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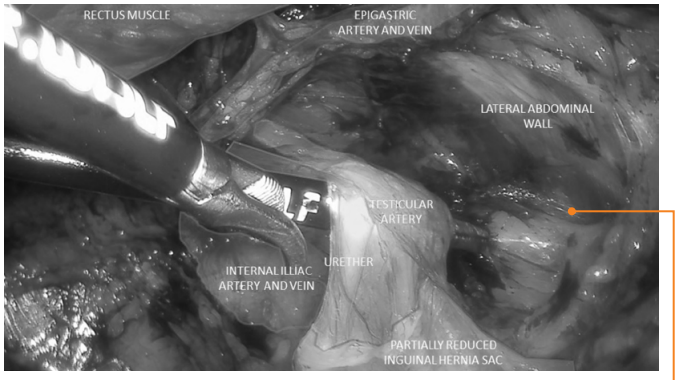

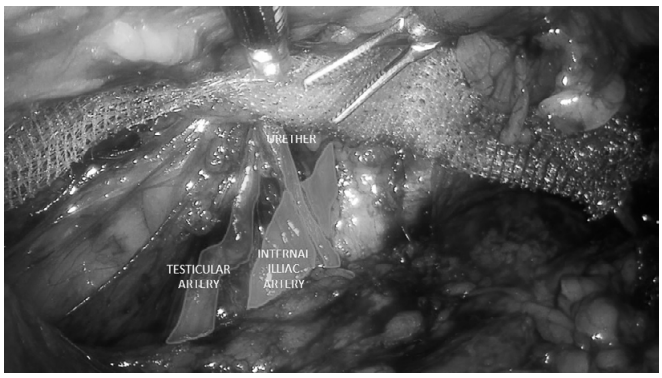

ARTICLES

RAMB REVISTA DA ASSOCIAÇÃO MÉDICA BRASILEIRA
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SUMMARY

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ARTICLES

REVIEW ARTICLES

Gray zone: mortality profile of newborns at the limit of viability

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The discussion about extreme prematurity and the limits of human viability currently constitutes an important theme with great relevance, with an imperative need to implement new clinical strategies for this population.

In recent years, some publications have shown that medical management varies based on local legislation, resources, and dilemmas relating to the interruption or non-introduction of invasive treatment measures[1,2,3]

Gestational age is considered the best feature in estimating the survival of these newborns, but other variables play an important role in the prognosis. In the United States, a consensus obtained by several medical entities, including the American Academy of Pediatrics[4], was the definition of a peri-viable birth as a birth that occurs between $20^{0/7}$ and $25^{6/7}$ weeks. Upon analyzing the mean of these gestational ages, survival ranged from 0% to more than 50%. The authors pointed out that gestational age and fetal weight are related to other variables, such as gender, multiple gestations, antenatal treatment with steroids, antibiotics, or neuroprotectors. Until more accurate models of neonatal prognosis are developed, gestational age remains the main predictor for guiding the conduct in the limit of fetal viability[4,5]. The interval between $23^{0/7}$ and $24^{6/7}$ weeks is considered the "gray zone", in which recommendations suggest individual neonatal resuscitation according to the parents' wishes[5].

In neonates with a gestational age younger than 25 weeks, there is insufficient evidence to determine the prognostic factors of survival at birth or within the first 30 days of life. Variables such as gestational age accuracy, presence or absence of chorioamnionitis, and level of care complexity in neonatal ICU are considered [6,7].

In the latest published research on the topic, a national study in Korea[9] the authors analyzed the variation of mortality according to age and causes of death

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in 621 newborns with gestational age of 23 and 24 weeks, dividing the patients into two groups, according to the mortality rate: group one, with mortality less than 50% and group two, with mortality equal to or greater than 50% of live births with borderline gestational age. The authors highlight the lower mortality in group one but do not describe the observed differences in the care structure between the two.

The Hospital das Clínicas of the Faculty of Medicine of the University of São Paulo, Brasil, is a national referral center for pregnant women at risk and intensive neonatal treatment at the quaternary level. Among 9476 live births in the period from 2012 to 2017, there were 28 newborns admitted to the neonatal ICU with gestational age between $23^{0/7}$ and $24^{6/7}$ weeks. The mortality rate in these neonates was 85.7% in the postnatal age after 28 days of life, with only four survivors. The majority of deaths (60%) occurred before seven days of life, with a predominance of early neonatal mortality.[8]

The main cause of death was infection, with a relevant role of maternal chorioamnionitis

There was a survival rate similar to group 2 from the study of Park et al[9] (14.3%), with comparable Apgar values, presence of chorioamnionitis and low body temperature at admission to the neonatal intensive care unit. In the article by Park et al[9], the method of evaluation of gestational age was based only on the date of the last menstruation. Fetal ultrasound performed early, together with the date of the last menstruation, would be better indicated because of the greater precision, especially in a population where any variation causes a great impact on the management

Upon consideration of the above, some seminal questions arise: What would be the evolution of these patients in developing countries where there is a legal limitation to suspend treatment? How do developing countries approach preterm newborns in the gray area?

We suggest a national and a multinational multi-center study to analyze the clinical differences of extreme prematures with gestational age between 23 and 24 weeks. The centers would be analyzed according to their characteristics and the characteristics of the patients.

Conflicts of interest

The authors declare they have no conflict of interest

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REFERENCES

1. Wyllie J, Perlman JM, Kattwinkel J, Wyckoff MH, Aziz K, Guinsburg R, Kim H-S, Liley HG, Mildenhall L, Simon WM, Szyld E, Tamura M, Velaphi S, on behalf of the Neonatal Resuscitation Chapter Collaborators. Part 7: Neonatal resuscitation: 2015 International Consensus on Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science With Treatment Recommendations. Resuscitation 2015;95:e169-e201.
2. Mercer BM . Periviable Birth and the Shifting Limit of Viability. Clin Perinatol 2017:44;283-6 http://dx.doi.org/10.1016/j.clp.2017.02.002
3. Rysavy MA, Li L, Bell EF, et al. Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Between-hospital variation in treatment and outcomes in extremely preterm infants. N Engl J Med 2015;372:1801-11.
4. Raju TN, Mercer BM, Burchfield DJ, et al. Periviable birth: executive summary of a joint workshop by the Eunice Kennedy Shriver National Institute of Child Health and Human Development, Society for Maternal-Fetal Medicine, American Academy of Pediatrics, and American College of Obstetricians and Gynecologists. Obstet Gynecol 2014;123:1083-96.
5. Pignotti MS, Donzelli G. Perinatal care at the threshold of viability: an international comparison of practical guidelines for the treatment of extremely preterm births. Pediatrics. 2008;121(1):e193-8.
6. Ishii N, Kono Y, Yonemoto N, et al, Neonatal Research Network, Japan. Outcomes of infants born at 22 and 23 Weeks' gestation. Pediatrics 2013;132:62-71.
7. Seaton SE et al. Babies born at the threshold of viability: chances in survival and workload over 20 year. Arch Dis Child Fetal Neonatal 2013; 98(1). F.15-20.
8. Matsushita FY, Krebs VLJ, de Carvalho WB, Use of inotropes in a tertiary neonatal intensive care in Brazil: A critical analysis, presented to American Academy of Pediatrics National Conference & Exhibition, Orlando, 2-6 November
9. Park JH, Chang YS, Sung S, Park WS, Network KN. Mortality Rate-Dependent Variations in the Timing and Causes of Death in Extremely Preterm Infants Born at 23–24 Weeks' Gestation. Pediatr Crit Care Med. 2019 Apr 2. [Epub ahead of print]

Chromoblastomycosis: a neglected disease

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KEYWORDS: *Chromoblastomycosis. Dermatomycoses. Skin Diseases, Infectious.*

Neglected tropical diseases (NTDs) include a series of tropical and subtropical endemic diseases worldwide. They usually affect individuals living in low-income regions of Asia, Africa, and Latin America. NTDs affect populations with little political voice and low visibility. According to the World Health Organization (WHO), the prevalence of NTDs is linked to poverty and social fragility. Those who suffer the most from NTDs are the most impoverished populations, often living in remote rural areas, urban slums, and areas of conflict. With little health care attention and political support, NTDs are not under the radar of public health systems, nor are they part of their priorities. Several endemic diseases, including diseases caused by helminths, protozoa, bacteria, and viral infections, except for fungal diseases, are defined as "neglected diseases" by the WHO[1]. Recently, we received a case of advanced chromoblastomycosis (CMB) in our service. The state of progression of the disease motivated us to write this latter due to our great concern regarding the assistance of patients affected by this disease in our country. Our patient was a male, 54 years old, coming from a rural area in the North Region of the country who reported having a lesion in the right leg, in progression for 25 years; he had never had access to medical treatment for this condition. The limb affected was already completely disabled at the time of service. It worries us to find such serious cases in our country still because this is an important endemic mycosis, with affordable diagnosis and that requires treatment in the early stages because it can lead to patient disability[2]. CMB remains a prevalent disease in Brasil and is endemic in many areas, especially in the Northern Region, where 872 cases have been reported

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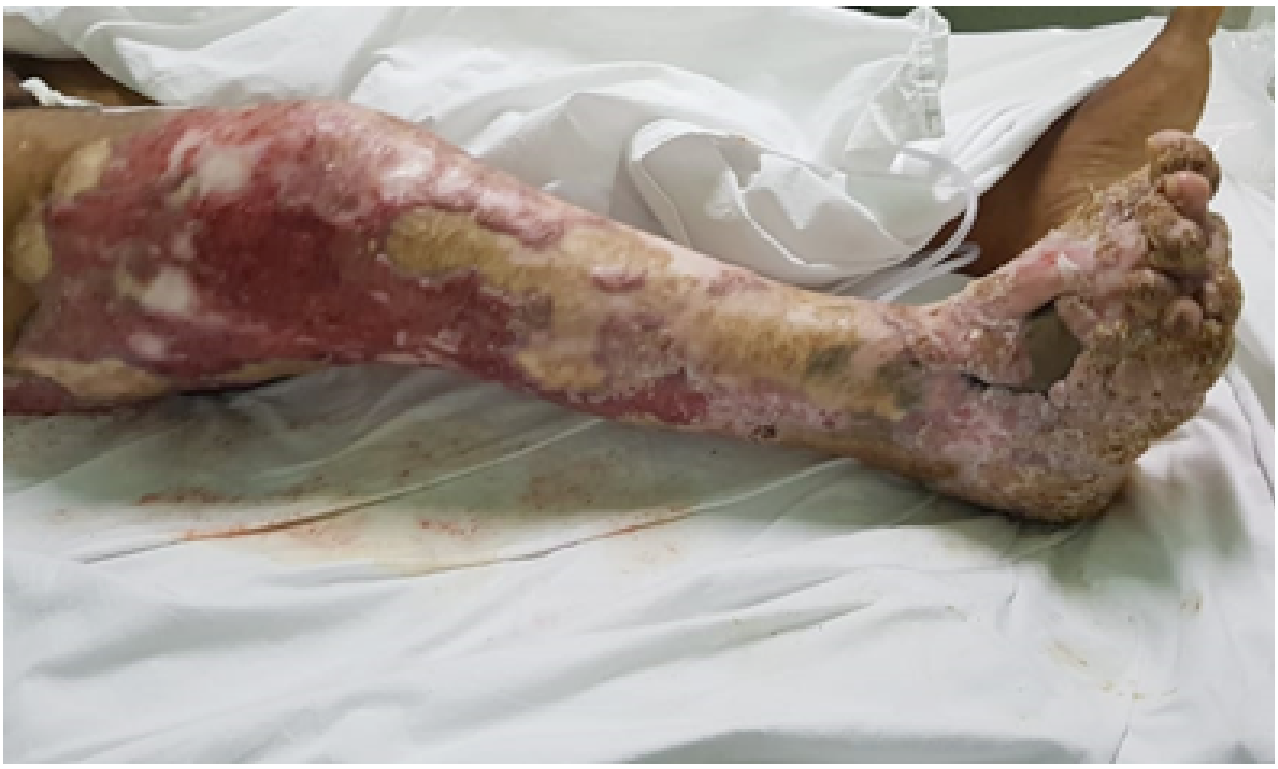

