. At the three-month follow-up, the PUL group had a 50% reduction in relation to the initial Auasi score (22.1 to 11.0 points - p <0.001), which remained stable up to 12 months[11] **(A)**.

The Auasi change was 88% higher for the PUL group than for the sham control. Also, Qmax increased significantly from 8.1 to 12.4 mL/s compared to baseline at three months, and this result was still maintained at 12 months (p <0.001). The difference for Qmax between the two groups was favorable to PUL and showed statistical significance (p = 0.005). There was no difference between the two groups in relation to residual post-urination volume (p = 0.30)[11] **(A)**.

A three-year analysis of this study showed a mean improvement from the baseline significant for the total IPSS (41.1%), quality of life (48.8%), Qmax (53.1%) and IPSS. Symptomatic improvement was regardless of the prostate size. There were no "again" events of ejaculatory or erectile dysfunction, and all evaluations of sexual function showed stability or average improvement after PUL. Fifteen of the 140 patients in the PUL group (10%) required reoperation due to treatment failure up to three years[12] **(A)**.

Another RCT compared PUL with TURP randomizing 80 patients with lower urinary tract symptoms secondary to BPH (45 PUL, 35 TURP). At 12 months, IPSS improvement was -11.4 for PUL and -15.4 for TURP (p = 0.05). There was no retrograde ejaculation among patients with PUL, while 40% of patients in the RUP group lost the ability to ejaculate (p <0.0001). Surgical recovery was measured using a validated instrument and confirmed that recovery quality was higher with PUL (p <0.01). The increase in Qmax was higher in the RTU group (+13.7 ± 10.4 mL/s) compared to PUL (4.0 ± 4.8 mL/s) after 12 months of the procedure[13] **(B)**.

A meta-analysis of prospective and retrospective studies showed an overall improvement after PUL, including IPSS (-7.2 to -8.7 points), Qmax (3.8 to 4.0 mL/s) and quality of life (QoL -2.2 to -2.4 points). Sexual function was preserved with a small improvement estimated at 12 months (standardized mean gain of 0.3-0.4)[14] **(B)**.

The most common complications reported in the postoperative period included hematuria (16-63%), dysuria (25-58%), pelvic pain (5-17.9%), urgency (7.1-10%), transient incontinence 3.6-16%) and ITU (2.9-11%). Most of the symptoms were from mild to moderate severity and were resolved within two to four weeks after the procedure[11.12] **(A)** [13.14] **(B)**.

The obstruction caused by median lobe enlargement could not be effectively treated by PUL, and efficiency in large prostates has not yet been demonstrated. Long-term studies are needed to evaluate the duration of effect compared to other techniques 5 **(D)**[14] **(B)**.

5. Intraprostatic Injection of Botulinum Toxin type A (BoNT-A)

The main mechanism of action of BoNT-A is inhibition of the release of neurotransmitters from cholinergic neurons by cleavage of synaptosome-associated protein 25 (Snap-25). BoNT-A also appears to act at several other levels, modulating the neurotransmissions of sympathetic, parasympathetic and sensory nerve terminals in the prostate, leading to reduced growth and promotion of prostatic apoptosis[15] **(D)**.

A recent systematic review with meta-analysis showed no difference in the efficacy of BoNT-A com-

pared to placebo, concluding that there is no evidence of clinical benefit. Three studies were included, with a total sample of 522 patients (260 in the BoNT-A group and 262 in control group). The duration of the studies ranged from 8 to 24 weeks. The standardized mean difference grouped at the change in the IPSS for the BTX-A group versus the placebo group was -1.02 (IC 95% C - 1.97, - 0.07). The other outcomes (Qmax, prostate volume and post-urination residual volume) were not statistically different between the two groups. The placebo effect in the single-group analysis ranged from 0% to 27.9% for IPSS and from -1.1 to 28.7% for Qmax (lower to higher, respectively)[16] **(A)**.

RECOMMENDATION

In patients with BPH:

Tumt is an outpatient procedure and an alternative for elderly patients with comorbidities who are at elevated anesthesiological risk or are unfit for invasive treatment. **(A)**

Tumt is comparable to TURP in improving symptoms; is associated with decreased morbidity, but with less improvement in urinary flow.

TURP has lower retreatment rates compared to Tumt. **(A)**

Tuna is not suitable for prostates > 75 mL or isolated obstruction of the bladder neck and cannot effectively treat median lobe. **(D)**

Tuna is a minimally invasive alternative, with reduced morbidity compared to TURP, but with less efficiency. **(B)**

Retreatment rates are lower with TURP compared to Tuna. **(A)**

Temporary stents may provide short-term relief of lower urinary tract symptoms secondary to OPB in patients temporarily unsuitable for surgery or after minimally invasive treatment. **(C)** Regarding adverse events, the high rate of stent migration is noted. **(C)**

Prostatic urethral lift improves IPSS, Qmax and quality of life. **(A)**

There is a low incidence of sexual side effects with use of prostatic Urethral Lift. **(A)**

A median lobe enlargement obstruction cannot be effectively treated with prostatic urethral lift, and efficiency in large prostates has not yet been demonstrated. **(B)**

There is currently no evidence to support the use of BoNT-A in patients with lower urinary tract symptoms due to BPH. **(A)**

ANNEX I

Clinical question

What is the efficiency and safety of different minimally invasive therapies in the treatment of low urinary tract symptoms in benign prostatic hyperplasia?

Eligibility criteria

The main reasons for exclusion were: did not respond to PICO and study design.

Narrative reviews, case reports, case series, and preliminary results were initially excluded.

Search for articles

Database

The basis of scientific information consulted was Medline (via PubMed) and references of the selected studies.

Identification of descriptors

P	Patients with lower urinary tract symptoms due to benign prostatic hyperplasia
I	Minimally invasive therapy
C	Other therapy
O	Clinical outcomes, adverse events

Search strategy

Medline/PubMed - (Lower Urinary Tract Symptoms OR benign prostatic obstruction OR benign prostatic hyperplasia OR benign prostatic hypertrophy OR BPH OR Prostatic Hyperplasia) AND (minimally invasive treatment OR minimally invasive therapy OR minimally invasive therapy* OR MIST OR Microwaves OR Transurethral Needle Ablation OR Catheter Ablation OR embolization therapeutic OR Stent* OR Stents* prostatic stent* OR prostatic urethral lift OR intraprostatic injection OR Bacterial Toxins OR Botulinum Toxins, Type A)

Central (Cochrane) - (Lower Urinary Tract Symptoms OR benign prostatic hyperplasia) AND (minimally invasive treatment OR minimally invasive therapy OR Microwaves OR Transurethral Needle Ablation OR prostatic stent OR prostatic urethral lift OR intraprostatic injection OR Bacterial Toxins OR Botulinum Toxins, Type A)

Lilacs via BVS - (Lower Urinary Tract Symptoms OR benign prostatic hyperplasia) AND (minimally invasive treatment OR minimally invasive therapy)

Critical Evaluation

Relevance - clinical importance

This guideline was prepared through a clinically relevant question to gather information in medicine to standardize the conduct and assist in decision making during minimally invasive therapy in the treatment of low urinary tract symptoms by benign prostatic hyperplasia.

Reliability - Internal validity

Obtaining the evidence to be used followed the following steps: elaboration of the clinical question, structuring the question, searching for the evidence, critical evaluation and selection of the evidence, exposure of the results and recommendations.

The bases of scientific information referred to were Medline via PubMed, Central (Cochrane) and Lilacs via BVS. Manual search from references of narrative reviews, as well as selected works, was performed.

The selection of the studies, the evaluation of the titles and abstracts obtained with the search strategy in the information bases referred to was conducted independently and blinded, obeying the inclusion and exclusion criteria, separating the works with potential relevance. When the title and abstract were not illuminating, the article was searched in its entirety. Only works which complete texts were available were considered for critical evaluation. There was no restriction on the year of publication.

Languages: Portuguese, English, Spanish.

Application of results - External validity

The level of scientific evidence was classified by type of study according to Oxford[17] (Table 1).

TABLE 1: GRADE OF RECOMMENDATION AND STRENGTH OF EVIDENCE

A: Experimental or observational studies of better consistency.
B: Experimental or observational studies of lower consistency.
C: Uncontrolled case reports/studies.
D: Opinion lacking critical evaluation, based on consensus, physiological studies or animal models.

The selected evidence was defined as a randomized controlled clinical trial (RCT), which was submitted to an appropriate critical evaluation checklist (Table 2). The critical evaluation of ECR allows classification according to the Jadad score 18, considering the Jadad < three (3) trials as inconsistent (grade B), and those with a score ≥ three (3), consistent (grade A).

When the selected evidence was defined as a comparative study (observational cohorts or non-randomized clinical trial), it was subjected to an appropriate critical evaluation checklist (Table 3), allowing the classification of the study according to the New Castle Ottawa score Scale 19, considering cohort studies consistent with score ≥ 6 and inconsistent <6.

TABLE 2 - DIRECTIONS FOR CRITICAL EVALUATION OF RANDOMIZED CONTROLLED CLINICAL TRIALS

Study Data Reference, Study Design, Jadad, Strength of evidence	**Sample calculation** Estimated differences, power, level of significance, total of patients
Selection of patients Inclusion and exclusion criteria	**Patients** Recruited, randomized, prognostic differences
Randomization Description and allocation blindfolded	**Patient follow-up** Time, losses, migration
Treatment Protocol Intervention, control and blind method	**Analysis** Intent of treatment, intervention and control analyzed
Items considered Primary, secondary, instrument of measure of the outcome of interest	**Result** Benefit or damage on absolute data, benefit or damage on average

Method of extraction and analysis of results

For results with available evidence, the population, intervention, outcomes, presence or absence of benefit and/or damage and controversies will be defined in a specific way, whenever possible.

The results will be preferentially exposed in absolute data, absolute risk, number needed to treat (NNT) or number to produce damage (NNH), and possibly in mean and standard deviation (Table 4).

TABLE 3 - DIRECTIONS FOR CRITICAL EVALUATION OF COHORT STUDIES

Representativeness of subjected and selection of non-subjected (max 2 points)	Exposure Definition (max 1 point)	Demonstration that the outcome of interest was not present at the start of the study (max 1 point)	Comparability on the basis of design or analysis (max 2 points)	Outcome evaluation (max 1 point)	Appropriate follow-up time (max 2 points)	Score and level of evidence

TABLE 4 - SPREADSHEET USED TO DESCRIBE AND PRESENT THE RESULTS OF EACH STUDY MEAN

Evidence included
Study Design
Selected population
Follow-up time
Outcomes considered
Expression of results: percentage, risk, odds, hazard ratio, mean

Results

Recovered work (05/2018)

TABLE 5 - NUMBER OF WORKS RETRIEVED WITH THE SEARCH STRATEGIES USED FOR EACH SCIENTIFIC INFORMATION BASE

INFORMATION BASE	WORK NUMBER
Primary	
PubMed-Medline	1,007
Central (Cochrane)	242
Lilacs via BVS	3

Application of evidence - Recommendation

The recommendations will be prepared by the authors of the review, with the initial characteristic of synthesis of the evidence, being submitted to the validation by all the authors participating in the preparation of the guideline.

The available evidence will follow some principles of exposure - will be by outcome and will have as components: number of patients, type of comparison, magnitude and precision (standard deviation and IC 95%).

It will have its estimated strength (Oxford[17]/ Grade[20]) in 1b and 1c (grades A) or strong and in 2a, 2b and 2c (grades B) or moderate or weak or very weak.

Conflict of Interests

There is no conflict of interest related to this review to be declared by any of the authors.

Final Declaration

The Guidelines Project, an initiative of the Brazilian Medical Association in conjunction with the Specialty Societies, aims to reconcile medical information in order to standardize behaviors that aid the physician's reasoning and decision making. The information contained in this project should be submitted to the evaluation and criticism of the physician responsible for the conduct to be followed in view of the reality and clinical condition of each patient.

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Wernicke's encephalopathy in a patient with non-Hodgkin's lymphoma post-Autologous HSCT

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http://dx.doi.org/10.1590/1806-9282.64.10.882

SUMMARY

Wernick's Encephalopathy (WE) is an acute neuropsychiatric syndrome caused by thiamine deficiency post hematopoietic stem cell transplant (HSCT). WE is associated with high mortality and morbidity rates, but due to its rare occurrence, it is rarely considered in patients submitted to this procedure. Considering that, the manuscript reports the clinical characteristics and the possible factors that predisposed the occurrence of WE in a patient with non-Hodgkin's lymphoma post-Autologous HSCT. We conclude that WE should be considered in patients submitted to autologous HSCT associated with prolonged use of TPN and malnutrition.

KEYWORDS: *Wernicke's encephalopathy; non-hodgkin's lymphoma; autologous hsct; thiamine deficiency.*

Wernicke's encephalopathy (WE) is an acute neuropsychiatric syndrome caused by thiamine deficiency and is associated with significant morbidity and mortality[1]. WE is a rare condition, characterized by altered mental status, as well as ocular, walking, and balance abnormalities. Although WE usually results from chronic alcohol dependence, nonalcoholic causes are reported in 20% to 50% of patients. WE rarely develops in patients with cancer. [2,3] There have been few case reports of WE in patients with malignant lymphomas [3]. The occurrence of HSCT-related WE is also rare, mainly in autologous HSCT [4]. WE occurrence is associated with drug use, prolonged Total Parenteral Nutrition (TPN), vomiting, and malnutrition. [3,4]

A 36-year-old female, married, small farmer, with no history of alcoholism was diagnosed with a bulky inguinal diffuse large B-cell non-Hodgkin lymphoma IIIB - (10cm). She was admitted to the University Hospital to undergo an autologous HSCT. The mobilization was performed with Granulocyte Colony-Stimulating Factor, and 2.5 x 10^6 CD34 cells/kg

DATE OF SUBMISSION: 20-Feb-2018
DATE OF ACCEPTANCE: 24-Feb-2018
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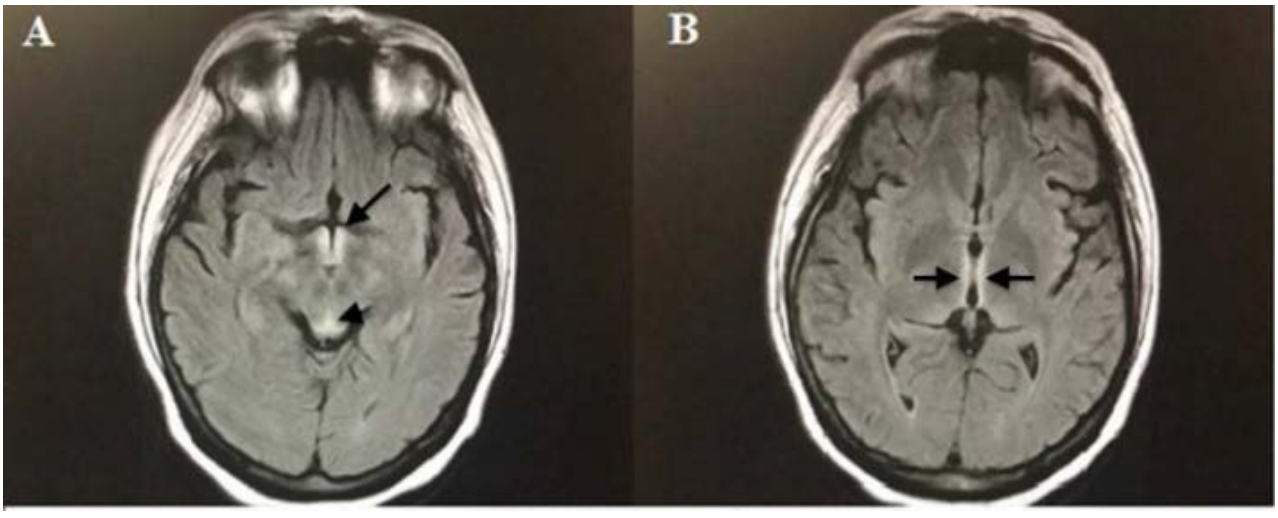
were collected. The conditioning regimen for autologous TCTH was BEAC (BCNU, etoposide, cytarabine, cyclophosphamide). The infusion of hematopoietic stem cells was performed, and on D+2, she had a recurrence of fever, abdominal pain, and vomiting. On D+3 the patient's overall condition was worse, with contraction of diuresis, hypotension, and abdominal distension. Volume expansion with saline solution and vasoactive drugs were performed. On D+4, a large amount of gastric residue was observed after nasogastric intubation. On the D +5 there was an improvement of the patient's overall condition with weaning of the vasoactive drug and a gastric residue (GR) of 3650mL, followed by 1950mL on D+6, and 2,150mL on D+7. Abdominal pain and abdominal distension persisted, and TPN was introduced on D+7. On D+12 the nasogastric tube (NT) was closed, and a restricted liquid diet was allowed. She had difficulty in accepting the oral diet, and the NT was maintained. On D+18, recurrence of fever was observed, and the patient still had abdominal pain and distension and difficulty in walking. On D+22, weaning from TPN was performed, and there was an improvement in the abdominal condition.

Neutrophil grafting occurred only on D+23. On D+24 the patient partially tolerated oral diet but still required blood transfusions. She developed apathy and drowsiness. On D+30, a new febrile peak was observed, with the reintroduction of Meropenem. A clinical scenario characterized by sensorineural fluctuation, the absence of focal signs, difficulty in ambulation and horizontal nystagmus was observed. The electroencephalogram (EEG) did not show any specific findings. On D+35 cerebrospinal fluid cytology revealed negative findings for malignancy. There was no evidence of central nervous system infection. PCR tests for herpes, dengue, and chikungunya fever were negative. Magnetic resonance imaging (MRI) of the brain showed increased signal in the fluid-attenuated inversion recovery (FLAIR) sequences around the Sylvian aqueduct and in the medial parts of both the thalamus and mammillary bodies (Figures 1a and 1b). Encephalitis Protocol (Meropenem, vancomycin, sulbactam sodium / ampicillin sodium, acyclovir) was empirically introduced. Thiamine replacement was initiated with 1500mg IV for 3 days, followed by 900mg/day. Improvement in the level of consciousness and nystagmus was quickly observed, while she persisted with temporal/spatial disorientation and recent amnesia. She was afebrile at discharge with hematologic recovery. The patient continued with progressive improvement of disorientation and amnesia but had pain in the lower limbs (neuropathy). During the follow-up, neurological changes and oral ingestion gradually improved.

Neurologic complications are frequently observed in patients during HSCT, being reported in 30% to 39% of cases[5]. These complications may be infectious, cerebrovascular, toxic, immuno-mediated or metabolic[5]. The complications may be due to drugs, thiamine deficiency, among others. Wernicke's encephalopathy (WE) is an acute neuropsychiatric syndrome

FIGURE 1. AXIAL FLAIR IMAGES OF THE BRAIN DEMONSTRATING AREAS OF SIGNAL CHANGE CHARACTERISTIC OF WERNICKE'S ENCEPHALOPATHY: IN (A), SYMMETRIC HYPERINTENSITIES IN THE MAMILLARY BODIES (ARROW) AND PERIAQUEDUCTAL GRAY MATTER; IN (B), SYMMETRIC HYPERINTENSITIES IN THE MEDIAL REGIONS OF THE THALAMUS.



caused by thiamine deficiency that causes mental alterations, ocular, and balance abnormalities. Reports of WE cases in the literature associated with HSCT, mainly allogeneic transplantations, have been poorly described. Among the indicators of predisposition to WE in HSCT is prolonged total parenteral nutrition (TPN), since the latter is thiamine-deficient.[5,6] Some authors have considered the prolonged use of TPN as the main risk factor for HSCT associated with Wernicke's Encephalopathy, but the duration of TPN required for the disease to manifest is unknown.[7] Patients receiving long-term TPN, and intravenous solutions require higher amounts of thiamine to metabolize carbohydrate intake, which may rapidly consume thiamine stocks.[3] Studies show that a state of depletion can develop within 18-20 days in patients receiving a strict diet without thiamine.[5] Most of the reports concluded that prolonged TPN was the primary risk factor for HSCT-associated WE. [4]

Our patient with NHL underwent an autologous HSCT. Busulfan is not used in the conditioning regimen. She received TPN for approximately 2 weeks associated with episodes of vomiting. TPN includes multivitamin and mineral supplementation. The patient received prolonged TPN without thiamine, and WE symptoms appeared on day +35. Wernicke's encephalopathy was diagnosed based on the history of consistent use of TPN and CNS symptoms with symmetrical T2/FLAIR hypersignal in the hypothalamic region, mammillary bodies and walls of the 3rd ventricle, medial region of the thalamus (Figure 1), although the level of thiamine was not assessed.

Several drugs routinely used in HSCT are associated with neurological abnormalities, including busulfan[4] cyclosporine A[5] and tacrolimus.[6] We must consider that patients with HSCT are at high risk of acute encephalopathy due to chronic malnutrition, nausea induced by chemotherapy and vomiting[8], by neurological alterations, including disorientation, mental state alteration, visual disturbances, and coma.[5] However, it is yet unclear whether the use of these drugs during HSCT are risk factors for triggering such complications.

The consensus of the European Federation of Neurological Societies (EFNS) is that whole-blood thiamine (vitamin B1) measurement should be performed immediately prior to the administration of thiamine to confirm suspected or manifested WE, and MRI should be used to support the diagnosis.[9] We conclude that WE should be considered in patients submitted to autologous HSCT associated with prolonged use of TPN and malnutrition.

CONFLICTS OF INTEREST

The authors declare having no conflict of interest.

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Application of confocal endomicroscopy in the diagnostic elucidation of pancreatic cyst

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http://dx.doi.org/10.1590/1806-9282.64.10.885

KEYWORDS: *Pancreatic cyst. Pancreatic neoplasms/diagnostics. Echo-endoscopy/methods. Confocal Microscopy/methods*

INTRODUCTION

Treatment of patients with pancreatic cysts is challenging and follow-up remains controversial. Some guidelines allow the standardization of the treatment of these patients, especially in cases of intraductal papillary mucinous neoplasia (IPMN).

New technologies have been developed and applied for diagnostic assistance and therapeutic management. Confocal laser endomicroscopy (CLE) represents a technique in which a probe is used, promoting the *in-vivo* microscopic image in real time of the tissue studied[1]. The CLE technique through EUS-guided needle aspiration CLE – nCLE has been shown to be promising, especially in IPMN cases.

The purpose of this study is to report the application of nCLE technology in a patient with pancreatic cyst, already with a previous diagnosis of IPMN at follow-up.

CLINICAL CASE

Patient, female, 74 years old, married, from São Paulo, hypertensive, diabetic, on atenolol, enalapril and glycated. She presented with nonspecific abdominal pain and cystic lesion in the pancreas, with communication with the main pancreatic duct, compatible with IPMN and in follow-up since 2011. Until 2016, she underwent an annual follow-up with magnetic resonance imaging showing cystic lesion with localized lobulated contours in the pancreatic body,

DATE OF SUBMISSION: 27-Jan-2018
DATE OF ACCEPTANCE: 16-Feb-2018
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measuring approximately 27 mm, in communication with the main pancreatic duct, which had a gauge of 23 mm. In 2017, there was a slight increase in its dimensions to 30 mm (Figure 1). Echoendoscopic evaluation with echo-guided puncture and nCLE were performed in search of signs of malignancy.

In September 2017, echoendoscopy showed an anechoic image of 30 mm x 16 mm, oval, with regular contours, precise limits, without nodules or solid components in the interior, with posterior acoustic reinforcement, in communication with the main pancreatic duct. An echo-guided puncture with 19G needle was performed and nCLE was carried out, observing the coffee beans aspect (pancreas), digitiform projections, and no signs of malignancy were found (Figures 2 and 3). The aspect was suggestive of IPMN of the intestinal type. At the end of the examination, the cyst was completely emptied, recovering 3 ml of fluid and translucent liquid. An antibiotic prophylaxis was performed for seven days with ciprofloxacin. The dosage of markers was amylase 111770 U/L, lipase 482960 U/L, CEA 8.3 ng/mL and CA 19.9 58.3 ng/mL. Cytological analysis was negative for neoplasia.

There were no complications related to the procedure. The multidisciplinary team decided on clinical follow-up.

DISCUSSION

In recent years, the incidental finding of pancreatic cysts in imaging studies has been increasing, probably due to equipment sophistication, the development of new technologies and a larger number of imaging studies carried out[2,3]. The prevalence of pancreatic cystic lesions in imaging studies may vary between 3% and 19%[4-6]. At autopsies, the number is even higher, 24%[2-5]. Pancreatic cysts may be congenital, inflammatory or neoplastic. It is estimated that less than half of the cases are of intra-ductal papillary mucinous neoplasia (IPMN), of which only a small share will develop into invasive carcinoma[2,4].

Several tests can be used to detect and evaluate pancreatic cysts, such as abdominal ultrasonography, computed tomography, nuclear magnetic resonance and echoendoscopy[7]. New technologies have been developed, such as CLE, promoting the microscopic image *in vivo* and in real time, with magnification in about a thousand times[1]. The CLE technique with a probe inserted into the EUS-guided needle-based CLE (nCLE) is promising, especially in IPMN cases.

In a multicentre study[1], the diagnostic capacity of nCLE was evaluated in 29 cases of pancreatic cystic lesions, with clinical information omitted. The rates of sensitivity, specificity, accuracy and intra-observer and inter-observer agreement were evaluated. The results for cystic lesions were 95%, 94% and 95%, respectively, with Kappa agreement of 0.81 for intra-observer and 0.86 for inter-observer. The authors considered the agreement in both cases to be almost perfect. The results of this study demonstrate that the nCLE technique in cases of pancreatic cystic lesions can be very useful in the conduct and follow-up, as was the case of the patient reported.

Some guidelines have attempted to standardize the treatment of patients with IPMN, such as the one published in 2012 by the International Association of Pancreatology[8]. According to it, patients with secondary ductus IPMN with no worrisome features can be followed. Through nCLE, it was possible to infer the histological type of IPMN, that is, intestinal[9]. This histological type generally presents a more favourable evolution when compared to the oncocytic, pancreatobiliary and gastric types[8].

In a Swedish study[10], published in 2017, the survival and the risk of progression of IPMN under surveillance were analysed in a group of 395 patients

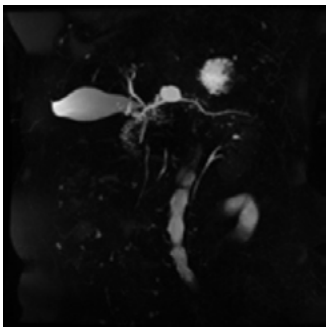

FIGURE 1

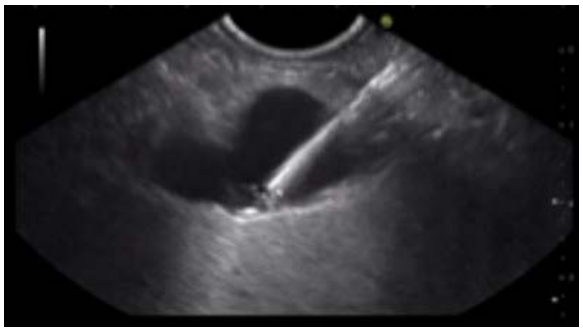

FIGURE 2

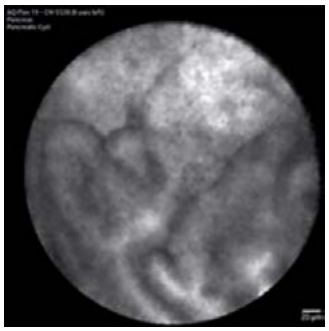

FIGURE 3

between January 2008 and December 2013. The authors found that the main pancreatic duct with ≥6 mm and cephalic location, including the uncinate process, are associated with an increased risk of progression, especially after five years of diagnosis. According to the guideline of the International Association of Pancreatology[8], there is no evidence to support that surveillance can be spaced or discontinued even in the face of morphological stability of the lesion. The patient in question maintained morphological stability for five years. The current recommendation is that surveillance should be continued at shorter intervals, especially in the period after five years, which is known when the risk of progression increases. However, the routine use of nCLE may suggest a new guidance regarding the follow-up interval. Prospective studies with adequate casuistry can prove this assertion.

CONCLUSION

Treatment of patients with incidental pancreatic cysts is based on clinical, radiological and biochemical findings. With the advent of echoendoscopy and nCLE, it became possible to aggregate information on the microscopic characteristic of cyst lining, which may be useful in the management and therapeutic strategy of this group of patients.

PALAVRAS-CHAVE: *Cisto pancreático. Pancreatic Neoplasms/diagnóstico. Endossonografia/métodos. Microscopia confocal/métodos.*

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ORIGINAL ARTICLE

Assessment of oxidative damage and enzymatic antioxidant system activity on the umbilical cord blood and saliva from preterm newborns with risk factors for early-onset neonatal sepsis

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http://dx.doi.org/10.1590/1806-9282.64.10.888

SUMMARY

BACKGROUND: *To determine the concentration of the Lipid Peroxidation Marker: Malondialdehyde (MDA), and Antioxidant Markers: Superoxide Dismutase (SOD), Glutathione Peroxidase (GPX), Catalase (CAL) in umbilical cord blood and in unstimulated saliva in the first 24 and 48 hours of life in the PTNB of mothers with and without risk factors for early-onset neonatal sepsis.*

METHODS: *Cross-sectional study with the signing of informed consent by the pregnant women and application of a standard questionnaire classifying the PTNB in Group 1 or 2.*

RESULTS: *Twenty-one PTNB were studied. Regarding gender, birth weight, need for oxygen, use of phototherapy, diagnosis of assumed sepsis, presence of fetal distress, number of pregnancies, type of delivery, use of corticosteroids, premature rupture of membranes, maternal fever, chorioamnionitis, APGAR at the 5th and 10th minute of life. Statistical analysis was performed with the Mann-Whitney test (p = 0.019) on the GPX variable of umbilical cord blood in the group of mothers with risk factors for early-onset neonatal sepsis. There was no statistical difference in the MDA, SOD, and CAT variables of the group with risk factors and in any variable of the group without risk factors.*

CONCLUSION: *There was an increase of the GPX concentration in the blood from the umbilical vein in the group with risk factors for early-onset neonatal sepsis. There was no statistical significance in the comparison of saliva and umbilical cord blood. There was no statistically significant difference in MDA, SOD, CAT.*

KEYWORDS: *Oxidative stress. Infant, Premature. Malondialdehyde. Glutathione peroxidase. Superoxide dismutase.*

INTRODUCTION

Over the last 30 years, research on neonatal sepsis has not brought significant improvements for the early diagnosis of the condition. Its incidence remains high[1], and it doubled from 4.5 to 9.7 cases for every 1,000 births between 1995 and 2005[2]. Thus, it is of the utmost importance that the risk factors for early-onset neonatal sepsis (EOS) are identified, enabling a quick diagnosis[3]. Several risk factors that

DATE OF SUBMISSION: 14-Jan-2018
DATE OF ACCEPTANCE: 20-Jan-2018
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can contribute to neonatal infection have been identified, and they can be categorized as environmental, neonatal, maternal.

Amongst the environmental factors are: use of infected hospital equipment, cross-contamination of preterm newborns (PTNB) through their carers, the absence of breastfeeding. Amongst neonatal factors, are important: the maturity of the PTNB immune system, and the oxidative damage represented by the free radicals (that act in moderate concentrations in physiological defense responses against pathogens and the induction of mitosis). It is known that over the first 72 hours of life there is an extreme production of free radicals in the PTNB due to the hyperoxia resulting from the transition from intrauterine life (hypoxic medium) to extrauterine, which can result in an important mediator of cell and tissue damage when associated with the low maturity of the NB antioxidant system, especially that of PTNB, thus favoring the onset of neonatal sepsis[4].

Amongst the maternal factors, there are:

1 – Rupture of membranes[3] 18 hours.

2 – Maternal fever over 38°C.

3 – Chorioamnionitis.

PTNB are more prone to develop oxidative stress than children and adults, due to their higher susceptibility to infections, inflammations, and their immature immune system. Oxidative damages are related primarily to "reactive oxygen species" through the exposure to high concentrations of oxygen. The transition from the intrauterine environment, with a partial pressure of oxygen (PaO2) of 20-25 mmHg, to the extrauterine environment, with PaO2 around 100 mmHg[6], increases the production of reactive oxygen radicals, which can be detected in the blood through the umbilical cord[7].

The combination of a high concentration of oxygen, hypoxic periods during labor, and oxidative stress cause an imbalance in the antioxidant system[8]. The hydroxyl radical (OH-), which is highly reactive, can damage the DNA, cause lipid peroxidation, changes in the protein structure and in the vascular permeability, and arterio-capillary fibrosis secondary to lipid peroxidation[9-12].

The malondialdehyde (MDA) is the most important marker for lipid peroxidation. This aldehyde is a highly toxic molecule. Its interaction with the DNA and proteins is often referred to as potentially atherogenic and mutagenic[13], and its primary source is the enzymatic peroxidation of polyunsaturated lipids and the non-enzymatic peroxidation, which involves the participation of reactive oxygen radicals, transition metals, and other free radicals[14]. It can be measured through the umbilical cord blood[15], peripheral blood[16], and saliva[17].

Amongst the antioxidant enzymes described, is the superoxide dismutase (SOD), a metalloprotein with a strong enzymatic antioxidant power. It has three different isoforms: the first, containing copper and zinc, is present in the cytoplasm; the second, containing manganese, is present in the mitochondria; and the third is present in the intracytoplasmic environment of the NB and passes on to the extracellular environment upon reaching adulthood[18,19]. Catalase (CAT), also known as hydroperoxidase, is present in the intracellular environment and is mostly produced mostly in the liver, transforming hydrogen peroxide into water[20].