FIGURE 1. SIDE VIEW OF THE RIGHT LEG OF A PATIENT AFFECTED BY CBM.

in recent decades. Such cases exemplify the neglect faced by patients with this condition in Brasil. CMB is a chronic fungal infection of the skin and subcutaneous tissue caused by the transcutaneous traumatic inoculation of a specific group of dermal fungi that occur mainly in tropical and subtropical areas of the world. It affects mainly men who are rural workers, and it has been increasingly found among other types of professionals. The fungus penetrates the skin after inoculation, and the agent most frequently isolated is the *Fonsecaea pedrosoi*. When left undiagnosed in the early stages, patients with CBM require prolonged treatments with systemic antifungal agents, sometimes associated with physical methods that are not always effective[3]. CBM is one of the most prevalent fungal infections worldwide, and it is the most common pathology among diseases caused by melanocyte fungi. CBM must be considered a neglected disease, because if on the one hand, poverty and limited access to health care contribute to the permanence of serious cases such as this, its effects perpetuate the condition of poverty and inequality in endemic areas, in a cyclic dynamic. CBM makes those affected disabled to work, which entails economic consequences. Its overall burden is comparable or greater than that of mycetoma and, like mycetoma, it is basically an occupational fungal disease. Due to its global prev-

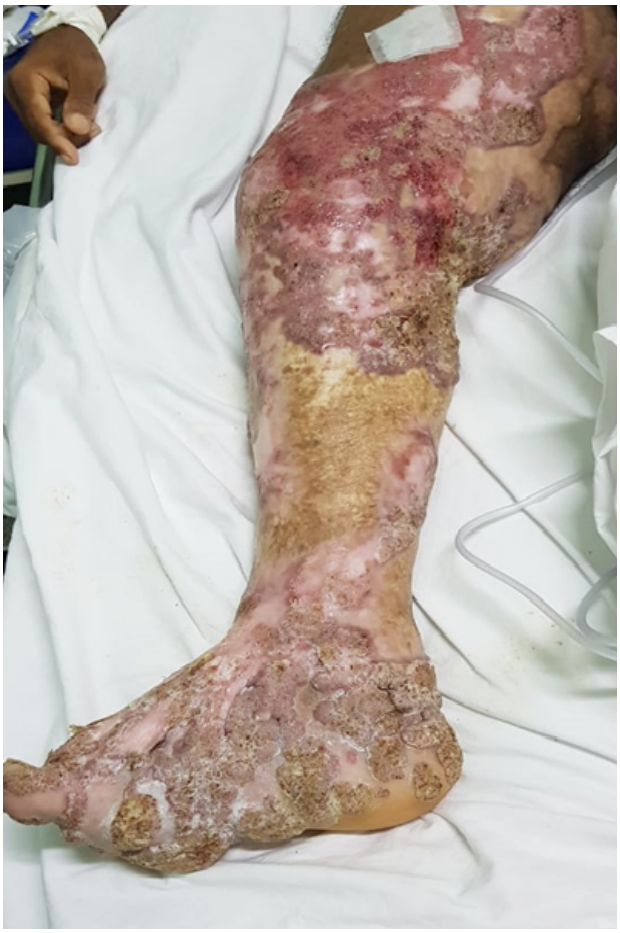

FIGURE 2. TOP VIEW OF THE RIGHT LEG OF A PATIENT AFFECTED BY CBM.

alence, its impact on poverty, and its resistance, this condition must be considered an actual neglected disease, at least in our country, even if not recognized as such by the WHO, so that we can prevent other cases like this[4].

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PALAVRAS-CHAVE: *Cromoblastomicose. Dermatomicoses. Dermatopatias Infecciosas.*

REFERENCES

1. Savioli L, Daumerie D. World Health Organization Department of Control of Neglected Tropical Diseases. Sustaining the drive to overcome the global impact of neglected tropical diseases: second WHO report on neglected tropical diseases. Geneva: World Health Organization; 2013.
2. Queiróz AJR, Pereira Domingos F, Antônio JR. Chromoblastomycosis: clinical experience and review of literature. Int J Dermatol. 2018;57(11):1351-5.
3. Correia RTM, Valente NYS, Criado PR, Martins JEC. Cromoblastomicose: relato de 27 casos e revisão da literatura. An Bras Dermatol. 2010;85(4):448-54.
4. Queiroz-Telles F, Hoog S, Santos DW, Salgado CG, Vicente VA, Bonifaz A, et al. Chromoblastomycosis. Clin Microbiol Rev. 2017;30(1):233-76.

Erectile dysfunction: drug treatment

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

Erectile dysfunction is the recurrent and persistent inability of having and/or maintain a sufficient penile erection for satisfactory sexual intercourse. It is considered a disease that impairs psychosocial health and quality of life.

By means of the PICO methodology, each clinical question was structured, using the following descriptors: (P) Patients with erectile dysfunction, (I) Injectable treatment associated with PDE5i, penile revascularization, use of a penile implant, (O) Adverse events/ International index of erectile function. We performed a systematic review of the literature for each clinical question, with no time restriction, in the MEDLINE database, using 59 papers to answer all the questions. The details about the methodology and the results are set out in Appendix I.

INTRODUCTION

Erectile dysfunction is the recurrent and persistent inability of having and/or maintaining a sufficient penile erection for satisfactory sexual intercourse[1](D). It is a prevalent disease that compromises the psychosocial health and quality of life[2-4](B).

Its causes are disorders of vascular, neurogenic, structural, hormonal, or psychogenic nature, or induced by drugs or by trauma[5](D).

The assistant physician must identify and treat the reversible causes, such as the psychogenic, associated with hormone deficiencies and those arising from the use of drugs. In the absence of a response, the treatment should be discussed with the patient. The involvement of the partner is always interesting since it promotes dialog and improves the chances of success and satisfaction with the treatment. Often times, the treatment of the male problem may not be enough to restore a satisfying sex life for the couple. The choice of treatment or the option of non-intervention should be shared with the patient or, preferably, with the couple, taking into account individual aspects. Preference should be given, initially, to oral pharmacotherapy[5](D).

RESULTS

1. What are the oral drugs most currently used for the treatment of erectile dysfunction?

The phosphodiesterase type 5 inhibitors (PDE5i) now constitute the most widely used oral therapy and act by promoting the relaxation of the muscle cells of the cavernous tissue, a necessary condition for obtaining an erection[6](A).

The most commonly used are:

- Sildenafil
- Tadalafil
- Vardenafil
- Lodenafil

2. What is the absolute contraindication for the use of phosphodiesterase type 5 inhibitors (PDE5i)?

The absolute contraindications of PDE5i are hypersensitivity to the components of the drug and concomitant use with nitrates[7](A).

3. What is the average duration of action of the main PDE-5 inhibiting drugs?

- Onset of action[8](D):
- Sildenafil: 30-60 min
- Tadalafil: 15-45 min
- Vardenafil: 15-30 min
- Lodenafil: 40 minutes

Duration of action:

- Sildenafil: 4-6h, up to 12h
- Tadalafil: 24-36h
- Vardenafil: 4-6h
- Lodenafil: 6h

4. Can PDE-5 inhibitors be used in patients who use drugs to control blood pressure or in users of alpha-blockers?

The use of PDE5i concomitantly with alpha-blockers or anti-hypertensive drugs can accentuate the hypotensive effect, without, however, contraindication of the simultaneous use of such drug classes[9-13](A). A study[13](A) demonstrated that the pressure variation after the use of anti-hypertensive medication with sildenafil was small, -3.6 mmHg in systolic pressure, while the placebo with anti-hypertensive had a variation of -0.8 mmHg.

5. What are the precautions that should be used for the employment of PDE-5 inhibitors in patients with liver failure, kidney failure, and in users of antiretroviral drugs?

Liver failure: In patients with liver cirrhosis (class A and B of Child-Pugh), the *clearance* of PDE5i is reduced, resulting in an increase in the drug's plasma levels. The pharmacokinetics of sildenafil in patients with Child-Pugh class C liver failure was not studied[14](B).

Kidney failure: In volunteers with severe kidney failure (creatinine clearance ≤ 30 mL/min), the PDE5i clearance is reduced, leading to an increase in the serum levels of the drug[15](A).

The concomitant administration of PDE5i and ritonavir or saquinavir (antiretroviral drugs), which is also a potent inhibitor of the P450 cytochrome, results in an increase in the plasma concentration of PDE5i. Sildenafil does not have any effect on the pharmacokinetics of ritonavir[15](A).

6. What are the possible causes when there is an inadequate response to the treatment of erectile dysfunction with PDE-5 inhibitors?

Comorbidities: Some comorbidities, such as diabetes and cardiovascular diseases, can induce endothelial dysfunction, which is a risk factor for erectile dysfunction[16](B).

Inappropriate use: Use of suboptimal doses, use with a full stomach and sexual intercourse outside the time of action of the drug may contribute to the ineffectiveness of the medication[17-19](B). A study[17](B) demonstrated that of 100 consecutive patients nonresponders to PDE5i, 56 used the drug in a suboptimal way, of which 45 used a dose below the recommended.

Incorrect diagnosis: Hypogonadism, hyperprolactinemia, and disorders of sensitivity may be causes of erectile dysfunction[20-22](B). Of the patients with hypogonadism and associated erectile dysfunction without an initial response to PDE5i, 72% will respond to treatment with testosterone replacement[22](B).

Lack of sexual stimulation: Without sexual stimulation, PDE5i is ineffective since this drug only acts upon stimulus[23](A).

Psychological disorders: Anxiety disorders or other psychological issues may interfere in sexual function[24](A).

7. Can the use of long-acting PDE-5 inhibitors be associated with short-acting PDE-5 inhibitors for the treatment of severe erectile dysfunction?

In patients with failed PDE5i monotherapy and severe erectile dysfunction, it is possible to try the joint use of short and long-acting PDE5i. There is no increase in the incidence of side effects with this combination[25](A).

8. Is there clinical evidence for the use of phytotherapics or vitamin supplements in the treatment of erectile dysfunction?

Some elements of traditional medicine can be employed in the treatment of erectile dysfunction but without scientific proof.

Yohimbine: a meta-analysis with clinical trials showed an improvement of erectile dysfunction, compared with placebo (odds ratio: 3.85; IC 95%: 6.67-2.22). Adverse reactions were infrequent and transient[26](A).

Red ginseng: a meta-analysis with randomized clinical trials showed an improvement of erectile dysfunction, compared with placebo (odds ratio of 2.40; IC 95%: 1.65-3.51). However, the assessment of the quality of the studies was low on average[27](A).

Tribulus Terrestris: a randomized, double-blind, clinical trial showed no effects on the international index of erectile function (IIEF-5)[28](A).

Ginkgo Biloba: shows improvement of erectile dysfunction, mainly for erectile dysfunction induced by antidepressants[29.21](A).

ERECTILE DYSFUNCTION: INJECTABLE TREATMENT

9. In which clinical situations are penile injections (intracavernous pharmacotherapy) indicated for the treatment of erectile dysfunction?

The use of intracavernous injections can be indicated in patients with failure or contraindications to PDE5i therapy or even if there is personal preference[8](D). The success rate of intracavernous therapy is high. It is effective in getting an erection suitable for penetration in 60-90% of men with erectile dysfunction, depending on the agent used[30](D). It requires no nerve integrity and, therefore, may be an alternative for men with spinal cord injury or post-radical prostatectomy. Despite its invasive nature, previous studies showed that the level of satisfaction could be greater with intracavernous therapy when compared with the PDE5i in men who used both methods. Even though it is considered a second-line therapy, intracavernous pharmacotherapy remains essential as part of the diagnostic arsenal of the vascular causes of erectile dysfunction and can play an important role in rehabilitation after radical prostatectomy[31](B).

10. What are the main local and/or systemic complications associated with penile injections?

The most frequent complications are local, while systemic complications are infrequent and generally mild[32-35](C):

Local complications:
- bleeding/bruising at the injection site;
- penile pain;
- fibrosis of the corpus cavernosum;
- penile tortuosity;
- priapism.

Systemic complications:
- arterial hypotension.

11. Should the risks and benefits of the injectable treatment be discussed with the patient?

Yes. If the patient does not understand the procedure and its implications, there is a risk of treatment interruption [36](B).

12. Should an initial test of the injectable treatment be conducted at the clinic?

The test has little diagnostic value regarding the vascular status of the penis. If indicated, a Doppler study can offer further information[37](C). However, the practical instruction on the use of this therapeutic alternative in a clinic setting enables titration of dosage and may reduce the occurrence of complications related to the therapy.

13. That drugs, drugs, or doses should be indicated to the injectable treatment?

Alprostadil (prostaglandin E1) can be used as monotherapy or in combination with other medications (phentolamine and papaverine)[38](B).

14. What is the rate of treatment abandonment for penile injections and its reasons?

The discontinuity occurs in approximately half of patients and, in more than 50% of these, it occurs in the first two months. The main causes of treatment abandonment are the desire for definitive treatment, low response (due to the progression of vascular disease), fear of needles, or complications[36,39-41](B).

15. What is the contraindication to the use of intracavernous pharmacotherapy?

The contraindications to intracavernous pharmacotherapy are predispositions to priapism, such as sickle cell anemia, hypersensitivity to agents, coagulopathies, and penile fibrosis[8](D).

16. How often can/should the injectable treatment be carried out?

The injections may be repeated up to three times a week, with an interval of 24 hours between each injection[42](C).

17. Can the injectable treatment be carried out in association with the oral treatment for erectile dysfunction?

Yes. In patients who do not respond to the inject-

able treatment, a combination of injectable pharmacotherapy and PDE5i may be employed[43](A).

18. When should the injectable treatment be suspended? What is the alternative in its failure?

Penile fibrosis may suggest disease the onset of Peyronie's disease. In these cases, suspend the use of injectable therapy and consider the use of a penile implant[8](D).

SURGICAL TREATMENT OF ERECTILE DYSFUNCTION

19. Is there currently any indication for coronary venous ligation for venous-occlusive dysfunction?

The venous ligation for the treatment of erectile dysfunction due to venous insufficiency is not an alternative for the treatment of erectile dysfunction because it presents very low long-term effectiveness (31% in 45 months) with a risk of complications such as hematoma, local pain, and temporary penile paresthesia [44](C).

20. What is the ideal candidate for the penile revascularization surgery?

Young patients without risk factors for erectile dysfunction, which have arterial deficit due to trauma[45](D).

21. What are the results obtained from penile revascularization surgery in the literature over the past 20 years?

Young men (under 30 years) have a higher success rate in the long term (*odds ratio*, 3.7; 95% *confidence interval*, 2.2 to 6.4; P = .001). The overall success rate in five years is around 64-67%[46-48](A)[49](C).

22. What is the main complication of penile revascularization surgery?

Penile hypervascularization[49](C).

23. What are currently the main indications for penile implants?

Patients with failure to oral or injectable pharmacological therapy who opt for a definitive solution[8](D).

24. What are the preoperative cares that must be adopted to prevent infection?

All care measures regarding the procedure aseptic technique must be adopted. Antibiotic prophylaxis against Gram-positive and Gram-negative bacteria should be used. The use of antibiotic-impregnated implants can also assist in the reduction of infectious complications[50-53](A). A pre-operative routine with mandatory requirements for the penile implant can significantly reduce the rate of infection. Among important measures, are the requirement of negative preoperative urine culture; washing and genital brushing with chlorhexidine 2% two days before surgery; prophylaxis started one hour before the incision; attention to the levels of glycated hemoglobin in diabetic patients (see item 25); brushing of the hands of the surgical team for 5 minutes; genital sanitation for 10 minutes, preferably with chlorhexidine; use of topical antibiotics to irrigate the corpus cavernosum; surgical synthesis in multiple layers, giving preference to absorbable and monofilament threads; minimize the flow of people in the surgical room after incision until the bandaging[54](B).

25. Is there any glycated Hb threshold that constitutes a contraindication to penile implant in the diabetic population?

In a prospective study with multivariate analysis, the glycated hemoglobin value of 8.5% suggests a high risk of infectious complications in penile implants[55](A).

26. What are the pros and cons of a malleable implant (semi-rigid)?

Advantages: low risk of chronic pain, easy to use, surgical implantation technically easier than for inflatable implants[56-59](C).

Disadvantages: the penis remains upright at all times. Its orientation can be modified depending on the need (to urinate, adjust clothes, sexual intercourse)[60](C).

27. What are the pros and cons of a inflatable implant (2 and 3 pieces)?

Advantages: they are softer than the semi-rigid ones, better cosmetic appearance (more "natural" look)[61](B).

Disadvantages: possibility of malfunction, requiring surgical reintervention in some situations[61](B).

28. What are currently the main complications of penile implants and their treatments?

Infection and mechanical failure. The treatment

generally demands the removal of the implant and, in the case of infectious complications, systemic antibiotic therapy[51](B).

SYNTHESIS OF EVIDENCE

Erectile dysfunction is a prevalent condition with several etiologies. The treatment of patients with erectile dysfunction should focus initially on diagnosing reversible causes of erectile dysfunction. This includes a multidisciplinary approach. Cardiovascular risk factors should be investigated and properly treated. As a therapeutic option, the phosphodiesterase-5 inhibitors are the most commonly used drug. In the failure of oral therapy, intracavernous injection with prostaglandin and/or papaverine and/or phentolamine can be used, although with high rates of discontinuity. As a definite alternative, a penile implant can be used.

Restorative therapies have aroused increasing interest in various areas of medicine, and erectile dysfunction is one of them. Among them, the use of low-intensity extracorporeal shock wave therapy (Li-ESWT), therapy with platelet-rich plasma, and the use of stem cells.

Studies in animals have shown that Li-ESWT improves the hemodynamic profile and mitigates the pathological changes related to diabetes in the penis. A few other studies in humans show improvement in erectile function and in response to inhibitors of the phosphodiesterase type 5 enzyme (PDE5i). Thus, it might represent an attractive and innovative alternative, if it is effectively able to interfere in the symptoms or in the natural history of erectile dysfunction. The mechanism of such an action still requires further investigation, but it is probably due to the improvement of endothelial dysfunction and damage caused to peripheral nerves. This technique promotes the formation of new blood vessels, which induce intracavernous neovascularization and the improvement of endothelial function[62-64].

However, there are no results from multicenter, placebo-controlled studies with long follow-up to confirm this therapeutic alternative as truly effective and safe.

There is no scientific evidence that endorses the use of platelet-rich plasma or stem cells as an alternative therapy for men with erectile dysfunction[65.66].

APPENDIX I

The evidence used was retrieved by the following steps: elaboration ofthe clinical question, structuring of the question, search for evidence, presentation of results, and recommendations.

Clinical Questions

- What are the oral drugs most currently used for the treatment of erectile dysfunction?
- What is the absolute contraindication for the use of phosphodiesterase type 5 inhibitors (PDE5i)?
- What is the average duration of action of the main PDE-5 inhibiting drugs?
- Can PDE-5 inhibitors be used in patients who use drugs to control blood pressure or in users of alpha-blockers?
- What are the precautions that should be used for the employment of PDE-5 inhibitors in patients with liver failure, kidney failure, and in users of antiretroviral drugs?
- What are the possible causes when there is an inadequate response to the treatment of erectile dysfunction with PDE-5 inhibitors?
- Can the use of long-acting PDE-5 inhibitors be associated with short-acting PDE-5 inhibitors for the treatment of severe erectile dysfunction?
- Is there clinical evidence for the use of phytotherapics or vitamin supplements in the treatment of erectile dysfunction?
- In which clinical situations are penile injections (intracavernous pharmacotherapy) indicated for the treatment of erectile dysfunction?
- What are the main local and/or systemic complications associated with penile injections?
- Should the risks and benefits of the injectable treatment be discussed with the patient?
- Should an initial test of the injectable treatment be conducted at the clinic?
- That drugs, drug,s or doses should be indicated to the injectable treatment?
- What is the rate of treatment abandonment for penile injections and its reasons?
- What is the contraindication to the use of intracavernous pharmacotherapy?
- How often can/should the injectable treatment be carried out?
- Can the injectable treatment be carried out in association with the oral treatment for erectile dysfunction?

- When should the injectable treatment be suspended? What is the alternative in its failure?
- Is there currently any indication for coronary venous ligation for venuso-occlusive dysfunction?
- What is the ideal candidate for the penile revascularization surgery?
- What are the results obtained from penile revascularization surgery in the literature over the past 20 years?
- What is the main complication of penile revascularization surgery?
- What are currently the main indications for penile implants?
- What are the preoperative cares that must be adopted to prevent infection?
- Is there any glycated Hb threshold that constitutes a contraindication to penile implant in the diabetic population?
- What are the pros and cons of a malleable implant (semi-rigid)?
- What are the pros and cons of a inflatable implant (2 and 3 pieces)?
- What are currently the main complications of penile implants and their treatments?

Structured clinical question

The PICO approach was structured according to the clinical question.

Below, the description of the specific structures.