Glutathione peroxidase (GPX) is an ANTIOXIDANT enzyme present virtually in all cells. Its primary biological function is to protect the organism from oxidative damage through the detoxification of peroxides in the presence of selenium[21-23].

Since PTNB do not have adequate oxidant levels due to the interruption of the transfer between the fetus and the placenta, the endogenous production is insufficient[24,25]. However, the GPX activity in PTNBs is two to six times higher, while the CAT activity is three times lower in neonatal tissues in comparison with adults[26].

Considering that neonatal sepsis still remains as the leading cause for NB mortality, the objective of this study is to measure and compare the concentration of malondialdehyde (marker for lipid peroxidation), superoxide dismutase, glutathione peroxidase, catalase (antioxidant markers) in the umbilical cord blood and saliva from PTNB from mothers with and without risk factors for early-onset neonatal sepsis.

METHODS

This is a cross-sectional study in which information was collected from the mothers in the delivery room and the Neonatal Intensive Care Unit (NICU) of the Municipal Hospital of Barueri, São Paulo.

A total of 21 PTNB were studied between 30-36 weeks of gestation born at the Maternity and from the Neonatology department of the Municipal Hospital of Barueri, São Paulo, from June to July 2016.

We used parameters of normality according to

the birth weight (BW), sex, and gestational age (GA) through Capurro.

Prior to the delivery, we contacted the legal guardian or the pregnant woman to explain the study and collect the signature for the Informed Consent Form. Gestational age was confirmed through Capurro. For all PTNB, we collected data according to the standard questionnaire, which contained : 1 – Maternal and obstetric history (age, weight, height, number of prenatal consultations, number of previous pregnancies, type of delivery, abortions, existence of systemic diseases, smoking, alcohol consumption, use of medication during pregnancy. 2 – Risk factors for neonatal infection: premature rupture of membranes > 18 hours, maternal fever above 38°C, chorioamnionitis. 3 – Neonatal history (sex, date of birth, weight, and height at birth, Apgar scores at the 5th and 10th minute of life, use of supplemental oxygen, diagnosis of suspicion of or proven early-onset neonatal sepsis, the need of phototherapy).

The PTNB were arranged into two groups. Group I: PTNB from mothers with risk factors for early-onset neonatal sepsis; Group II: PTNB from mothers with no risk factors for infection.

Collection of Material

After delayed umbilical cord clamping and the placenta expulsion for each PTNB delivery, we collected 10 ml of blood from the umbilical cord through umbilical vein puncture in a heparin tube in the delivery room. From the NB, we collected 1 ml of unstimulated saliva to prevent the influence of external factors, such as stress, at 24 and 48 hours of life, using direct suction through a vacuum in a dry tube. The samples were centrifuged and stored in a freezer at -70°C and later forwarded to the Oral Biochemistry Laboratory of the Department of Biomaterial and Oral Biology of the Faculty of Odontology of the University of São Paulo (USP), under the responsibility of Dr. Fernando Neves Nogueira. Spectrophotometry analysis of GPX[27] and SOD[28] were carried out. The MDA analysis was carried out through an estimate of the reactive substances of thiobarbituric acid[29], and the CAT analysis through the decomposition of the hydrogen peroxide[30].

Statistical analysis

Based on evidence found in the literature[31] that the risk group for early-onset neonatal sepsis would have an average level of MDA of 10.1 and standard deviation of 2.8, and the group with no risk factors an average of 4.2 and a standard deviation of 2.5 for a 5% probability of committing an alpha error and a statistical power of 80%, it would be necessary to have 7 PTNB in each group.

We studied 21 PTNB between 30-36 weeks of gestation.

We applied the Shapiro-Wilk normality test. For the variables that rejected it, we applied the Mann-Whitney nonparametric test. For all other variables, the parametric t-test was used to obtain the averages.

We only found a statistically significant difference in the glutathione peroxidase variable from the umbilical cord blood (Table 3). In this variable, we can say that the risk factor group presents higher values of glutathione peroxidase than the group with no risk factors.

For all other variables, we found no statistically significant difference between the groups.

Results

This is a prospective cross-sectional study in which were included 21 premature infants according to the inclusion criteria.

We collected data from the PTNB at birth.

The variables selected were: sex, classification according to the weight at birth, Apgar score at the 5th and 10th minute of life, use of 100% oxygen, phototherapy, premature rupture of membranes, maternal fever, chorioamnionitis, assumed sepsis, neonatal stress, number of previous pregnancies, type of delivery, smoking, alcohol consumption, and use of corticosteroids.

As for sex, 47.6% were males and 52.4% females (Table 1). Regarding the classification of birth weight, 19% were SGA, 71.4% AGA, and 9.5% LGA (Table 1). As for the use of 100% supplemental oxygen, 61.9% used it, and 38.1% did not (Table 1). As for the use of phototherapy, 100% did not go through it (Table 1). As for the diagnosis of assumed sepsis, 9.5% had the diagnosis, and 90.5% did not (Table 1). As for the variable of fetal distress, 14.3% presented it, and 85.7% did not (Table 1). As for the number of previous pregnancies, 33.3% had one pregnancy, 38.1% had two. 19% had four, 9.5% had five (Table 1). As for the type of delivery, 33.3% had a vaginal birth, 28.6% an elective cesarean, and 38.1% an emergency cesarean (Table 1). As for smoking and alcohol consumption, 100% of the mothers declared not having either habits (Table

TABLE 1 – SAMPLE CHARACTERIZATION: ABSOLUTE VALUES AND PERCENTAGES ACCORDING TO GROUP

Variables	With factors		Without factors		Total	
	n	%	n	%	n	%
Gender						
Men	5	41.7	5	55.6	10	47.6
Female	7	58.3	4	44.4	11	52.4
Classification per weight						
SGA	-	-	4	44.4	4	19.0
AGA	12	100.0	3	33.3	15	71.4
LGA	-	-	2	22.2	2	9.5
100% Oxygen						
Yes	9	75.0	4	44.4	13	61.9
No	3	25.0	5	55.6	8	38.1
Phototherapy						
Yes	-	-	-	-	-	-
No	12	100.0	9	100.0	21	100.0
Assumed sepsis						
Yes	2	16.7	-	-	2	9.5
No	10	83.3	9	100.0	19	90.5
Neonatal stress						
Yes	-	-	3	33.3	3	14.3
No	12	100.0	6	66.7	18	85.7
Number of pregnancies						
One	7	58.3	-	-	7	33.3
Two	3	25.0	5	55.6	8	38.1
Four	2	16.7	2	22.2	4	19.0
Five	-	-	2	22.2	2	9.5
Type of delivery						
Vaginal	7	58.3	-	-	7	33.3
Elec. Cesarean	3	25.0	3	33.3	6	28.6
Emer. Cesarean	2	16.7	6	66.7	8	38.1
Smoker						
Yes	-	-	-	-	-	-
No	12	100.0	9	100.0	21	100.0
Alcohol consumption						
Yes	-	-	-	-	-	-
No	12	100.0	9	100.0	21	100.0
Use of corticosteroids						
Yes	4	33.3	1	11.1	5	23.8
No	8	66.7	8	88.9	16	76.2
Premature rupture of membranes						
Yes	4	33.3	-	-	4	19.0
No	8	66.7	9	100.0	17	81.0
Maternal Fever						
Yes	-	-	-	-	-	-
No	12	100.0	9	100.0	21	100.0
Chorioamnionitis						
Yes	2	16.7	-	-	2	9.5
No	10	83.3	9	100.0	19	90.5
Total	12	100.0	9	100.0	21	100.0

1). As for the use of corticosteroids for lung maturation, 23.8% used, and 76.2% did not (Table 1).

As for risk factors for early neonatal sepsis: premature rupture of membranes – 19% had it, 81% did not (Table 1); maternal fever – 100% did not have it (Table 1); chorioamnionitis – 9.5% had it, 90.5% did not (Table 1).

The PTNB presented Apgar scores between 8-10 at the 5th minute of life, with a mean of 9, and between 9-10 at the 10th minute of life, with a mean of 9 as well (Table 2).

In the group with no risk factors, the MDA, CAT, SOD, and GPX behaved similarly. For the MDA and CAT, the highest values were found in the blood from the umbilical cord. As for SOD and GPX, the concentration in the saliva at 24 and 48 hours of life was greater than in the blood from the umbilical cord.

There was an statistically significant difference (P=0.019) in the group with risk factors for the variable of GPX in the blood from the umbilical cord. In this variable, we can say that the risk factor group presents higher values of GPX than the group with no risk factors.

For all other variables, we found no statistically significant difference between the groups.

DISCUSSION

In the present study, we analyzed 21 PTNB, out of which 12 (57.2%) were placed in Group I, with risk factors for early-onset neonatal sepsis, and 9 (42.8%) were placed in Group II, with no risk factors for neonatal sepsis.

There was a statistically significant difference (P=0.019) in the group with risk factors for the variable of GPX in the blood from the umbilical cord. In this variable, we can say that the group with risk factors for early-onset neonatal sepsis presents higher values of GPX than the group with no risk factors.

That unbalance is also found in cases of clinical or proven neonatal sepsis, in which levels of

TABLE 2 – SAMPLE CHARACTERIZATION: DESCRIPTIVE STATISTICS ACCORDING TO GROUP

Groups	Variables	n	Lower value	Higher value	Mean	Average	Standard deviation
With factors	Birth weight (g)	12	1,390.00	3,270.00	2,355.00	2,308.33	587.62
	5th minute Apgar	12	8.00	10.00	9.00	9.25	0.75
	10th minute Apgar	2	9.00	10.00	9.50	9.50	0.71
Without factors	Birth weight (g)	9	1,270.00	3,855.00	2,235.00	2,356.67	768.14
	5th minute Apgar	9	8.00	10.00	9.00	9.22	0.67
	10th minute Apgar	1	9.00	9.00	9.00	9.00	-
TOTAL	Birth weight (g)	21	1,270.00	3,855.00	2,300.00	2,329.05	653.09
	5th minute Apgar	21	8.00	10.00	9.00	9.24	0.70
	10th minute Apgar	3	9.00	10.00	9.00	9.33	0.58

TABLE 3 – DESCRIPTIVE STATISTICS AND RESULTS OF THE COMPARATIVE TESTS – BLOOD FROM THE UMBILICAL CORD

Variables (blood)	Groups	Mean	Average	Standard deviation	p-value
Protein	With factor	190.8165	195.9479	34.1258	0.570**
	Without factor	189.4390	228.6762	96.5989	
MDA	With factor	0.0271	0.0281	0.0052	0.286**
	Without factor	0.0313	0.0295	0.0041	
Catalase	With factor	0.1896	0.1922	0.0374	0.366*
	Without factor	0.1487	0.1628	0.0875	
Glutathione peroxidase	With factor	0.2285	0.2465	0.1793	0.019**
	Without factor	0.0791	0.0792	0.0220	
SOD	With factor	0.0051	0.0054	0.0016	0.067*
	Without factor	0.0039	0.0041	0.0014	

* t-Test for averages . **Mann-Whitney Test

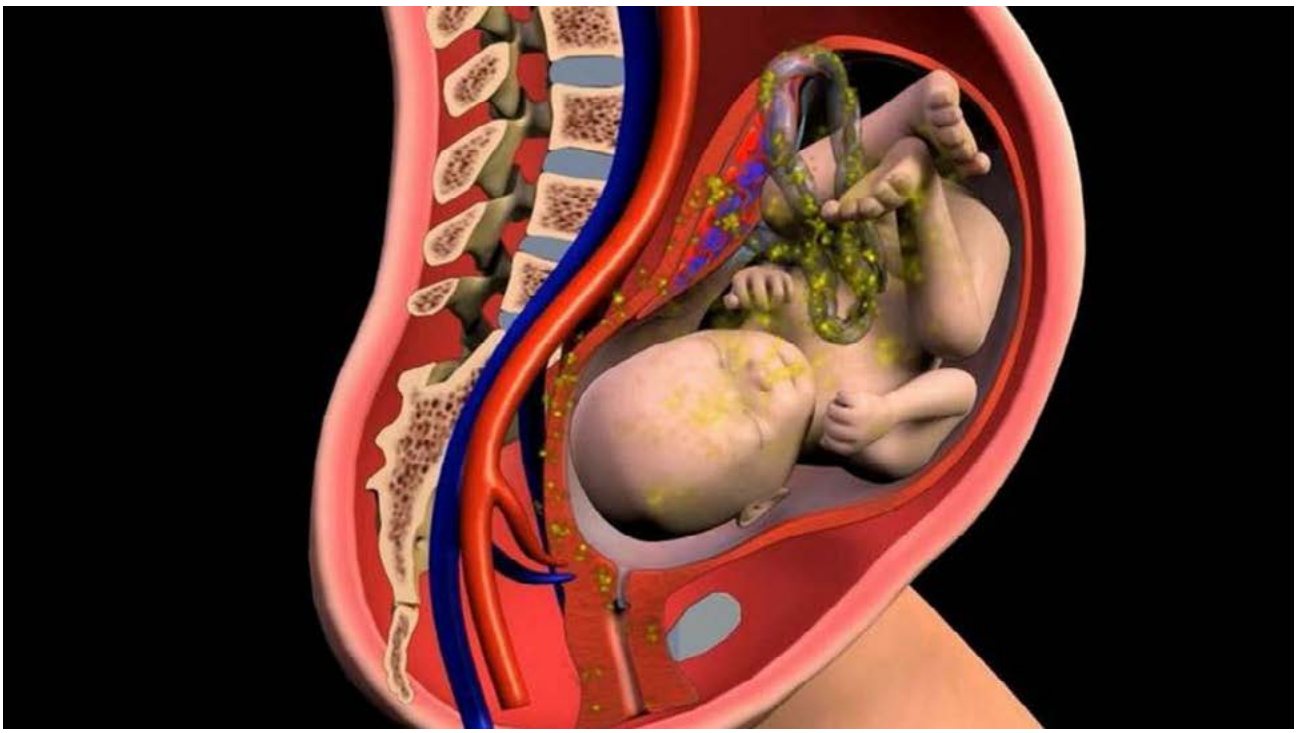

FIGURE 1

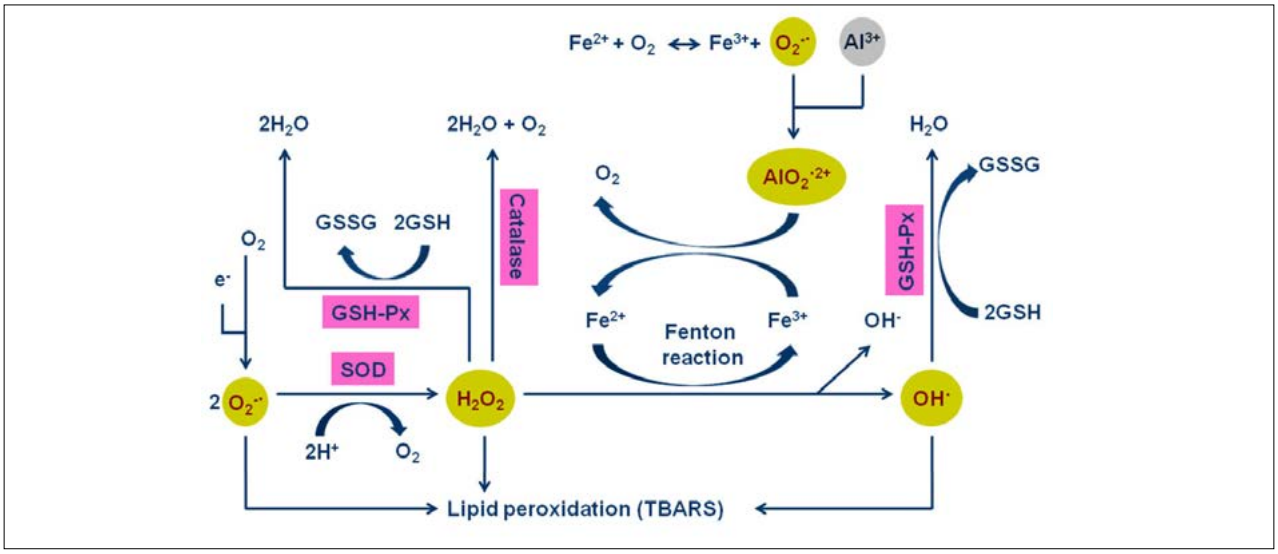

FIGURE 2

lipid peroxidation rise. There is also a significant increase of GPX in these cases of clinical neonatal sepsis[32]. A pediatrics study with 84 children – 42 with clinical sepsis and 42 healthy - showed that the concentration of GPX was higher (P<0.001) in the presence of sepsis than in patients from the control group[33].

In this group, were also found higher concentrations of MDA and CAT in the blood from the umbilical cord, with no statistical difference in the saliva at 24h and 48h of life. In conformity with the literature, a study describes the comparison between MDA concentrations in preterm newborns (31 PTNB) and term NB (29). It was found an MDA concentration two times greater (P=0.002) in PTNB than in term NB[34]. This difference can be explained, for the intracellular defenses against oxidative damage are lower in PTNB than in term NB[35].

The concentration of SOD in the blood from the umbilical cord was lower in comparison with the concentration found in the saliva at 24h and 48h of life. Consistent with the literature, which presented a reduction of SOD activity in the blood from the umbilical cord, showing the possibility

of the enzyme antioxidant protection mechanism against the increase of lipid peroxidation[36].

In the group with no risk factors, MDA and CAT in the blood from the umbilical cord behaved with similar concentrations in the saliva from 24h and 48h of life. In a study were collected 30 blood samples from the umbilical cords from healthy NB, and the MDA analyzed showed low concentrations during labor[37]. However, the GPX and SOD concentrations were lower in the blood from the umbilical cord in comparison with the saliva at 24h and 48h of life. Several authors have shown reduced concentrations of GPX and SOD in comparison with CAT in PTNB[38,39].

Another study also describes that the concentration of GPX in health PTNB is linked to the gestational age: the more premature the newborn is, the lower the GPX concentration[40].

CONCLUSION

The data from this study indicate an increase in the concentration of GPX in the blood from the umbilical vein in PTNB from the group of mothers with risk factors for sepsis in comparison with the other group. There was no statistical significance in the comparison between the saliva and the blood from the umbilical cord. There was no statistically significant difference in the MDA, SOD, and CAT variables between both groups, regarding the saliva at 24h and 48h of life and the blood from the umbilical cord.

The levels of GPX in the blood from the umbilical cord might be a diagnostic tool for more quickly identifying the suspicion of early-onset neonatal sepsis in PTNB of a mother with risk factors for early-onset neonatal sepsis without putting the patient under further stress.

RESUMO

OBJETIVOS: *Determinar a concentração do marcador de peroxidação lipídica: Malondialdeído (MDA) e dos marcadores antioxidantes: Superóxido Dismutase (SOD), Glutationa Peroxidase (GPX), Catalase (CAL) no sangue do cordão umbilical e na saliva não estimulada nas primeiras 24 e 48 horas de vida nos RNPT de mães com e sem fatores de risco para sepse neonatal precoce.*

METODOLOGIA: *Estudo transversal com a assinatura do termo de consentimento livre esclarecido pela gestante e aplicação de um questionário padrão classificando o RNPT no Grupo 1 ou 2.*

RESULTADOS: *Foram estudados 21 RNPT. Quanto ao gênero, peso ao nascimento, necessidade de oxigênio, uso de fototerapia, diagnóstico de sepse presumida, presença de sofrimento fetal, número de gestações, tipo de parto, uso de corticoide, rotura prematura de membranas, a presença de febre materna, a presença de corioamnionite, Apgar no 50 e 100 minuto de vida, a análise estatística foi feita com o teste de Mann-Whitney (p=0,019) na váriável GPX do sangue do cordão umbilical no grupo das mães com fatores de risco para sepse neonatal precoce. Não houve diferença estatística nas outras variáveis MDA, SOD, CAT do grupo com fatores de risco e em nenhuma variável do grupo sem fatores de risco.*

CONCLUSÃO: *O aumento de duas vezes a concentração da GPX no sangue da veia umbilical dos RNPT do grupo das mães com fatores de risco para sepse neonatal precoce. Sem significância estatística na comparação entre a saliva e o sangue do cordão umbilical. Não houve diferença estatisticamente significante nas variáveis MDA, SOD e CAT.*

PALAVRAS-CHAVE: *Estresse oxidativo. Recém-nascido prematuro. Malondialdeído. Glutationa peroxidase. Superóxido dismutase.*

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ORIGINAL ARTICLE

Growth achieved and correlation with blood pressure levels in schoolchildren

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http://dx.doi.org/10.1590/1806-9282.64.10.896

SUMMARY

INTRODUCTION: *The prevalence of systemic arterial hypertension in childhood has increased progressively*

OBJECTIVE: *To analyze blood pressure and height reached by children in public schools in the South of Brazil.*

METHODS: *This is a sectional study of a randomized sample of 1,082 students of six to seven and nine to ten years old used to evaluate blood pressure and height. Blood pressure levels were classified by percentile for gender, age and stature according to the North American reference of* National High Blood Pressure Education Program Working Group on High Blood Pressure *in Children and Adolescents.*

RESULTS: *Schoolchildren presented adequate growth, which was, on average, higher than the WHO reference values. Blood pressure levels were altered in 13.8% of children, with a higher frequency in the nine and ten year olds (17.6%). The isolated analysis of systolic and diastolic Blood pressure revealed a similar trend, with blood pressure levels higher than the greater the value of the Z-score for stature.*

CONCLUSION: *The schoolchildren in the study evidenced adequate growth and an elevated prevalence of pre-hypertension and arterial hypertension, which tended to be higher in older children and/or those that achieved a greater stature growth.*

KEYWORDS: *Body Height. Obesity. Arterial Pressure. Child.*

INTRODUCTION

Systemic Arterial Hypertension in children has become a point of increasing interest due to its elevated prevalence which has been connected, at least partially, to the growth in the prevalence of obesity in children. However, recent evidence suggests that other factors may also be associated with elevated blood pressure (BP), such as a sedentary lifestyle, eating habits and, in particular, the excessive ingestion of salt [1-6].

Several surveys among American children and adolescents during the periods of 1988–1994 and 1999–2002 have shown a slight mean increase of 1.3 mmHg in systolic arterial pressure, whereas diastolic blood pressure increased substantially (8.4 mmHg). In the same period, the prevalence of elevated blood pressure increased from 2.7% to 3.7% [7,8]. Other studies show discrepancies between the secular trend of BP elevation and the prevalence of

DATE OF SUBMISSION: 19-Jan-2018
DATE OF ACCEPTANCE: 20-Jan-2018
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obesity in children and teenagers. A less accentuated increase of arterial pressure means has been verified over the last decade compared to the epidemiologic evolution of obesity [9,10].

In contrast, the proportion of children diagnosed with hypertension during hospitalisation has doubled over the last 20 years, indicating that hypertension in children can no longer be considered a diagnosis to be 'monitored', since, in pediatric patients, the disease can have important consequences in terms of clinical manifestations, complexity and global treatment cost [11].

In Brazil, it is possible that the same phenomenon is occurring because obesity has increased in a marked manner and has become a major public health problem. The results of the Family Budget Survey, in 2009, revealed that one in three children aged 5 to 9 years was over their recommended weight [12]. The research also showed that, since 1989, among children of 5–9 years old in families with low incomes, in 20% of these, there was a strong growth in excess weight (8.9% to 26 5%), while in families with higher incomes, the increase was from 25.8% to 46.2% in the same period [3,13].

Considering that the secular growth trend is observed in Brazil[2] and that height is one of the parameters used for the evaluation of blood pressure in children, a change in the burden of the disease due to hypertension is to be expected, including among children.

This makes it relevant to assess the BP and achieved growth, as well as their correlation in children attending public schools in the capital of Santa Catarina (SC), who are approaching the upper limit of the secular growth trend [14].

The objective of this research was to evaluate the growth of schoolchildren in public schools and their correlation with blood pressure levels.

METHODS

In 2012, a sample of 1,343 children was randomly selected from the list of classes of schoolchildren supplied by the Public Department of Primary Education of Santa Catarina State. The initial sample was obtained by conglomerate raffling of classes until each of the required had 252 children for analysis by age and sex. From the 1,324 initially sampled children, 1,082 (81.7%) were included in the final sample evaluated: 611 aged 6 or 7 years and 471 aged 9 or 10 years.

Of the 242 excluded children, 229 (17.3% of the initial sample) were outside the age range of the study or had morbid problems known to have a correlation with growth or blood pressure, and only 13 (1.0%) were excluded because they did not consent to participate.

Considering a power of 80%, an alpha of 5%, a difference of 1/4 z score on median blood pressure between the groups of age and sex, the sample size estimated as necessary was 252 children per group.

The variables collected were age, gender, blood pressure, stature, and weight. The evaluation of growth and blood pressure took place at the school on scheduled days using standardized instruments for gathering and filing data, always under the researcher's supervision.

One examiner conducted all of the blood pressure measurements. After the child was at rest for five minutes, an aneroid sphygmomanometer was used to measure blood pressure, with cuffs in child sizes (size 15.0 x 6.5 cm), adolescent sizes (dimension 16.0 x 8.0 cm) and adult sizes (dimension 21.5 x 12.0 cm). The aneroid sphygmomanometer was a *Missouri Mikatos* and had been previously calibrated according to recommended techniques. The BP measures, when altered, were repeated three times on the same day and were obtained the average arterial pressure [15].

Blood pressure levels were individually classified by percentile according to the North American standards for gender, age, and stature[15] and were considered altered (pre-hypertension) when higher than the 90th percentile, and elevated (hypertension) when higher than the 95th percentile.

The values for systolic (SBP) and diastolic arterial pressure (DBP) were analyzed statistically as continuous and/or discrete variables. The former (continuous) was utilized to calculate the average and standard deviation; the latter was used to categorize BP into two groups: altered BP and adequate BP. The correlation between growth rate and pre-hypertension (higher than the 90th percentile) and arterial hypertension (higher than the 95th percentile), classified as altered BP, was analyzed for all cases.

For height measurements, we used a *WISO®* portable stadiometer fixed to the wall, graduated in centimeters and millimeters, and annotated with a precision of centimeter tenths. The students were measured standing barefoot, wearing light clothing and with hair lying flat on the head, standing erect, with the head positioned so as to leave the Frankfurt

plane horizontal, legs straight, feet together, arms hanging loosely at the sides and heels, calves, buttocks, shoulder blades and back of the head kept in contact with the flat surface of the wall. Before reading the measurement, the child was placed firmly while the spindle stadiometer was placed at the top of the head (vertex)[15-17].

For the statistical analysis, we used the SPSS (Statistical Package for the Social Sciences) 15.0, *MedCalc* 12.1.4.0 and *GraphPad Prism* 5.04 software. Frequency, proportions, mean, standard deviations (SD), distribution curves and correlation coefficients were calculated.

All the children whose parents or guardians refused to consent to participation and those who were outside the age range of the study and/or had morbid problems known to have a correlation with growth and/or blood pressure, were excluded from the study.

The Ethics Research Committee of the Public Health School of the University of São Paulo approved the study in February of 2012, following the ethical principles for research with human beings (National Health Council 196/96).

RESULTS

The sample was divided into two groups according to age: Group 1 (G1) (six and seven-year-olds) and Group 2 (G2) (nine and ten-year-olds). The proportion of males and females was similar in both groups, and the average age was 6.5 years in the first group, with a standard deviation (SD) of 0.31, and 9.6 years in the second group, with an SD of 0.26 (Table 1).

Figure 1 depicts the distribution of school children, independently of gender and age, according to the z-score of stature. The group as a whole grew, and the median (0.24) shifted towards the upper reference values. The average z-score of stature for age was 0.23 (SD 1.09) for males and 0.26 for females (SD 1.09), indicating a non-significant difference (p = 0.67). In relation to age, the mean of z-score of stature was 0.25 (SD 1.08) in G1 and 0.25 (SD 1.09) in G2, again indicating a non-significant difference (p = 0.989).

FIGURE 1 – FREQUENCY OF SCHOOLCHILDREN ACCORDING TO THE Z-SCORE OF STATURE.

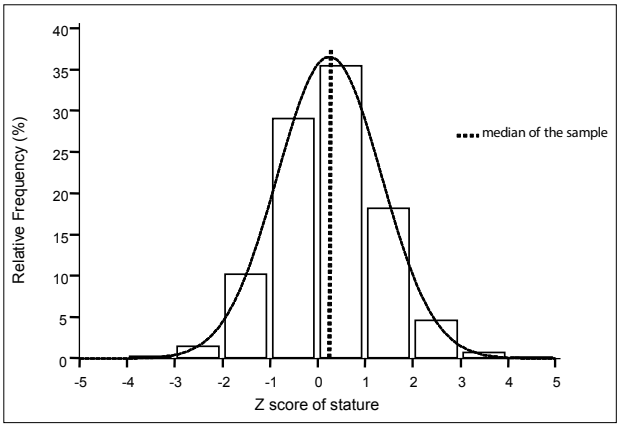


TABLE 1 – DISTRIBUTION OF FREQUENCIES OF SCHOOLCHILDREN ACCORDING TO GENDER AND AGE GROUP.

Age Group	Gender		Total n (%)
	Male n (%)	Femalen (%)	
G1	303 (49.6) (55.4)	308 (50.4) (57.6)	611(100.0) (56.5)
G2	244 (51.8) (44.6)	227 (48.2) (42.4)	471(100.0) (43.5)
Total	547 (50.5) (100.0)	535 (49.5) (100.0)	1082 (100.0) (100.0)

TABLE 2 - DISTRIBUTION OF SCHOOLCHILDREN ACCORDING TO THE CLASSIFICATION OF THEIR CHANGE IN THE SYSTOLIC BLOOD PRESSURE (SBP) AND DIASTOLIC BLOOD PRESSURE (DBP).

Blood Pressure	SAP n (%)	DAP n (%)
Prehypertension	36 (34,6)*	38 (48,1)**
Hypertension	68 (65,4)*	41 (51,9)**
Total	104 (100,0)	79 (100,0)

*p=0,018 (statistically significant). **p=0,08891

The mean systolic blood pressure of the children as a whole was 97.2 mmHg, (95% CI 96.5 to 97.8 mmHg), and the mean diastolic was 61.6 mmHg (95% CI 61.1 to 62.1 mmHg) for the whole group. The prevalence of pre-hypertension or hypertension was 13.7% (148/1082 children). There was an increase of SBP in 9.5% (104 children) and DBP in 7.3% (79 children) in the group. The proportion (65.4%) of children with systolic hypertension was higher than was the proportion (34.6%) with pre-hypertension (p< 0.018). On the contrary, the diastolic pressure showed no statistically significant difference between the proportions of pre-hypertension and hypertension (Table 2).

In the school group analysis as a whole, a statistically significant correlation was observed between the level of SBP and DBP with Z-score of height in the children (Table 3). The Pearson's correlation coefficients between stature and systolic and diastolic arterial pressure were significant for both genders (Table 3).

A statistically significant correlation was also observed with SBP or DBP and the z-score for stature when the analysis was stratified by age group (Table 4), and the comparison of correlation coefficients between the two age groups did not show statistical significance.

The prevalence of higher levels of SBP and DBP was significantly greater in older children, independent of stature. The older age group presented a prevalence ratio of 1.66 when compared to the younger age, a difference that was statistically significant (Table 5).

The presence of changes in blood pressure as a function of height occurred statistically more frequently (p = 0.0414) between the half of children with greater height compared to the half of lower height, 15.8% (85/540) and 11.5% (62/542) respectively.

The analysis of the correlation between "blood pressure level (S and D) and height", Figure 2, was tested both among children of lower zBMI (equal to or less than median zBMI of children in the sample) and higher zBMI (above sample median) including overweight and obesity, and in both the correlation was statistically significant and direct.

DISCUSSION/CONCLUSION

In comparison with the distribution of proposed values by the World Health Organisation in 2007, children attending public schools in Florianopolis in 2012 presented adequate stature growth, independent of gender or age. Notably, the median stature was higher than the reference values were, indicat-

TABLE 3 – CORRELATION OF SYSTOLIC ARTERIAL PRESSURE (SBP) AND DIASTOLIC ARTERIAL PRESSURE (DBP) WITH STATURE, ACCORDING TO SEX.

Gender	SAP	DAP
	r* (CI95%) p	r* (CI95%) p
Male	0.22(0.14 a 0.30) p<0.0001	0.21(0.14 a 0.30) p<0.0001
Female	0.16(0.07 a 0.24) p<0.0003	0.13(0.05 a 0.22) p=0.0021
All	0.19(0.13 a 0.24) p<0.0001	0.18(0.12 a 0.23) p<0.0001

*r= Pearson Correlation Coefficient

TABLE 4 – CORRELATION OF SYSTOLIC BLOOD PRESSURE (SBP) AND DIASTOLIC BLOOD PRESSURE (DAP) WITH STATURE, ACCORDING TO AGE GROUP.