PICO for Question 4:

P - Patients with erectile dysfunction
I - Use of PDE5i associated with anti-hypertensive drug
C - Does not apply
O - Adverse events

PICO for Question 5:

P - Patients with erectile dysfunction and liver failure/liver failure/use of antiretroviral drugs
I - Use of PDE5i
C - Does not apply
O - Adverse events/plasma concentration of PDE5i

PICO for Question 8:

P - Patients with erectile dysfunction
I - Udenafil, Mirodenafil Tadalafil, Vardenafil, Lodenafil, Avanafil
C - Sildenafil
O - Effectiveness of the treatment

PICO for Question 15:

P - Patients with erectile dysfunction
I - Penile injections
C - Does not apply
O - Therapy interruption

PICO for Question 18:

P - Patients with erectile dysfunction
I - Injectable treatment associated with PDE5i
C - Sildenafil
O - Adverse events/International index of erectile function

PICO for Question 22:

P - Patients with erectile dysfunction
I - Penile revascularization
C - Does not apply
O - Adverse events/International index of erectile function

PICO for Question 29:

P - Patients with erectile dysfunction
I - Penile implant
C - Does not apply
O - Adverse events

Search strategy

The scientific database searched was Medline via PubMed. A manual search was conducted on reviews in references (narrative or systematic) and on the selected papers.

Strategy described according to the clinical question:

Question 4: Date of last search: 22/03/2019

Search: ("erectile dysfunction"[MeSH Terms] OR ("erectile"[All Fields] AND "dysfunction"[All Fields]) OR "erectile dysfunction"[All Fields]) AND ("phosphodiesterase 5 inhibitors"[Pharmacological Action] OR "phosphodiesterase 5 inhibitors"[MeSH Terms] OR "phosphodiesterase 5 inhibitors"[All Fields]) AND ("antihypertensive agents"[Pharmacological Action] OR "antihypertensive agents"[MeSH Terms] OR ("antihypertensive"[All Fields] AND "agents"[All Fields]) OR "antihypertensive agents"[All Fields]) AND ("hypertension"[MeSH Terms] OR "hypertension"[All Fields]) NOT ("hypertension, pulmonary"[MeSH Terms] OR ("hypertension"[All Fields] AND "pulmonary"[All Fields]) OR "pulmonary hypertension"[All Fields] OR ("hypertension"[All Fields] AND "pulmonary"[All Fields]) OR "hypertension, pulmonary"[All Fields])

Question 5: Date of last search: 22/03/2019

Search: ("phosphodiesterase inhibitors"[Pharmacological Action] OR "phosphodiesterase inhibitors"[MeSH Terms] OR ("phosphodiesterase"[All Fields] AND "inhibitors"[All Fields]) OR "phosphodiesterase inhibitors"[All Fields]) AND ("kidney diseases/metabolism"[Mesh Terms] OR "liver/metabolism"[Mesh Terms] OR "liver diseases/metabolism"[Mesh Terms] OR ("thiazoles"[MeSH Terms] OR "thiazoles"[All Fields]))

Question 8: Date of last search: 22/03/2019

Search: ("phosphodiesterase inhibitors"[Pharmacological Action] OR "phosphodiesterase inhibitors"[MeSH Terms] OR ("phosphodiesterase"[All Fields] AND "inhibitors"[All Fields]) OR "phosphodiesterase inhibitors"[All Fields]) AND ("treatment outcome"[MeSH Terms] OR ("treatment"[All Fields] AND "outcome"[All Fields]) OR "treatment outcome"[All Fields]) AND ((Clinical Trial[ptyp] OR Review[ptyp]) AND "humans"[MeSH Terms])

Question 15: Date of last search: 22/03/2019

Search: "erectile dysfunction"[MeSH Terms] OR ("erectile"[All Fields] AND "dysfunction"[All Fields]) OR "erectile dysfunction"[All Fields] OR ("dysfunction"[All Fields] AND "erectile"[All Fields]) OR "dysfunction, erectile"[All Fields] AND ("alprostadil"[MeSH Terms] OR "alprostadil"[All Fields]) AND ("treatment outcome"[MeSH Terms] OR ("treatment"[All Fields] AND "outcome"[All Fields]) OR "treatment outcome"[All Fields])

Question 18: Date of last search: 22/03/2019

Search: ("erectile dysfunction"[MeSH Terms] OR ("erectile"[All Fields] AND "dysfunction"[All Fields]) OR "erectile dysfunction"[All Fields] OR ("dysfunction"[All Fields] AND "erectile"[All Fields]) OR "dysfunction, erectile"[All Fields]) AND ("alprostadil"[MeSH Terms] OR "alprostadil"[All Fields]) AND ("phosphodiesterase 5 inhibitors"[Pharmacological Action] OR "phosphodiesterase 5 inhibitors"[MeSH Terms] OR "phosphodiesterase 5 inhibitors"[All Fields])

Question 22: Date of last search: 22/03/2019

Search: (("treatment outcome"[MeSH Terms] OR ("treatment"[All Fields] AND "outcome"[All Fields]) OR "treatment outcome"[All Fields] OR ("treatment"[All Fields] AND "outcomes"[All Fields]) OR "treatment outcomes"[All Fields]) AND "Vascular Surgical Procedures"[Mesh]) AND "Erectile Dysfunction/surgery"[Mesh] AND ("1999/01/01"[PDAT] : "2019/12/31"[PDAT])

Question 29: Date of last search: 22/03/2019

Search: ("erectile dysfunction"[MeSH Terms] OR ("erectile"[All Fields] AND "dysfunction"[All Fields]) OR "erectile dysfunction"[All Fields] OR ("dysfunction"[All Fields] AND "erectile"[All Fields]) OR "dysfunction, erectile"[All Fields]) AND ("prosthesis implantation"[MeSH Terms] OR ("prosthesis"[All Fields] AND "implantation"[All Fields]) OR "prosthesis implantation"[All Fields]) AND ("postoperative complications"[MeSH Terms] OR ("postoperative"[All Fields] AND "complications"[All Fields]) OR "postoperative complications"[All Fields] OR ("complications"[All Fields] AND "postoperative"[All Fields]) OR "complications, postoperative"[All Fields])

Eligibility criteria

The selection of the studies and the evaluation of the titles and abstracts obtained from the search strategy in the database consulted were independently and blindly conducted by two researchers with expertise in the development of systematic reviews, in total accordance with the inclusion and exclusion criteria established and described in the PICO. The studies with potential relevance were separated, accordingioh the studies design.

We included in our evaluation systematic reviews with meta-analysis of randomized clinical trials, and before and after studies, considering the best evidence available to answer the clinical questions. Narrative reviews were considered for full reading with the purpose of retrieving references which may have had been during the initial search strategy.

We included studies available without restriction to the language.

Only studies with texts available in its entirety were considered for critical evaluation.

Results

Question 4 - 48 papers
Question 5 - 2,587 papers
Question 8 - 2,581 papers
Question 15 - 162 papers
Question 18 - 184 papers
Question 22 - 24 papers
Question 29 - 264 papers

The level of scientific evidence was classified by type of study, according to Oxford[67](Table 1).

TABLE 1. GRADES FOR RECOMMENDATION AND LEVELS OF EVIDENCE

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

The selected evidence was defined as a randomized controlled clinical trial (RCT) and submitted to an appropriate critical evaluation checklist (Table 2). The critical evaluation of RCTs allows to classify them according to the Jadad score[68], considering Jadad trials < three (3) as inconsistent (grade B) and those with score ≥ three (3 , consistent (grade A), and according to the Grade[70] score (strong or moderate evidence).

When the evidence selected was defined as a comparative study (observational cohorts, or non-randomized clinical trial), it was subjected to an adequate critical assessment checklist (Table 3), allowing for the classification of the study according to the Newcastle Ottawa Scale[69], which considered consistent cohort studies with scores ≥ 6, and inconsistent < 6.

TABLE 2. PROCESS FOR CRITICAL EVALUATION OF RANDOMIZED CONTROLLED TRIALS

Study data Reference, study design, Jadad, level of evidence	Sample size calculation Estimated differences, power, significance level, he ttotal number of patients
Patient selection Inclusion and exclusion criteria	Patients Recruited, randomized, prognostic differences
Randomization Description and blinded allocation	Patient follow-up Time, losses, migration
Treatment protocol Intervention, control, and blinding	Analysis Intention to treat, analyzed intervention and control
Outcomes considered Primary, secondary, measurement instrument for the outcome of interest	Results Benefits or harmful effects in absolute data, benefits or harmful effects on average

Method of extraction and result analysis

For results with available evidence, the population, intervention, outcomes, presence or absence of benefits and/or harmful effects, and controversy will be specifically defined whenever possible.

The results will be presented preferably in absolute data, absolute risk,the number needed to treat (NNT) or number needed to harm (NNH) and, eventually, in mean and standard deviation values (Table 4)

TABLE 4. SPREADSHEET USED FOR DESCRIBING AND PRESENTING THE RESULTS OF EACH STUDY

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

Application of evidence - Recommendation

The recommendations will be elaborated by the authors of the review, with the initial characteristic of synthesis of evidenc,g subject to validation by all authors who participated in creating the Guideline.

The global synthesis will be based on the evidence described. Its strength will be estimated (Oxford[67]/Grade[70]) as 1b and 1c (grade A) or strong, and as 2a, 2b and 2c (grade B) or moderate weak, or very weak.

Conflict of interest

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

Final declaration

The Guidelines Project, an initiative of the Brazilian Medical Association in partnership with the Specialty Societies, aims to reconcile medical information in order to standardize approaches that can aid the physician's reasoning and decisio- making process. The information contained in this project must be submitted to the evaluation and criticism of the physicia , responsible for the conduct to be followed, given the reality and clinical condition of each patient.

TABLE 3. PROCESS FOR CRITICAL EVALUATION OF COHORT STUDIES

Representativeness of the exposed and selection os the non-exposed (Max. 2 points)	Exposure definition (Max. 1 point)	Demonstration that the outcome of interest was not presentatn the beginning of the study (Max. 1 point)	Comparability on the basis of the design or the analysis (Max. 2 points)	Outcome assessment (Max. 1 point)	Adequate follow-up time (Max. 2 points)	Scores and level of evidence

REFERENCES

1. NIH Consensus Conference. Impotence. NIH Consensus Development Panel on Impotence. JAMA. 1993 Jul 7;270(1):83-90.
2. Feldman HA, Goldstein I, Hatzichristou DG, Krane RJ, McKinlay JB. Impotence and its medical and psychosocial correlates: results of the Massachusetts Male Aging Study. J Urol. 1994 Jan;151(1):54-61.,
3. Fisher WA, Eardley I, McCabe M, Sand M. Erectile dysfunction (ED) is a shared sexual concern of couples I: couple conceptions of ED. J Sex Med. 2009 Oct;6(10):2746-60.
4. Salonia A, Castagna G, Saccà A, Ferrari M, Capitanio U, Castiglione F, Rocchini L, Briganti A, Rigatti P, Montorsi F. Is erectile dysfunction a reliable proxy of general male health status? The case for the International Index of Erectile Function-Erectile Function domain. J Sex Med. 2012 Oct;9(10):2708-15.
5. Gratzke C, Angulo J, Chitaley K, Dai YT, Kim NN, Paick JS, Simonsen U, Uckert S, Wespes E, Andersson KE, Lue TF, Stief CG. Anatomy, physiology, and pathophysiology of erectile dysfunction. J Sex Med. 2010 Jan;7(1 Pt 2):445-75.
6. Chen L, Staubli SE, Schneider MP, Kessels AG, Ivic S, Bachmann LM, Kessler TM. Phosphodiesterase 5 inhibitors for the treatment of erectile dysfunction: a trade-off network meta-analysis. Eur Urol. 2015 Oct;68(4):674-80.
7. Goldstein I, Lue TF, Padma-Nathan H, Rosen RC, Steers WD, Wicker PA. Oral sildenafil in the treatment of erectile dysfunction. Sildenafil Study Group. N Engl J Med. 1998 May 14;338(20):1397-404. Erratum in: N Engl J Med 1998 Jul 2;339(1):59.
8. Chen L, Staubli SE, Schneider MP, Kessels AG, Ivic S, Bachmann LM, Kessler TM. Phosphodiesterase 5 inhibitors for the treatment of erectile dysfunction: a trade-off network meta-analysis. Eur Urol. 2015 Oct;68(4):674-80.
9. Giuliano F, Jackson G, Montorsi F, Martin-Morales A, Raillard P. Safety of sildenafil citrate: review of 67 double-blind placebo-controlled trials and the postmarketing safety database. Int J Clin Pract. 2010 Jan;64(2):240-55.,
10. Keating GM, Scott LJ. Vardenafil: a review of its use in erectile dysfunction. Drugs. 2003;63(23):2673-703.,
11. Chung E, Broc GB. A state of art review on vardenafil in men with erectile dysfunction and associated underlying diseases. Expert Opin Pharmacother. 2011 Jun;12(8):1341-1348.,
12. Sanford M. Vardenafil orodispersible tablet. Drugs. 2012 Jan 1;72(1):87-98.
13. Zusman RM, Prisant LM, Brown MJ. Effect of sildenafil citrate on blood pressure and heart rate in men with erectile dysfunction taking concomitante antihypertensive medication. Sildenafil Study Group. J Hypertens. 2000 Dec;18(12):1865-9.
14. Muirhead GJ, Wilner K, Colburn W, Haug-Pihale G, Rouviex B. The effects of age and renal and hepatic impairment on the pharmacokinetics of sildenafil. Br J Clin Pharmacol. 2002;53 Suppl 1:21S-30S.
15. Muirhead GJ, Wulff MB, Fielding A, Kleinermans D, Buss N. Pharmacokinetic interactions between sildenafil and saquinavir/ritonavir. Br J Clin Pharmacol. 2000 Aug;50(2):99-107.
16. Vlachopoulos C, Rokkas K, Ioakeimidis N, Aggeli C, Michaelides A, Rousakis G, Fassoulakis C, Askitis A, Stefanadis C. Prevalence of asymptomatic coronary artery disease in men with vasculogenic erectile dysfunction: a prospective angiographic study. Eur Urol. 2005 Dec;48(6):996-1002; discussion 1002-3.
17. Hatzichristou D, Moysidis K, Apostolidis A, Bekos A, Tzortzis V, Hatzimouratidis K, Ioannidis E. Sildenafil failures may be due to inadequate patient instructions and follow-up: a study on 100 non-responders. Eur Urol. 2005 Apr;47(4):518-22; discussion 522-3.
18. Hatzimouratidis K, Moysidis K, Bekos A, Tsimtsiou Z, Ioannidis E, Hatzichristou D. Treatment strategy for "non-responders" to tadalafil and vardenafil: a real-life study. Eur Urol. 2006 Jul;50(1):126-32; discussion 132-3.
19. Rutchik SD, Baudiere M, Wade M, Sullivan G, Rayford W, Goodman J. Practice patterns in the diagnosis and treatment of erectile dysfunction among Family practice physicians. Urology. 2001 Jan;57(1):146-50.
20. Guay AT, Perez JB, Jacobson J, Newton RA. Efficacy and safety of sildenafil citrate for treatment of erectile dysfunction in a population with associated organic risk factors. J Androl. 2001;22:793–797.
21. Koulikov D, Fridmans A, Chertin B, Shenfeld O, Farkas A, Spitz IM. Is sildenafil citrate associated with an amelioration of the symptomatology of androgen decline in the aging male? J Urol. 2007;177:2267–2271.
22. Hwang TI, Chen HE, Tsai TF, Lin YC. Combined use of androgen and sildenafil for hypogonadal patients unresponsive to sildenafil alone. Int J Impot Res. 2006;18:400–404.
23. Boolell M, Allen MJ, Ballard SA, Gepi-Attee S, Muirhead GJ, Naylor AM, Osterloh IH, Gingell C. Sildenafil: an orally active type 5 cyclic GMP-specific phosphodiesterase inhibitor for the treatment of penile erectile dysfunction. Int J Impot Res. 1996 Jun;8(2):47-52.
24. Allen MS, Walter EE. Erectile Dysfunction: An Umbrella Review of Meta-Analyses of Risk-Factors, Treatment, and Prevalence Outcomes. J Sex Med. 2019 Mar 1. pii: S1743-6095(19)30354-6.
25. Cui H, Liu B, Song Z, Fang J, Deng Y, Zhang S, Wang H, Wang Z. Efficacy and safety of long-term tadalafil 5 mg once daily combined with sildenafil 50 mg as needed at the early stage of treatment for patients with erectile dysfunction. Andrologia. 2015 Feb;47(1):20-4.
26. Ernst E, Pittler MH. Yohimbine for erectile dysfunction: a systematic review and meta-analysis of randomized clinical trials. J Urol 1998;159:433-6.
27. Jang DJ, Lee MS, Shin BC, Lee YC, Ernst E. Red ginseng for treating erectile dysfunction: a systematic review. Br J Clin Pharmacol. 2008 Oct;66(4):444-50.
28. Santos CA Jr, Reis LO, Destro-Saade R, et al. Tribulus terrestris versus placebo in the treatment of erectile dysfunction: A prospective, randomized, double blind study. Actas Urol Esp 2014;38:244-8.
29. Sohn M, Sikora R. Ginkgo biloba Extract in the Therapy of Erectile Dysfunction. Journal of Sex Education and Therapy. 1991. 17:1, 53-61.
30. Mulhall JP. Intracavernosal injection therapy: a practical guide. Tech Urol 1997; 3: 129–34)
31. Mulhall JP, Simmons J. Assessment of comparative treatment satisfaction with sildenafil citrate and penile injection therapy in patients responding to both.BJU Int. 2007 Dec;100(6):1313-6)
32. Linet OI, Ogrinc FG. Efficacy and safety of intracavernosal alprostadil in men with erectile dysfunction. The Alprostadil Study Group. N Engl J Med 1996; 334: 873–7
33. Tal R, Mulhall JP. Intracavernosal injections and fibrosis: myth or reality? BJU Int 2008; 102: 525–6
34. The long-term safety of alprostadil (prostaglandin-E1) in patients with erectile dysfunction. The European Alprostadil Study Group. Br J Urol. 1998 Oct;82(4):538-43.
35. Delongchamps NB, Legrand G, Zerbib M, Peyromaure M. Unstable angina following intracavernous injection of alprostadil: a case study. BMJ Case Rep. 2009
36. Gupta R, Kirschen J, Barrow RC 2nd, Eid JF. Predictors of success and risk factors for attrition in the use of intracavernous injection. J Urol. 1997 May;157(5):1681-6.
37. Hatzichristou DG, Hatzimouratidis K, Apostolidis A, Ioannidis E, Yannakoyorgos K, Kalinderis A. Hemodynamic characterization of a functional erection. Arterial and corporeal veno-occlusive function in patients with a positive intracavernosal injection test. Eur Urol. 1999;36(1):60-7.
38. Fallon B. Intracavernous injection therapy for male erectile dysfunction. Impotence. Urologic Clinics of North America 1995; 22: 833.
39. Eardley I, Donatucci C, Corbin J, El-Meliegy A, Hatzimouratidis K, McVary K, Munarriz R, Lee SW. Pharmacotherapy for erectile dysfunction. J Sex Med. 2010 Jan;7(1 Pt 2):524-40.
40. Vardi Y, Sprecher E, Gruenwald I. Logistic regression and survival analysis of 450 impotent patients treated with injection therapy: long-term dropout parameters. J Urol. 2000 Feb;163(2):467-70.
41. Sundaram CP, Thomas W, Pryor LE, Sidi AA, Billups K, Pryor JL. Long-term follow-up of patients receiving injection therapy for erectile dysfunction. Urology. 1997 Jun;49(6):932-5.
42. Shabsigh R, Padma-Nathan H, Gittleman M, McMurray J, Kaufman J, Goldstein I. Intracavernous alprostadil alfadex (EDEX/VIRIDAL) is effective and safe in patients with erectile dysfunction after failing sildenafil (Viagra). Urology. 2000 Apr;55(4):477-80.
43. McMahon CG, Samali R, Johnson H. Treatment of intracorporeal injection nonresponse with sildenafil alone or in combination with triple agente intracorporeal injection therapy. J Urol. 1999 Dec;162(6):1992-7; discussion 1997-8.
44. Da Ros CT, Teloken C, Antonini CC, Sogari PR, Souto CA. Long term results of penile vein ligation for erectile dysfunction due to cavernovenous disease. Tech Urol 2000; 6 (3): 172-4.
45. Dabaja AA, Teloken P, Mulhall JP. A critical analysis of candidacy for penile revascularization. J Sex Med. 2014 Sep;11(9):2327-32.
46. Vardi Y, Gruenwald I, Gedalia U, Nassar S, Engel A, Har-Shai Y. Evaluation

of penile revascularization for erectile dysfunction: a 10-year follow-up. Int J Impot Res. 2004 Apr;16(2):181

47. Babaei AR, Safarinejad MR, Kolahi AA. Penile revascularization for erectile dysfunction: a systematic review and meta-analysis of effectiveness and complications. Urol J. 2009 Winter;6(1):1-7. Review.

48. Kawanishi Y, Kimura K, Nakanishi R, Kojima K, Numata A. Penile revascularization surgery for arteriogenic erectile dysfunction: the long-term efficacy rate calculated by survival analysis. BJU Int. 2004 Aug;94(3):361-8.

49. Kayıgil O, Okulu E, Aldemir M, Onen E. Penile revascularization in vasculogenic erectile dysfunction (ED): long-term follow-up. BJU Int. 2012 Jan;109(1):109-15.

50. Hellstrom WJ, Montague DK, Moncada I, Carson C, Minhas S, Faria G, Krishnamurti S. Implants, mechanical devices, and vascular surgery for erectile dysfunction. J Sex Med. 2010 Jan;7(1 Pt 2):501-23.

51. Carson CC 3rd, Mulcahy JJ, Harsch MR. Long-term infection outcomes after original antibiotic impregnated inflatable penile prosthesis implants: up to 7.7 years of followup. J Urol. 2011 Feb;185(2):614-8.

52. Darouiche RO, Bella AJ, Boone TB, Brock G, Broderick GA, Burnett AL, Carrion R,

53. Serefoglu EC, Mandava SH, Gokce A, Chouhan JD, Wilson SK, Hellstrom WJ. Long-term revision rate due to infection in hydrophilic-coated inflatable penile prostheses: 11-year follow-up. J Sex Med. 2012 Aug;9(8):2182-6.