Age	SAP	DAP
	r* (CI95%) p	r* (CI95%) p
G1	0.18(0.10 a 0.25) p<0.0001	0.17(0.10 a 0.25) p<0.0001
G2	0.22(0.14 a 0.31) p<0.0001	0.19(0.10 a 0.27) p=0.0001

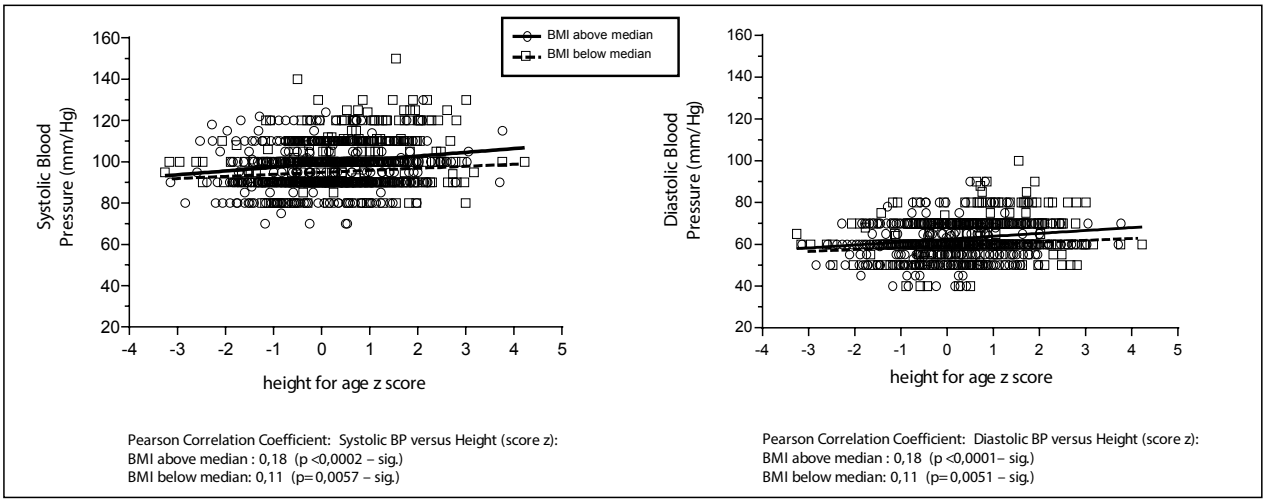
*r= Pearson Correlation Coefficient

TABLE 5 – DISTRIBUTION OF SCHOOLCHILDREN ACCORDING TO THE PRESENCE OF PRE-HYPERTENSION OR HYPERTENSION AND AGE.

Age	Normal Pressure n(%)	BP Alteration n(%)	Total
G1	546 (89.4)	65 (10.6)	611 (100.0)
G2	388 (82.4)	83 (17.6)	471 (100.0)

Fisher's Exact Test: p=0.0012 . Relative Risk=1.66 (CI95%= 1.23–2.24)

FIGURE 2- : SYSTOLIC AND DIASTOLIC BLOOD PRESSURE OF SCHOOLCHILDREN *VERSUS* HEIGHT Z SCORES, ACCORDING TO THEIR BODY MASS INDEX (BMI).



ing that these children probably had already reached values near the expected growth limit as a result of the secular trend of growth [11,18,19].

Considering that the analysis including overweight and obesity, in both groups, the SBP and DBP always increases with rising BMI z score, showing an effect probably independent of overweight (figure 2), in this article the option was to analyze the correlations of blood pressure with height.

Although the observed systolic and diastolic blood pressure averages were very similar to the reference values [15] an elevated prevalence of arterial pre-hypertension and hypertension was verified in comparison with other studies in children [5,8,20].

In relation to age group, a higher prevalence of arterial pre-hypertension or hypertension was observed among older school children, independent of gender. In G2, the relative risk of high blood pressure was 1.66 times higher compared to G1. The 95% CI (1.23–2.24) shows that the attributable risk of increased blood pressure according to age (between six and ten years), at best, is a 23% and at worst a 124% greater risk factor. It suggests that there seems to be an increase in the prevalence of BP alteration with age or, if this is not true, that the reference data, North American, may not be the most appropriate for assessing children of other populations.

The relationship between BP and age, with a higher prevalence of hypertension among older children, is already described in the literature as probably related to hormonal and metabolic changes that occur at the beginning of puberty [21-24].

The fact that our study includes children of nine to ten years of age, meaning that some participants were pre-pubescent or at the beginning of puberty, may partially explain the tendency of elevation of the prevalence of alteration of BP because some studies recognize the association between obesity and early puberty [21,22].

However, this hypothesis regarding the evolution of BP as the normal consequence of the beginning of puberty does not seem to be valid, since it is evidenced by several authors that pubescent children with BP alterations present a higher risk of developing cardiovascular diseases (CVD) in adulthood, when compared to smaller pre-pubescent children of nine years of age[21,25,26].

Our sample showed a direct correlation between greater stature and BP levels intra-group of age or gender, but as the comparison between groups of age and gender evidenced no difference in growth achieved by the children, a specific influence of the child height growth on BP values is evident, regardless of gender or age.

With or without the normal phenomena of puberty, the detection of elevated blood pressure in schoolchildren should always be the object of special attention, due to the increased risk of CVD that might be present.

In accordance with our results, other studies that showed a correlation of blood pressure with growth and pubertal development observed that there is a relationship between BP elevation rates and growth, also suggesting that growth, as a whole, could influence BP [19,26,27]

The results of this study suggest that school-age children who have a height growth greater than the WHO benchmark are subject to a higher risk of pre-hypertension or arterial hypertension, with levels that correlate with the achieved height, regardless of their Body Mass Index.

RESUMO

INTRODUÇÃO: *A prevalência de hipertensão arterial sistêmica na infância aumentou progressivamente.*

OBJETIVO: *Analisar a pressão arterial e a altura alcançada pelas crianças nas escolas públicas do sul do Brasil.*

MÉTODOS: *Estudo transversal de uma amostra aleatória de 1.082 alunos de 6 a 7 e de 9 a 10 anos de idade, para avaliar a pressão arterial e a altura. Os níveis de pressão arterial foram classificados por percentil segundo gênero, idade e estatura, de acordo com a referência norte-americana do* Grupo de Trabalho do Programa Nacional de Estudo em Pressão Arterial *sobre a pressão arterial elevada em crianças e adolescentes.*

RESULTADOS: *Escolares apresentaram crescimento adequado, que foi, em média, superior aos valores de referência da OMS. Os níveis de pressão arterial foram alterados em 13,8% das crianças, com maior frequência aos 9 e 10 anos de idade (17,6%). A análise isolada da pressão arterial sistólica e diastólica revelou uma tendência similar, com níveis de pressão arterial elevados nas crianças com maiores valores de escore Z para a estatura.*

CONCLUSÃO: *Os escolares no estudo apresentaram um crescimento adequado e uma prevalência elevada de pré-hipertensão e hipertensão arterial, que tendem a ser maiores em crianças mais velhas e/ou naquelas que alcançaram maior crescimento de estatura.*

PALAVRAS-CHAVE: *Estatura. Obesidade. Pressão arterial. Criança.*

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ORIGINAL ARTICLE

Comparison of students' motivation at different phases of medical school

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http://dx.doi.org/10.1590/1806-9282.64.10.902

SUMMARY

INTRODUCTION: *Knowledge about student motivation allows educators to broaden their understanding and to establish strategies that make it possible to enhance motivation.*

OBJECTIVES. *To investigate the levels of student motivation at different phases of medical education, comparing incoming students' motivation with that of those at the end of their studies, as well as during the different preclinical, clinical, and clerkship cycles.*

METHOD: *Cross-sectional study including students from a Brazilian public university. The questionnaire included sociodemographic data and the Academic Motivation Scale (AMS). Student motivation was compared at different phases of the medical course.*

RESULTS: *710 students were included. Students in the preclinical phase (1st–2nd years) had higher levels of integrated regulation AMS (e.g., "Education is a privilege."), introjected regulation AMS (e.g."I come because it is what is expected of me."), and intrinsic motivation AMS (e.g."School is a pleasure"). Students in the clinical phase (3rd–4th years) had higher levels of amotivation (e.g., "I'm wasting my time at school.") and external regulation AMS (e.g., "I'm coming to school to earn a degree"). AMS levels of external regulation, introjected relation, and integrated regulation were different for Clerkship students (5th–6th years) compared to preclinical students, but not for clinical students. Comparing only the first and last years, incoming students had higher levels of integrated regulation AMS and lower levels of amotivation AMS and external regulation AMS.*

CONCLUSION: *Important motivational changes were found during different phases of medical school, with higher levels of motivation during the course's initial semesters. These findings can aid in developing educational strategies to stimulate self-determined education.*

KEYWORDS: *Schools, Medical. Personal Autonomy. Students, Medical. Motivation. Behavior and Behavior Mechanisms.*

INTRODUCTION

In recent years, the role of motivation in the teaching-learning process for medical students has been widely discussed. Motivation constitutes one of the indispensable affective components for effective learning in medical education, as well as for higher quality study patterns, better well-being, improved performance, and for training worthy professionals[1,2].

Motivation is defined by the Oxford dictionary (https://en.oxforddictionaries.com/) as the "Desire or willingness to do something; enthusiasm". Although there are several definitions of motivation, a more solid theory for this concept was lacking until the 80s when the Self-Determination Theory (SDT) was created[3]. This is an important theory that defines

DATE OF SUBMISSION: 22-Jan-2018
DATE OF ACCEPTANCE: 22-may-2018
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motivation as psychological energy directed at a particular goal[3], sustaining that motivation possesses different spectra, from amotivation (lack of motive to pursue an activity), passing through extrinsic motivation (the activity is an instrument to achieve a desirable result or to avoid the undesirable) and reaching intrinsic motivation (doing something for the pleasure inherent in the activity itself) [4-6]. The motivation's quality varies due to an innate process of internalization that can be stimulated or inhibited by external and internal factors. Satisfaction of three basic psychological needs is needed to stimulate and maintain intrinsic motivation: autonomy, competence, and belonging [7].

Studies have attempted to evaluate the response to different patterns of motivation. In relation to medical students, a Dutch study found a positive correlation between high intrinsic motivation and more study time, deeper study strategies, and less exhaustion [8]. Corroborating those findings, a Brazilian study found that motivation is associated with valuing the course, level of academic achievement, and greater student self-confidence [9].

However, beyond evaluating which factors are associated with motivation, we need to understand motivational changes during the course's different phases, thus permitting the planning of new educational strategies and curriculum changes. In fact, studies have been undertaken in an attempt to understand university students' motivational patterns. In a study of 856 undergraduate students in 30 courses at an American college, a decline in both intrinsic motivation and extrinsic motivation was found [10]. These results are similar to those of other studies conducted in areas both related and unrelated to healthcare [11,12]. Specifically, in relation to medical students, a Brazilian study [13] evaluated 85 first year students and found an increase in the indices of anxiety and a decrease in academic motivation after 12 months, demonstrating that, in a short period of time, it is already possible to demonstrate motivational changes in students. According to the authors, the possible cause for these findings may be related to the curriculum of the first year, in which the disciplines deal primarily with concepts that are not directly related to the practice of the profession chosen by the freshmen student.

Knowledge about student motivation allows educators to broaden their understanding and to establish strategies that can increase their potential, as well as reduce factors that foster amotivation. Despite this relevance, few studies have evaluated student motivation during the different phases of medical studies. SDT has also been little used in this context. Considering this need, our study attempts to investigate the levels of student motivation at different phases of their medical education, comparing incoming students' motivation with that of those at the end of their studies, as well as during the different preclinical, clinical, and clerkship cycles. With that strategy, our objective has been to see which types of motivation do or do not undergo changes and what the probable hypotheses for those changes would be.

METHODS

Participants and Design of the Study

A Cross-sectional study including students during the six years (12 semesters) of the medical school at the Federal University of Juiz de Fora (UFJF, acronym in Portuguese) was carried out in 2016. All students enrolled in one of the three phases of medical school (preclinical – 1st and 2nd years, clinical – 3rd and 4th years, and clerkship – 5th and 6th years) were invited to participate. Students who were not in Brazil due to exchange activities, who were doing their clerkship in another city, who were not present when data was collected, or who did not wish to participate were excluded.

The project was approved by the UFJF University Hospital's Ethics in Research Committee, registered under the number 13767322015. All participants signed an informed consent.

Procedures

Data collection was done in medical course classrooms, before or after educational activities. The questions were applied as follows: students were given both questionnaires after a brief explanation of study's objectives and the complete fill of the consent form. Students were guaranteed that their information would be confidential.

INSTRUMENTS

The self-applied questionnaire took about 20 minutes to answer and included the following data:

Sociodemographic data: age, ethnicity, civil status, and course year in which enrolled.

Academic Motivation Scale (AMS): we used the

scale developed by Vallerand et al. [14,15], which was translated, validated, and adapted for Portuguese after undergoing factorial analysis [9,16]. This questionnaire, used to evaluate student motivation for learning, is made up of 31 items. The global scale had a Cronbach alpha of 0.817 in the present study. Likert scale answers range from one to seven, with one being "does not correspond at all" and seven being "corresponds exactly". The scale is subdivided into seven subscales:

- Amotivation (Cronbach alpha: 0.864 for this study) – composed by six items (e.g. "Honestly, I don't know why I come to school", "I don't see why I should come to school"),
- Extrinsic motivation through external regulation (Cronbach alpha: 0.684 for this study) – composed by five items (e.g., "I come to school to not get marked absent", "I come to school to earn a degree"),
- Extrinsic motivation through introjected regulation (Cronbach alpha: 0.795 for this study) – composed by six items (e.g., "I come because it is what's expected of me", "to show myself that I am an intelligent person");
- Extrinsic motivation through identified regulation (Cronbach alpha: 0.560) for this study) – composed by two items (e.g. "I come because frequenting classes is necessary for learning", "because I think that requiring attendance is needed so students will take the course seriously")
- Extrinsic motivation through integrated regulation (Cronbach alpha: 0.794 for this study) – composed by four items (e.g. "because education is a privilege", "because studying broadens our horizons"),
- Intrinsic motivation (Cronbach alpha: 0.744 for this study) – composed by three items (e.g., "because the university is a pleasure for me", "because I really like going to school")
- Extrinsic motivation through social regulations (Cronbach alpha: 0.567 for this study) – composed by four items (e.g. "I come to school to get out of the house", "to see my friends is the main reason I come to the university".)

Data Analysis

Descriptive analysis was carried out using frequency and percentage or mean and standard deviation. Inferential analysis was then performed. Students were divided into three groups according to their undergraduate phase (preclinical – 1st and 2nd years, clinical – 3rd and 4th years, and clerkship – 5th and 6th years). To compare student motivation among different phases of the undergraduate medical course, we compared the scores of each of the Academic Motivation Scale's subdimensions using independent t-test or one-way ANOVA for independent samples with a Tukey post hoc test. Two analyses were performed, the first comparing preclinical, clinical, and clerkship students and the second comparing students in the first semester with students in the last semester of the medical course. All analysis was performed using SPSS version 21 (SPSS Inc.). A $p<0.05$ was considered significant.

RESULTS

A total of 710 students were included (response rate 70.5%), from which 265 (37.3%) were enrolled in the preclinical years, 233 (32.8%) in the clinical years, and 212 (29.9%) in the clerkship. Most students were female (55.4%), single (98.0%), and white (66.9%) with a mean age of 22.11 (SD: 3.11) years.

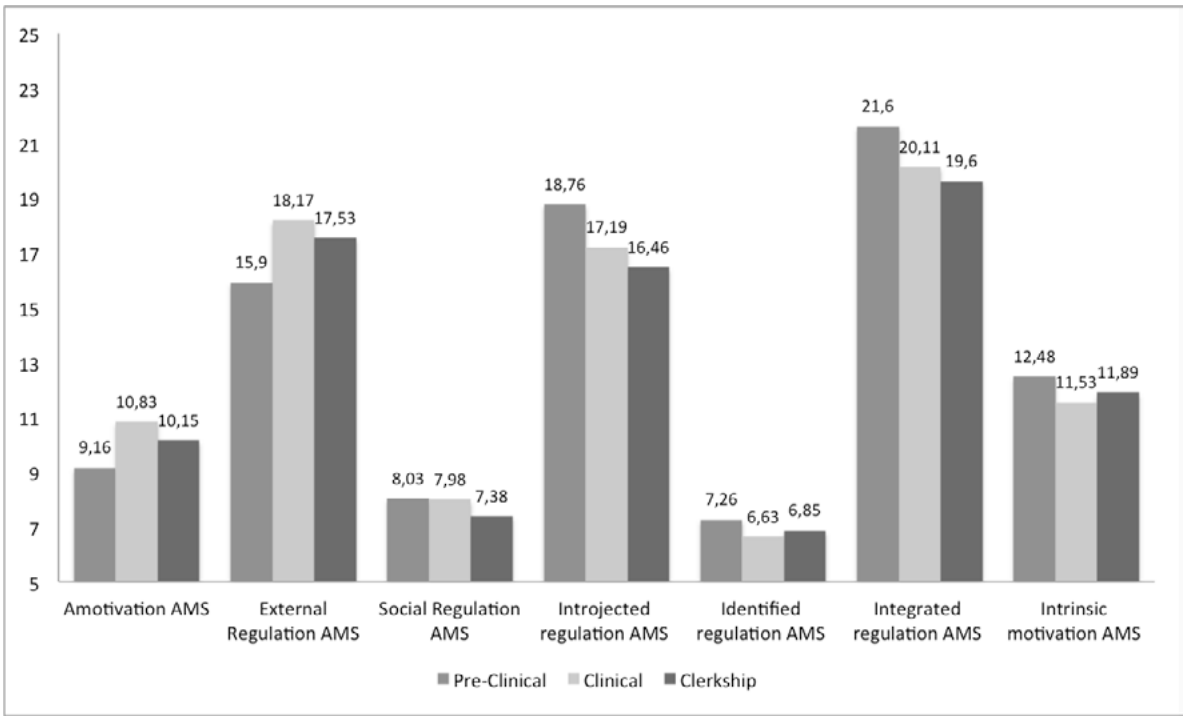
Figure 1 shows the comparison among the different subdimensions of the Academic Motivation Scale among the course's distinctive phases. Comparing to the preclinical phase, the clinical phase had higher scores in the amotivation AMS ($p=0.007$) and external regulation AMS ($p<0.001$) and lower scores in the integrated regulation AMS ($p=0.007$), and intrinsic motivation AMS ($p=0.044$). Likewise, the clerkship phase had higher scores in the external regulation AMS ($p=0.024$) and lower scores in the introjected regulation AMS ($p=0.006$), and integrated regulation AMS ($p<0.001$). Differences between the clinical period and clerkship were not verified.

Figure 2 compares the academic motivation scale's different subdimensions between the course's first and last semesters. The last semester had lower scores in the amotivation AMS ($p<0.001$) and in the external regulation motivation AMS ($p=0.006$), and and higher scores in the integrated regulation AMS ($p<0.001$).

DISCUSSION

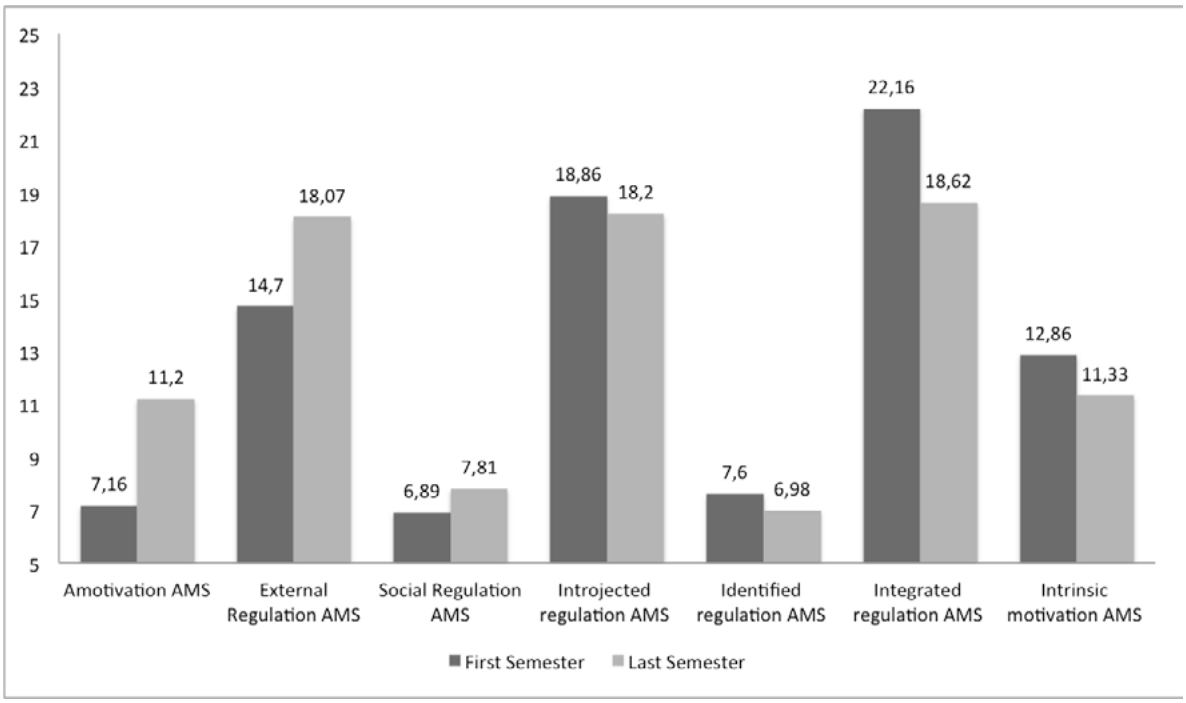
This study has shown that there are significant differences in motivation in medical course phases. Students in the preclinical phase have higher levels

FIGURE 1: LEVELS OF MOTIVATION IN THE PRECLINICAL, CLINICAL, AND CLERKSHIP PHASES OF MEDICAL SCHOOL#



* p<0.05 #Amotivation AMS (e.g. "I am wasting my time in school"), External regulation AMS (e.g. "I come to school to complete my degree"), Social regulation AMS (e.g. "I come to school to get out of the house"), Introjected regulation AMS (e.g. "I come because it is what is expected of me"), Identified regulation AMS (e.g. "I come because I must attend classes to learn"), Integrated regulation AMS (e.g. "Education is a privilege"), Intrinsic motivation AMS (e.g. "school is a pleasure")

FIGURE 2: LEVELS OF MOTIVATION COMPARING THE FIRST AND LAST SEMESTERS OF MEDICAL SCHOOL#



*p<0.05 # Amotivation AMS (e.g. "I am wasting my time in school"), External regulation AMS (e.g. "I come to school to complete my degree"), Social regulation AMS (e.g. "I come to school to get out of the house"), Introjected regulation AMS (e.g. "I come because it is what is expected of me"), Identified regulation AMS (e.g. "I come because I must attend classes to learn"), Integrated regulation AMS (e.g. "Education is a privilege"), Intrinsic motivation AMS (e.g. "school is a pleasure")

of integrated regulation AMS (e.g. "Education is a privilege"), introjected motivation AMS (e.g. "I come because it is what's expected of me") and intrinsic motivation AMS (e.g. "school is a pleasure"). On the other hand, students in the clinical phase have higher levels of amotivation AMS (e.g. "I'm wasting my time at school") and external regulation AMS (e.g., "I come to school to earn a degree"). Clerkship students had results that were non-significant in relation to the clinical period, but different in relation to the preclinical in external regulation AMS, introjected regulation AMS, and integrated regulation AMS. Comparing only the first and last semester of the course, incoming students had higher levels of integrated regulation AMS, and lower levels of amotivation AMS and external regulation AMS. We will discuss some explanations for these findings below.

In relation to high motivation in first period (semester) students, this result concurs with other studies [4,9] and is related to factors that might be difficult for the educational institution to modify. Greater intrinsic motivation at the outset of the course comes from the very choice to pursue a career in medicine as a doctor, which is influenced by factors inherent on student's sociocultural profile (e.g. age, ethnicity, support from parents and professors, higher socioeconomic level), personality traits (self-transcendence and self-directedness), higher socioeconomic level, greater altruism, and the novelty experienced at the beginning of the course, as well as the search for challenges [4,9,17,18].

Once a student enters medical school, motivation begins to depend not only on the student's inherent characteristics, but also on questions related to the learning environment, curriculum, and strategies provided by the medical school. In this context, our study has shown that there are marked differences among the course's phases. Comparing with the scientific literature in the area, Brouse et al.[10] and Hakan & Münire[11] have shown a decrease in the self-determined motivation over the course of undergraduate studies, which can be also observed in our study during the clinical period, with higher values of amotivation (e.g. "I'm wasting my time at school") and of extrinsic motivation through external regulation (e.g. "I come to school to earn a degree") and lower levels of extrinsic motivation through integrated regulation (e.g. "Education is a privilege"). This corroborates the idea that higher education courses in general fail to stimulate and maintain the more intrinsic forms of motivation. The drop in intrinsic motivation from the preclinical to clinical phases is in line with Del-Ben et al.[13]'s findings which suggested that one of the mechanisms is the lack of greater clinical contextualization between theoretical disciplines and medical practice. In our context, the clinical phase still has classes that are mostly theoretical, a high course load, and little contact with patients, which can explain students' low motivation.

In the preclinical and clerkship relationship, we note the lack of difference in the fields of amotivation (e.g. "I'm wasting my time at school"), intrinsic motivation (e.g., "school is a pleasure"), extrinsic motivation through social regulations (e.g. "I come to school to get out of the house"), and extrinsic motivation through identified regulation (e.g., "I come to school because I must attend classes to learn"). The lack of difference between preclinical and clerkship phases and, in contrast, the drop in these variables during the clinical phase, can be explained by the increase in clerkship students' responsibilities, related to higher intrinsic motivation, as pointed out by another study [19] involving primary care clerkship students in the United Kingdom. Its results demonstrated that greater responsibility for patients and clinical practice positively contribute to reinforcing and sustaining the student's self-determined profile. Another possibility is insertion itself in the field of practice, which in and of itself can stimulate self-determined motivation.

Nevertheless, the fact that students in the course's final semester have higher amotivation (e.g. "I'm wasting my time at school") and extrinsic motivation through external regulation (e.g. "I come to school to earn a degree") than beginning students is an important finding. These results are of concern if we consider that this last educational phase involves intense clinical contact and proximity to the moment of "becoming a doctor". These findings differ from those of other studies mentioned in literature which show that last year students tend to be more altruistic and think about alleviating patient suffering in detriment to the secondary benefits of a medical career [20,21]. We can speculate that, in the Brazilian context, great competitiveness and the lack of openings for medical residences can result in higher levels of stress [18] and, consequently, amotivation for clinical activities in detriment to greater hours of study time due to the extremely cognitive tests in the selection process for spaces in residency programs.

This notable change in medical students during

different phases and motivational decline with the passage of time can be explained by a curricular structure based more on the cognitive component of learning [22], in that it minimizes the importance of motivational factors and enhances competitiveness, hence contributing to the increase in amotivation and forms of extrinsic motivation [23]. Thus, the need, especially during the clinical period, for an andragogic proposal to approach motivation through techniques like offering an environment of choices and freedom in order to facilitate empowerment so that students can assume greater responsibility for their learning process; to furnish a well-structured orientation so students are successful, without failing to value their work, allowing them to feel comfortable and free to express their opinions and foment their interest in subjects and tasks [5,24].

Another potential explanation is the learning environment that students are exposed. Positive (i.e. active learning, professional environment, ethical teachers) and negative aspects of this environment (i.e. hazing, unethical behaviors, hidden curriculum) may be responsible to worse outcomes in medical education, including mental health problems, loss of empathy and burnou[25,26]. However, few studies have already found that a worse learning environment can be correlated to worse levels of motivation[27,28]. Further studies should be carried out in order to elucidate the possible causes of this relationship.

This study has some limitations that we should consider. Being a cross-sectional study, variations between the university periods can be a bias for each specific group's motivation profile. The same question can be identified in the vast majority of studies of motivation in medical education [4]. Studies that focus on longitudinal follow up could throw light on fluctuations in the motivational profile over the course of medical studies. Further, factors that influence motivation as a mutable variable, presented by Kusurkar et al.[4] – such as the type of curriculum, form of evaluations, precocious insertion in the field of practice, greater perception of autonomy (as in primary care scenarios), and the sensation of greater well-being – were not investigated in this study, with their presence or absence in the preclinical, clinical, and clerkship periods being related to the variations encountered.

On the other hand, this study contributes to knowledge of the variability of medical students' motivational profile during undergraduate studies. As the SDT is little known in the field of medical education, in spite of advances in understanding motivation for learning [7], this study increases its visibility for understanding and application in medical teaching. Furthermore, this study permits actions designed to develop self-determined motivation in these students be planned and executed in accordance with the peculiarities of each period.

CONCLUSION

In conclusion, our findings reinforce the fact that there are important motivational changes among the different phases of medical school, with there being greater levels of motivation during the course's initial period. These findings can aid in developing educational strategies to stimulate self-determined motivation, fomenting the intrinsic part of motivation so that the disposition to practice and to learn will be stimulated in students in a more genuine way, with the view of prolonging their desire to learn after graduating through contributions by educators during their academic life, bringing improvements to students' medical careers.

RESUMO

INTRODUÇÃO: *O conhecimento sobre a motivação dos estudantes possibilita aos educadores ampliar sua compreensão e estabelecer estratégias que possam potencializá-la.*

OBJETIVOS: *Investigar a motivação do estudante em diferentes momentos da formação médica, comparando-se a motivação de alunos ingressantes e do final do curso, assim como nas diferentes fases pré-clínica, clínica e internato.*

MÉTODOS: *Estudo transversal incluindo estudantes de uma universidade pública brasileira. O questionário incluiu dados sociodemográficos e a Escala de Motivação Acadêmica (EMA). A motivação dos estudantes foi comparada nas diferentes fases do curso.*

RESULTADOS: *Foram incluídos 710 estudantes de medicina. Houve diferenças significantes entre a motivação nas diferentes fases do curso de medicina. Estudantes nas fase pré-clínica (1º e 2º anos) possuíam maiores níveis de EMA regulação integrada (e.g. "Educação é um privilégio"), EMA regulação introjetada (e.g. "venho porque é isso que esperam de mim") e EMA motivação intrínseca (e.g. "universidade é um prazer"). Já estudantes da fase clínica (3º e 4º anos) possuíam maiores níveis de EMA desmotivação (e.g. "estou perdendo meu tempo na universidade") e EMA regulação externa (e.g. "venho à universidade para conseguir o diploma"). Os estudantes do internato (5º e 6º anos) obtiveram resultados não significantes em relação ao período clínico, mas diferentes em relação ao*

pré-clínico na EMA regulação externa, EMA regulação introjetada e EMA regulação integrada. Comparando-se apenas o primeiro com o último semestre do curso, os alunos ingressantes possuíam maiores níveis de EMA regulação integrada e menores níveis de EMA desmotivação e EMA regulação externa.

CONCLUSÃO: *Foram encontradas mudanças motivacionais importantes entre as diferentes fases da formação médica, tendo maiores níveis de motivação nos períodos iniciais do curso. Esses achados podem auxiliar o desenvolvimento de estratégias educacionais que estimulem a educação autodeterminada.*

PALAVRAS-CHAVE: *Faculdades de medicina. Autonomia pessoal. Estudantes de medicina. Motivação. Comportamento e mecanismos comportamentais.*

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ORIGINAL ARTICLE

Opioid tapering and weaning protocols in pediatric critical care units: a systematic review

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http://dx.doi.org/10.1590/1806-9282.64.10.909

SUMMARY

OBJECTIVE: *Opioid abstinence syndrome is common in the pediatric intensive care environment because sedation is often needed during the children's treatment. There is no specific guideline regarding the management of these patients; and lately, methadone is an important drug for the prevention of abstinence symptoms during the weaning of opioids. This study gathers the available research to establish the initial dose of methadone, the rate of taper and tools to recognize this syndrome and act promptly.*

METHODS: *A systematic review was made from data of four different databases. Forty-nine articles of observational and experimental studies were selected based on the inclusion criteria (critical pediatric patients in acute use of opioids) and exclusion criteria (previous chronic use of opioids, other medications). The data regarding specific themes were separated in sections: initial dose of methadone, use of protocols in clinical practice, abstinence scales and adjuvant drugs.*

RESULTS: *The articles showed a great heterogeneity of ways to calculate the initial dose of methadone. The pediatric intensive care units of the study had different weaning protocols, with a lower incidence of abstinence when a pre-defined sequence of tapering was used. The Withdrawal Assessment Tool - 1 was the most used scale for tapering the opioids, with good sensitivity and specificity for signs and symptoms.*

CONCLUSION: *There is still little evidence of other medications that can help prevent the abstinence syndrome of opioids. This study tries to promote a better practice during opioid weaning.*

KEYWORDS: *Review. Critical Care. Analgesics, Opioid. Child. Substance Withdrawal Syndrome.*

INTRODUCTION

Pediatric intensive care includes situations of physiological stress and emotional distress, like invasive procedures (arterial and venous catheterization, orotracheal intubation), care of skin lesions, and others. The child is susceptible to a low degree of cooperation and physical and mental suffering in this environment. Due to these reasons, the use of analgesics and sedatives is an important concern in the care of critically ill children [1].

The main agents used include opioids and benzodiazepines, drugs that on a prolonged use can have serious consequences for the patient, such as muscular atrophy, delirium, and abstinence[1,2].

The prolonged use of sedatives can also cause tol-

DATE OF SUBMISSION: 19-Jan-2018
DATE OF ACCEPTANCE: 20-Jan-2018
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erance, which can be defined as the decrease of the drug's efficiency over time or the need of greater doses to achieve the same effect, a physiologic dependence which is how the body responds needing the maintenance of a certain agent to avoid the development of withdrawal [3].

Abstinence Syndrome (AS) can be described as symptoms and signs associated with the process of discontinuing analgesics and sedatives, characterized by agitation, gastrointestinal and autonomic dysfunction. In this context, the development of strategies and drugs that can improve these collateral effects is of particular interest of the critical care physician[2,3].

Protocols guiding the use of opioids and benzodiazepine are a form of standardizing the clinical practice by providing tools to identify signs and symptoms of tolerance, dependence, and withdrawal[4,5], allowing for prompt action to minimize the physiological impact of the administration of sedatives in adequate doses and taper them safely.

Our group performed a Systematic Review of the medical literature in search for the best available evidence on methadone use for opioid weaning as a way to improve patient care in the pediatric intensive care setting. Our main focus was on the initial methadone dose used for the weaning process, the importance of weaning protocols and well-stablished dosage tapering schemes.

METHODS

Searching Criteria:

Two independent researchers performed a literature search on electronic databases (PubMed, EMBASE, SCOPUS, Web of Science) on July 2016. No time period restriction was applied. The terms used for research were: "substance withdrawal syndrome", "withdrawal syndrome", "opioid", "infant", "child", "adolescent", "pediatric", "critically ill". References cited on the selected studies were also searched for additional articles for potential inclusion.

Inclusion and Exclusion Criteria:

Of the publications found on the search described above were included for the review the ones that fulfilled the following inclusion criteria: studies performed on critically ill pediatric patients (1 month to 18 years old) and admitted in intensive care units. All articles focused on the chronic use of opioids and other drugs or published in languages other than English, Spanish and Portuguese were excluded.

Analysis of included Studies:

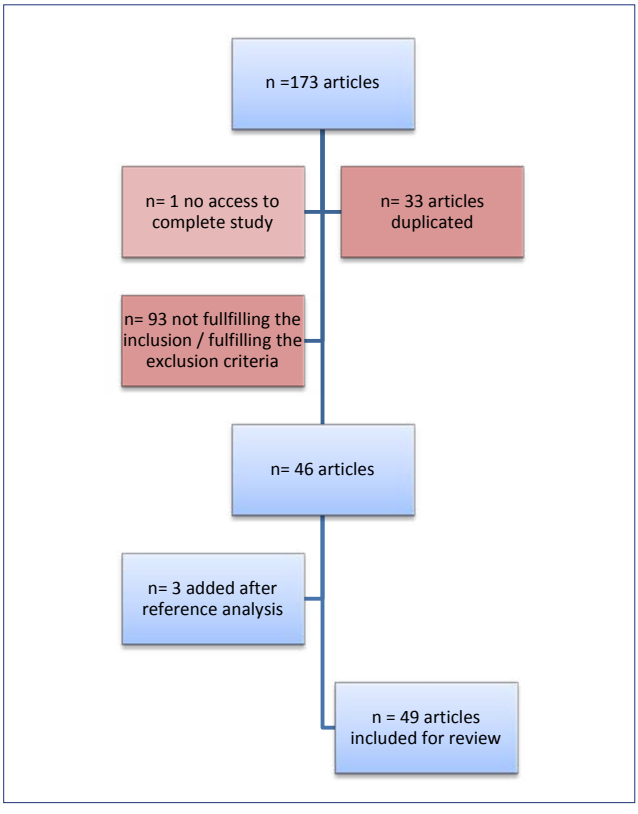
All studies that fulfilled the criteria above were reviewed by 2 independent researchers. After allocation on specific categories, the studies were qualitatively classified by the Jadad and Newcastle-Ottawa scales for their level of evidence[6,7]. A recommendation level for the proposed practice was issued based on the evidence available.

RESULTS

Study Selection

A total of 173 studies were selected after the database search. Of these, 33 were excluded after duplication removal, and 1 for lack of access to the complete article. Of the 139 remaining studies, after an analysis of inclusion and exclusion criteria, 46 articles remained for the review. After an in-depth analysis of the references of the included studies, another 3 articles were selected on an ancestry approach (Figure 1).

FIGURE 1: STUDY SEARCH AND SELECTION OF ARTICLES



The 49 studies included were heterogeneous on their study categories and study objectives (a total of 2 systematic reviews, 10 narrative reviews, 10 clinical trials, 5 cohorts, 7 case-control studies, 7 longitudinal studies, and 8 case-reports/case-series).

The included articles were divided by the 2 independent researchers into categories, based on their study focus: initial methadone dosage, opioid weaning protocol, abstinence scales, and adjuvant therapies.

Initial Methadone Dosage

Of the 49 selected articles, 10 addressed the topic of the initial dose of methadone used for abstinence treatment and prevention. A total of 7 of them were included for systematic analysis: 2 clinical trials, 3 cohorts, 2 case-control studies. Three studies were excluded due to the low level of evidence and references to a previously included study protocol.

Table 1 below shows the analysis of the results of all the studies included in this category:

The studies included analyzed different ways to determine the initial dosage of methadone used for opioid tapering. In spite of the great heterogeneity of the dose determination methods, a tendency to the use of low doses of methadone in the initial abstinence prevention therapy can be observed with no statistically significant differences in the incidence of abstinence symptoms or other outcomes. In this way, after systematic analysis, the use of low methadone initial doses (0,1 mg/kg/dose q6h) can be recommended (Grade B).

TABLE 1. STUDIES REGARDING INITIAL METHADONE DOSE. INCLUSION CRITERIA: USE OF CONTINUOUS FENTANYL OR MORPHINE FOR AT LEAST 5 DAYS AND/OR USE OF METHADONE DURING OPIOID TAPER. EXCLUSION CRITERIA: CNS ABNORMALITIES THAT INFLUENCED THE INTERPRETATION OF THE SIGNS AND SYMPTOMS RELATED TO THE OPIOID WITHDRAWAL SYNDROME.

Author	Study design (evidence)	Population	Initial dose	Withdrawal	Outcomes	Example (weight: 10kg, fentanyl 10mcg/h)
Bowens et al. (8)	Prospective Randomized Trial (IB)	n=78, 1-36 mo	"low-dose" (0,1mg/kg/dose) n=34 vs "high-dose" [0,1mg/kg/dose x recent fentanyl (mcg/kg/h)] n=26	No difference of incidence between the groups	No significant difference of oversedation incidence. Patients that failed to complete the assigned taper (18/78) had longer PICU length of stay and more days of ventilation	Methadone dose: 1mg
Lugo et al. (9)	Retrospective (III)	n=22, 6,1 ± 5,4 yrs	0,5 ± 0,22 mg/kg/dia q6h. Group (1) n=15 no need of dose increase; Group (2) n=7 dose was increased to 0,91 ± 0,37 mg/kg/dia	There was no withdrawal	No significant difference regarding the duration of taper	Methadone dose: 1,25mg
Johnson et al. (10)	Retrospective (III)	n=55, 0,03-12,2 yrs	initial "low-dose" <0,84mg/kg/dia n=27 vs initial "high-dose" ≥ 0,84mg/kg/dia n=28	No difference of incidence between the groups	No significant difference regarding length of stay in PICU	Methadone dose: 2,1mg
Siddappa et al. (11)	Retrospective (III)	n=30, 0,1 - 16,2 yrs	methadone (mg) = 3 x daily fentanyl dose (mg)	There was a significant difference of withdrawal incidence: 78% (7/9) of those who used ≤ 80% of the initial dose had symptoms in comparison with 14% (3/21) of the patients with the initial dose of >80%		Methadone dose: 0,72mg
Jeffries et al. (12)	Retrospective (III)	n=43, 0,25-201 mo	methadone (mg) = morphine (mg)	42% had withdrawal symptoms		
Meyer et al. (13)	Prospective (III)	n=29, 1 day - 19,8 yrs	enteral methadone (mg) = morphine IV (mg) x 2 = fentanyl (mcg/h) x 120	14% (4/29) had withdrawal symptoms		Methadone dose: 1,2mg
Robertson et al. (14)	Prospective comparison of protocol vs control groups (III)	n=20, 6 mo - 18 yrs	enteral methadone (mg) = morphine (mg) = daily fentanyl (mcg) x 10	No difference of incidence between the groups (protocol vs control)		Methadone dose: 2,4mg

Tapering Protocols

Of the 49 selected articles, 5 addressed the topic of the opioid tapering protocol. Were included: 3 clinical trials, 2 cohort/case-control articles that studied the use of a pre-established protocol to guide methadone dose reduction and abstinence prophylaxis and treatment.

Table 2 below summarizes the results of the included studies in this category:

Berens et al. [15] compared two groups with previous use of opioids ≥ 5 days in relation to their time to methadone tapering (5 days x 10 days), with no statistically significant differences between the two approaches in relation to abstinence incidence and

TABLE 2. STUDIES REGARDING METHADONE WEANING PROTOCOLS. INCLUSION CRITERIA: USE OF CONTINUOUS FENTANYL OR MORPHINE FOR AT LEAST 5 DAYS AND/OR USE OF METHADONE DURING OPIOID TAPER. EXCLUSION CRITERIA: CNS ABNORMALITIES THAT INFLUENCED THE INTERPRETATION OF THE SIGNS AND SYMPTOMS RELATED TO THE OPIOID WITHDRAWAL SYNDROME.

Author	Study (evidence)	Population	Protocol	Rescue	Abstinence score	Groups	Incidence of abstinence	Secondary outcomes
Berens et al. (15)	Clinical trial (IB)	n=37, ≤ 18 yrs	Switch over fentanyl or morphine to enteral methadone using an initial dosage ("attack dosage ") and a maintenance daily dosage. Comparison between 5 day weaning protocol reducing 20% of initial dosage + 5 days of placebo vs. 10 days weaning protocol reducing 10% of initial dosage.	Additional dose of methadone equal to the same dose administered the day before or 0,025mg/kg of morphine IV or 0,05mg/kg of methadone incrementally every 30 minutes	Neonatal Abstinence Score - Finnegan (NAS), modified Ramsay.	5 days (n=16) vs. 10 days (n=21)	No difference in incidence of abstinence between groups.	Duration of mechanical ventilation, vasopressor therapy, pediatric intensive care unit (ICU) length of stay and pediatric risk of mortality scores did not differ between the two groups
Steineck et al. (16)	Case - control (III)	n=52, 1m - 16 yrs	Weaning protocol based on the risk of development of abstinence by the duration of opioid and by the accumulate dose of fentanyl. Initial dose ranges from 0,05 to 0,2mg/kg/dose every 8 or 6 hours with reduction rate of 10% to 33% per day.	Additional dose of 0,05mg/kg of morphine IV every 2 hours if abstinence score between 9-11 or 0,1mg/kg if score ≥ 12	Modified Neonatal Abstinence Score - Finnegan (NAS)	pharmaceutic guided protocol (n=20) vs. regular protocol (n=32)	No difference in incidence of abstinence between groups.	A shorter weaning time of methadone (24,7 days vs. 15 days; p=0,003) and shorter length of hospital stay (38%) in the intervention group
Neunhoeffer et al. (4)	Clinical trial (IIB)	n=337, 0-18 yrs	Taper based on duration of opioid exposure: < 5 day – decrease of 50% of initial dose every 24hours; > 5 days – decrease of 10 to 20% every 24hours.	Dose of morphine, fentanyl and midazolam is adjusted according to COMFOT-B and NISS scale. The reduction is suspended for 24 hours if SOS ≥ 4	COMFORT-B, Nurse Interpretation Sedation Scale (NISS), Sophia Observation Withdrawal (SOS).	before protocol (n=165) vs. after protocol (n=172)	Lower incidence of abstinence after implementation of protocol (12,8% vs. 23,6%, p=0,005)	No difference in duration of mechanical ventilation, in days in ICU or total dosage of opioid.
Best et al. (17)	Prospective cohort (III)	n=145, 2 wks - 17 yrs	Group comparisons were made between patients with an intermittent weaning pattern, defined as a 20% or greater increase in daily opioid dose after the start of weaning, and the remaining patients defined as having a steady weaning pattern.	Not specified in the study	FLACC, Wong-Baker Faces, numerical scales to evaluate analgesia; State Behavior Scale; WAT-1.	intermittent pattern (n=66) vs. steady pattern (n=79)	Lower incidence of abstinence (WAT-1 ≥ 3) in the steady group: 46% vs. 85%, p<0,001. The tapering time of steady group was also shorter.	Comparison between protocol group and non-protocol group: lower length of mechanical ventilation (5,9 vs. 9,1 days, p<0,001), lower length of ICU stay (9,3 days vs. 12,8 days, p<0,001) and lower length of hospital stay (14 days vs. 21,5 days, p<0,001).
Robertson et al. (14)	Clinical trial (III)	n=20, 6 m - 18 yrs	Weaning protocol based on duration of opioid exposure: 7 to 14 days: decrease of 20% every day (taper time of 5 days) > 14 day: decrease of 20% every 2 days (taper time of 10 days)	Not specified in the study	Scale based on signs and symptoms from Neonatal Abstinence Score.	protocol (n=10) vs. non protocol (n=10)	No difference in incidence of abstinence between groups.	Lower weaning time in protocol group (9 days vs. 20 days, p<0,001).

ICU length-of-stay showing that a reduction of 20% or 10% of the initial dose had similar results. On the other hand, Steineck et al. [16] compared tapering based on a protocol-based approach with usual care, with no difference on the incidence of abstinence, but with a statistically significant reduction on methadone tapering time and hospital length-of-stay. In this study the transition of intravenous (IV) opioids to enteral was made in 24 to 48 hours, and the doses were decreased daily depending on the previous duration of the IV treatment (< 5 days: q8h to q12h to q24h to suspension; ≥ 5 days: 20% to 10% of initial dose q6h and after q8h to q12h to q24h to suspension).

Neunhoeffer et al. [4] compared two periods (before and after the implementation of an abstinence control protocol), showing a reduction in the incidence of abstinence (12,8% x 23,6%; p = 0,005) with no difference in the hospital length-of-stay. The same approach was performed by two other groups (18), performing analysis on a population before and after the implementation of an abstinence management protocol, showing reductions in the methadone tapering time and hospital length-of-stay.

The evidence points towards safe and fast daily weaning protocols especially in those patients with shorter use of opioids (≤ 5 days) without the increase of abstinence symptoms [15,16,19].

Despite the differences in the protocols implemented on the different studies, the systematical approach to the monitoring of abstinence symptoms and the adjustment of methadone dosage, as well as reduction schemes, can be helpful in the management of opioid withdrawal. Based on the studies above, we can recommend the use of abstinence management protocols, based on the use of assessment scales and pre-defined methadone dose tapering; the weaning rates cannot yet be specified by the available data (Grade: B).

Abstinence Evaluation Scales and Adjuvant Therapies:

Due to the low level of evidence of the studies evaluated, the same systematic approach applied above was not possible. In this way, it was decided to perform a narrative review on these two topics, in a way to provide some basis for these practices. Of the searched articles, 31 analyzed aspects of symptom evaluation and/or adjuvant therapies.

Abstinence Scales (Narrative Review):

The three main scores will be briefly presented below:

Finnegan's neonatal abstinence score: the first widely used abstinence scale in the pediatric setting was developed based on the observation of neonates exposed to opioids during gestation (20). It is composed of 21 evaluation items of neurological, gastrointestinal and autonomic symptoms, generating a numeric score, on which a pharmacologic intervention is warranted on values ≥ 8 (21). Its main limitation is the lack of validity outside neonatal period [21].

Sophia observational withdrawal symptoms scale (SOS): The SOS is composed of 15 items, including vital signs, gastrointestinal, neurologic and autonomic symptoms. The score was developed through a prospective observational study on 76 intensive care patients under 16 years-old who received at least 5 days of continuous sedation (fentanyl, midazolam or morphine) [22]. The lack of multicenter validation is considered the main limitation of SOS [23].

Withdrawal Assessment Tool-1 (WAT-1): Constitutes the most widely used abstinence evaluation tool in a pediatric intensive care setting, because of its easier bedside application, composed of 11 items (including gastrointestinal, neurologic and autonomic symptoms) (24). It was validated on a subsequent multicenter, presenting a sensibility of 87.2% and specificity of 88%, for values ≥3 [(25)].

Although the impossibility of performing a proper systematic recommendation, it was clear to our group that the better external validity and systematic approach on the confection favor the use of WAT-1 as an abstinence assessment tool in a pediatric intensive care setting. However, more evidence is needed to establish the best assessment method.

Adjuvant Therapies (Narrative Review):

Were found 5 case-reports/series-of-cases describing the adjuvant use of dexmedetomidine [26-28], naloxone and clonidine[29], oral morphine[30] and one retrospective study (n = 9) that analyzed the efficacy of subcutaneous fentanyl [31], on the management of abstinence after prolonged use of opioids. All the reports were about specific populations, such as post-cardiac surgery subjects (27,28), which makes external validity an issue. The small population of patients exposed to these interventions makes it impossible to issue a recommendation on the use of any of

these adjuvant therapies, which makes more studies necessary to assess the potential of some of these interventions.

DISCUSSION/CONCLUSION

The use of opioids is well-established in the critical care setting with the goal of analgesia and sedation, reducing stress and distress of the pediatric patient [1,3,32]. The prolonged use of these agents has the potential to lead to abstinence[33-35], a clinical syndrome that can increase the length-of-stay and decrease ventilation-free day of patients, culminating with worst prognosis [2,8].

Our systematic review tries to give emphasis to this growing issue and to promote better scientific-based practices for the management and prevention of abstinence. In spite of the poor level of evidence and lack of substantial and well-controlled trials, some observations could be made.

The use of protocols of opioid tapering and pharmacological management had a tendency of reduction of the total duration of methadone tapering and hospital length-of-stay [4,14-17,36]. The most systematic and objective approach favored a quicker reduction of the daily doses of methadone, promoting a shorter time to its discontinuation, in spite of the great variability of the implemented schemes. More research is needed to further support this observation, especially through clinical trials or prospective observational studies, preferably in a multicenter setting.

The initial dose of methadone is still a big issue, with different ways of calculating and determining it leading to either low or high doses (above 0.1 mg/kg q6h) [8-14]. Although there is not a consensus on the topic, and the results of the papers presented above having showed no statistical differences, the use of low methadone doses can be recommended based on the theoretical benefit of less potential adverse reactions[9-12,37,38] more commonly associated to higher doses of opioids. Nevertheless, this observation regarding adverse reaction is not supported by the articles included in our systematic analysis. A recent meta-analysis and systematic review by Dervan et al.[39] showed that initial doses are widely variable throughout the medical literature, ranging from 1 to 17-times the previously used doses of fentanyl through opioid equivalence.

Monitoring signs and symptoms of abstinence can be made by a wide arsenal of scales and tools, varying from service to service, depending on its clinical routine [40]. There is still a gap of knowledge with good control and systematically designed to asses this question. In light of such a lack of evidence on medical literature, WAT-1 appears to be the most promising and best-defined evaluation tool [24,25], leading to its recommendation and implementation on many pediatric intensive care units. However, there is still a promising field for further research on the topic, especially comparatively analyzing the different methods of evaluation.

Adjuvant therapies, such as the use of dexmedetomedine[28], show promising impressions. However, the great variability of the study population and the small number of patients on which they were tested[26-29] make the external validity an issue, making it crucial to further investigate it using a bigger population and on a more controlled approach.

RESUMO

OBJETIVO: *A síndrome de abstinência de opioides é comum no ambiente de terapia intensiva pediátrica porque a sedação é frequentemente necessária durante o tratamento das crianças. Não existe uma diretriz específica sobre o manejo desse paciente e, ultimamente, a metadona tem sido uma droga importante para a prevenção dos sintomas de abstinência durante o desmame dos opioides. Este estudo reúne as pesquisas disponíveis para estabelecer a dose inicial de metadona, taxa de redução e ferramentas para reconhecer essa síndrome e agir prontamente.*

MÉTODOS: *Uma revisão sistemática foi feita a partir de dados de quatro diferentes bases de dados. Quarenta e nove artigos, de estudos observacionais e experimentais, foram selecionados com base nos critérios de inclusão (pacientes críticos pediátricos em uso de opioides agudamente) e critérios de exclusão (uso crônico prévio de opioides, outros medicamentos). Os dados referentes a temas específicos foram separados em seções: dose inicial de metadona, uso de protocolos na prática clínica, escalas de abstinência e drogas adjuvantes.*

RESULTADOS: *Os artigos mostraram uma grande heterogeneidade de formas de calcular a dose inicial de metadona. As unidades de terapia intensiva pediátrica do estudo apresentaram diferentes protocolos de desmame, com menor incidência de abstinência quando foi utilizada uma sequência predefinida de redução gradual. A Ferramenta de Avaliação de Retirada - 1 foi a escala mais utilizada durante a redução dos opioides, com boa sensibilidade e especificidade para sinais e sintomas.*

CONCLUSÃO: *Ainda há poucas evidências de outros medicamentos que possam ajudar a prevenir a síndrome de abstinência dos opioides. Este estudo tenta promover uma prática melhor durante o desmame dos opioides.*

PALAVRAS-CHAVE: *Revisão. Cuidados críticos. Analgésicos opioides. Criança. Síndrome de Abstinência a Substâncias.*

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ORIGINAL ARTICLE

The artery of Mouchet: blood supply of the septomarginal trabecula in 50 human hearts

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http://dx.doi.org/10.1590/1806-9282.64.10.916

SUMMARY

The septomarginal trabecula is a muscular structure which transmits the right branch of the atrioventricular bundle. It is usually supplied by a branch from the second anterior septal artery. Anastomoses between the right and left coronary arteries may happen on the septomarginal trabecula. They are of great significance in order to prevent ischemia during a myocardial infarction. Surgeries such as Konno's and Ross' procedures implies in knowledge of these vessels anatomy. The coronary arteries of 50 human hearts were injected with latex and subsequentely dissected with the purpose of identifying the arterial branch that supplied the septomarginal trabecula. The trabecular branch arose from the second anterior septal artery in 38% of cases, and the branch arose from the first anterior septal artery in 26%. One of the hearts had its septomarginal trabecula supplied by the conus arteriosus arteryliterature. Anastomoses between the right and left coronary arteries were found inside the septomarginal trabecula. The right branch of the atrioventricular bundle is subject to a great number of clinical conditions and is often manipulated during surgery, thus, the study of the septal branches of the coronary arteries and the trabecular branch is essential.

KEYWORDS: *Heart/anatomy & histology. Coronary circulation. Heart ventricles/anatomia & histologia.*

INTRODUCTION

The septomarginal trabecula (ST) was originally described by Leornardo Da Vinci in 1573 as a fleshy pons inside the right ventricle which originated from the interventricular septum wall and reached the right ventricular anterior wall, either ending on this wall or at the base of the anterior papillary muscle. Da Vinci named it as "arcuate trabecula"[1,2].

The ST is described in modern literature as an extremely important myocardial projection that rises from the septal wall of the right ventricle below to the pulmonary orifice and reaches the anterior papillary muscle[3-5]. Its function was believed to prevent over distension of the right ventricle, although nowadays it is stated that the ST is responsible to transmit the right branch of the atrioventricular bundle to the right ventricle, and consequently, the electric impulse[2,3,5,6].

The interventricular septum is supplied by septal branches of the anterior interventricular artery and septal branches of the posterior interventricular artery. The ST is usually supplied by the second anterior septal branch of the anterior interventricular artery, although this disposition is prone to vary[5,7-12]. The anterior interventricular artery is a branch of the left coronary artery, while the posterior interventricular artery is a branch of the right coronary artery[3,5,6].

Considerations regarding the blood supply of the ST and study of the vascular aspects of the interventricular septum can be of fundamental importance to myocardial revascularization, visualization of those vessels in imaging exams and understanding

DATE OF SUBMISSION: 22-Jan-2018
DATE OF ACCEPTANCE: 27-Jan-2018
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circulatory phenomenon of myocardial hypertrophy[12,13]. Furthermore, pathologies of ischemic nature can compromise the ST and consequently the right branch of the artrioventricular bundle[4].

This study aims to perform macroscopic analysis of the septal branches of the right and left coronary arteries on human hearts, emphasizing the blood supply of the ST.

MATERIALS AND METHODS

The coronary arteries of 50 fresh human hearts were injected with latex (green for the right coronary artery and red for the left coronary artery) and fixed in a solution of 3% formaldehyde, 3% absolute alcohol, and 2% ethylene glycol. The causa mortis of the donors was unrelated to the cardiovascular system and the hearts were free of damage. The present study was conducted at the Anatomy Laboratory of the Rio de Janeiro University and the Morphology Department of the Fluminense Federal University.

The right coronary artery and its main branches (conus arteriosus artery, sinoatrial nodal artery, marginal arteries, posterior interventricular artery, and the atrioventricular nodal branch) as well as the left coronary artery and its main branches (circumflex artery, anterior interventricular artery, diagonal branches) were carefully dissected.

With the help of a surgical microscope, the anterior septal branches of the anterior interventricular artery were dissected. Collateral branches of the right coronary artery that anastomosed with the anterior septal branches were also dissected. The number of anterior septal branches and which branch gave the ST supply was observed and measured with the aid of a digital caliper.

The left coronary artery was divided in three segments according to the ventricular area: superior, middle and inferior.

Descriptive statistics (mean and standard deviation) were analyzed with GraphPad Prism 6 software. All pictures were taken with a Sony Alpha ILCE-3000K (20.1 Megapixels).

RESULTS

The number of anterior septal branches ranged from six to fifteen. All hearts had at least one arterial branch to the ST.

In 26% of cases (13 hearts), the blood supply came from the first anterior septal artery. In 38% (19 hearts), the artery arose from the second anterior septal artery. Seven hearts (14%) had the ST supplied by a branch from the third anterior septal artery. Abnormally, the artery that supplied the ST arose in one case from the conus arteriosus artery (Figure 1). Other results are summarized in Table 1.

Two branches of the trabecular artery were found in 21 cases (42%). In 20 cases (40%) there was one branch. In 5 cases (10%) there were three branches. In 3 cases (6%), four branches. Only one case (5%) possessed five branches inside the ST, which was not previously described in the literature. The results are summarized in Table 2.

The vessels reached the ST through three different patterns: the first pattern was the usual sub-endocardic disposition; the second pattern was through an intramuscular disposition, beneath the center of the ST; and the third pattern was also with an intramuscular disposition, although it was beneath the inferior

FIGURE 1

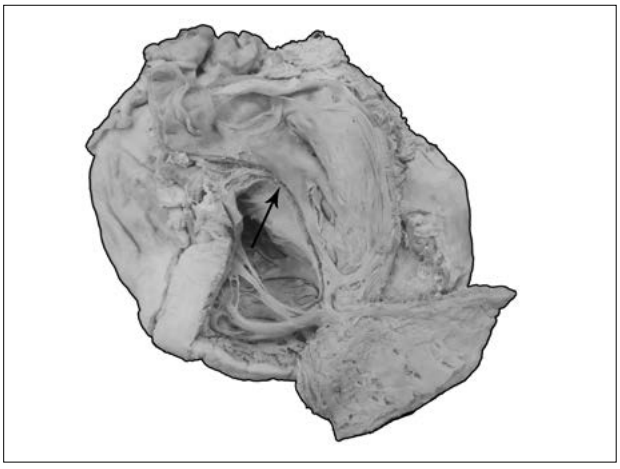

TABLE 1. ORIGIN OF THE TRABECULAR ARTERY.

Origin	Number of cases	%
1st anterior septal	13	26%
2nd anterior septal	19	38%
3rd anterior septal	07	14%
4th anterior septal	01	2%
5th anterior septal	03	6%
6th anterior septal	01	2%
7th anterior septal	02	4%
2nd and 6th anterior septal	01	2%
Left coronary artery	01	2%
1st anterior septal with Right coronary branches	01	2%
Conus arteriosus artery	01	2%
TOTAL	50	100%

TABLE 2. NUMBER OF BRANCHES INSIDE THE ST.

Number of branches	Number of cases	%
01	20	40%
02	21	42%
03	05	10%
04	03	6%
05	01	2%
TOTAL	50	100%

surface of the ST. 58% of the sample had the pattern 1, 34% had the pattern 2, while 6% had the third pattern. One of the cases (2%) had two ST branches, one of them presented the pattern 1 and the other branch possessed the pattern 2.

The ventricular area ranged from 65 to 100 mm, with a mean of 85 mm. Thus, the three segments (superior, middle and inferior) had a mean of 28.3 mm. In 38 hearts (76.%), the ST supply came from the middle segment. In 9 hearts (18%) it came from the superior segment. In only three cases (6%), the branches for the ST blood supply came from the inferior segment.

The length of the ST branch in different hearts varied from 10 to 68 mm.

DISCUSSION

The ST is classified as a trabecula of second order by Sappey[14], Cunningham[15], Llorca[16], and Testut and Latarjet5. It can be seen at the ninth week of fetal age, together with the anterior papillary muscle4.

Dewitt17 observed the disposition and arrangement of the conduction system in mammals' hearts and concluded that there is a constant presence and distribution of the right branch of the atrioventricular bundle and its relation to the ST in all studied hearts.

The blood supply of the anterior pillar is target to only a few anatomical variations, as stated by Winckler[18] and Llorca[16] although both authors emphasizes the fact that the anterior interventricular artery gives origin to a dozen of branches that penetrates the ventral portion of the interventricular septum. Cunningham15, Schlesinger et al.[19], and Testut and Latarjet5 also support this description.

These vessels have a well-defined vascular territory. The anterior septal arteries that arise from the superior third of the anterior interventricular artery possess a descending and posterior trajectory, while the vessels that originates from the middle third have a horizontal trajectory, and the arteries that arise from the inferior third have an ascending and posterior, slightly recurrent trajectories[5,15,16,20]. Those vessels, according to Schlesinger et al.[19], Farrer-Brown and Rowles[21], and Hadziselimović[22], runs to the interventricular septum and forms anastomosis with posterior septal arteries.

Usually, the second anterior septal artery reaches the base of the anterior papillary muscle on the right ventricle and gives origin to a small branch that penetrates the ST, although this branch can originate from the first, third or fourth anterior septal arteries[7-11,16,18,23]. Names such as "artere du pilier anterieru du ventricle droit de Mouchet"[5,6,10], "ramus limbi dextri de Gross"[5,23], "anterior pilar artery"[6] "ramus trabeculae supra-marginalis"[18], "artere de la branche droite du faisceau de His"[18] have been used to refer to the trabecular branch of the second anterior septal artery.

Our results showed that the "trabecular artery" can arise in rare cases from the fifth, sixth, and seventh anterior septal arteries, as well as directly from the right coronary artery, from the conus arteriosus artery, or even from two anterior septal arteries (second and sixth), facts never reported in the literature.

Testut and Latarjet[5] stated that in rare cases, the first and third anterior septal arteries would be responsible for the ST supply, in contraposition, our results showed that in 26% it arose from the first and in 14% it came from the third.

The anterior septal branches would be also responsible for the most part of the IVS blood supply, together with the ST, the right branch of the atrioventricular bundle, and the Purkinje fibers, due to the low pressure values of the right ventricle[24].

Hadziselimović[22] studied 71 human hearts (from neonatal to 81 years old and of both sexes) and his results showed that intramyocardic anastomosis were found in all portions of the heart, particularly on the interventricular septum and adjacent areas.

Moscovici[25] in a study of 80 human hearts also stated the presence of intercoronary anastomosis on the interventricular septum, and their importance regarding the subendocardic plexus. The author also gives importance to the vessels that reach the papillary muscles through their implantation on the ventricular wall, as these vessels use the fleshy trabeculas and myocardic pons to reach them, according to his results, those arteries would also provide intercoronary anastomoses.

Despite the presence of the anterior septal arteries, Correia[23] reported the presence of smaller branches from the anterior interventricular artery that penetrated the ST at different locations, thus, the author

proposed that the ST blood supply should be classified as segmental.

In a study of 651 human hearts, Schlesinger et al.[19] found that in 50% of his sample had an artery rising from the right coronary sinus, together with the right coronary artery: the "conus artery", as he named. The territory supplied by this vessel, according to the author, was the superior portion of the interventricular septum, close to the supraventricular crest, although this territory can also extend itself to larger portions of the septum, thus, its role is fundamental regarding collateral circulation in cases where the right and left coronary arteries are obstructed. Furthermore, the author reported six large arteries that penetrated the interventricular septum (branches of the anterior interventricular artery) with an intimate trajectory with the right margin of the IVS's endocardium.

Schlesinger et al.[19] also stated that some of the anterior interventricular artery branches - especially the ones next to the cardiac apex - diverted their usual trajectory in order to supply or anastomose with nutricious branches of large trabeculas or large myocardic bands (such as the supramarginal crest). Regarding the anastomotic branches, the author states that their origins can be from the anterior interventricular artery, the circumflex artery or the right coronary artery. The branches of the anterior interventricular artery usually have 70 to 800 mm of length, according to Schlesinger et al.[19],

A study by Zapedowski[26] showed that the territory supplied by posterior septal branches would receive collateral vessels from the right and left coronary arteries, thus, again, proving that the anastomosis on the interventricular septum and the papillary muscles can play a large role in pathophysiological and morphological changes.

Hadziselimovic et al.[27] investigated 200 human hearts through coronariography and dissections. Their results displayed the role of the many anastomoses between the collateral and terminal branches of both coronary arteries in respect to coronary artery diseases.

A study conducted by Melo et al.[28] showed that the human heart possess 7 anterior septal branches on average, although some hearts displayed less than 5 branches, while others possessed 13 branches, in contrast with a study performed by Hosseinpour et al.[29] which had the presence of 4 anterior septal branches, on average.

In 2% of cases, the ST blood supply originated from an anastomosis between the first anterior septal artery and branches of the right coronary artery. There are no descriptions regarding this variation in the literature, although Campbell7 stated the existence of anastomoses between the right and left coronary arteries in 20% of his specimens. According to the author, those anastomoses could reduce the consequences of ischemia on the right branch of the atrioventricular bundle in cases of anterior septal arteries obstruction. Likewise, a great number of authors reiterate the clinical significances of those anastomoses[19,24,25,27] .

Pino and Prates[1,30] stated that the origin of the trabecular artery was mainly from the superior segment of the sternocostal face, in contraposition, our results showed that this vessel often came from the middle segment. Furthermore, the authors did not found arteries arising from the inferior segment.