54. Katz BF, Gaunay GS, Barazani Y, Nelson CJ, Moreira DM, Dinlenc CZ, Nagler HM, Stember DS. Use of a preoperative checklist reduces risk of penile prosthesis infection. J Urol. 2014 Jul;192(1):130-5.

55. Habous M, Tal R, Tealab A, Soliman T, Nassar M, Mekawi Z, Mahmoud S, Abdelwahab O, Elkhouly M, Kamr H, Remeah A, Binsaleh S, Ralph D, Mulhall J. Defining a glycated haemoglobin (HbA1c) level that predicts increased risk of penile implant infection. BJU Int. 2018 Feb;121(2):293-300.

56. Patil AY, Nerli RB, Dixit NS, Hiremath MB. Satisfaction with the semirigid penile prosthesis among couples from a Semiurban Indian population. J Sci Soc 2018;45:26-9.

57. Salonia A, Burnett AL, Graefen M, Hatzimouratidis K, Montorsi F, Mulhall JP, Stief C. Prevention and management of postprostatectomy sexual dysfunctions part 2: recovery and preservation of erectile function, sexual desire, and orgasmic function. Eur Urol. 2012 Aug;62(2):273-86.

58. Martínez-Salamanca JI, Mueller A, Moncada I, Carballido J, Mulhall JP. Penile prosthesis surgery in patients with corporal fibrosis: a state of the art review. J Sex Med. 2011 Jul;8(7):1880-9.

59. Montague DK. Penile prosthesis implantation in the era of medical treatment for erectile dysfunction. Urol Clin North Am. 2011 May;38(2):217-25.

60. Patil AY, Nerli RB, Dixit NS, Hiremath MB. Satisfaction with the semirigid penile prosthesis among couples from a Semiurban Indian population. J Sci Soc 2018;45:26-9.

61. Bozkurt IH, Arslan B, Yonguc T, Kozacıoglu Z, Degirmenci T, Gunlusoy B, Minareci S. Patient and partner outcome of inflatable and semi-rigid penile prosthesis in a single institution. Int Braz J Urol. 2015 May-Jun;41(3):535-41.

62. Sokolakis I, Hatzichristodoulou G. Clinical studies on low intensity extracorporeal shockwave therapy for erectile dysfunction: a systematic review and meta-analysis of randomised controlled trials. Int J Impot Res. 2019 Jan 21.

63. Lu Z, Lin G, Reed-Maldonado A, Wang C, Lee YC, Lue TF. Low-intensity. Extracorporeal Shock Wave Treatment Improves Erectile Function: A Systematic Review and Meta-analysis. Eur Urol. 2017 Feb;71(2):223-233.

64. Sokolakis I, Dimitriadis F, Teo P, Hatzichristodoulou G, Hatzichristou D,Giuliano F. The Basic Science Behind Low-Intensity Extracorporeal Shockwave Therapy for Erectile Dysfunction: A Systematic Scoping Review of Pre-Clinical Studies. J Sex Med. 2019 Feb;16(2):168-194.

65. Epifanova MV, Gvasalia BR, Durashov MA, Artemenko SA. Platelet-Rich Plasma Therapy for Male Sexual Dysfunction: Myth or Reality? Sex Med Rev. 2019 Mar 19.pii: S2050-0521(19)30008-3.

66. Pozzi E, Muneer A, Sangster P, Alnajjar HM, Salonia A, Bettocchi C,Castiglione F, Ralph DJ; Trauma, Reconstructive Urology Working Party of the European Association of Urology (EAU) Young Academic Urologists (YAU). Stem-cell regenerative medicine as applied to the penis. Curr Opin Urol. 2019 Apr 16.

67. Levels of Evidence and Grades of Recommendations - Oxford Centre for Evidence Based Medicine. Disponível em URL: http://cebm.jr2.ox.ac.uk/docs/ old_levels. Htm

68. Jadad AR, Moore RA, Carroll D, Jenkinson C, Reynolds DJ, Gavaghan DJ, et al. Assessing the quality of reports of randomized clinical trials: is blinding necessary? Control Clin Trials 1996; 17:1-12.

69. Wells G, Shea B, O'Connell D, Robertson J, Peterson J, Welch V, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomssed studies in meta-analyses. Disponível em: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp

70. Goldet G, Howick J. Understanding GRADE: an introduction. J Evid Based Med 2013; 6:50-4.

"Sexually transmitted infections – laboratory diagnosis"

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Question: What are the laboratory diagnostic methods for urethritis? (Page 747)

Answer: Infectious urethritis is caused by sexually transmissible pathogens. There should be clinical suspicion in cases of sexually active individuals with complaints of dysuria, urethral pruritus and/or urethral discharge. The diagnosis can be confirmed by the presence of one of the following findings: urethral secretions; bacterioscopy by Gram staining of purulent secretions obtained through urethral smear showing ≥two polymorphonuclear leukocytes on an immersion slide; testing for leukocyte esterase and/or presence of ≥10 leukocytes per field in the urinary sediment of the first urine (Page 750).

REFERENCE

1. Hachul M, Medeiros MVV, Simões R, Bernardo WM.Sexually transmitted infections – laboratory diagnosis. Rev Assoc Med Bras 2019; 65(6):745-754

Therapeutic effects of dimethyldiguanide combined with clomifene citrate in the treatment of polycystic ovary syndrome

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SUMMARY

OBJECTIVE: *In view of the high incidence of polycystic ovary syndrome (PCOS) and the unsatisfactory therapeutic effects of dimethyldiguanide or clomifene citrate alone, our study aimed to investigate the therapeutic effects of dimethyldiguanide combined with clomifene citrate in the treatment of PCOS.*

METHODS: *A total of 79 patients with POCS and 35 healthy females were included, and endometrial biopsies were obtained. The sterol regulatory element-binding protein-1 (SREBP1) expression in endometrial tissues was detected by qRT-PCR. POC patients were randomly divided into group A (n=40) and group B (n=39). Patients in group A were treated with dimethyldiguanide combined with clomifene citrate, while patients in group B were treated with clomifene citrate alone. The number of mature follicles and cervical mucus score, follicular development rate and single follicle ovulation rate, cycle pregnancy rate, early miscarriage rate, ovulation rate, endometrial thickness, positive rate of three lines sign, follicle stimulating hormone level and luteinizing hormone level were compared between the two groups.*

RESULTS: *The expression level of SREBP1 was higher in PCOS patients than that in the healthy control. SREBP1 expression was inhibited after treatment, while the inhibitory effects of combined treatment were stronger than those of clomifene citrate alone. Compared with clomifene citrate alone, the combined treatment improved cervical mucus score, follicle development rate, single follicle ovulation rate, endometrial thickness, positive rate of three lines sign, and follicle-stimulating hormone level.*

CONCLUSION: *The therapeutic effect of combined treatment is better than clomifene citrate alone in the treatment of PCOS.*

KEYWORDS: *Polycystic ovary syndrome. Clomiphene. Metformin. Sterol Regulatory Element Binding Protein 1.*

INTRODUCTION

Polycystic ovary syndrome, or PCOS, is one of the most common endocrine disorders, affecting more than 7 % of females during their reproductive age[1]. At present, PCOS is mostly defined as hyperandrogenism-induced chronic anovulation in females who are not suffering from pituitary gland or adrenal diseases[2]. PCOS is not only closely correlated with the occurrence of anovulatory infertility but is also one of the major risk factors for the development of various types of ovary cancer[3]. Although abnormal gonadotropin secretion has been proved to closely correlate with hyperandrogenism and anovulation, the pathogenesis of PCOS still hasn't been fully elucidated[4]. At hormone level, the excessive production of luteinizing hormone during PCOS can increase testosterone level, leading to the arrest of ovarian folliculogenesis[5]. Although various types of drugs have been developed to treat PCOS, treatment outcomes are usually unsatisfactory.

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Therefore, the development of an effective and safe treatment drug for PCOS is always in high demand.

Previous studies have shown that dimethyldiguanide[6] and clomiphene citrate[7] have protective effects for patients with PCOS, and both of them can be used to induce ovulation and pregnancy. However, their efficacies should be further improved to meet the treatment requirement. In this study, dimethyldiguanide were combined with clomiphene citrate to treat patients with PCOS. We found that the therapeutic effects of combined treatment are stronger than those of clomiphene citrate alone.

METHODS

Patients

A total of 79 patients with polycystic ovary syndrome (PCOS) in reproductive age were selected from a reproductive hospital affiliated to Shandong University from august 2012 ~ august 2014. The age ranged from 24 to 39 years, with a mean age of 27.11±4.28 years. All those patients underwent an endometrial biopsy to detect potential ovarian lesions. Inclusion criteria: (1) patients who did not take contraception or were pregnant during the past year; (2) patients who met the diagnostic criteria of PCOS[8]; (3) patients who did not receive reproductive hormones or ovulation induction treatment during the past 3 months; (4) lipiodolized angiography showed that at least one side of the uterine tube was unobstructed; (5) patients who signed informed consent; (6) male partner had a sperm test a+b total>107. Exclusion criteria: (1) patients with other disorders of the ovary; (2) patients with a history of ovarian surgery; (3) patients with high levels of androgen caused by other diseases. At the same time, 35 healthy females were selected to serve as a control group. Inclusion criteria of the control group: females with normal physiological conditions; 2) females who matched the age distribution of the patient group. Exclusion criteria: 1) females who were not willing to participate; 2) females who failed to cooperate with researchers. Healthy women had difficulties in getting pregnant, while endometrial biopsies showed no existence of lesions. The age of those healthy females ranged from 23 to 40 years, with a mean age of 26.99 ± 3.57 years. No significant differences in age, BMI, and other basic information were found between the two groups. This study has been approved by the Ethics Committee of Shandong University. All participants signed informed consent.

Grouping and treatment

Patients were randomly divided into group A (n=40) and group B (n=39). Patients in group A were treated with dimethyldiguanide (long-acting 2.5 g metformin, once per day, Bristol-Myers Squibb) combined with clomifene citrate (50 mg per time, once per day, hengshan pharm) for 5 days (day 3 to day 7 of the menstrual cycle). Patients in group B were only treated with clomifene citrate during the same period. From the 8th day of the menstrual cycle, a daily vaginal ultrasound examination was performed to monitor follicular development. Human chorionic gonadotropin (hCG) was injected via intramuscular injection in patients of group A and B when the mean diameter of the largest follicles monitored was >=20 mm. No significant differences in general information were found between group A and B (Table 1).

Observation indices

Vaginal ultrasound examination was performed by the same professional physician before and after the treatment to calculate the positive rate of three lines sign, measure the endometrial thickness, and count bilateral ovarian follicles (measured 3 times for the average). Based on the endometrial thickness and ovarian follicle number, the cycle pregnancy rate (one cycle), early abortion rate, periodic ovulation rate, single follicle ovulation rate, follicular growth rate, and other indicators were calculated. The number and size of follicles were monitored since the 10th day of the menstrual cycle by vaginal ultrasound. Cervical mucus examination and scoring were performed from the 14th day of the menstrual cycle, and the formation of typical ovate crystals and oval body indicated ovulation. Levels of follicle-stimulating hormone and luteinizing hormone were measured 3 days after treatment.

Real-time quantitative PCR

Total RNA was extracted from endometrial tissues using Trizol reagent (Invitrogen, USA). RNA samples were tested by NanoDrop™ 2000 Spectrophotometers (Thermo Fisher Scientific, USA), and the ones with a A260/A280 ratio between 1.8 and 2.0 were used to synthesize cDNA through reverse transcription. The following primers were used in PCR reactions: 5'-GCGGAGCCATGGATTGCAC11-3' (forward) and 5'-CTCTTCCTTGATACCAGGCCC-3' (reverse) for SREBP1; GACCTCTATGCCAACACAGT (forward) and AGTACTTGCGCTCAGGAGGA (reverse) for β-actin. PCR reaction conditions were: 95 °C for 30 s, followed

by 40 cycles of 95 °C for 12 s and 60 °C for 32 s. Data were analyzed using the 2-ΔΔCT method. The relative expression level of SREBP1 was normalized to endogenous control β-actin.

Statistical analysis

SPSS19.0 (SPSS Inc., USA) was used to perform the analysis. Data followed a normal distribution. Measurement data were recorded by (`x±s), and comparisons between two groups were performed by t-test. Count data were processed by the Chi-square test. P-value < 0.05 was considered to be statistically significant.

RESULTS

Sterol regulatory element-binding protein-1 (SREBP1) expression was upregulated in endometrial tissues of patients with PCOS

Expression of SREBP1 in endometrial tissues of both PCOS patients and normal healthy females was detected and compared. As shown in Fig. 1, the

TABLE 1A. COMPARISON OF GENERAL INFORMATION BETWEEN GROUP A AND B

Groups	Cases	BMI	Age	Course of disease (years)
A	40	21.61±3.82	27.46±3.48	3.7±1.3
B	39	20.88±3.44	26.87±4.01	3.3±1.5
c2 or F		0.48	1.07	1.04
p		0.89	0.36	0.35

TABLE 1B. COMPARISON OF THE NUMBER OF MATURE FOLLICLES AND CERVICAL MUCUS SCORE BETWEEN THE TWO PATIENT GROUPS (X±S)

Groups	Cases	Number of mature follicles	Cervical mucus score
A	40	3.17±0.68*	11.61±2.43*
B	39	2.32±0.41	7.19±3.51

Notes:*compared with group B, p<0.05

TABLE 1C. COMPARISON OF OVULATION INDUCTION EFFECT BETWEEN THE TWO PATIENT GROUPS [N(%)]

Groups	Cases	Cycle pregnancy rate	Early miscarriage rate	Ovulation rate	Single follicle ovulation rate	Follicular development rate
A	40	9 (23.07%)	3 (7.69%)	16 (41.03%)	17 (43.59%)	14(35.90%)
B	39	9 (22.5%)	7 (17.5%)	17(42.5%)	11 (27.5%)	7(17.5%)

Notes:*compared with group B, p<0.05

TABLE 1D. COMPARISON OF ENDOMETRIAL THICKNESS AND POSITIVE RATE OF THREE LINES SIGN BETWEEN THE TWO PATIENT GROUPS

Groups	Group	Endometrial thickness <7mm	Positive rate of three lines sign
A	40	7 (17.95%)*	12 (30.77%)
B	39	19 (47.5%)	7 (17.5%)

Notes:*compared with group B, p<0.05

TABLE 1E. COMPARISON OF HORMONE LEVELS BETWEEN TWO PATIENT GROUPS (X±S)

Groups	Cases	Follicle-stimulating hormone (IU/L)	Luteinizing hormone (IU/L)
A	40	8.19±1.20*	4.55±1.24#
B	39	4.03±1.51	5.01±1.77#
Control	35	9.17±1.33	2.87±0.31

Notes:*compared with group B, p<0.05; # compared with the control group, p<0.05

expression level of SREBP1 was significantly higher in endometrial tissues of PCOS patients than that in normal healthy females ($p < 0.05$).

Expression of SREBP1 in different groups before and after the treatment

Before treatment, no significant differences in the expression level of SREBP1 mRNA were found between group A and B (Fig. 2a). Compared with the pre-treatment levels, the expression level of SREBP1 mRNA was significantly reduced in both group A ($p < 0.05$, Fig. 2b) and group B ($p < 0.05$, Fig. 2c). After the treatment, no significant differences in the expression level of SREBP1 mRNA were found between group A and the control group (Fig. 2d), while the expression level of SREBP1 mRNA was significantly higher in group B than that in group A or the control group. Those data indicate that both combined treatment and clomifene citrate alone can inhibit the expression of SREBP1 mRNA in endometrial tissues, while the inhibitory effect of the combined treatment was stronger than that of clomifene citrate alone.

Comparison of mature follicles number and cervical mucus score between two patient groups

After the treatment, the number of mature follicles in group A was higher than that in group B ($p < 0.05$). In addition, cervical mucus score was also higher in group A than that in group B ($p < 0.05$).

Comparison of ovulation induction effect between two patient groups

After the treatment, the follicular development rate was significantly higher in group A than that in group B ($p < 0.05$). In addition, the single follicle ovulation rate was also slightly higher in group A than that in group B at 48 h to 60 h after injection of hCG. However, no significant differences in cycle pregnancy rate, early miscarriage rate, and ovulation rate were found between the two groups.

Comparison of endometrial thickness and positive rate of three lines sign between two patient groups

After treatment, the number of PCOS patients with endometrial thickness < 7mm in group A was significantly smaller than that in group B ($p < 0.05$). In addition, the positive rate of three lines sign was also slightly higher in group A than that in group B, but the difference was not statistically significant.

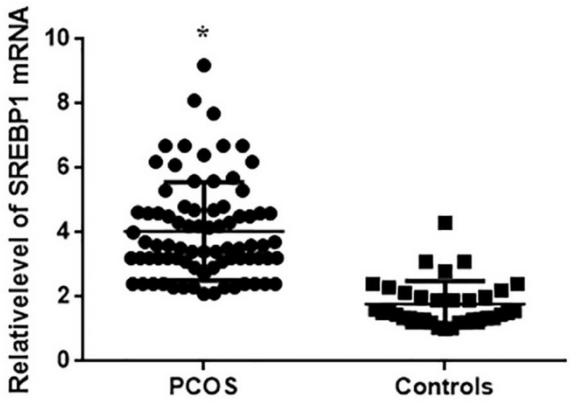
Comparison of hormone levels between the two patient groups and the control group

After treatment, the level of follicle-stimulating hormone in group A was significantly higher than that in group B ($p < 0.05$), and reached the level of the control group. However, no significant differences in the level of luteinizing hormone were found between two patient groups after the treatment; however, the luteinizing hormone level in group A and B was still significantly higher than in the control group.

DISCUSSION

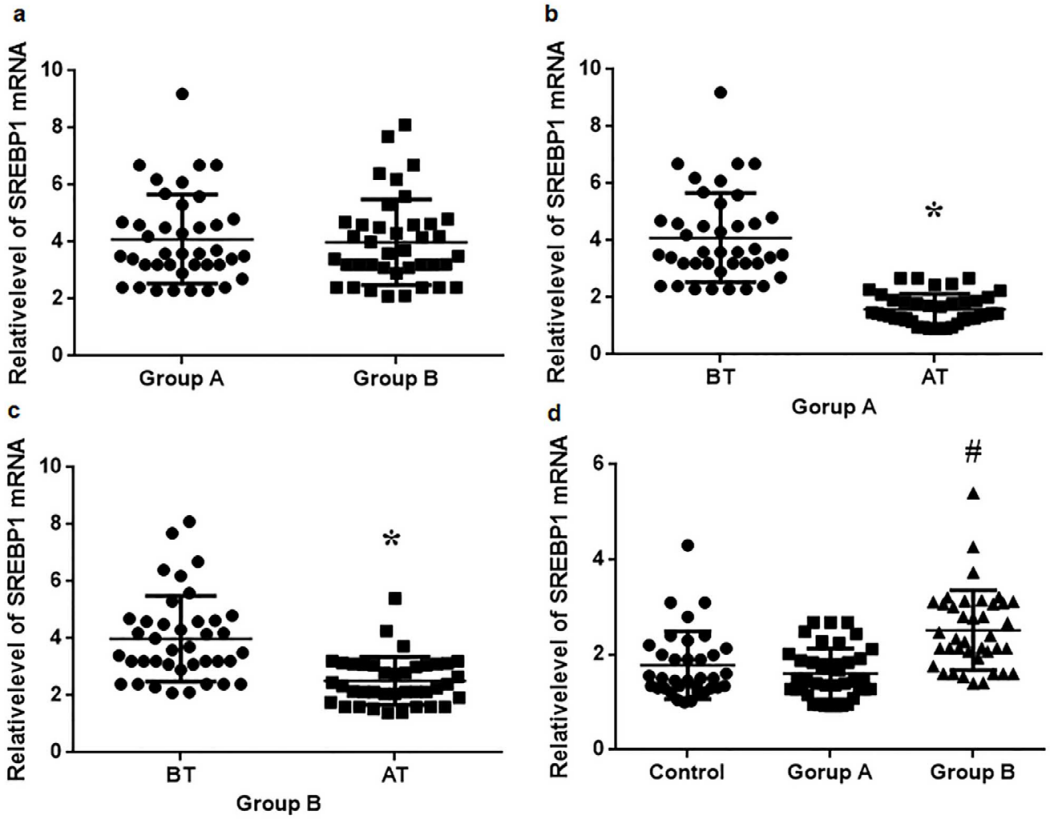
The development of PCOS is a complex process with various internal and external factors involved. It has been well accepted that the abnormal production of sexual hormones is closely correlated with the occurrence, development, and progression of this disease. Hyperandrogenism in women with PCOS is characterized by an increased level of tosterone[9]. Besides that, increased luteinizing hormone/follicle-stimulating hormone ratio caused by reduced production of follicle-stimulating hormone and increased production of luteinizing hormone is also a common hormonal feature of PCOS[9]. Recent studies have also shown that excessive secretion during PCOS is responsible for the reduced secretion of follicle-stimulating hormone and the production of testosterone, as well as the increased luteinizing hormone/follicle-stimulating hormone ratio, which is a key factor for the arrest of ovarian folliculogenesis[8,9]. Dimethyldiguanide has been proved to regulate the abnormal secretion of follicle-stimulating hormone and luteinizing hormone in patients with PCOS[6]. Similar therapeutic effects of clomiphene citrate were also observed in the clinical treatment of PCOS[7]. In our study, the level of follicle-stimulating hormone in patients with combined treatment was significantly higher than in patients who underwent the clomiphene citrate treatment alone and reached the level of the control group. However, no significant differences in the level of luteinizing hormone were found between the two patient groups after the treatment, but the luteinizing hormone level in the two patient groups was still significantly higher than that in control group. Those data suggest that, compared with the clomiphene citrate treatment alone, the combined treatment can significantly increase the

FIGURE 1. EXPRESSION OF SREBP1 IN ENDOMETRIAL TISSUES OF BOTH PCOS PATIENTS AND NORMAL HEALTHY FEMALES



Notes:*compared with normal healthy females, p<0.05

FIGURE 2. EXPRESSION OF SREBP1 IN DIFFERENT GROUPS BEFORE AND AFTER TREATMENT



a Expression of SREBP1 in group A and B before treatment; **b** Expression of SREBP1 in group A before and after treatment; **c** Expression of SREBP1 in group B before and after treatment; **d** Expression of SREBP1 in group A, group B, and control group after treatment.