The present work showed the presence of one to five arteries inside the ST. In 21 hearts (42% of cases), it was found 2 arteries inside the trabecula, in accordance to the results of Mouchet[10,11], Correia[23], and Pino and Prates[1,30]. Other authors such as Lascano[31] and Truex and Conpenhaver[32] described the presence of one main branch accompanied by smaller vessels. Furthermore, Pino and Prates[1,30] found four arteries inside the ST, whereas the results of the present study showed a ST with five arteries on its inside, a fact never reported in the literature.

As previously stated, the vessels that supplied the ST reached the band in three different patterns, although a review of the literature only showed the description of the subendocardic pattern[1,8-11,16,18,30,33]. In the present study, it was found two new patterns: an intramuscular pattern in which the vessel ran through inside the ST (34% of cases) and another intramuscular pattern in which the artery ran through the inferior margin of the trabecula (6% of cases).

The mean distance between the origin of the coronary vessels and the trabecular branch of Pino and Prates[1,30] studies had similar results to ours, although the authors only found branches from the first five anterior septal arteries.

A study performed by Possatti et al.[12] in 40 hearts showed that the ST blood supply came from a branch of the first anterior septal branch in 52.5% of cases, from the second in 42.5% of cases and from the third in 5% of cases, in accordance to Pino and Prates1. This is not in accordance with the results found in

the present study, as the second anterior septal artery was the main responsible for the ST blood supply (38% of cases) and the first anterior septal artery was responsible in 26% of cases.

According to Hosseinpour et al.[29] there are slight differences among the pattern and trajectory of the anterior septal branches in normal hearts in comparison to congenitally malformed hearts, especially with hearts that had ventricular septal defects.

Clinical features of the ST and its blood supply involves the fact that it is deeply related to the atrioventricular bundle. Due to this anatomy, removal of this structure in order to treat low defects on the interventricular septum may cause dynamic changes and disruption of the conduction system4. Furthermore, variations on the length and girth of the ST may cause surgical difficulty[4].

Knowledge of the anterior septal branches anatomy is significant to operations such as Ross procedure, Konno procedure (correction of Fallot's tetralogy), resection of obstructive muscular subaortic stenosis, and enlargement of restrictive ventricular septal defects, as they require incisions on the upper portion of the interventricular septum[28,29]. Injury of these vessels can cause myocardial damage and arrhythmia, due to its relation with the ST and right branch of the atrioventricular bundle, in rare cases, iatrogenic injuries can cause sudden death[29].

Aortic stenosis associated with myocardial bridges has shown disappearance of the ASB during systole and their reappearance during diastole[12]. The anterior septal branches are also clinically relevant to angioplasties, as it was shown that those branches can be used to myocardial revascularization if their diameter was at least 2 mm wide[12].

CONCLUSIONS

In summary, the ST is usually supplied by branch from the anterior interventricular artery. Our work showed that the first and second anterior septal branches have an important role regarding this vascular supply, since they originated the trabecular artery in most cases. The conus arteriosus artery can exceptionally provide the ST blood supply, as well as anastomotic branches between the right and left coronary arteries, facts never reported in the literature.

We believe this work has added to the literature new findings regarding the interventricular septum, ST and right branch of the atrioventricular bundle blood supply.

The increasing rates of coronary artery diseases, the constant advances in imaging exams and surgical procedures implies in a more detailed study of the distribution, branching pattern and anastomoses of coronary vessels.

ACKNOWLEDGEMENTS

The authors wish to pay respects and posthumously honor Professor Mauricio Moscivi which was responsible for guiding and supervising Dr. Carlos Chagas Master's thesis, presented in the current study. Furthermore, the authors wish to honor his memory as Anatomy Professor of the Federal University of Rio de Janeiro.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interests.

RESUMO

A trabécula septomarginal é uma estrutura muscular que transmite o ramo direito do feixe atrioventricular. É usualmente suprida por um ramo da segunda artéria septal anterior. Anastomoses entre as artérias coronárias direita e esquerda podem ocorrer na trabécula. São de grande significância especialmente na prevenção de isquemia durante um infarto do miocárdio. Procedimentos cirúrgicos como o de Konno`s e Ross implicam conhecimento anatômico desses vasos. As artérias coronárias de 50 corações humanos foram injetadas com látex e dissecadas com o propósito de identificar o ramo arterial que supria a trabécula septomarginal. Em somente 38% dos casos o ramo foi proveniente da segunda artéria septal anterior, enquanto que em 26% dos casos a artéria se originou da primeira septal anterior. Um dos corações teve a trabécula septomarginal suprida por um ramo originário da artéria do cone arterioso. Além disso, foram encontradas anastomoses entre as artérias coronárias no interior da trabécula septomarginal. Em suma, o ramo direito do feixe atrioventricular está sujeito a inúmeras condições clínicas e é alvo de manuseio em cirurgias, logo, o estudo dos ramos septais das artérias coronárias, em especial o ramo trabecular é essencial.

PALAVRAS-CHAVE: *Coração/anatomia e histologia. Circulação coronária. Ventrículos do coração/anatomia e histologia.*

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ORIGINAL ARTICLE

Cognition, functionality and symptoms in patients under home palliative care

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http://dx.doi.org/10.1590/1806-9282.64.10.922

SUMMARY

OBJECTIVE: *Evaluating the degree of cognition, functionality, presence of symptoms and medications prescribed for patients under palliative home care.*

METHOD: *Descriptive, cross-sectional study where 55 patients under palliative home care were interviewed. Cognition was evaluated using the Mini-Mental state examination (MM), with patients being separated into two groups: with preserved cognitive ability (MM≥24), or altered (MM <24). The functionality was verified by the Palliative Performance Scale (PPS) and the patients were divided into two groups: PPS≤50 and PPS≥60. The symptoms presence was evaluated by ESAS (Edmonton Symptom Assessment System) being considered as mild (ESAS 1-3), moderate (ESAS 4-6) or severe (ESAS 7-10) symptoms. Medications prescribed to control the symptoms were registered. Statistical analysis used Student's t test (p <0.05).*

RESULTS: *Most of the 55 patients were women (63.6%), 70.9% of these had MM> 24, 83.6% had PPS <50 and 78.2% presented chronic non-neoplastic degenerative disease. There was a significant relationship between PPS≤50 and MM≤24. Symptoms were present in 98% of patients. Asthenia was more frequently reported and was not treated in 67% of the cases. Severe pain was present in 27.3%: 46% without medication and 13% with medication, if necessary. Most patients with severe dyspnea used oxygen.*

CONCLUSIONS: *Most of the analysed patients had their cognition preserved, presented low functionality and 98% reported the presence of symptoms. Severe pain was present in almost 1/3 of the patients without effective treatment. Re-evaluate palliative home care is suggested to optimize patient's quality of life.*

KEYWORDS: *Palliative Care. Symptom Assessment. Pain. Home Nursing.*

INTRODUCTION

Population aging associated with increased control of chronic-degenerative diseases leads to a systemic commitment of the patient, with greater dependence and consequent loss of quality of life, which leads to an increase in hospital admissions and a high financial expense on healthcare[1].

The hospital is not the place for such patients because it increases the risk of infection, causes isolation from the family, and exposure to technological resources that do not bring them benefits[1]. In this reality, palliative care (PC) is inserted as a necessary measure that provides humanized medical care directed to the sick person and not only to the disease, and whose main goal is the quality of life. WHO estimates that 20 million people every year require PC at the end of life, and that another 20 million need this care in the years before death[2].

The medical world opinion today is preferably curative/restorative. Consequently, patients with chronic degenerative diseases are often submitted to unnecessary examinations and treatment, with loss of quality of life. These patients, if questioned, would

DATE OF SUBMISSION: 24-Jan-2018
DATE OF ACCEPTANCE: 27-Jan-2018
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not want any kind of invasive therapy. However, the paternalistic view of medicine does not offer these patients the right to exercise their autonomy. On the other hand, the palliative view allows the patient to live as actively as possible, redeem their pending issues and exercise their autonomy[3]. This philosophy is aligned with home treatment, which allows patients to be in their space, to determine their schedules, to live with friends and family, maintaining their daily life routine[3].

One of the challenges of palliative medicine is to respect the autonomy of the human being, combining their perception and way of living to the disease and death. For the evaluation of the patient's autonomy, the determination of their cognitive status becomes important. The Mini-Mental State Examination (MM) is the most widely used scale for this purpose, with high sensitivity and specificity. In Brazil, there are different versions of the MM, with several cut-off points4. The Almeida version[5] points out the value of 24 for the cut-off level of the MM as the most suitable for educated elderly people. According to the authors, patients with MM equal or above 24 are with cognitive capacity preserved and able to exercise their autonomy. On the other hand, patients below this value have impaired cognitive ability[5].

Functionality is an important item for the prognostic evaluation of the patient, and the palliative performance scale (PPS) is used for this purpose[6,7]. It is described that patients with PPS equal to or less than 50 have a prediction of survival around six months and therefore should preferentially receive PC[6,8].

Adequate symptom control is one of the anchors of PC and for such, there is a need for a systematic evaluation that allows identification of the physical, psychic and social symptoms of these patients[6,9]. In order to verify an adequate control of the symptoms, the Edmonton Symptom Assessment System (ESAS)[10] is indicated. The greatest limitation in the use of this scale is that requires the patients communication ability.[6,9].

Considering the need for PC to be optimized in home care, to encourage respect for patients' autonomy and for healthcare professionals to be adequately trained to control the symptoms of patients under such care, this study was idealized, which aimed to evaluate the degree of cognition, functionality and the presence of symptoms in the patients under home PC of an health plan in the city of Florianópolis. Secondarily, medications used to control the identified symptoms were noted.

METHODS

Cross-sectional and descriptive study, approved by the Ethics Committee on Research in Human Beings of the University of Southern Santa Catarina (Unisul), under report No. 1,193,273, which evaluated adult patients over 18 years of age enrolled in a private health plan, who were under home PC in the city of Florianópolis, in the year of 2016. In this plan, PC is provided at the request of the attending physician and are carried out by a multi-professional team, through home visits according to the periodicity established by the professional in charge of the case. Inclusion criteria were patients over 18 years old and able to answer questions. At the time of the study, the total number of adult patients under PC was 215. Sixty-five patients who would receive visits from the researcher in charge were randomly selected. This number was obtained taking into account a sample margin of error of 10%, with a confidence level of 95% and a maximum percentage of 40%. During the study period, 10 patients were excluded due to death or discharge. Fifty-five patients were evaluated between March and August 2016. The interviews were carried out by the author of this study, who, as an observer, did not appear at any time as a provider of any professional services, such condition being informed to the subjects of the study prior to all interviews. All the patients have signed a informed consent form (TCLE), being guaranteed the secrecy to collected data. Preceding the interview, the researcher made telephone contact with all the patients to know the possibility of accepting participation in the study and to schedule the visits, always carried out at the patient's home. During the visit, after obtaining the TCLE, the questionnaire was applied, which included questions about demographic and clinical data.

For the clinical evaluation of the patients' cognitive status, and indirect evaluation of their autonomy, the MM was administered at the time of the visit. Patients were then separated into two groups, those considered to have preserved cognitive ability, which had a value of MM≥24, and that of patients who had an MM<24 and, therefore, had altered cognitive ability[5].

Patients' functionality, an important item for prognostic identification, was verified by the Pallia-

tive Performance Scale (PPS), which is based on five dimensions: ambulation, activity and evidence of disease, self-care, intake, and conscious level. The score of this scale ranges from 0 to 100, in intervals of 10 points, and the higher the score, the better the performance status[6]. Patients were divided into two groups, with PPS≤50 and PPS≥60[8,11].

The presence of symptoms was analysed using ESAS10. This scale can evaluate the presence and intensity of nine symptoms: pain, asthenia, nausea, depression, anxiety, drowsiness, appetite changes, dyspnea and malaise. The patients were grouped according to the intensity of each symptom investigated: 0 absent; 1-3 mild; 4-6 moderate and 7-10 severe. It was found in the patients' records whether they were receiving medication for their symptoms and whether they were using oxygen. For statistical analysis, Student's t test (Windows Excel) was used, being considered a $p < 0.05$.

RESULTS

Analysing the clinical-demographic characteristics of the patients evaluated, 35 (63.6%) were female and 20 (36.4%) were male, with ages ranging from 25 to 105 years old and a mean of 71.3±19.3, and 60% of the patients were over 71 years old. In relation to schooling, 5 (9.1%) patients had 0-3 years of schooling, 15 (27.3%) 4-8 years and 35 (63.6%) over 8 years of schooling. Regarding mental capacity/MM (Mini-Mental State Exam), 16 (29.1%) had MM<24 and 39 (70.9%) MM≥24. Regarding the PPS functionality, 46 (83.6%) had PPS≤50 and 9 (13.4%) had PPS≥60. There was a significant relationship between altered cognitive ability (MM<24) and female ($p < 0.01$), older age ($p < 0.01$) and low functionality ($p < 0.03$). It should be noted that there were a small number of patients with PPS≥60. Regarding the diagnosis of the underlying disease, 12 (21.8%) patients had neoplastic disease and 43 (78.2%) had non-neoplastic chronic-degenerative disease.

Regarding the evaluation of the symptoms, 54 (98%) of the 55 patients studied reported some type of symptom. Figures 1, 2 and 3 indicate the results regarding the presence of symptoms and the suitability of the prescribed treatment for the control of these symptoms. It can be verified that almost all the patients reported some type of malaise sensation, and the majority, 39 (70.9%), considered this malaise as mild or moderate. In general, the most prevalent symptoms were asthenia (n=37/67%), drowsiness (n=31/56%), anxiety (n=30/54%), pain (n=28/51%), depression (n=26/47%), dyspnea (n=25/45%), appetite change (n=25/45%) and nausea (n=11/20%). The most prevalent symptom severity was asthenia (n=20/36%), followed by dyspnea (n=18/33%) and anxiety (n=16/29%). It is noteworthy that 15 (27.3%) patients reported severe pain.

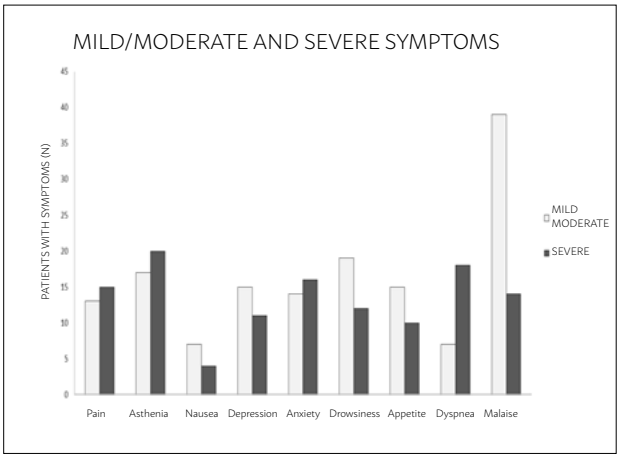

FIGURE 1. Presence of mild/moderate and severe symptoms (ESAS), in relation to the number of patients studied.

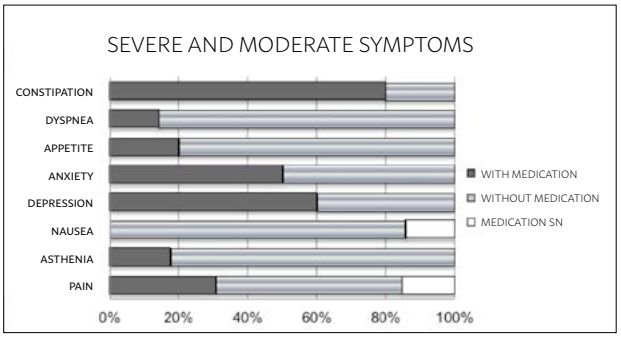

FIGURE 2. Relationship between the presence of mild/moderate symptoms (ESAS) and the prescription of medication to control these symptoms.

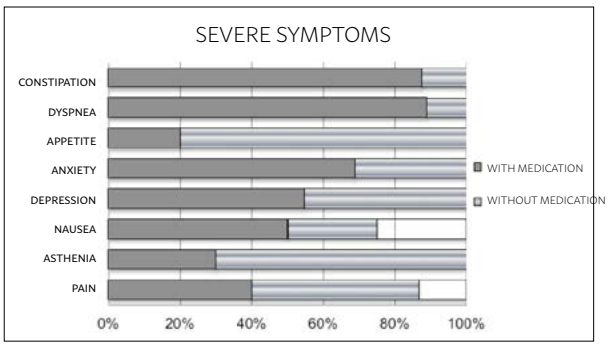

FIGURE 3. Relationship between the presence of severe symptoms (ESAS) and prescription of medication for the control of the symptoms studied.

DISCUSSION

In this study, a significant relationship can be observed between the impaired cognitive state (MM<24) of the patients analysed, female sex, older age and low functionality. These data are within the expected, because with advancing age, worsening functionality and cognitive status are part of aging. Harris et al.[8] demonstrated that age and sex also contribute to the prognosis, with patients under 65 years old being less likely to die within six months and male patients being more likely to die within six months. It should be noted that the majority of patients analysed (70.9%) presented MM≥24 and, therefore, preservation of cognitive ability, essential for the patient to be able to exercise their autonomy. The MM may present a bias because people may have difficulty performing the test. However, since in this study patients had good education level, this did not occur.

In the sample analysed, there was no difference between the degree of cognition of the patients and the level of education or the diagnosis of the underlying disease. The majority of patients suffered from chronic non-neoplastic degenerative disease, which corroborates the current trend regarding the aging of the population and consequent increase in prevalence of these diseases[1]. It is worth noting that the diagnosis of the disease also interferes with the prognosis. Patients with severe cardiac disease, but with PPS still high, are likely to suffer cardiorespiratory arrest, and patients with certain neoplastic diseases, with any PPS value, have a high probability of death within six months. On the other hand, it is difficult to predict adequate life expectancy for patients with neurological disease or dementia, who may present a very low PPS and no quality of life, but still live for a long time[8].

Regarding the functionality (PPS) of the patients studied, it can be seen that the majority (83.6%) had low functionality and, therefore, an important impairment of their quality of life and social life. A significant correlation between PPS≤50 and altered cognitive ability was also identified. Therefore, it can be said that the majority of the patients who composed the sample of this study would prefer PC preferentially or exclusively. A study that evaluated patients with non-neoplastic and neoplastic chronic-degenerative diseases pointed out a life expectancy for those with PPS<50 around 90 days[12]. Other papers, including only patients with neoplasm, demonstrated an even shorter survival of these patients (34 days)[13]. Thus, it can be concluded that the lower the PPS value, the shorter the patient's survival[8,13]. It should be emphasized that prognostic evaluation is important for the definition of the treatment, and more specifically the planning of end-of-life care, which should be guided by the values of the patients and their relatives[7,13].

One of the foundations of PC is symptom control, which allows comfort and quality of life to the patient. At the stage where the patient only needs palliative medical care, events must be predicted for decisions to be shared with the patient/family binomial[6,14]. The control of pain and suffering is considered a fundamental human right, being an important therapeutic competence and ethical responsibility of healthcare professionals[14]. Since 1999, WHO has recommended as priority the effective control of pain, which is considered the fifth vital sign[2]. In palliative medicine, there is the definition of "total pain", so that this symptom is evaluated in its physical, emotional, social and spiritual dimension[14].

The multicentre study Support[15], conducted in 1995 with 10,000 patients with terminal disease, showed that half of them died with moderate or severe pain, without any analgesic prescription. A recent study with hospitalized patients, not on PC, showed patients with various non-medicated symptoms, including pain[16]. On the other hand, studies with chronic patients under PC demonstrate a good control of symptoms and improvement of the quality of life of these patients[17,18].

When the patients are under home PC, relatives are responsible for their care, therefore they must be adequately informed about the real condition of their beloved relative and the prognosis of their disease[19]. They also need to receive technical support for care, especially regarding the supply of drugs to control symptoms[17]. This will avoid unnecessary use of care in emergency sectors[20]. Inadequate control of symptoms will lead to patient and family suffering, rendering it unsafe, which will certainly contribute to a difficult mourning, and may lead to conflicts with healthcare professionals and consequent lawsuits[1].

A fact observed in this study was the high prevalence of poorly controlled symptoms in the patients analysed, especially regarding the control of mild, moderate or severe pain. The adequate control of pain must ask for individualized prescription, scheduled medication and rescue doses, if necessary.[21]. Unfortunately, in this study only 30% of the patients were receiving scheduled medication, 45% of which received

no drugs to control pain and 20% received prescription "if necessary". Another poorly controlled symptom in almost 70% of the patients studied was asthenia. A study that evaluated the symptoms of 168 patients hospitalized in a university hospital showed similar results, indicating poor control of pain reported as moderate. The prescriptions for pain control were predominantly "if necessary", prevailing the use of non-opioid analgesics and weak opioids. In the same study, anxiety was not treated in over 75% of the patients[16].

Regarding the evaluation of dyspnea, it was verified that 30 patients were asymptomatic, one of which was submitted to non-invasive ventilation, another was nebulized with bronchodilators and a third one used oxygen associated with nebulization with bronchodilators. Severe dyspnea was reported by 18 patients (32.7%), of which two patients received no medication to control this symptom. Non-invasive ventilation was provided for three patients with severe dyspnea, and 11 were on oxygen therapy. The use of oxygen was detected in 61.11% of the patients, with nebulization associated with bronchodilators, the second most commonly prescribed medication for dyspnea control (n=12).

Thus, a high prevalence of severe dyspnea was observed, in which about 70% of the patients were being medicated with the administration of oxygen. It is worth noting that the use of oxygen in the final stage of life, and more specifically for the control of dyspnea, is controversial, since, besides the benefit of this medication not being proved, it generates a high cost for the treatment. On the other hand, simple measures like the act of opening a window or connecting a ventilator can decrease the patient's dyspnea by stimulating receptors on the face. It is emphasized that the standard treatment indicated for the control of terminal dyspnea is the use of opioids[22]. This medication was not prescribed for any of the patients with severe dyspnea who participated in this study.

It is worth mentioning that the inadequate control of one symptom may negatively interfere in the development or aggravation of another correlated symptom[9,23]. It is described that, for each symptom spontaneously reported by the patient, nine are no longer communicated[16]. For the mentioned reasons, the studies recommend the systematization of the evaluation of the symptoms and their control, the ESAS scale being the most used for this purpose[18,19]. Evaluation and re-evaluation, carried out using the ESAS and PPS scales, should be done at all visits and is essential for the monitoring of symptom control and prognosis, which may vary with the evolution of the disease, serving as a parameter to determine the most appropriate treatment for each moment[6,19]. Studies indicate that the presence of uncontrolled symptoms leads to a deterioration in the quality of life and its control leads to a rapid improvement in patients' well-being, suggesting that the use of checklists can have a fundamental influence on the quality of care[23,24].

Many symptoms are poorly controlled dued to the doctor's fear of double effect drugs. However, there is an ethical and legal definition regarding the PC to be administered in the final stage of life. The Medical Ethics Code states *that in cases of incurable and terminal illness, the physician should offer all PC available, but avoid unnecessary therapies.* It is described in Resolution n. 1805/2006 of the Federal Council of Medicine[25] that, *in the terminal stage of serious and incurable diseases, the doctor can suspend treatments and must guarantee the necessary care to relieve the symptoms that lead to the suffering, within an integral care, respecting the patient's wishes.* Therefore, the patient has the right to a care that allows them to not suffer, with their symptoms controlled, and as the physician has an ethical duty to provide this type of care[26]. It also influences in the inadequate conduction as to the treatment of patients with terminal illness the lack of technical training of the professionals involved. Brugnolli et al.[27] showed that, although physicians knew the concept of PC, they did not know how to actually apply it in practice. Other studies show the difficulties of physicians in controlling symptoms, alleviate, treating pain adequately and talking about death[26]. Collins et al.,[28] in their study of "a decade after the Support study," show that the end-of-life quality desired by patients and families should incorporate shared decisions, continuity of care, emotional and spiritual support, and adequate symptom control. These items should be associated with comfort care and the organizational support of healthcare services[19].

CONCLUSION

Most patients had preserved cognition, had low functionality and reported the presence of symptoms. Severe pain and major dyspnea were present in almost 1/3 of the patients, not being prescribed effective treatment to them. It is suggested that home PC should be reassessed in order to optimize patients' quality of life.

RESUMO

OBJETIVO: *Avaliar o grau de cognição, a funcionalidade, a presença de sintomas e as medicações prescritas para pacientes sob cuidados paliativos (CP) domiciliares.*

MÉTODO: *Estudo transversal, descritivo, em que foram entrevistados 55 pacientes sob CP domiciliares. A cognição foi avaliada pelo miniexame do estado mental (MM), sendo os pacientes separados em dois grupos: com capacidade cognitiva preservada (MM≥24) ou alterada (MM<24). A funcionalidade foi verificada pela performance paliativa (PPS), sendo os pacientes divididos em dois grupos: PPS≤50 e PPS≥60. A presença de sintomas foi analisada pelo ESAS, sendo considerados sintomas leves (ESAS 1-3), moderados (ESAS 4-6) ou graves (ESAS 7-10). Foram anotadas as medicações prescritas para o controle dos sintomas. Para análise estatística, foi utilizado teste t student's (p<0,05).*

RESULTADOS: *Dos 55 pacientes entrevistados, a maioria era de mulheres (63,6%), 70,9% tinham MM>24, 83,6% tinham PPS<50 e 78,2% apresentavam doença crônica degenerativa não neoplásica. Houve relação significante entre PPS≤50 e MM≤24. Sintomas estavam presentes em 98% dos doentes. Astenia foi mais frequentemente apontada, não sendo tratada em 67% dos casos. Dor grave estava presente em 27,3%: 46% sem medicação e 13% com medicação se necessário. A maioria dos pacientes com dispneia grave usava oxigênio.*

CONCLUSÃO: *A maioria dos pacientes tinha cognição preservada, apresentava baixa funcionalidade e referia a presença de sintomas. Dor grave estava presente em quase 1/3 dos pacientes, não lhes sendo prescrito tratamento eficaz. Sugere-se que sejam reavaliados os CP domiciliares com o objetivo da otimização da qualidade da vida dos pacientes.*

PALAVRAS-CHAVE: *Cuidados paliativos. Avaliação de sintomas. Dor. Assistência domiciliar.*

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ORIGINAL ARTICLE

Detection of intracellular *Helicobacter pylori* in *Candida.* SPP from neonate oral swabs

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http://dx.doi.org/10.1590/1806-9282.64.10.928

SUMMARY

BACKGROUND: *There is evidence of detection of Helicobacter pylori (H. pylori) in the stool of newborns and in the yeast that colonizes the oral cavity of this age group. However, there is a lack of research to confirm it. This study proposes to determine the existence of the bacteria at an early age, specifically in newborns.*

OBJECTIVE: *To identify intracellular H. pylori in oral yeasts and to detect antigens of the bacteria in newborn stools.*

METHODOLOGY: *Cross-sectional and descriptive study. Samples were obtained from infants (oral swab and meconium). Identification of yeast species was performed using the following techniques: CHROMagar Candida, Germinal Tube Test and API Candida Identification System, then the yeasts were observed by light microscopy and fluorescence. Detection of H. pylori antigen in meconium and PCR were performed to amplify specific genes of the bacterium (rRNA16S, cagA, vacA s1a, vacA s1b, vacA s2, vacA m1, vacA m2 and dupA).*

RESULTS: *Intracellular H. pylori was detected in yeast of the species Candida glabrata (C. glabrata) isolated from an oral swab of a newborn.*

CONCLUSION: *The results of this study evidenced the existence of intracellular H. pylori in newborns.*

KEYWORDS: *Helicobacter pylori. Infant, Newborn. Candida glabrata. Candida albicans. Mouth.*

INTRODUCTION

Infections caused by *H. pylori* are one of the most common types in children and adults[1]. Most of the diseases caused by *H. pylori* in adults are the result of an infection acquired during childhood[2], with intra-familial being one of the main routes of transmission of the bacteria[3].

Studies have shown that the presence of *H. pylori* in individuals are related to their socio-economic level and the level of development of their country of residence, since countries with low development levels have a prevalence of up to 84%[5] of *H. pylori* infection amongst children, while in highly developed countries, such as Australia, that number is lower than 15%[6]. Chile is ranked amongst highly developed countries, according to the United Nations classification[7], along with Brazil and Turkey, which present a reported prevalence of 47.5%[8] and 23.9%[9], respectively. According to Jamile et al.[4], in 2013, there was a reported prevalence of 18.1% of *H. pylori* infections among school children aged between 8-15 in a school in Santiago, Chile. Currently, there are no studies in Chile that explore the epidemiology of *H. pylori* in the neonate population, but there are studies in the pediatric population[10].

DATE OF SUBMISSION: 29-Jan-2018
DATE OF ACCEPTANCE: 16-Feb-2018
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H. pylori can cause several gastrointestinal diseases, such as chronic and acute gastritis, duodenal and gastric ulcers, and MALT lymphoma (lymphoma involving the mucosa-associated lymphoid tissue)[1,11]. In addition, it is the main risk factor for the development of gastric cancer[12]. On the other hand, *H. pylori* is associated with problems beyond the intestines, such as growth below normal, malnutrition and anemia, especially in children.[10] For these reasons, the diagnosis and treatment of this bacteria in children is of the utmost importance.

The diagnosis of *H. pylori* infection has proved to be more challenging in children than in adults[13]. Non-invasive diagnostic methods have been used for this age group, despite its relatively low diagnostic accuracy, since invasive procedures, such as endoscopy, are difficult to apply in newborns and/or small children. Amongst non-invasive methods, the measurement of antigens in the stool is easy to apply and has shown excellent results[13].

There are several studies that demonstrate that bacteria can survive in vacuoles of eukaryotes, such as free-living amebas, as is the case with *Enterobacter aerogenes*, *Aeromonas hydrophila*, *Legionella pneumophila*, which were found to be able to survive in vacuoles of *Acanthamoeba castellanii*, through the inhibition of phage-lysosome fusion[14]. They observed the same phenomenon with Pseudomonas aeruginosa, which grew inside the cells in amebas of different genres isolated from hospital tubes[15]. With this background, Siavoshi et al.[16], determined a relationship between *H. pylori* and the oral cavity yeast, since the later organism is found as a commensal on the surface of the mucosa of the human oral cavity, intestines, and vagina. In the same way, that research group isolated yeast from the oral cavity of dyspeptic patients and were able to amplify specific PCR genes of *H. pylori* (*ARNr 16S, cagA, vacA* e *ureAB*) and observed through cellular optical microscopy intracellular *H. pylori* inside the vacuoles of the *Candida* yeast. They concluded that *H. pylori* possibly used the eukaryote as protection from distress conditions. In addition, the infection of neonates by *Candida albicans* species occurs very early, through vertical transmission when they go through the birth canal, causing an oral infection with yeast[17.] That fact led to the objective of this investigation, which is to identify intracellular *H. pylori* in yeats from oral swabs and *H. pylori* antigens from newborn stools.

PATIENTS AND METHODS

Type of study:

This is a quantitative study, observational and descriptive, of cross-sectional time, approved by the Ethics Committee under the code 06/15-22.

Study population

Term newborns (37 weeks or more), born at the Guillermo Grant Benavente Hospital, between August and December 2015, in the City of Concepcion, Biobío, Chile.

Inclusion criteria

Term newborns, born at the Guillermo Grant Benavente Hospital, from women who accepted to participate in the study by signing the informed consent form in order for us to obtain samples from their newborns. The Informed Consent Form could also be signed by the newborn's tutor.

Exclusion criteria

Premature newborns, newborns whose mothers or tutors refused to sign the informed consent form at the moment the samples were collected, and term newborns who were hospitalized at the moment the samples were collected.

Oral swab collection

The samples oral collected through oral swabs in all 53 newborns, at the day of release from the maternity ward, by scraping the cheeks, gums, and under the tongue. The samples were then placed in Stuart tubes previously identified and stored in a hermetically container at room temperature. Later, the samples were transferred to the Bacterial Pathogenicity Laboratory of the Biosciences Faculty of the University of Concepcion, where they were analyzed[18].

Stool sample collection

The meconium from the participant newborns was collected in previously identified wide mouth containers. The samples at the day of release from maternity ward and were stored in a hermetically sealed container with ice. They were then transferred to the Bacterial Pathogenicity Laboratory to be analyzed[16].

Processing of samples to isolate yeast

Each sample from the oral swabs of newborns was sown in Petri plates 94X16 containing 20 ml of

Sabouraud Dextrose Agar prepared according to the manufacturer's instructions and supplemented with chloramphenicol, incubated at 37° C for 24-48 hours.

Then, with a curve handle, an inoculum was removed from the colonies and spread in lines in 94x16 Petri plates containing CHROMagar *Candida* medium prepared in accordance with the manufacturer's recommendations, incubated at 37 °C for 72 hours, and checked at every 24 hours to see if there were growth and differentiation of yeasts by color. Tests of germ tube were conducted in green colonies to differentiate between *C. albicans* and *C. dubliniensis* of non-albicans *Candida*.

Confirmation of the *Candida* species was performed using the API® system for the identification of the *Candida* yeast, following the manufacturer's recommendations (BIOMÉRIEUX, France).

Detection of intracellular H. *pylori* in yeast:

For the intracellular detection of *H. pylori* in the yeast identified in oral samples from newborns, a drop of saline solution at 0.9% was deposited in a 22.4X76.2 mm container, then using a curve handle, an inoculum of a colony was deposited at random, and a 22x22 mm cover was placed over the sample for observation in the microscope with 40X objective, figure1. Then, *H. pylori* were detected with immunofluorescence using rabbit polyclonal IgG anti- *H. pylori* antibodies marked with FITC, whose concentration was 5,000 mg/ml, with a wavelength of 528 nm. The ATCC 90028 strain of C. *albicans* was used as a negative control and the ATCC 43504 strain of *H. pylori* infection as positive control[16].

The images were obtained with DIC (differential interference contrast) and fluorescence with laser stimulation Ar488 nm and emission between 490-560 nm. The fluorescent images correspond to a with a 2-4 µm thick plan. The acquisition software used was Zen 2012.

To perform the intracellular *H. pylori* genotyping of yeasts from oral swabs from newborns, their DNA was extracted using the UltraClean ® Microbial DNA Isolation kit. The DNA was quantified, and the ARNr16S, *cagA, dupA, vacAs1a, vacAs1b, vacAs2, vacAm1, vacAm2* genes were amplified using the SaphireAmp® Fast PCR Master Mix Kit (TAKARA BIO INC, Japan).

Once the genes were amplified, we conducted electrophoresis in agarose gels (Lonza, USA) at 2%[19].

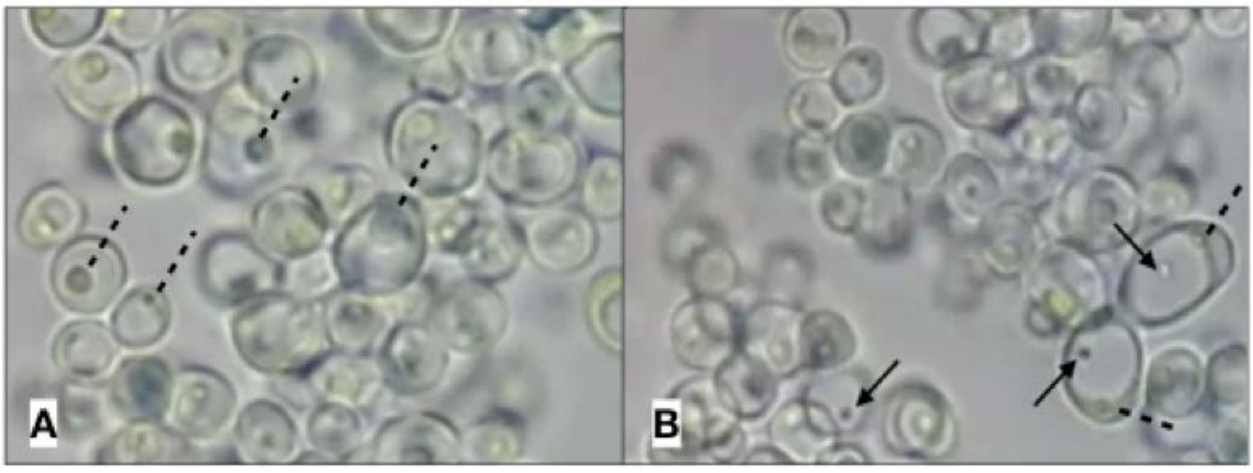
Detection of H. *pylori* antigens in meconium:

We used the Premier Platinum HpSA® Plus Kit (Meridian Bioscience Europe, Italy) following the manufacturer's recommendations. The reaction was read in a spectrophotometer (TECAN, Switzerland); the controls used in this trial were those supplied with the kit.

Data Analysis:

Of 108 newborns, 43 mothers abandoned the study, 2 mothers went home before the official release, and 4 newborns remained hospitalized. There-

FIGURE 1: DIRECT EXAMINATION OF YEAST SAMPLES FROM NEONATE ORAL SWABS, WITH 100X MAGNIFICATION. IMAGE (A) CORRESPONDS TO THE ATCC 90028 STRAIN, USED AS THE CONTROL, AND IMAGE (B) CORRESPONDS TO THE YEASTS FROM THE CLINICAL SAMPLE. THE DOTTED LINES INDICATE THE NUCLEUS OF THE YEAST, AND THE DATES INDICATE INTRACELLULAR INCLUSIONS.



fore, the total number of newborns enrolled in the study was 53, of which 28 were males and 25 females.

The data obtained from the samples were entered into an Excel database and were analyzed using SPSS version 19.0. For this study, only descriptive analyzes were performed, and the qualitative variables were presented as absolute frequency and relative percentage.

TABLE 1: IDENTIFICATION OF YEAST SPECIES FROM NEONATAL ORAL SWABS.

Neonatal oral swabs	Frequency	Percentage (%)
C. albicans	2	3.8
C. glabrata	2	3.8
Negative	49	92.4
Total	53	100

TABLE 2: DETECTION OF *H. PYLORI* IN NEONATAL ORAL YEAST-INFECTIONS THROUGH CRP.

ARNr16S for H. *pylori*	Frequency	Percentage (%)
Negative	52	98.1
Positive	1	1.9
Total	53	100

TABLE 3: RELATIONSHIP BETWEEN YEAST SPECIES FOUND IN NEONATAL ORAL SWABS AND TYPE OF DELIVERY.

	C-section		Vaginal	
Oral swab	N	%	N	%
C. albicans	0	0	2	100
C. glabrata	1	50	1	50
Negative	12	24.5	37	75.5
Total	13	24.5	40	75.5

TABLE 4: IDENTIFICATION OF NEONATES INFECTED WITH INTRACELLULAR *H. PYLORI* THROUGH ANTIGENS IN STOOL AND CRP.

Variable		Frequency	Percentage (%)
H. *pylori* antigen			
	Negative	53	100
ARNr16S for *H. pylori*			
	Negative	52	98.1
	Positive	1	1.9
Total		53	100

RESULTS

The total number of oral swabs samples from newborn babies was 53, of which the majority, 92.4% (n=49), had negative results for yeast. Out of the positive samples, we found the same percentage for C. albicans, 3.8% (n=2) and *C. glabrata*, 3.8% (n=2) % (Table 1).

We managed to detect intracellular inclusion by direct examination using optical microscopy of the yeast samples, obtained from the oral mucosa of newborns, in only one of these samples (corresponding to 1.9%, n=1), as shown in Figure 1. It is noteworthy that the strain of yeast detected then corresponded to the species *C. glabrata*. After applying immunofluorescence, we confirmed that this inclusion corresponded to the *H. pylori* species, also through amplification of 16S rRNA gene of *H. pylori* (Table 2).

Once we confirmed the intracellular inclusion corresponded to the *H. pylori* species, we proceeded to carry out the genotyping of the strain detected. The genotype of intracellular H pylori strains in the *C. glabrata* species, based on the genesis of virulence dupA cagA and vacA was cagA-, dupA-, vacAs1a.

Depending on the type of delivery, we can say that there was only one positive culture for oral yeasts in newborns from cesareans, corresponding to the *C. glabrata* species, and three positive cultures in neonates born from vaginal delivery, two cultures corresponding to the *C. albicans* species and one to *C. glabrata*. For all cultures positive for yeasts, we were able to detect and identify only in one of them the intracellular presence of *H. pylori*; that was in the yeast of the *C. glabrata* species from a newborn by vaginal delivery (Table 3).

With respect to the detection of *H. pylori* antigens in meconium (first evacuation of the newborn), we found no positive samples (Table 4).

DISCUSSION

In Chile, 73% of the adult population have antibodies against *H. pylori*. The infection is acquired in childhood, presenting the highest rates of infection before the age of 10 years[17,20].

In this study, we determined the endosymbiotic relationship between *H. pylori* and yeasts of the *Candida* strains isolated in samples from oral swabs. In addition, we analyzed the existence of *H. pylori* antigens in meconium obtained from term newborns to correlate the presence of intracellular *H. pylori* in

yeasts and the possibility of detection of such antigens in stool samples, a similar study to that by Siavoshi et *al.*[21].

There is evidence that the age at which the infection is acquired could be an important determinant of its course. When the infection occurs in small children, it presents itself as pangastritis, which is associated with the possibility of developing ulcers and carcinoma. When the infection occurs in older children, it is associated with antral-predominance gastritis, with the possibility of developing duodenal ulcers[21]. In turn, the complications caused by the infection are due to a combination of factors, which include bacterial virulence, the characteristics of the host and the environment[22.23].

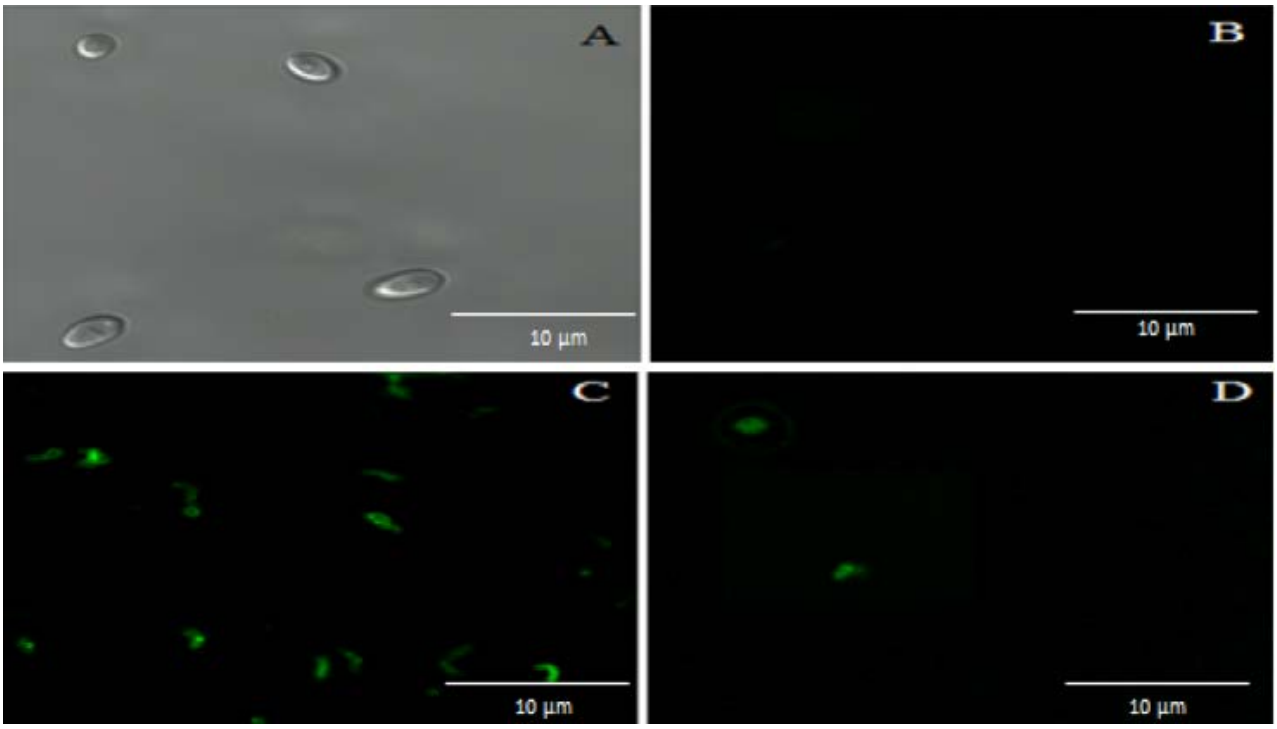
According to a recent report, up to 28% of pregnant women can have vaginal *Candida spp*[24]. This is due to an increase in the estrogen level, which increases glycogen in the vagina, increasing the adhesiveness of vaginal epithelial cells and strengthening the formation of mycelium with the presence of specific receptors of estrogen. In addition, reduced local immunity can also facilitate vaginal colonization and a subsequent infection[25].

Between 75-90% of vulvovaginitis in pregnant women is caused by *C. albicans*, while the other yeasts are called *non-albicans* species, among which is the *Candida glabrata*[26], also found in the oral swabs from newborns in this study and which can be congenitally acquired, i.e., through the presence of micro-organisms on a colonized vagina transported to the uterine cavity[27]. This occurs, in the majority of cases, through intact membranes or the subclinical rupture of membranes prior to the delivery. Once the membranes are penetrated, the micro-organism spreads from the amniotic fluid to the fetal skin and, subsequently, to the respiratory and digestive tract [27].

The newborn can swallow or aspire to the infected amniotic fluid at the time of delivery, whether it as a vaginal delivery or cesarean section [27].

In this study, 12 newborns were delivered by cesarean section; of these, one had a positive result for *C. glabrata*; and of 40 newborns through vaginal delivery, 1 had a positive result for *C. glabrata* and 2 for C. albicans. This suggests that there may be a greater transmission of yeasts from mother to newborn during the passage through the birth canal, which would not happen during cesarean sections. This

FIGURE 2: IMMUNOFLUORESCENCE STUDY WITH *H. PYLORI* ANTIBODIES LABELED WITH FITC. IMAGE A: BRIGHTFIELD MICROSCOPY OF THE NEGATIVE CONTROL *C. ALBICANS* ATCC 90028 STRAIN. IMAGE B: IMMUNOFLUORESCENCE MICROSCOPY WITH *C. ALBICANS* ATCC 90028 STRAIN, WITH NO SIGNS OF FLUORESCENCE. IMAGE C: DARK-FIELD MICROSCOPY OF *H. PYLORI* ATCC 43504 STRAIN (POSITIVE CONTROL). IMAGE D: IMMUNOFLUORESCENCE MICROSCOPY THAT SHOWS THE PRESENCE OF INTRACELLULAR *H. PYLORI* IN YEAST SAMPLES FROM NEONATAL ORAL SWABS. PLAN APOCHROMAT OBJECTIVE 63 X NA 1.4 PLUS ZOOM UP TO 2X



may be due to a greater contact of the newborns with the mothers' vaginal fluids while passing through the birth canal or the contact with the amniotic liquid while rupturing membranes in labor, compared with cesarean sections without going into labor and with no premature rupture of membranes, in which case there is no contact with vaginal secretions.

However, in this case, a positive culture is justified by the rupture of membranes before the cesarean section.

There is evidence from the 1980's that show *C. albicans* as the main agent of fungal infections in newborns, in 80 to 90% of cases [27,28]. However, the recent literature already shows there is a tendency of the emergence of other species, such as *C. parapsilosis* and *C. glabrata* [27], as we have seen in this study.

Some researchers consider the vertical transmission as a way of newborns acquiring an *H. pylori* infection from their mothers during their passage through the birth canal [16]. So far, there have been no reports that bacteria have been isolated directly from the vaginal mucosa. However, a symbiotic relationship has been proposed between the *Candida* species and *H. pylori* bacteria[28,29]. Siavoshi et al.[21], in 2005, isolated yeast from the oral cavity and were able to determine through PCR the presence of *H. pylori* genes (*ARNr 16S, cagA, vacA,* and *ureAB*) and observed through cellular optical microscopy the intracellular *H. pylori* inside the vacuoles of the *Candida* yeast. They concluded that *H. pylori* possibly used the *Candida* as protection from distress conditions. Those findings are in agreement with our study since we detected intracellular *H. pylori* in *C. glabrata* isolated from oral swabs from a newborn using PCR to amplify specific genes of the bacteria.

The research of the gene of virulence in clinical isolates of *H pylori*, as the latter described, constitutes an important tool for characterizing and detecting specific strains that present a greater potential of pathogenicity. The genotyping of *H. pylori* strains found intracellularly in *C. galabrata* and based on virulence genes *dupA, cagA*, and *vacA* had the following results: *cagA-, dupA-, vacAs1a*. It is important to highlight that the vacuolating cytotoxin *VacA* is secreted around 50% of *H. pylori* strains and causes vacuolar degeneration of gastric epithelial cells and ulceration of the gastric mucosa. [30,31].

Genotyping studies of strains of *H. pylori* from Chilean patients showed that the presence of the cagA gene or the allelic variant *vacAs1* has no predictive value for estimating risks of severe gastric pathologies. However, when the strain has the *cagA+/ vacA s1m1* genotype, there is a higher risk of developing peptic ulcer[19,32].

The diagnosis of *H. pylori* infection has proved to be more challenging in children than in adults[32]. Reviews have described the existence of few studies that evaluate the effectiveness of different non-invasive methods to establish the diagnosis of infection in children[17,33].

In this way, one of the objectives of this study was to detect *H. pylori* antigens in the meconium (HpSA) of term newborns. This method, compared with the search for *H. pylori* antigens through serology, is not invasive, corresponding to the painless collection of a sample, which is easy to obtain and presents good results (94% sensitivity and 97% specificity).

In this study, none of the 53 newborns had positive results for *H. pylori* antigens in their meconium, which correlates with what was described in the Chilean study by O'Ryan et al.10, in which only one of 102 stool samples collected at 3 months of life had positive results. In that study, they evaluated the persistent or transitory infection by *H. pylori* in a population of children with follow-up until 5 years of life, in addition to evaluating the dynamics of infection in the group.

The fact that we did not find infected newborns in our group may be due to the time in which the sample was taken, three days after birth, and the small amount of meconium collected. Therefore we suggest an increased sample size and number of samples in future studies.

CONCLUSIONS

The *H. pylori* infection was detected intracellularly in *C. glabrata* by immunofluorescence, which was performed using rabbit polyclonal antibodies IgG anti- *H. pylori* marked with FITC, and was confirmed by the presence of *H. pylori*-specific genes in oral swabs from newborns.

The genotype of intracellular *H. pylori* in the *C. glabrata* species in oral swabs from newborns, based on virulence genes *cag*A, *dup*A e *vac*A, was *cag*A-, *dup*A-, *vac*As1a, determined through PCR.

It will be necessary to monitor these newborns to detect when in their lives they are infected with *H.*

pylori and use other microbiological techniques, such as real-time PCR, for the detection of yeasts in oral swab samples, as well as increase the amount of meconium in the samples and, additionally, perform a search for antigens in stool samples posterior to the meconium of newborns.

This study demonstrates the presence of *H. pylori* in oral swab samples from newborns in an endosymbiotic relationship with *C. glabrata*.

ACKNOWLEDGMENTS

Scientific Initiation project at the University of Concepción, code 215.084.016-1.OIN.

Ethical aspects: The authors of this research declare that they have no conflict of interest. This research was approved by the Health Service Ethics Committee of Concepción-Chile, with the code 15-22-06 and was approved on July 5, 2015.

RESUMO

ANTECEDENTES: *Há evidências de detecção de Helicobacter pylori (H. pylori) em fezes de recém-nascidos, como também dentro de leveduras que colonizam a cavidade oral dessa faixa etária. No entanto, faltam investigações que confirmem esses achados.*

OBJETIVO: *Identificar H. pylori intracelular em leveduras de origem oral e detectar antígenos dessa bactéria em fezes neonatais.*

METODOLOGIA: *Estudo transversal e descritivo. As amostras foram obtidas de bebês (zaragatoa oral e mecônio). As identificações das espécies de leveduras foram realizadas utilizando as seguintes técnicas: CHROMagar Candida, teste de tubo germinativo e sistema de identificação API Cândida. As leveduras foram observadas por microscopia óptica e fluorescência. Realizou-se a detecção de antígeno de H. pylori em mecônio e PCR para a amplificação de genes específicos desta bactéria (rRNA16S, cagA, vacA s1a, vacA s1b, vacA s2, vacA m1, vacA m2 e dupA).*

RESULTADOS: *Foi detectado H. pylori intracelular em leveduras da espécie Candida glabrata (C. glabrata) isoladas a partir de zaragatoas oral de um recém-nascido.*

CONCLUSÃO: *Os resultados deste estudo evidenciaram a existência interna de levedura de H. pylori em recém-nascidos.*

PALAVRAS-CHAVE: *Helicobacter pylori. Recém-nascidos. Candida glabrata. Candida albicans. Boca.*

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ORIGINAL ARTICLE

Ultrasonography features of abdominal perimuscular connective tissue in elite and amateur basketball players: an observational study

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http://dx.doi.org/10.1590/1806-9282.64.10.936

SUMMARY

The purpose of this study was to assess and compare with rehabilitative ultrasound imaging (RUSI) the perimuscular connective tissue (PMCT) and interrecti distance (IRD) between elite and amateur basketball players. A sample of 22 healthy basketball players was included and divided into two groups: elite basketball players from Spanish 1st division (n = 11) and amateur basketball players from an entertainment Spanish division (n = 11). Ultrasound images of the external oblique (EO), internal oblique (IO), transversus abdominis (TrAb), rectus anterior (RA) and IRD PMCT were measured and analysed by the ImageJ software. Measurements of abdominal wall muscles PMCT present statistically differences (P < .05) for an increase of perimuscular connective tissue of external oblique (PMCTEO), perimuscular connective tissue of transversus abdominis (PMCTTA) of the left side and an increase of PMCTEO on the right side in favor of the elite group. Rather, the study showed statistically differences (P < .05) for a decrease of perimuscular connective tissue between the internal oblique and transversus abdominis (PMCTIO-TA), and a decrease in PMCT total summation of the left side with elite group in respect to amateur group. This study reported an increase of left PMCTEO, left PMCTTAA, right PMCTEO as well as a decrease of left PMCTIO-TA and in PMCT total summation on the left side.

KEYWORDS: *connective tissue, oblique abdominis, rectus abdominis, transversus abdominis, ultrasonography, basketball.*

INTRODUCTION

Muscles and perimuscular connective tissue (PMCT) of the abdominal wall develop an important role stabilizing and supporting the spine.[1] The spine is surrounded in the midline by rectus abdominis (RA), laterally 3 overlapping layers conformed by the external oblique (EO), internal oblique (IO) and transversus abdominis (TrAb).[2] Moreover, these muscles and PMCT have an important role transferring loads from lower limbs to upper limbs and balancing abdominal pressures.[3] In subjects with lumbopelvic pain (LPP), Whittaker et al.[2] found a thicker abdominal PMCT.

DATE OF SUBMISSION: 29-Jan-2018
DATE OF ACCEPTANCE: 16-Feb-2018
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Many methods exist to assess the morphology and characteristics of the abdominal wall muscles, including magnetic resonance[4], eletromiography,[5,6] and rehabilitative ultrasound imaging (RUSI). These assessment tools have been used to evaluate the thickness, cross sectional area (CSA) and PMCT related to musculoskeletal conditions that may influence the physical therapy approach.[7] Considering the lower limbs, a recent study found a reduced CSA of the peroneus longus and a negative correlation for the circular perimeter of connective tissue in patients with ankle sprains.[8] Additionally, Taniguchi et al.[9] reported a decreased thickness in vastus medialis muscles in subjects with knee osteoarthritis. CSA and thickness of the flexor hallucis brevis and abductor hallucis were reduced in subjects with hallux valgus.[10] Regarding the upper limb supraspinatus muscle, thickness have been related with subacromial impingement syndrome.[11] Furthermore, CSA of intrinsic hand muscles can be evaluate with RUSI and could be reliable to predict muscle strength in subjects with nerve injury.[12] With respect to cervical muscles, Javanshir et al.[13] observed that deep cervical flexor muscles may be evaluated during programs for individuals with neck pain. Temporomandibular joint disorders have showed an altered function of the masseter, temporalis and sternocleidomastoid muscles.[14] Additionally, ultrasonography examinations can be appropriate to assess muscles and PMCT changes in individuals with patology.[15] Whittacker et al.[2] found a thicker PMCT and a wider interrecti distance (IRD) in patients with LPP compared with a healthy group. Moreover, multifidus and abdominal wall muscles have been linked with a decreased CSA in patients with LPP.[2, 16]

RUSI may consider a non-invasive, relatively affordable and portable tool, which provides information of morphology, and size of muscles and PMCT.[2] Following Whittacker et al.[2] criteria, ultrasonography assessments were carried out at rest for muscular tissue, PMCT and IRD of the abdominal wall. Moreover, RUSI evaluation of the trunk and abdominal wall may predict risk of injuries in professional football players.[4]

To date, RUSI comparison of abdominal wall PMCT of elite and amateur players has not been carried out. Therefore, the purpose of this study was to assess and compare with RUSI the PMCT and IRD between elite and amateur basketball players.

METHODS

Study design

An observational study was developed following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.[17]

Sample size calculation

A sample size was calculated using the difference between two independent groups with G*Power 3.1.9.2 software and based on the IR distance (cm) of a pilot study with 2 groups (mean ± SD), 10 elite basketball players (1.35 ± 1.01 cm) and 10 amateur basketball players (0.78 ± 0.28 cm). Indeed, 1-tailed hypothesis, effect size of 0.99, α error probability of 0.05, power (1-β error probability) of 0.90 and allocation ratio (N2/N1) of 1 was carried out for the sample size calculation. Therefore, a total sample size of 20 subjects, 10 for each group, was calculated.

PARTICIPANTS

A sample of 22 healthy basketball players (age: 21.0 ± 6.0 y; height: 1.88 ± 09.30 m; weight: 85.9 ± 13.5 kg; body mass index, BMI: 22.6 ± 2.6 kg/m^2) was included and divided into two groups: professional elite basketball players from Spanish 1st Division league club (n =11) and amateur basketball players from an entertainment Spanish division (n = 11). Inclusion criteria for the present study consisted of individuals aged 18 to 35 years old; male; players with right-handed dominance, right-handed throw, and left-foot jump dominance; the professional group had to meet at least three of the following requirements: a) at least three years as a professional player; b) playing at least one year in the national team; c) professional players in youth categories; d) having won an international championship with his team or in a national team.

Exclusion criteria were any musculoskeletal disease in the lumbopelvic region[2], skin disease, hypocapnia,[18] neurological signs, lower limb pathology (i.e.; fracture, osteoarthritis) and a body mass index (BMI) greater than 31kg/m^2.[2] Furthermore, hypocapnia was considered when Nijmegen questionnaire values were higher than 24.[19]

Ethical considerations

The study was approved by A Coruña University Ethics Committee, Spain, and participants signed the

informed consent form. The study also adhered to the ethical standards of the Declaration of Helsinki.[20]

Sociodemographic and respiratory distress data

Before the ultrasonography procedure, age (y), height (m), weight (kg) and BMI (kg/m^2) were recorded. Moreover, respiratory distress values were registered with the Nijmegen questionnaire.[2, 19]

Ultrasonography of the abdominal wall

All imaging procedures were carried out by 1 operator (J.A.P), who was a physiotherapist with 3 years of RUSI experience. Following Whittaker et al.[2] procedure, the operator was not blinded during the ultrasonography examination. A diagnostic ultrasound device (Toshiba Aplio 500 Platinum, Toshiba American Medical Systems; CA, USA) with a 7 to 14-MHz-range linear transducer (18L7 PLT-1204BT type; 40-mm footprint) was used for B mode ultrasound imaging. All images were performed in supine position. For PMCT of the EO, IO and TrAb, the operator was situated in mid-axillary line, using the reference point located between inferior border of subcostal line and iliac crest. PMCT of RA muscle the transducer was placed aligned with the umbilicus; and IRD was evaluated just under the umbilicus (Figure 1).[2] Measurements were collected at right and left sides at the end of expiration with the transducer in the same place. For the statistical analysis, the mean of 3 repeated values for each measure was used. PMCT was defined as the distance between the inside borders of each connective tissue layer. IRD was defined as the distance between the inside borders of both RA.[2] ImageJ software (version 2.0; US National Institutes of Health, Bethesda, Maryland, USA) was utilized for measuring all images offline.[21]

Statistical analysis

SPSS 22.0 software (IBM SPSS Statistics for Windows; NY: IBM Corp.) was employed for the data analysis. An α error of 0.05 (95% confidence interval) and desired power of 80% (β error of 0.2) were used. First, the Shapiro-Wilk test was utilized to assess normality. Second, a descriptive analysis was performed for the total sample together, as well as in both groups separately. Finally, a comparative analysis between both groups was performed. For the parametric data, mean ± standard deviation (SD) and Student´s t-test for independent samples were applied. For the non-parametric data, the median ± interquartile range (IR) and Mann-Whitney U test were used.

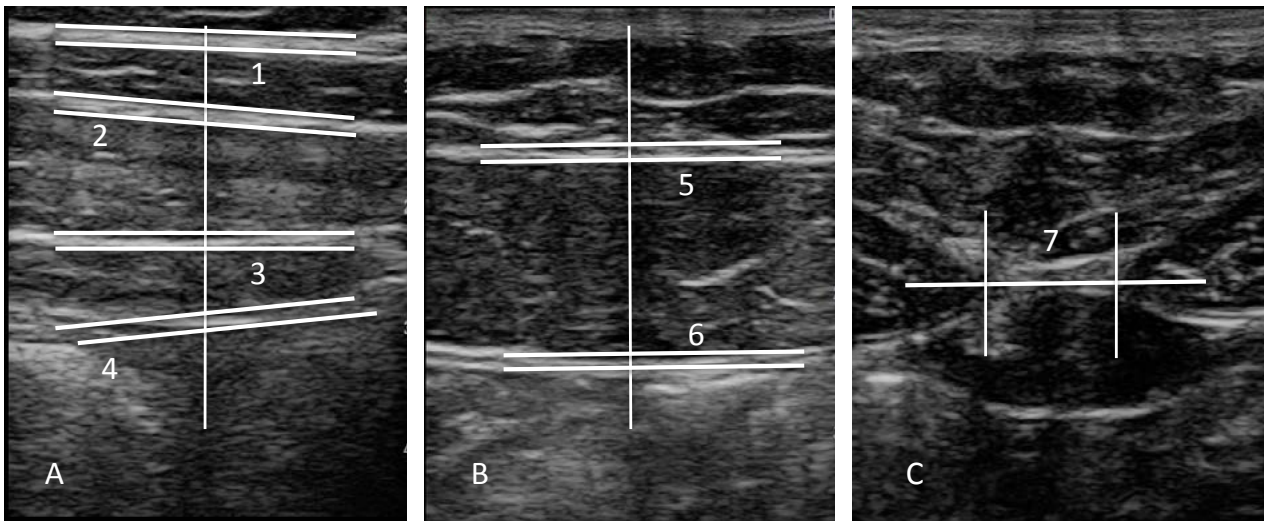

FIGURE 1. DESCRIPTIONS

1. Transducer places during ultrasound evaluation of the abdominal wall.
2. PMCT thickness and IRD measurements of the abdominal wall. 1=PMCTO; 2=PMCTEO-IO; 3=PMCTIO-TA; 4=PMCTTA; 5=PMCTSUPRA; 6=PMCTDEERA; 7=IRD. Abbreviations: IRD, interrecti distance; PMCTEO, perimuscular connective tissue external oblique; PMCTEO-IO, perimuscular connective tissue external oblique- internal oblique; PMCTIO-TA, perimuscular connective tissue internal oblique- transversus abdominis; PMCTTA, perimuscular connective tissue transversus abdominis; PMCTRA, perimuscular connective tissue rectus anterior; PMCTSUPRA, perimuscular connective tissue superficial rectus anterior; PMCTDEERA, perimuscular connective tissue deep rectus anterior.

RESULTS

Regarding the Table 1, sociodemographic data did not show statistically significant differences (P > .05) for age between both groups. Instead, statistically significant differences (P < .05) were observed in Nijmegen scores in favor of the elite basketball players.

Considering Table 2, measurements of abdominal wall muscles PMCT present statistically differences (P < .05) for an increase of perimuscular connective tissue of external oblique (PMCTEO), perimuscular connective tissue of the transversus abdominis (PMCTTA) of the left side and an increase of PMCTEO on the right side in favor of elite group. Rather, the study showed statistically differences (P < .05) for a decrease of perimuscular connective tissue between the internal oblique and transversus abdominis (PMCTIO-TA), and a decrease in PMCT total summation of the left side with elite group compared to amateur group.

TABLE 1. SOCIODEMOGRAPHIC DATA AND RESPIRATORY DISTRESS SCORES OF THE BASKETBALL PLAYERS ‡

Data	Amateur (n = 16)	Elite (n= 16)	P-value
Age, y	21.0 ± 3.0 †	23.0 ± 9.0 †	.748 ‡
Weight, kg	79.63 ± 10.29 *	92.18 ± 13.94 *	.026**
Height, m	1.84 ± 0.07 *	1.92 ± 0.10 *	.035**
BMI, kg/m2	21.55 ± 2.28 *	23.72 ± 2.67 *	.054**
Nijmegen test *	3.72 ± 3.13 *	12.54 ± 3.77 *	<.001 **

* Mean ± standard deviation (SD) was applied. ** Student´s t-test for independent samples was performed. † Median ± interquartile range (IR) was used. ‡ Mann-Whitney U test was utilized

DISCUSSION

To date, this new study may be considered as the first study to make a comparison of the abdominal wall PMCT between elite and amateur basketball players. To our knowledge, the only study of RUSI examination of PMCT abdominal wall muscles at rest was carried out in subjects with and without LPP.[2] Langevin and Sherman[22] have hypothesized that PMCT plays an important role in subjects with LPP. Additionally, greater thickness (22%) of PMCT was found in patients with LPP.[23] A dominant patterns like left-foot jump dominance may predispose an extra mechanical stress as well

TABLE 2. ULTRASOUND IMAGING OF THE INTERRECTI DISTANCE AND PERIMUSCULAR CONNECTIVE TISSUE.

Measurement	Amateur (n=16) †	Elite (n=16) †	P-value
Distance (cm)			
IRD	1.04 ± 0.51 (0.64–1.96) †	1.28 ± 0.69 (0.54–3.64) †	0.001‡
Thickness (cm)			
Right PMCTEO	2.00 ± 4.00 (0.01–11.00) †	0.45 ± 0.18 (1.15–0.81) †	0.001‡
Right PMCTEO-IO	0.11 ± 0.05 (0.07–0.33) †	0.10 ± 0.07 (0.19–0.11) †	0.847‡
Right PMCTIO-TA	0.12 ± 0.04 (0.07–0.24) *	0.04 ± 0.04 (0.09–0.25) *	0.371**
Right PMCTTA	0.09 ± 0.09 (0.06–0.29) †	0.13 ± 0.04 (0.09–0.22) †	0.193‡
Right PMCTRA	0.16 ± 0.03 (0.11–0.24) *	0.14 ± 0.02 (0.11–0.18) *	0.334**
Right PMCTSUPRA	0.09 ± 0.02 (0.06–0.14) *	0.08 ± 0.02 (0.05–0.12) *	0.446**
Right PMCTDEERA	0.06 ± 0.03 (0.04-0.10) †	0.06 ± 0.01 (0.04–0.07) †	0.898‡
Total Right PMCT	0.09 ± 0.04 (0.06–0.22) †	0.11 ± 0.03 (0.08–0.17) †	0.438‡
Left PMCTEO	0.11 ± 0.05 (0.06–0.23) †	0.47 ± 0.12 (0.36–0.65) †	0.001‡
Left PMCTEO-IO	0.11 ± 0.29 (0.07–0.17) *	0.11 ± 0.28 (0.08–0.16) *	0.520**
Left PMCTIO-TA	0.90 ± 0.01 (0.06–0.13) †	0.13 ± 0.06 (0.10–0.28) †	0.001‡
Left PMCTTA	0.09 ± 0.03 (0.08–0.15) †	0.13 ± 0.03 (0.10–0.21) †	0.001‡
Left PMCTRA	0.16 ± 0.05 (0.12–0.28) *	0.14 ± 0.04 (0.08–0.20) *	0.256**
Left PMCTSUPRA	0.09 ± 0.07 (0.20–0.14) †	0.08 ± 0.05 (0.05–0.13) †	0.401‡
Left PMCTDEERA	0.06 ± 0.02 (0.04-0.12) *	0.05 ± 0.01 (0.03–0.09) *	0.369**
Total Left PMCT	0.41 ± 0.15 (0.31–0.61) †	0.10 ± 0.03 (0.08–0.14) †	0.001‡

Abbreviations: IRD, interrecti distance; PMCTEO, perimuscular connective tissue external oblique; PMCTEO-IO, perimuscular connective tissue external oblique- internal oblique; PMCTIO-TA, perimuscular connective tissue internal oblique- transversus abdominis; PMCTTA, perimuscular connective tissue transversus abdominis; PMCTRA, perimuscular connective tissue rectus anterior; PMCTSUPRA, perimuscular connective tissue superficial rectus anterior; PMCTDEERA, perimuscular connective tissue deep rectus anterior. * Mean ± standard deviation (SD) (minimum-maximum) was applied. ** Student´s t-test for independent samples was performed. † Median ± interquartile range (IR) (minimum-maximum) was used. ‡ Mann-Whitney *U* test was utilized.

as physiological changes in this tissues.[22] Fibrosis of the connective tissues are known to occurs as a result of repeated trauma and inflammation.[24, 25] Based on these findings, it is possible to hypothesize the relation between too many training sessions, high intensity, high loads, abnormal movement patters and adaptive connective tissue changes. In addition, right-handed dominance, right-handed throw, and left-foot jump dominance may be related with the abdominal wall connective tissue morphology. Further studies may be necessary in order to correlate these player´s characteristics with the connective tissue morphology.

Regarding the studies performed by Hides et al.[26] and Leung et al.[27], a motor control RUSI examination of the abdominal wall muscles could be considered as an important index to predict the risk of injuries in soccer players. Likewise, studies developed by Hides et al.[28] showed that a motor control program improves the ability to drawn-in abdominal wall in soccer players and normalize excessive tension of abdominal muscles in response to a low load task.[29]

Our sample was composed of healthy subjects and showed a higher IRD for the elite group contrary to LPP subjects.[2] Nijmegen test registered fourfold higher score of respiratory distress in favor elite basketball players. Moreover, the scores are coinciding with the Nijmegen values between patients with and without LPP.[2]

LIMITATIONS AND FUTURES STUDIES

Several limitations should be contemplated in this study. First, the sample was composed of healthy players, so it would be interesting in future investigations to study players with pathology, such as LPP.[2] Second, muscle contraction changes were not studied and the ultrasound exploration during abdominal hollowing, functional tasks, dynamic movements, straight leg raise test or Valsalva manoeuvre might be interesting.[15, 27, 30]

At last, ultrasonography M-Mode and power Doppler mode may be useful for the study of PMCT characteristics providing functional thickness examinations and a direct visualization of inflammation within the layers and tissues.[31,32]

Further research is recommended to determine characteristics of the abdominal wall structures like muscle and PMCT in elite athletes different from basketball. Moreover, it would be very interesting to examine other sports and populations with RUSI.

CONCLUSIONS

This study reported an increase of left PMCTEO, left PMCTTAA, right PMCTEO, as well as a decrease of left PMCTIO-TA and in PMCT total summation on the left side.

Conflicts of Interest and Source of Funding

There are no conflicts of interest or Source of Funding.

RESUMO

O objetivo deste estudo foi avaliar e comparar com ultrassonografia de reabilitação (IUR) o tecido conjuntivo perimuscular da parede abdominal (PMPA) e interrecti distância (IRD) entre elite e jogadores de basquete amadores. Uma amostra de 22 jogadores de basquete saudáveis foi incluída e dividida em dois grupos: jogadores de basquete de elite da 1ª divisão espanhola (n=11) e jogadores de basquete amadores de uma divisão de entretenimento espanhol (n=11). As imagens de ultrassom do oblíquo externo (OE), oblíquo interno (OI), transverso abdominal (TrAb), recto anterior (RA) e IRD PMPA foram medidas e analisadas pelo software ImageJ. Medições dos músculos da parede abdominal O PMPA apresentam diferenças estatisticamente (P<0,05) para o aumento do tecido conjuntivo perimuscular de oblíquo externo (PMOE), tecido conjuntivo perimuscular de transverso abdominal (PMTA) do lado esquerdo e aumento do PMOE do lado direito a favor do grupo de elite. Em vez disso, o estudo mostrou diferenças estatisticamente (P<0,05) para uma diminuição do tecido conjuntivo perimuscular entre o oblíquo interno e transverso abdominário (PMOI-TA) e uma diminuição no somatório total de PMTA do lado esquerdo do grupo de elite em relação ao amador grupo. Este estudo relatou um aumento do PMTOE esquerdo, PMTA esquerdo, PMCTOE direito, bem como uma diminuição do PMCTOI-TA esquerdo e no somatório total do PMTA no lado esquerdo.

PALAVRAS-CHAVE: *Tecido conjuntivo. Abdome oblíquo. Reto abdominal. Abdominal transverso. Ultrassonografia.*

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REVIEW ARTICLE

Relevance of hMLH1 -93G>A, 655A>G and 1151T>A polymorphisms with colorectal cancer susceptibility: a meta-analysis based on 38 case-control studies

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http://dx.doi.org/10.1590/1806-9282.64.10.942

SUMMARY

Sodium-glucose cotransporter-2 inhibitors (SGLT2i) are drugs that act by maintaining glycosuria. Recent studies have shown promisObjective: There has been increasing interest in the study of the association between human mutL homolog 1 (hMLH1) gene polymorphisms and risk of colorectal cancer (CRC). However, results from previous studies are inconclusive. Thus, a meta-analysis was conducted to derive a more precise estimation of the effects of this gene.

Methods: A comprehensive search was conducted in the PubMed, EMBASE, Chinese Biomedical Literature databases until January 1, 2018. Odds ratio (OR) with 95% confidence interval (CI) was used to assess the strength of the association.

Results: Finally, 38 case-control studies in 32 publications were identified met our inclusion criteria. There were 14 studies with 20668 cases and 19533 controls on hMLH1 -93G>A, 11 studies with 5,786 cases and 8,867 controls on 655A>G and 5 studies with 1409 cases and 1637 controls on 1151T>A polymorphism. The combined results showed that 655A>G and 1151T>A polymorphisms were significantly associated with CRC risk, whereas -93G>A polymorphism was not significantly associated with CRC risk. As for ethnicity, -93G>A and 655A>G polymorphisms were associated with increased risk of CRC among Asians, but not among Caucasians. More interestingly, subgroup analysis indicated that 655A>G might raise CRC risk in PCR-RFLP and HB subgroups.

Conclusion: Inconsistent with previous meta-analyses, this meta-analysis shows that the hMLH1 655A>G and 1151T>A polymorphisms might be risk factors for CRC. Moreover, the -93G>A polymorphism is associated with the susceptibility of CRC in Asian population.

Keywords: colorectal cancer, hMLH1, polymorphism; Meta-analysis.

DATE OF SUBMISSION: 19-Jan-2018
DATE OF ACCEPTANCE: 24-Mar-2018
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INTRODUCTION

Colorectal cancer (CRC) is one of the most frequent malignant tumours of the digestive tract in human, especially in the Western world.[1,2] CRC ranks among the three most common cancers in terms of both cancer incidence and cancer-related deaths in most developed countries. More than one million cases of CRC are diagnosed in the worldwide every year.[1-3] The cross cultural and migrant studies suggest that the majority of CRC cases (≈85%) is related to environmental factors including smoking, drinking, meat consumption, less activity, exposure to aryl amines, and heterocyclic amines.[4-6]

The MMR genes encode a family of highly conserved proteins, including MLH1, MSH2, MSH6, and PMS2.[7,8] MMR systems promote genetic stability by repairing DNA replication errors, inhibiting recombination between non-identical DNA sequences, and participating in responses to DNA damage.[9,10] MLH1 protein physically interacts with other MMR components; although the exact role of MLH1 gene remains elusive, MLH1-deficiency is associated with cancer predisposition.[11] To date, most of the causative mutations have been identified in MLH1 gene. Mutations in the gene for MLH1 are estimated to account for nearly 40% of the more than 400 known MMR gene mutations, and prevalence of mutations in MLH1 in Western countries is between 1 of 1000.[12] Standardized incidence ratios (SIRs) for carriers of hMLH1 mutations, when compared with the general population is 68 and the relative risk for CRC for first-degree relatives of mutation carriers compared with first degree relatives of non-carriers is 8.1.[13,14]

Molecular epidemiological and pooling analyses studies have reported the association of hMLH1 -93G>A, 655A>G and 1151T>A with CRC risk,[15-46] but the results remain inconsistent and inclusive. Inconsistencies in results may be caused by differences in study design, population, or different statistical methods. Meta-analysis is a powerful tool for summarizing the different studies. It can not only overcome the problem of small size and inadequate statistical power of genetic studies of complex traits, but also provide more reliable results than a single case-control study. However, the previous meta-analysis on hMLH1 polymorphisms with CRC risk has shown conflicting conclusions. Because, several published studies were not included in the meta-analysis and additional original studies with larger sample sizes have been published since then. We therefore performed a meta-analysis to make a more precise assessment the association between hMLH1 polymorphisms with CRC risk, by adding more studies implemented in recent years.

METHODS

Search Strategy

A comprehensive literature search was performed using the MEDLINE (National Library of Medicine), EMBASE (Excerpta Medica), CANCERLIT (National Cancer Institute), Web of Science (Thomson-Reuters), Cochrane Library, Chinese Biomedical Literature Database and Google scholar for all relevant articles published up to January 1, 2018 that evaluated the association between the hMLH1 gene polymorphisms and risk of CRC. The following terms were included in the search: ''Colorectal cancer" or ''CRC", ''human mutL homolog 1" or ''hMLH1",''-93G>A" or ''rs1800734", ''655A>G" or ''p.Ile219Val" or ''rs1799977", ''1151T>A" or ''p.Val384Asp" or ''rs63750447", "polymorphism", "mutation", "variant", "gene", "genotype", "SNP", and "allele". The search was not restricted by the publication year or language. Furthermore, in order to identify potentially relevant studies, we manually searched reference lists of eligible studies, reviews and related meta-analyses. If there were multiple reports of the same sample or overlapping data only the study with the largest sample sizes or the most recent one was included following careful examination.

Inclusion and Exclusion Criteria

Studies were selected according to the following inclusion criteria: (1) full-text published studies; (2) epidemiological studies with case-control or cohort design; (3) investigating the association between hMLH1 polymorphisms and CRC risk; (4) providing sufficient genotype data or information that could help infer the results in the studies to calculate the odd ratios (ORs) with a 95% confidence interval (CI). The exclusion criteria were as follows: (1) studies with only case population (no control population); (2) studies without detail genotype frequencies, which were unable to calculate odds ratio; (3) duplicate of previous publication.

Data Extraction

Information was carefully extracted from all eligible studies independently by two investigators ac-

cording to the inclusion criteria. For each study the following information was extracted: name of first author, publication year, country of origin, ethnicity, polymorphisms, source of controls, genotyping method, number of cases and controls, genotype frequency in cases and controls, minor allele frequencies (MAFs) in control subjects, and Hardy-Weinberg equilibrium test in control subjects. Diverse ethnicities were categorized as Caucasian, Asian, African and Mixed, which included more than one race. Disagreements were resolved in consultation with the third reviewer.

STATISTICAL ANALYSIS

The strength of associations was assessed by using odds ratios (ORs) and 95% confidence interval (CIs). The significance of the pooled OR was determined by the Z-test; a P value of <0.05 was considered significant. The OR of hMLH1 polymorphisms and CRC risk was estimated for each study. The pooled ORs were performed for allele (B vs. A), homozygote (BB vs. AA), heterozygote (AB vs. AA), dominant (BB+BA vs. AA) and recessive (BB vs. AB+ AA) models. A Chi square-test based Q-statistic test and an I^2 statistics (I^2= 100 %×(Q-df)/Q) were performed to assess the heterogeneity between studies.[47] A significant Q-statistic (P<0.10) indicated heterogeneity across studies. Venice criteria for the I^2 statistics: "I^2<25% represents no heterogeneity, I^2=25–50% represents moderate heterogeneity, I^2=50–75% represents large heterogeneity and I^2>75% represents extreme heterogeneity". Dependent on the results of heterogeneity test among individual studies, the fixed effect model (Mantel–Haenszel method) or random effect model (DerSimonian–Laird method) was utilized to summarize the pooled OR.[1,48] Furthermore, to detect the source of between-study heterogeneity, subgroup analyses (Meta-regression) by ethnicity, genotyping method and source of controls were performed. A Hardy–Weinberg equilibrium (HWE) was assessed for each study using the goodness-of-fit test (Chi square-test or Fisher exact test) only in control groups, and deviation was considered when P<0.01. The one-way sensitivity analyses were performed to survey the stability of the results, namely, a single study in the meta-analysis was omitted each time to reflect the influence of the individual data set to the pooled OR. Publication bias was evaluated by visual inspection of the funnel plot and Egger's linear regression test, and the significance level was set at 0.05 for both.[49,50] If publication bias observed, the Duval and Tweedie non-parametric "trim and fill" method was assessed to adjust for it. All the statistical analyses were performed by Comprehensive Meta-Analysis (CMA) software (Version 2.20; Biostat, USA). P<0.05 (two-tailed) was considered statistically significant.

RESULTS

Study Selection and Characteristics

After deleting of duplicates, 361 articles were excluded from screening the titles and abstracts, as these were unrelated to hMLH1 polymorphisms, or CRC risk. Further, 40 articles was excluded for no genotypic information, reviews, letters, case report, clinical, and animal studies. Totally, 38 case-control studies in 32 publications [15-46] containing 4092 cases and 5909 controls were included in the meta-analysis. The studies were published from 1998 to 2017. The main characteristics of the selected studies and the genotype distribution of the hMLH1 gene polymorphisms are summarized in Table 1. Of the 30 studies, eight were conducted in Asians (Japan, Korea, China, Kazakhstan, Malaysia, and Iran), 5 in Caucasians (Canada, Czech, USA, UK, Spain, Denmark, and Sweden), and one in a mixed population (Mexico). Of them, there were 19 studies with 20,668 CRC cases and 19,533 controls for -93G>A (rs1800734) polymorphism, eleven studies with 5,786 CRC cases and 8,867 controls for hMLH1 655A>G (rs1799977) polymorphism, and eight studies with 1,409 CRC cases and 1,637 controls for hMLH1 1151T>A (rs63750447) polymorphism. For the ethnicities, 12 studies of Caucasians and six studies of Asians were included on the hMLH1 -93G>A (rs1800734). As to hMLH1 655A>G (rs1799977) polymorphism, six studies of Caucasians and four studies of Asians were included. The eight studies on hMLH1 1151T>A (rs63750447) polymorphism were all based on the Asians. The distribution of the genotypes in the control subjects was in agreement with HWE except three studies.

Quantitative Synthesis

hMLH1 -93G>A (rs1800734) Polymorphism

The main results of the meta-analysis for all 19 case-control studies [15-33] on hMLH1 -93G>A polymorphism are presented in Table 3. The results of pooling all studies showed that there was no statistically

TABLE 1: MAIN CHARACTERISTICS OF STUDIES INCLUDED IN THIS META-ANALYSIS.

First Author	Country (Ethnicity)	SOC	Genotyping Technique	Case/Control	Cases					Controls					MAFs	HWE
					Genotypes			Allele		Genotypes			Allele			
-93G>A (rs1800734)					GG	AG	AA	G	A	GG	AG	AA	G	A		
Ito 1999 15	Japan (Asian)	PB	PCR-SSCP	27/84	8	10	9	26	28	22	46	16	90	78	0.464	0.355
Shin 2002 16	Korea (Asian)	HB	PCR-SSCP	139/157	33	61	45	127	151	42	74	41	158	156	0.496	0.472
Raptis 2007 17	Canada (Caucasian)	PB	TaqMan	929/1098	554	331	44	1439	419	687	352	59	1726	470	0.214	0.118
Chen 2007 18	USA(Caucasian)	NA	Pyroseq	99/286	44	47	8	135	63	169	99	18	437	135	0.236	0.497
Tulupova 2008 19	Czech (Caucasian)	HB	TaqMan	619/611	359	216	44	934	304	365	209	37	939	283	0.231	0.336
Samowitz 2008 20	USA(Caucasian)	PB	DS	1006/1963	610	344	52	1564	448	1170	688	105	3028	898	0.228	0.768
Koessler 2008 21	UK(Caucasian)	PB	TaqMan	2288/2276	1407	778	103	3592	984	1392	777	107	3561	991	0.217	0.914
Allan 2008 22	UK(Caucasian)	NA	PCR-RFLP	1518/589	878	566	74	2322	714	369	196	24	934	244	0.207	0.750
Campbell 2009 23	USA(Caucasian)	PB	PCR-RFLP	1600/1963	952	553	95	2457	743	1170	688	105	3028	898	0.228	0.768
van Roon 2010 24	Netherland(Caucasian)	NA	DS	39/920	12	20	7	44	34	554	331	44	1425	415	0.225	0.542
Whiffin 2011 25	UK(Caucasian)	PB	KASPae	10409/6965	6408	3504	497	16320	4498	4395	2261	309	11051	2879	0.206	0.401
Savio 2012 26	Canada (Caucasian)	PB	PCR-RFLP	252/845	150	96	6	396	108	528	264	53	1320	370	0.218	0.011
Muniz-Mendoza 2012 27	Mexico (Mixed)	HB	PCR-RFLP	100/115	47	44	9	138	62	39	55	21	133	97	0.421	0.834
Nizam 2013 28	Malaysia (Asian)	HB	PCR-RFLP	104/104	22	50	32	94	114	33	33	38	99	109	0.524	0.520
Martinez-Uruena 2013 29	Spain (Caucasian)	HB	PCR-RFLP	383/236	233	131	19	597	169	129	102	5	360	112	0.237	0.002
Djansugurova 2015 30	Kazakhstan (Asian)	HB	PCR-RFLP	249/244	126	94	29	346	152	101	115	28	317	171	0.350	0.581
Li 2015 31	China(Asian)	NA	PCR-RFLP	451/629	88	198	165	374	528	218	301	110	737	521	0.414	0.728
Zhang 2016 32	China(Asian)	HB	TaqMan	312/300	66	139	107	271	353	52	154	94	258	342	0.570	0.413
Mik 2017 33	Poland (Caucasian)	NA	PCR-RFLP	144/151	74	45	25	193	95	53	61	37	167	135	0.447	0.024
655A>G(rs1799977)				20668/19533	AA	AG	GG	A	G	AA	AG	GG	A	G		
Kim 2004 34	Korea (Asian)	PB	TaqMan	107/330	100	7	0	207	7	311	18	1	640	20	0.030	0.191
Mei 2006 35	China (Asian)	HB	PCR	160/150	144	14	2	302	18	141	9	0	291	9	0.030	0.704
Raptis 2007 17	Canada (Caucasian)	PB	TaqMan	929/1098	451	391	87	1293	565	514	485	99	1513	683	0.311	0.309
Berndt 2007 36	USA(Caucasian)	PB	TaqMan	211/2090	100	94	17	294	128	968	896	226	2832	1348	0.322	0.386
Christensen 2008 37	Denmark(Caucasian)	PB	SBE-tags	380/770	172	170	38	514	246	364	327	79	1055	485	0.314	0.660
Nejda 2009 38	Spain (Caucasian)	HB	PCR-RFLP	140/125	41	72	27	154	126	64	44	17	172	78	0.312	0.044
Campbell 2009 23	USA(Caucasian)	PB	PCR-RFLP	1601/1944	764	678	159	2206	996	937	848	159	2722	1166	0.299	0.087
Picelli 2010 39	Sweden (Caucasian)	PB	DS	1781/1701	819	781	181	2419	114	832	708	161	2372	1030	0.302	0.636
Muniz-Mendoza 2012 27	Mexico (Mixed)	HB	PCR-RFLP	102/100	71	26	5	168	36	81	19	0	181	19	0.095	0.293
Milanizadeh 2013 40	Iran (Asian)	HB	PCR-RFLP	219/248	25	62	132	112	326	248	54	119	227	269	0.346	≤0.001
Peng 2016 41	China (Asian)	PB	PCR-HRM	156/311	151	5	0	151	5	307	4	0	618	4	0.006	0.909
1151T>A(rs63750447)				5786/8867	TT	AT	AA	T	A	TT	AT	AA	T	A		
Wang 1998 42	China (Asian)	NA	PCR-SSCP	26/80	22	4	0	48	4	77	3	0	157	3	0.018	0.864
Wang 2000 43	China (Asian)	HB	PCR-SSCP	101/100	88	13	0	189	13	94	6	0	194	6	0.030	0.757
Kim 2004 34	Korea (Asian)	PB	TaqMan	107/330	100	7	0	207	7	313	17	0	643	17	0.025	0.631
Zhang 2005 44	China (Asian)	PB	DHPLC	90/268	82	8	0	172	8	251	17	0	519	17	0.031	0.591
Mei 2006 35	China (Asian)	HB	PCR	160/150	142	18	0	302	18	141	9	0	291	9	0.030	0.474
Ohsawa 2009 45	Japan (Asian)	NA	PCR-RFLP	670/332	630	39	1	1299	41	327	5	0	659	5	0.007	0.890
Wang 2010 46	China (Asian)	NA	DHPLC	99/66	83	16	0	182	16	63	3	0	129	3	0.022	0.850
Peng 2016 41	China (Asian)	PB	PCR-HRM	156/311	142	13	1	297	15	310	1	0	621	1	0.001	0.977

SOP, source of population; HB, Hospital-based study; PB, Population-based study; RT-PCR, Real-Time PCR; PCR-RFLP, PCR-restriction fragment length polymorphism; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MAFs, Minor Allele Frequencies; HWE, Hardy–Weinberg equilibrium; SBE-tags: Single base extension; HRM : High Resolution Melting.

TABLE 2. THE META-ANALYSIS OF HMLH1 -93G>A (RS1800734) POLYMORPHISMS AND CRC RISK.

Subgroup	Genetic Model	Type of Model	Heterogeneity		Odds Ratio				Publication Bias	
			I2 (%)	PH	OR	95% CI	Ztest	POR	PBeggs	PEggers
Overall (n=19)	A vs. G	Random	99.00	≤0.001	0.946	0.650-1.379	-0.287	0.774	0.161	0.936
	AA vs. GG	Random	80.83	≤0.001	1.220	0.950-1.566	1.555	0.120	0.363	0.769
	AG vs. GG	Random	98.69	≤0.001	0.914	0.546-1.532	-0.341	0.733	0.141	0.625
	AA+AG vs. GG	Random	75.86	≤0.001	1.066	0.954-1.191	1.127	0.260	1.000	0.838
	AA vs. AG+GG	Random	80.60	≤0.001	1.191	0.954-1.486	1.547	0.122	0.401	0.715
By ethnicity										
Caucasians (n=12)	A vs. G	Random	99.35	≤0.001	0.882	0.535-1.453	-0.492	0.623	0.303	0.875
	AA vs. GG	Random	66.00	0.001	1.061	0.859-1.310	0.549	0.583	0.192	0.716
	AG vs. GG	Random	99.16	≤0.001	0.826	0.422-1.619	-0.556	0.578	0.064	0.807
	AA+AG vs. GG	Random	68.02	≤0.001	1.056	0.958-1.164	1.089	0.276	0.631	0.703
	AA vs. AG+GG	Random	53.03	0.019	1.090	0.911-1.304	0.943	0.346	0.119	0.516
Asians (n=6)	A vs. G	Random	88.14	≤0.001	1.179	0.840-1.655	0.951	0.342	1.000	0.355
	AA vs. GG	Random	80.19	≤0.001	1.759	1.054-2.934	2.163	0.031	0.452	0.102
	AG vs. GG	Random	88.07	≤0.001	1.168	0.644-2.119	0.513	0.608	0.707	0.795
	AA+AG vs. GG	Random	84.60	≤0.001	1.139	0.708-1.833	0.535	0.592	1.000	0.593
	AA vs. AG+GG	Random	81.46	≤0.001	1.381	0.885-2.155	1.421	0.155	0.707	0.330
By Population-Based										
PB (n=7)	A vs. G	Random	99.63	≤0.001	0.720	0.349-1.486	-0.888	0.375	0.071	0.640
	AA vs. GG	Fixed	18.16	0.291	1.020	0.917-1.136	0.368	0.713	0.548	0.168
	AG vs. GG	Random	99.51	≤0.001	0.600	0.221-1.628	-1.004	0.316	0.071	0.863
	AA+AG vs. GG	Fixed	0.00	0.656	1.042	0.995-1.092	1.753	0.080	1.000	0.578
	AA vs. AG+GG	Fixed	37.36	0.143	1.030	0.927-1.145	0.558	0.577	0.548	0.446
HB (n=7)	A vs. G	Fixed	41.77	0.112	0.971	0.878-1.074	-0.573	0.566	0.548	0.392
	AA vs. GG	Random	78.04	≤0.001	1.526	0.963-2.418	1.800	0.072	0.229	0.101
	AG vs. GG	Random	81.22	≤0.001	0.943	0.650-1.368	-0.308	0.758	0.367	0.477
	AA+AG vs. GG	Random	55.17	0.037	0.890	0.711-1.114	-1.017	0.309	1.000	0.882
	AA vs. AG+GG	Fixed	35.45	0.158	1.087	0.895-1.321	0.840	0.401	0.763	0.763
By Genotyping Technique										
PCR-RFLP (n=9)	T vs. C	Random	89.42	≤0.001	0.984	0.784-1.234	-0.141	0.888	0.251	0.306
	AA vs. GG	Random	87.93	≤0.001	0.999	0.592-1.686	-0.004	0.997	0.602	0.232
	AG vs. GG	Random	85.77	≤0.001	1.031	0.782-1.360	0.217	0.828	0.916	0.950
	AA+AG vs. GG	Random	84.94	≤0.001	0.997	0.775-1.284	-0.022	0.983	0.251	0.624
	AA vs. AG+GG	Random	88.95	≤0.001	1.146	0.693-1.896	0.531	0.595	0.117	0.384
TaqMan (n=4)	A vs. G	Fixed	0.00	0.720	1.017	0.947-1.093	0.467	0.641	1.000	0.513
	AA vs. GG	Random	84.67	≤0.001	1.291	0.795-2.097	1.034	0.301	0.734	0.585
	AG vs. GG	Fixed	39.21	0.177	1.024	0.934-1.124	0.506	0.613	0.734	0.635
	AA+AG vs. GG	Fixed	13.49	0.325	1.022	0.935-1.117	0.475	0.635	0.734	0.762
	AA vs. AG+GG	Fixed	0.00	0.649	1.018	0.855-1.212	0.203	0.839	1.000	0.680

PCR-RFLP, PCR-restriction fragment length polymorphism; HWE, Hardy–Weinberg equilibrium.

significant association between hMLH1 -93G>A polymorphism and the risk of CRC.

To evaluate the potential effects of specific study characteristics on the association between hMLH1 -93G>A polymorphism and CRC risk; we pooled the ORs and 95% CIs by the subgroups analysis of ethnicity, control source, and genotyping technique. When stratified by ethnicity, significant association between hMLH1 -93G>A polymorphism and CRC risk was detected among the Asian population under the homozygote model (OR = 2.283, 95% CI 1.810-2.880, P < 0.001), but not among Caucasians. Furthermore, no significant associations were detected when the studies were stratified based on the source of control subjects and genotyping method (Table 2).

hMLH1 655A>G (rs1799977) Polymorphism

The main results of the meta-analysis for all eleven case-control studies [17,23,27,34-41] on hMLH1 655A>G polymorphism are presented in Table 4. The results of pooling all studies showed that there was a significant association between hMLH1 655A>G polymorphism and the risk of CRC under the heterozygote (OR = 1.493, 95% CI 1.147-1.944, P = 0.865, P = 0.003), dominant (OR = 1.298, 95% CI 1.085-1.553, P = 0.004) and recessive (OR = 1.150, 95% CI 1.020-1.297, P = 0.022) models.

In the subgroup analysis by ethnicity, we found a significant association between the hMLH1 655A>G polymorphism and the risk of CRC in Asians under the allele (OR = 2.251, 95% CI 1.758-2.884, P < 0.001), homozygote (OR = 10.262, 95% CI 6.419-16.405, P < 0.001), dominant (OR = 2.411, 95% CI 1.663-3.495, P < 0.001) and recessive (OR = 1.660, 95% CI 1.155-2.385, P < 0.001) models with a fixed effect, whereas there was no significant association in any of the genetic models with a random effect models in Caucasians. When stratified by source of controls, significant association between hMLH1 655A>G polymorphism and CRC risk was observed in hospital-based controls in the allele (OR = 2.153, 95% CI 1.763-2.628, P≤0.001), homozygote (OR = 5.873, 95% CI 1.911-18.04, P=0.036), heterozygote (OR = 2.955, 95% CI 1.111-7.859, P=0.036), dominant (OR = 2.513, 95% CI 1.876-3.367, P≤0.001), and recessive (OR = 1.671, 95% CI 1.216-2.297, P=0.036) models, but not in the in population-based controls. Furthermore, hMLH1 655A>G polymorphism was significantly associated with increased CRC risk in the subgroup of PCR-RFLP genotyping method in the allele model (OR = 1.725, 95% CI 1.038-2.866, P=0.036), dominant (OR = 1.961, 95% CI 0.999-3.847, P=0.05) and recessive (OR = 1.366, 95% CI 1.133-1.647, P=0.001) models. In contrast, no significant association was observed in TaqMan genotyping subgroup (Table 3).

hMLH1 1151T>A (rs63750447) Polymorphism

The main results of the meta-analysis for all case-control eight studies [34,35,41-46] on hMLH1 1151T>A polymorphism are presented in Table 5. Significant association between hMLH1 1151T>A polymorphism and CRC was observed in the allele (OR = 2.462, 95% CI 1.763-2.628, P≤0.001), homozygote (OR = 2.501, 95% CI 1.593-3.806, P≤0.001) and dominant (OR = 2.526, 95% CI 1.622-3.934, P≤0.001) models (Table 3).

Sensitivity analysis

Sensitivity analysis was conducted by deleting each study in turn from the pooled analysis to examine the stability of the results. However, no individual study changed the pooled OR qualitatively, indicating that the pooled results were statistically robust.

Publication bias

We have assessed publication bias qualitatively by Begg's funnel plot and quantitatively by Egger's test. The shapes of the funnel plot did not indicate any evidence of obvious asymmetry in all genotypes in overall population. However, the results of Egger's test statistically confirmed the evidence of publication bias in the dominant model for hMLH1 655A>G polymorphism (PBeggs= 0.146, PEggers=0.021). Therefore, we have used the Duval and Tweedie non-parametric "trim and fill" method to adjust for publication bias. However, meta-analysis with and without "trim and fill" did not draw different conclusion, indicating that our results were statistically robust. Moreover, neither Begg's funnel plot nor Egger's test detected obvious evidence of publication bias in subgroup analysis based on ethnicity, source of controls and genotyping methods by using of Begg's and Egger's test.

DISCUSSION/CONCLUSION

An increasing number of studies on genetic association studies, genome-wide association studies (GWASs), and relate meta-analyses have been published to clarify the association between gene polymorphisms and CRC.[32,33,41] Theoretically, polymorphisms in the hMLH1 gene could change the function of this gene, disturb the DNA repair and increase risk of CRC.[32,33] The role of hMLH1 polymorphisms in the risk of CRC is controversial. The association between hMLH1 gene polymorphisms and risk of CRC has been a topic of particular interest, but the results from individual studies had been inconsistent and controversial. To better define the possible association, we carried out a comprehensive meta-analysis of hMLH1 polymorphisms.

Overall, our meta-analysis indicates that 655A>G and 1151T>A polymorphisms are associated with increased CRC risk when all eligible studies were pooled into the meta-analysis, whereas -93G>A polymorphism was not significantly associated with CRC risk. In further stratified, significantly increased

TABLE 3. THE META-ANALYSIS OF HMLH1 655A>G AND 1151T>A POLYMORPHISMS AND CRC RISK.

subgroup	Genetic Model	Type of Model	Heterogeneity		Odds Ratio				Publication Bias	
			I2 (%)	PH	OR	95% CI	Ztest	POR	PBeggs	PEggers
655A>G										
Overall (n=12)	A vs. G	Random	98.36	≤0.001	1.101	0.638-1.901	0.344	0.731	0.303	0.755
	AA vs. GG	Random	90.65	≤0.001	1.562	0.919-2.655	1.647	0.099	0.350	0.547
	AG vs. GG	Random	87.70	≤0.001	1.493	1.147-1.944	2.979	0.003	0.086	0.054
	AA+AG vs. GG	Random	73.65	≤0.001	1.298	1.085-1.553	2.853	0.004	0.146	0.021
	AA vs. AG+GG	Fixed	19.33	0.260	1.150	1.020-1.297	2.289	0.022	0.640	0.414
By ethnicity										
Caucasians (n=6)	A vs. G	Random	99.15	≤0.001	0.713	0.335-1.518	-0.878	0.380	0.452	0.645
	AA vs. GG	Random	75.85	0.001	1.036	0.770-1.394	0.235	0.814	1.000	0.690
	AG vs. GG	Random	64.92	0.014	1.079	0.931-1.251	1.015	0.310	0.452	0.191
	AA+AG vs. GG	Random	66.83	0.010	1.086	0.940-1.255	1.177	0.264	0.707	0.280
	AA vs. AG+GG	Fixed	0.00	0.420	1.095	0.964-1.243	1.397	0.162	0.452	0.573
Asians (n=4)	A vs. G	Fixed	7.33	0.356	2.251	1.758-2.884	6.425	0.00	0.734	0.381
	AA vs. GG	Fixed	12.08	0.321	10.262	6.419-16.405	9.727	0.00	0.296	0.282
	AG vs. GG	Random	88.34	≤0.001	2.793	0.794-9.818	1.601	0.109	0.734	0.226
	AA+AG vs. GG	Fixed	39.39	0.175	2.411	1.663-3.495	4.644	0.00	0.734	0.352
	AA vs. AG+GG	Fixed	0.00	0.760	1.660	1.155-2.385	2.736	0.006	1.000	0.762
By Population-Based										
PB (n=7)	A vs. G	Random	98.79	≤0.001	0.807	0.411-1.586	-0.622	0.534	0.107	0.797
	AA vs. GG	Random	61.27	0.017	0.960	0.738-1.248	-0.306	0.760	0.548	0.392
	AG vs. GG	Fixed	0.799	0.423	1.037	0.959-1.121	0.901	0.367	0.265	0.150
	AA+AG vs. GG	Fixed	0.00	0.479	1.047	0.971-1.127	1.198	0.231	0.386	0.265
	AA vs. AG+GG	Fixed	0.00	0.677	1.081	0.950-1.230	1.182	0.237	0.367	0.365
HB (n=4)	A vs. G	Fixed	0.00	0.591	2.153	1.763-2.628	7.536	≤0.001	0.734	0.530
	AA vs. GG	Random	74.01	0.009	5.873	1.911-18.04	3.090	0.002	0.734	0.794
	AG vs. GG	Random	89.30	≤0.001	2.955	1.111-7.859	2.171	0.030	0.734	0.414
	AA+AG vs. GG	Fixed	0.00	0.415	2.513	1.876-3.367	6.176	≤0.001	0.308	0.166
	AA vs. AG+GG	Fixed	0.00	0.531	1.671	1.216-2.297	3.168	0.002	0.734	0.145
By Genotyping Technique										
PCR-RFLP (n=4)	A vs. G	Random	92.46	≤0.001	1.725	1.038-2.866	2.103	0.036	1.000	0.158
	AA vs. GG	Random	95.47	≤0.001	3.821	0.963-15.152	1.907	0.057	0.734	0.454
	AG vs. GG	Random	96.27	≤0.001	2.556	0.828-7.892	1.632	0.103	0.734	0.213
	AA+AG vs. GG	Random	90.74	≤0.001	1.961	0.999-3.847	1.958	0.050	1.000	0.083
	AA vs. AG+GG	Fixed	21.29	0.283	1.366	1.133-1.647	3.273	0.001	0.308	0.127
TaqMan (n=3)	A vs. G	Fixed	0.00	0.874	0.955	0.853-1.069	-0.797	0.425	1.000	0.876
	AA vs. GG	Random	77.78	0.011	0.660	0.285-1.530	-0.968	0.333	1.000	0.789
	AG vs. GG	Fixed	0.00	0.742	0.952	0.816-1.110	-0.633	0.527	0.296	0.261
	AA+AG vs. GG	Fixed	0.00	0.902	0.945	0.816-1.094	-0.760	0.447	0.296	0.037
	AA vs. AG+GG	Fixed	0.00	0.484	0.950	0.732-1.231	-0.390	0.696	1.000	0.780
1151T>A										
Overall (n=8)	A vs. G	Fixed	41.92	0.099	2.462	2.350-1.635	3.378	≤0.001	0.063	0.013
	AA vs. GG	Fixed	0.00	0.535	3.189	0.1-30.756	1.003	0.316	NA	NA
	AG vs. GG	Fixed	38.80	0.121	2.416	1.669-3.496	4.678	≤0.001	0.063	0.011
	AA+AG vs. GG	Fixed	41.31	0.103	2.654	1.610-4.375	3.828	≤0.001	0.220	0.055
	AA vs. AG+GG	Fixed	0.00	0.546	2.990	0.310-28.834	0.947	0.343	NA	NA

CRC risk was observed in Asians for -93G>A and 655A>G polymorphisms, but not in Caucasians. It should be considered that the apparent inconsistency of these results may underlie differences in population background, source of controls, lifestyle, disease prevalence, sample size, and also by chance as well as possible limitations due to the relatively small sample size. The current available data support the multifactorial nature of CRC, and both genetic and environmental factors play an important role in development of CRC. Thus, it is unlikely that the same gene polymorphisms may play different roles in cancer susceptibility, because cancer is a complicated multi-genetic disease, and different genetic backgrounds may contribute to the discrepancy.

Present meta-analysis results were not consistent with a previous meta-analysis on MLH1 -93G>A and 655A>G polymorphisms with CRC risk.[51,52] In 2012, Wang et al. included six case–control studies with 17,791 cases and 13,782 controls on MLH1 -93G>A polymorphism. Their results suggested that MLH1 -93G>A polymorphism was associated with increased risk of CRC under the heterozygote (OR= 1.06, 95% CI =1.01–1.11), and the dominant (OR = 1.06, 95% CI = 1.01–1.11) models.[51] In the more recently meta-analysis, Chen et al. included 13 case–control studies on hMLH1 -93G>A, nine studies on 655A>G, and seven studies on 1151T>A. They have reported that there is a significant association between hMLH1 1151T>A polymorphism and CRC risk, but not with hMLH1 655A>G and -93G>A polymorphisms. Additionally, they have found similar results by subgroup analyses according to quality score and genotyping methods.[52] However, their meta-analysis might be generated conflicting results, which had insufficient power in the meta-analysis because the number of studies was considerably smaller than that needed for the achievement of robust conclusions. In addition, due to small size meta-analysis, they could not rule out the possibility of publication bias. With more studies about hMLH1 polymorphisms and CRC have available recently, our updated meta-analysis, which has the largest sample size thus reported, we found that the 655A>G and 1151T>A polymorphisms were associated with risk of CRC. Moreover, we found that they wrongly calculated HWE test for both cases and controls in their meta-analysis. Therefore, cumulative meta-analyses have suggested that no significant association was observed between hMLH1 polymorphisms and CRC, as evidence accumulated by time.

Heterogeneity is a potential problem when interpreting the results for most meta-analyses, and finding out the sources of heterogeneity is one of important goals of meta-analyses.[53-55] In the present meta-analysis there was obvious between-study heterogeneity.[54,55] Three subgroup analyses were conducted by ethnicity, control source, and smoking status and the heterogeneity still existed. Despite some diversity in the studies about designs, sample sizes, inclusion criteria, and ethnicity, significant heterogeneity between studies was only observed for the -93G>A and 655A>G polymorphism. Thus, we performed subgroup analyses by ethnicities, genotyping methods and source of controls to explore the sources of heterogeneity. The results showed that the heterogeneity disappeared or decreased in several subgroups but remained in other subgroups, suggesting that other covariates might confound the association.

The limitations of this meta-analysis should not be ignored when interpreting the results. First, the meta-analysis was limited by the relatively small number of eligible studies for 1151T>A polymorphism, which may fail to provide enough statistical power to detect a possible or weak effect of the polymorphism on CRC and limited our ability to perform subgroup analyses. Second, only articles published in English or Chinese were selected, potentially causing a language bias. Third, our analysis was limited to Asian and Caucasian ethnicities, and it is uncertain whether these results are generalizable to other ethnicities. Forth, there was significant between-study heterogeneity for two genes in the overall and Caucasians. In addition, the unknown factors including lifestyles and environments may account for the heterogeneity in study results and the lack of significant findings in the overall and Caucasian populations. Fifth, some studies were hospital-based, while others were population-based. Thus, selection bias might exist. Finally, CRC is a multifactorial disease that results from complex interactions between various genes and environmental factors. Our results were based on unadjusted estimates; data were not stratified by other main confounding variables such as age, gender, lifestyle, diet, major systemic illness etc., because sufficient information was not available from those studies.

In summary, the study inconsistent with the previous meta-analyses suggested that hMLH1 -93G>A and 1151T>A polymorphisms may be associated with

the risk of CRC. Moreover, the -93G>A polymorphism is associated with the susceptibility of CRC in Asian population. However, to ascertain a definitive conclusion on hMLH1 1151T>A polymorphism, well-designed epidemiologic studies with larger sample size and more ethnic groups are suggested to further clarify the association. Moreover, gene-gene and gene-environment interactions studies should also be considered in future studies.

Conflict of interest

The authors declare no conflict of interest.

RESUMO

OBJETIVO: *Tem havido crescente interesse no estudo da associação entre polimorfismos do gene mutL homólogo 1 humano (hMLH1) e risco de câncer colorretal (CRC). No entanto, os resultados de estudos anteriores não são conclusivos. Assim, uma meta-análise foi conduzida para obter uma estimativa mais precisa dos efeitos desse gene.*

MÉTODOS: *Uma pesquisa abrangente foi realizada nas bases de dados PubMed, Embase, Chinese Biomedical Literature até 1º de janeiro de 2018. Odds ratio (OR) com 95% de intervalo de confiança (IC) foi utilizado para avaliar a força da associação.*

RESULTADOS: *Finalmente, foram identificados 38 estudos de casos e controles em 32 publicações, atendendo aos nossos critérios de inclusão. Houve 14 estudos com 20.668 casos e 19.533 controles em hMLH1 -93G>A, 11 estudos com 5.786 casos e 8.867 controles em 655A>G e cinco estudos com 1.409 casos e 1.637 controles em 1151T>Um polimorfismo. Os resultados combinados mostraram que os polimorfismos 655A>G e 1151T>A estavam significativamente associados ao risco de CRC, enquanto que o polimorfismo -93G>A não estava significativamente associado ao risco de CRC. Quanto à etnia, os polimorfismos de -93G>A e 655A>G foram associados ao risco aumentado de CRC entre os asiáticos, mas não entre os caucasianos. Mais interessante, a análise de subgrupos indicou que 655A>G pode aumentar o risco de CRC em subgrupos PCR-RFLP e HB.*

CONCLUSÃO: *Inconsistente com a meta-análise anterior, esta meta-análise mostra que os polimorfismos hMLH1 655A>G e 1151T>A podem ser fatores de risco para CRC. Além disso, o polimorfismo -93G>A está associado à susceptibilidade do CRC na população asiática.*

PALAVRAS-CHAVE: *Câncer colorretal. hMLH1. Polimorfismo. Meta-análise.*

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REVIEW ARTICLE

Mourning and Takotsubo cardiomyopathy: neuroendocrine implications and nutritional management

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http://dx.doi.org/10.1590/1806-9282.64.10.952

SUMMARY

This article aims to make reference to some recent mourning aspects considered risk factors for cardiovascular disease, specifically the Takotsubo cardiomyopathy. The objective was to describe the stress from the death of a loved one combining it to the possibility of occurrence of Takotsubo cardiomyopathy through the perception of a traumatic event by the cortex, which triggers the subcortical brain circuit affecting the endocrine response. Given the growing acknowledgement of this cardiomyopathy, it is possible to contextualize the nutritional behaviours and decisions surrounding it, whose benefits must exceed the condition of temporary cardiac dysfunction and extend to food choices that have some influence in the limbic system. It is a descriptive analysis that aims to problematize the theme into reflections based on this experience, considering the foundation with the science of nutrition.

KEYWORDS: *Takotsubo cardiomyopathy. Bereavement. Death. Nutritional Support.*

INTRODUCTION

Faced with death, the constancy of intense affective-emotional reactions is what classifies the event as a traumatic stressor[1]. The difficulty in coping with the death of a loved one favours the intensity and duration of reactions mediated by biophysiological and psychological mechanisms that may be more devastating to the organism[2], allowing the manifestation of Takotsubo cardiomyopathy (TC).

Although the pathophysiological mechanisms of TC are not fully understood,[3] its association with high stress has focused attention on the autonomic nervous system[4], whose evidence suggests that it is a reflex response controlled by this system[5] - since patients with Takotsubo have higher levels of catecholamine than those with acute myocardial infarction[6]; endomyocardial biopsy is typical of this elevation[7]; and myocardial dysfunction induced by increased catecholamine is the most likely mechanism for TC[8].

Because of this, the exacerbated response of catecholamine is pointed out as a central factor for the occurrence of this cardiomyopathy[5,7,9]. In this follow-up, consideration is given to the organic effects to the myocardium in line with the impact of death of a loved one, which has implications on hormonal mediators. Based on this premise, it was important to discuss the effects of recent mourning on the cardiovascular system from the perspective of Takotsubo cardiomyopathy and its repercussion on nutri-

DATE OF SUBMISSION: 18-Jan-2018
DATE OF ACCEPTANCE: 20-Jan-2018
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tional management, based on the neuroendocrine implications associated with the trauma of losing a loved one. It should be noted that TC is referenced by other names[5,8,10-15], among which the expression *Broken Heart Syndrome*[5] is included in the scope of this article.

METHODS

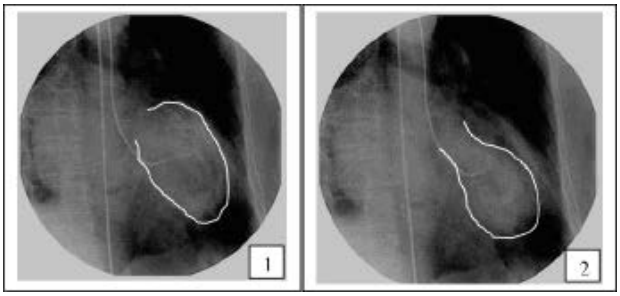
A search for scientific articles with the topics of Takotsubo Cardiomyopathy and Mourning was carried out. The following descriptors were searched in the Capes journal portal and the Science Direct and SciELO databases: "Takotsubo syndrome"; "Takotsubo cardiomyopathy"; "broken-heart syndrome". Other sources of research were: "death" and "stress"; "mourning" and "neuroendocrine responses"; "effects of bereavement"; "bereaved parents"; "grieving process". Publications that did not meet the criteria of interest were excluded from the theoretical reference. The bibliographic research was conducted from May to August 2017, without period restriction, given the need for unified information to support this review. It is a descriptive analysis, based on the main heart alterations of the TC, contextualizing the broken heart syndrome in the face of mourning and nutritional management. To further substantiate this descriptive analysis, the content was organized into three sections: Takotsubo cardiomyopathy in mourning; Takotsubo cardiomyopathy from the perspective of the loss of a loved one; and nutritional management in Takotsubo cardiomyopathy.

TAKOTSUBO CARDIOMYOPATHY IN MOURNING

Depending on the particularities of each person, the emotional regulation profile[16] and the affective bonds established between people, the death of a loved one can trigger a *strong psychological emotion* that can result in cardiac changes. Mourners look for emergency care[17,18] especially for the fear of having a heart attack. Mourning can increase the risk of acute myocardial infarction (AMI) by up to 21 times, an assertion based on a sample of 1,985 patients, where the increased risk is noted within the first 24 hours after the death of the loved one[19]. As angiographic data were not available, the investigators[19] claim that it cannot be ruled out the possibility that some cases refer to TC. Despite its rarity, an AMI may not exclude a cardiomyopathy of stress, and it could even be its trigger[11]. The TC precipitant commonly falls into the stressful physical or emotional condition[14]; however, the lack of a precedent does not exclude the diagnosis[5].

Given that there is an association between mourning and loss experience with heart disease,[20] and that in the first few days, weeks and months after the death of a loved one, cardiovascular disease represents an increase in the risk of death during mourning[19], subtle differences between heart attack and TC deserve appreciation, as the symptoms are similar[6]. In TC, coronary cineangiography does not show significant obstructions[10] - indicating that the primary cause is not related to obstructive coronary disease[9,21] and ventriculography reveals apical ballooning of the left ventricle due to akinesia, with basal hyperkinesia similar to dumbbells or Takotsubo[6] - characteristic image of the syndrome[10] (Figure 1).

FIGURE 1: VENTRICULOGRAPHY SHOWING THE DIASTOLE (1) AND SYSTOLE (2) OF TAKOTSUBO'S CARDIOMYOPATHY. (LEFT VENTRICLE IN SYSTOLE, WITH THE CLASSIC IMAGE OF THE SYNDROME THAT RESEMBLES THE JAPANESE TAKOTSUBO POT)



In the TC electrocardiogram (ECG) pattern, a greater number of derivations with ST segment horizontal depression in the inferior wall1[3] and/or T wave inversion[14,22] are identified, with a prolonged QT interval[23], although there are differences in this pattern[24,25] and the ECG may not be specific[14]. Laboratory tests reveal a slight elevation in the serum concentration of markers of myocardial injury[6,10,12] disproportionate to the area affected to the ECG[8,23]. Levels of B-type natriuretic peptides, which reflect left ventricular systolic dysfunction, are elevated[8], with values higher than those found in AMI[14,26]. Although the term stunned cardiac muscle is used to indicate cardiac injury in Takotsubo[4], the lack of elevation of the enzymes does not exclude the diagnosis[5,26].

Other biomarkers have been reported[27,28]. The

alpha tumour necrosis factor (TNF-α) and the soluble form of its receptors (sTNFR-1 and sTNFR-2) are involved in myocardial dysfunction[27-29], although its effects may include adaptive responses to cardiac protection[29]. The increase of TNF-α is influenced by the plasma concentrations of catecholamines[30]; in response to its stimulus, other proinflammatory cytokines are released, whose activation occurs early to cardiac alterations[27]. Despite the clinical importance of cardiac biomarkers, in post-traumatic stress conditions, their dosages should compose routine exams.

Among the care given to the mourners, which need to start early on, attention must be given to the cardiac conditions of these individuals. Greater attention to the established diagnostic criteria[25] in guidelines[23,31] for TC, aligned with the mourning, can provide the actual dimension of this association.

The literature has presented clinical cases that associate TC with mourning[32]. A possible link between cognitive emotional processing and vulnerability to Takotsubo syndrome was evidenced in a clinical case, after a domestic discussion, with a history of death of a loved one, six months before the symptoms[32]. For Redfors et al.[33], psychiatric conditions may predispose an individual to develop stress-induced cardiomyopathy in response to a strong stressor. Due to other evidences[34,35], a deeper understanding of this phenomenon imposes investigations on the interface: post-traumatic stress due to death of a loved one and biological predisposition to TC.

TAKOTSUBO CARDIOMYOPATHY FROM THE PERSPECTIVE OF THE LOSS OF A LOVED ONE

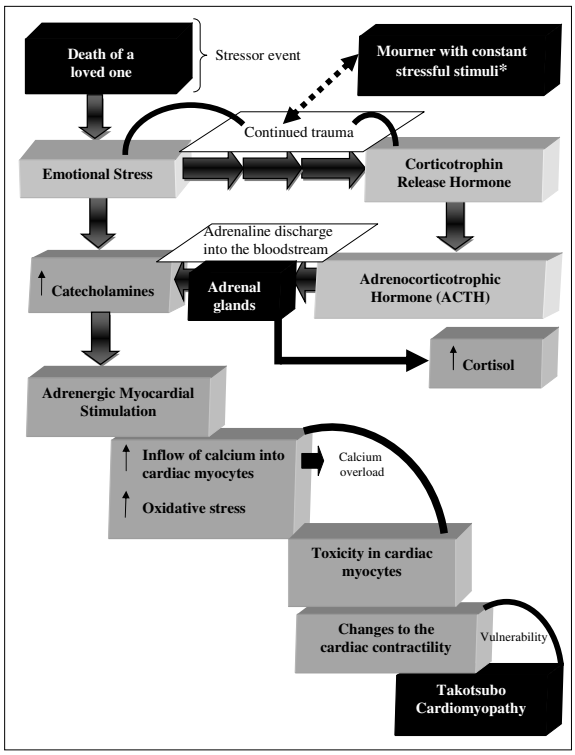
According to Crawford and Schaffer[36], the healthy heart has signalling mechanisms through which it responds to metabolic stress with a remarkable degree of efficiency to meet the high demand and plasticity in response to changes, varying in the supply of the energy substrate to provide for homeostatic, mechanical and electrical activities. Dysfunction in contraction and damage appear when this capacity is exceeded[36].

In an attempt to explain the pathogenesis of TC, several theories have been proposed. Although the pathophysiology is not fully elucidated, there is consensus about the action of catecholamines[5,26,37]. For Coupez et al.[37], the common denominator in TC is catecholaminergic stress. High concentrations of catecholamine may cause damage to the myocardium[4] through the supraphysiological activation of β1 and β2 beta-adrenergic receptors, by activating adenylate cyclase by interacting with stimulatory G protein, elevating the formation of intracellular cyclic adenosine monophosphate that activates protein kinase A – this phosphorylates membrane proteins, increasing the influx of calcium into cells and oxidative stress[7]. This would lead to disturbance of ventricular contraction and function, which may decrease the viability of cardiac myocytes[26]. The interaction of this mechanism with other individual predisposing factors may favour TC.

Among the factors that can trigger an overload of catecholamine in the body is the *death of the loved one* and the *trauma* that occurred during this event. The perception of a traumatic event by the cortex triggers the subcortical cerebral circuit, through the structures that control the emotions and the functions of the visceral systems, whose activation triggers the release of norepinephrine in the hypothalamus[2]. Therefore, the release of epinephrine from the marrow of the adrenal gland is induced[26]. Dealing with the news of death and the loss of a loved one is an *aggression of a psychogenic nature*. In this circumstance, the fundamental hormonal element of organic reactive response is the corticotrophin releasing hormone[38]; in response, the pituitary gland releases the adrenocorticotrophic hormone (ACTH), which stimulates the secretion of corticosteroid hormones and catecholamine by the adrenal glands[2]. Catecholamine induce myocardial adrenergic stimulation; the excess release induces toxicity in cardiac myocytes because it causes a calcium overload in myocytes[7], leading to alterations in cardiac contractility - a more accepted proposition for TC[26] (Figure 2).

Hypercortisolemia is associated with AMI[39] and other heart diseases[40]; it induces proteolysis and lipolysis[2]; and may involve the increase of visceral fat that is associated with metabolic disorders[41]. The organic imbalance appears when the changes are repetitive, coming from an excessive activation, plausible condition of occurrence in the bereavement of children and partners of a lifetime, because, in the psychic complexity of these mourning, the expected hormonal compensatory response will not always occur[2]. Not living with a child anymore (due to death) emotionally destabilizes parents - reactions and disorders arise as a direct consequence of stress or continuous trauma[42], altering the endocrine response,

FIGURE 2: VULNERABILITY TO TAKOTSUBO CARDIOMYOPATHY FROM THE PERSPECTIVE OF THE TRAUMA OF LOSING A LOVED ONE



*Stressful stimuli coming from anguish, deep sadness and thought focused on the cause of death, on the loss of the loved one and the absence of the loved one.

which in turn has reflexes in other metabolic pathways, deserving more investigations since it portrays a differentiated issue of mourning in confrontations and adaptations. On the other hand, the loss of a daily present partner[34], depending on affective attachment, emotional shock[9] and cardiac conditions, may favour the occurrence of TC, especially at more advanced ages.

Effects of *mourning state* and *grief time* on cortisol response revealed a significant interaction, indicating differences in cortisol response pathways in mourners and non-mourners[43]. The increase of cortisol by psychological disorders in non-mourners is less expressive than in mourners. The interaction of the significant loss of the loved one with the grief time evidenced the persistence in the increase of this hormone, having differences in values, as a result of the death[43]. It may be hypothesized that, in the case of recent mourning, the vulnerability to TC lies in the abnormal activation of the *hypothalamus-pituitary-adrenal axis* with the constancy of intense affective-emotional reactions; and the cause of the death of the loved one is an aggravating factor, because it intensifies the psychic trauma - "feeding" the psychological *distress*, since it recalls strong and painful memories that constitute constant stressful stimuli. Under conditions of excessive sympathetic stimulation, one must also consider the possibility of reduced parasympathetic modulation, whose impaired control may play a relevant role in TC[44].

NUTRITIONAL MANAGEMENT IN TAKOTSUBO CARDIOMYOPATHY

Initially, the goal is to alleviate cardiac overload by opting for the nutritional management that apply to this condition. Therefore, restriction of salt (sodium chloride) in cooking and of salt-based condiments, non-addition of salt in ready-made foods, restriction of industrialized foods with high sodium content in their composition and the control of water intake are crucial recommendations whose effects on the body must be carefully monitored. To give a pleasant flavour to the meal, preferably combine spices referenced with cardioprotective properties[45,46] with aromatic herbs.

Due to myocardial injury, include food sources of phenolic compounds, which, depending on their absorption and metabolism, can modulate the expression of various biomarkers, attenuating oxidative stress and reducing proinflammatory cytokines[47]. As for fruits, preference should be given to those with purple and red colors[48], citrus[49] and oleaginous,[50] because they contain a higher content of phenolic compounds. Calculate daily fructose intake to avoid that its excess can contribute to the increase of uric acid - via degradation of adenosine monophosphate (AMP) from adenosine triphosphate (ATP) generated in the metabolism of fructose, which is converted into uric acid. Although uric acid is considered an important antioxidant in human plasma, supranormal levels in the blood have repercussions on cardiovascular function[51]. Hyperuricemia inhibits the release of nitric oxide, which activates the renin-angiotensin system, constituting an important collaborator in determining cardiovascular risk[52], and it also has an impact on the diagnosis of the metabolic syndrome[53,54]. Moderate fructose consumption, ≤50 /day, has no harmful effect on health[55]; excesses can exert harmful effects on metabolic analogies to ethanol, differing only by non-metabolization in the central nervous system[56].

However, due to TC, it is prudent that the ingested fructose is between 25 and 30 g daily. Consult the fructose content in fruit types to establish the quantities and size of the portions and avoid processed foods containing sucrose or fructose-rich corn syrup. In order to keep the serum concentration of uric acid in the normal range, also designate, in daily portions, foods with a more expressive content of purines - the main precursor of uric acid.

Ensuring homeostasis of the enteric microbiota is another behaviour known for its beneficial effects[57-60]. The combined use of pre-and probiotic foods has a hypolipidemic effect[58] and is suggested to reduce plasma concentrations of toxins in plasma and, given the ability of bacteria to produce and recognize neurochemicals, it has been indicated as a therapeutic adjuvant for brain chemistry.[59,60] The replacement of probiotics (through diet) is done with the daily intake of products such as fermented milk and kefir, and of prebiotics as ingredients in the culinary - field with increasing evidences in nutritional benefits. It is recommended to include pre and probiotic products duly regulated by the competent bodies of each country to recommend those with substantiated claims.

Balance amounts and types of fats (monounsaturated, polyunsaturated and saturated) provided by dietary intake, through analysis of tests that results in individual guidelines[48] and restriction of trans fats[61]. Adequate consumption of polyunsaturated fatty acids contributes to a decrease in the hepatic production of VLDL (very low density lipoprotein)[58], improvement in antiarrhythmic effects, among other benefits[61]. Up to 10% of the diet caloric value of polyunsaturated fat diet[62] is recommended in combination with foods that contain antioxidants[48] - excessive intake may lead to increased lipid oxidation, decreasing HDL-c (*high density lipoprotein cholesterol*)[58]. Polyunsaturated fats, when exposed to heat and oxygen, favour processes of oxidation and other chemical changes. Culinary methods that use high temperatures and low humidity (*frying, roasting or grilling*) potentiate the formation of advanced glycation products (AGEs),[63] which, in excess, favour injuries to the tissues, predisposing the organism to the progression of diseases, including cardiovascular diseases, causing oxidative stress and increasing the expression of inflammatory mediators[64]. Gentle preparation methods with high water activity generate lower levels of these compounds[65] and restrict cooking fats[66]. Although the formation of AGEs is predominantly endogenous in the body, they can be introduced by exogenous sources[63] especially incorporated in foods[64]. The diet to attenuate myocardial injury should add to the dietary recommendations for fats and cardiovascular health[61] the control of dietary AGEs intake, in order not to exceed the body's capacity to degrade these compounds, benefiting recovery.

If there is heart failure and volume overload, the use of diuretics is administered[14,21,26]; in these cases, ensure the presence of food sources of potassium in the composition of the meals, coupled with adequate monitoring in the organic response to avoid other electrolyte disturbances.

In view of the reestablishment of cardiac functions, the conducts inherent to the limbic system must be instituted. The limbic system is a complex combination of various structures of the brain that receives sensory stimulation, which is translated into emotional and psychological expression, regulating various functions in the organism[67]. If the balance in the chemistry of this system interferes with emotional health, the contribution of precursors of neurotransmitters responsible for the feelings of well-being must be restored through diet[48]. When the person suffers a significant loss, such as the death of a child, the sadness can be intense and prolonged, characterizing a condition of mental depression or of adjustment disorder[2]. A balanced supply of these chemical transmitters is essential so that the body can respond better to the stressor event. For example, to benefit serotonin production, consider food sources of tryptophan in balance with carbohydrates (aiming to increase the uptake of tryptophan in the cerebrospinal fluid in competition for the transporter that crosses the blood-brain barrier)[48] and other nutrients (folic acid, pyridoxine and magnesium) for conversion of tryptophan into serotonin[2]. In mourners, the replacement of nutrients and food components that have a beneficial influence on mental health needs to be implemented and with the combination of foods that provides the best response.

However, the treatment of patients with TC should be individualized according to their clinical, psychic and nutritional conditions. This requires the application of a more specific clinical-nutritional protocol, paying attention to diet and food components referenced as protectors of heart injury, as well as traumatic situations triggering this syndrome, seeking to identify the basis of each person's disturbances. If TC resulted from the loss of a loved one, cover the emotional stressors associated with this pecu-

liarity, know the cause of death, the particularities of mourning, behavioural changes instituted after the death of the loved one, being aware of the changes that may have come up in sleep, diet, body weight and perimeter. Among the multiplicity of factors involved (psychic and physiological)in the genesis of the psychosomatic phenomenon, the situations of losses preceding the illness are more emphasized[68], which redirects attention to the mourners because of their vulnerability to becoming ill. Considering the connection of emotional stress with the heart, depending on the psychic conditions of the bereaved, in addition to grief therapy[20] and cardiac evaluation, it is reasonable to evaluate the need for pre-treatment with medication indication and referral to nutritional care, mainly in clinical cases of *inability* or *refusal to eat*. The way we deal with death and mourning has implications in eating behaviors, with serious consequences to health,[2] and restoring, through food, benefits to the mind and heart, is realizing that, in the face of individuals destroyed by loss and with symptoms of anhedonia, nutritional management require gradual adaptation. Cases of stress-induced cardiomyopathy have been described in individuals with eating disorders, who reach higher severity because they are associated with prolongation of the QT interval due to electrolyte imbalances and hypoglycaemia[69]. It is conceived that cardiovascular research in mourners needs to be carried out, especially in the presence of well-defined behavioural and mood changes, and when severe diet restriction is noted.

FINAL CONSIDERATIONS

Deepening the psychic issues of mourning requires a careful look at individual differences in reaction to death. Mourning is not a process that progresses in a linear way, therefore, it deserves the deepening in studies that ally its effects to cardiac alterations.

In mourners, due attention to the cardiac symptoms of Takotsubo cardiomyopathy should also be part of the clinical care protocols, in order to guarantee more specific investigations directed to this syndrome. Because this cardiomyopathy has a strong association with emotional stress, greater attention in this recognition could contribute to the notification of clinical cases in Brazil.

The science of nutrition is inserted in this context because it favours, together with hemodynamic therapy and coping with mourning, not only benefits for the reestablishment of cardiac function, but also extends to diet planning applied to mental health and nutritional health.

ACKNOWLEDGEMENTS

To the cardiologists LPM (Hemodynamic Service, Hospital São João Batista, Viçosa, Minas Gerais, Brasil) and FMQV (Clinicor/Viçosa) for the provision of Figure 1.

Conflict of Interest

The authors declare no conflict of interest.

RESUMO

INTRODUÇÃO: *Este artigo busca fazer referência a alguns aspectos do luto recente considerados fatores de risco para a doença cardiovascular, especificamente a cardiomiopatia de Takotsubo. Objetivou-se descrever o estresse proveniente da morte do ente querido aliando-o à possibilidade de ocorrência da cardiomiopatia de Takotsubo, mediante a percepção de um evento traumático pelo córtex que aciona o circuito cerebral subcortical tendo repercussões na resposta endócrina. Dado o crescente reconhecimento dessa cardiomiopatia, torna-se viável contextualizar as condutas nutricionais e as decisões que as norteiam, cujos benefícios devem ultrapassar a condição de disfunção cardíaca temporária e se estender às escolhas alimentares que exercem alguma influência no sistema límbico. Trata-se de uma análise descritiva que objetiva problematizar a temática em reflexões pautadas nessa vivência, considerando o alicerce com a ciência da nutrição.*

PALAVRAS-CHAVE: *Cardiomiopatia de Takotsubo. Luto. Morte. Conduta nutricional.*

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