Notes: *compared with the level before treatment, p<0.05; #compared with the control group and group A, p<0.05

enhancing effects on the secretion of follicle-stimulating hormone, but had no significant impact on the inhibitory effects of the secretion of luteinizing hormone. Our data provided references for the combined use of clomiphene citrate and dimethyldiguanide in the treatment of PCOS. Clomiphene citrate and dimethyldiguanide may be combined with other drugs that can reduce luteinizing hormone to improve the treatment further.

Numerous studies have shown that both dimethyldiguanide and clomiphene citrate have protective effects for patients with PCOS, and treatment with dimethyldiguanide or clomiphene citrate alone can significantly induce ovulation and pregnancy. In this study, compared with the clomifene citrate treatment alone, dimethyldiguanide combined with clomifene citrate significantly improved cervical mucus score, follicle development rate, single follicle ovulation rate, endometrial thickness and positive rate of three lines sign. Those data suggest that the combined treatment is superior to the clomifene citrate treatment alone in various indices for the treatment of PCOS. However, compared with patients who were treated with clomifene citrate alone, no significant improvement in pregnancy rate, early miscarriage rate, and ovulation rate was observed in patients with the combined treatment. Therefore, ways to further improve those indices will be the focus of future work.

Genetic factors also play pivotal roles in the treatment of PCOS. SREBF1 is a transcription factor that binds to the promoter regions of a variety of genes to regulate their expressions. Abnormal expression of SREBF1 exists in various disease models, such as Parkinson's, non-alcoholic fatty liver disease, among others. Recent studies have shown that 54G/C polymorphism of SREBF-1 gene is closely associated with the development of PCOS. In addition, abnormal upregulated SREBP1 is observed not only in endometrial cancer but also in PCOS. Consistent with previous studies, in this study, the expression level of SREBP1 was found to be significantly higher in the endometrial tissues of PCOS patients than that in normal healthy females, indicating the involvement of this gene in PCOS. Both combined treatment and clomifene citrate alone inhibited the expression of SREBP1 mRNA in endometrial tissues, while the inhibitory effect of combined treatment was stronger than that of clomifene citrate alone. Those data suggest that the combined treatment may inhibit the expression of SREBP1 to improve PCOS.

It has been reported that dimethyldiguanide can inhibit CYP-17 expression and reduce theca cell proliferation in androgenized rat models, thereby improving ovarian follicle dynamics[10]. Therefore, the improved therapeutic effects of the combined treatment are possibly mediated by the altered CYP-17 expression and theca cell proliferation. Our future studies will try to investigate this possibility. Our study provided a promising combined treatment for PCOS. However, it is worth noting that PCOS is a reproductive disorder as well as a complex metabolic disease; therefore, the treatment should be individualized. Some factors, such as patients' pregnancy plans and the risk of long-term complications should be considered before the selection of treatment strategies[11]. Therefore, more studies are still needed to explore the individualized treatment for PCOS further.

In is worth noting that our studies failed to investigate the gene polymorphism of SREBP1 in PCOS, so the opposite correlation cannot be excluded. Therefore, more studies are still needed.

CONCLUSION

In conclusion, the expression level of SREBP1 was higher in PCOS patients than that in the healthy control. SREBP1 expression was inhibited after treatment, while the inhibitory effects of combined treatment were stronger than those of clomifene citrate alone. Compared with clomifene citrate alone, the combined treatment improved the cervical mucus score, follicle development rate, single follicle ovulation rate, endometrial thickness, positive rate of three lines sign, and follicle-stimulating hormone level. Therefore, we conclude that the therapeutic effect of combined treatment is better than that of clomifene citrate alone in the treatment of PCOS. The small sample size limits our study. Further studies with bigger sample size are expected to confirm our conclusion.

RESUMO

OBJETIVO: *Tendo em vista a alta incidência de síndrome dos ovários policísticos (SOP) e os efeitos terapêuticos insatisfatórios da dimetildiguanida ou do citrato de clomifeno isoladamente, nosso estudo teve como objetivo investigar os efeitos terapêuticos da dimetildiguanida associada ao citrato de clomifeno no tratamento da SOP.*

MÉTODOS: *Um total de 79 pacientes com POCS e 35 mulheres saudáveis foram incluídos, e biópsias endometriais foram obtidas. A expressão da proteína de ligação do elemento regulador de esterol-1 (SREBP1) nos tecidos endometriais foi detectada por qRT-PCR. Pacientes POC foram divididos aleatoriamente em grupo A (n=40) e grupo B (n=39). Os pacientes do grupo A foram tratados com dimetildiguanida combinada com citrato de clomifeno, enquanto os pacientes do grupo B foram tratados apenas com citrato de clomifeno. O número de folículos maduros e muco cervical, taxa de desenvolvimento folicular e taxa de ovulação, taxa de gravidez, abortamento precoce, taxa de ovulação, espessura endometrial, taxa positiva de três linhas, nível de hormônio folículo estimulante e nível de hormônio luteinizante foram comparados entre os dois grupos.*

RESULTADOS: *O nível de expressão do SREBP1 foi maior nos pacientes com SOP do que no controle normal. A expressão de SREBP1 foi inibida após o tratamento, enquanto os efeitos inibidores do tratamento combinado foram mais fortes do que os do citrato de clomifeno isoladamente. Comparado com o citrato de clomifeno sozinho, o tratamento combinado melhorou significativamente a pontuação do muco cervical, a taxa de desenvolvimento folicular, a taxa de ovulação do folículo único, a espessura endometrial, a taxa positiva de três linhas de sinal e o nível de hormônio folículo estimulante.*

CONCLUSÃO: *O efeito terapêutico do tratamento combinado é melhor do que o citrato de clomifeno isolado no tratamento da SOP.*

PALAVRAS-CHAVE: *Síndrome do ovário policístico. Clomifeno. Metformina. Proteína de ligação a elemento regulador de Esterol 1.*

REFERENCES

1. Flannery CA, Rackow B, Cong X, Duran E, Selen DJ, Burgert TS. Polycystic ovary syndrome in adolescence: impaired glucose tolerance occurs across the spectrum of BMI. Pediatr Diabetes. 2013;14(1):42-9.
2. Zawadzki JK, Dunaif A. Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. Polycystic ovary syndrome. Boston: Blackwell Scientific; 1992. p.377-84.
3. Balen A. Polycystic ovary syndrome and cancer. Human Reprod Update. 2001;7(6):522-5.
4. Diamanti-Kandarakis E, Dunaif A. Insulin resistance and the polycystic ovary syndrome revisited an update on mechanisms and implications. Endocrine Rev. 2012;33(6):981-1030.
5. Franks S, Stark J, Hardy K. Follicle dynamics and anovulation in polycystic ovary syndrome. Human Reprod Update. 2008;14(4):367-78.
6. Morin-Papunen L, Rantala AS, Unkila-Kallio L, Tiitinen A, Hippeläinen M, Perheentupa A, et al. Metformin improves pregnancy and live-birth rates in women with polycystic ovary syndrome (PCOS): a multicenter, double-blind, placebo-controlled randomized trial. J Clin Endocrinol Metab. 2012;97(5):1492-500.
7. Chakravorty R, Athwal A, Sur D, et al. A prospective, randomized trial comparing the effects of letrozole versus clomiphene citrate for induction of ovulation and pregnancy rate in women with polycystic ovary syndrome. Fertil Sci Res. 2016,3(2):93-7.
8. Legro RS, Arslanian SA, Ehrmann DA, Hoeger KM, Murad MH, Pasquali R, et al. Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2013;98(12):4565-92.
9. Chen MJ, Ho HN. Hepatic manifestations of women with polycystic ovary syndrome. Best Pract Res Clin Obstet Gynaecol. 2016;37:119-28.
10. Mahamed RR, Maganhin CC, Sasso GRS, Jesus Simões M, Baracat MCP, Baracat EC, et al. Metformin improves ovarian follicle dynamics by reducing theca cell proliferation and CYP-17 expression in an androgenized rat model. J Ovarian Res. 2018;11(1):18.
11. Soares Júnior JM, Baracat MC, Maciel GA. Polycystic ovary syndrome: controversies and challenges. Rev Assoc Med Bras (1992). 2015;61(6):485-7.

Severe malnutrition after bariatric surgery and clinic manifestations of infection

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SUMMARY

This report describes the post-bariatric-surgery evolution of an obese patient who had low adherence to the diet and micronutrient supplementation. Four years after two bariatric surgeries, the patient was admitted due to transient loss of consciousness, slow thinking, anasarca, severe hypoalbuminemia, in addition to vitamin and mineral deficiencies. She had subcutaneous foot abscess but did not present fever. Received antibiotics, vitamins A, D, B12, thiamine, calcium, and parenteral nutrition. After hospitalization (twenty-eight days), there was a significant body weight reduction probably due to the disappearance of clinical anasarca. Parenteral nutrition was suspended after twenty-five days, and the oral diet was kept fractional. After hospitalization (weekly outpatient care), there was a gradual laboratory data improvement, which was now close to the reference values. Such outcome shows the need for specialized care in preventing and treating nutritional complications after bariatric surgeries as well as clinical manifestations of infection in previously undernourished patients.

KEYWORDS: *Obesity. Bariatric surgery. Protein Deficiency. Avitaminosis. Mineral Deficiency.*

INTRODUCTION

Obesity is defined as the excessive accumulation of body fat and classified based on a body mass index (BMI) above 30 kg/m². Its treatment involves a dietary approach, the practice of physical activities and, under specific conditions, the use of medication. When these therapeutic measures fail, bariatric surgery is an alternative to control and treat obesity.[1]

Bariatric treatment of obesity involves the reduction of the size of the stomach, besides intestinal shutting to reduce nutrient absorption.[2] In comparison to changes in lifestyle, behavior and pharmacological intervention, bariatric surgery is more effective in promoting weight loss, thus resulting in the clinical improvement of comorbidities associated with obesity.[3] Although it presents a low mortality rate, bariatric surgery increases the risk of surgical, metabolic, and nutritional complications.[1] In this report, we discuss a case of multiple combined nutritional deficiencies and atypical clinical manifestations of infection in the late postoperative period of bariatric surgery.

CASE PRESENTATION

This case was conducted in a public teaching hospital. The local Research Ethics Committee was notified, and informed consent from the patient was obtained.

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A 39-year-old woman was hospitalized for 28 days. She stayed in the emergency unit for two days and remained in the medical nutrition ward for the rest of the period.

Her weight before her first pregnancy (21 years ago) was 50 kg; after that, she gained weight, reaching 180 kg (BMI= 69 kg/m^2). Four years before the admission to our service, she was submitted to bariatric surgery. At that time, the Duodenal Switch procedure was performed, a restrictive and malabsorptive technique.[4] She lost 50 kg, and her weight stabilized at 130 kg. She did not do physical exercise and did not adhere to the diet prescribed. One year before admission to our center, she underwent another bariatric surgery with an additional weight loss of 40 kg. There was no information about this more recent procedure, although the patient reported further intestinal resection. Since the first surgery, the patient maintained four liquid evacuations per day, without steatorrhea and food debris present in the feces. Two months before admission, the number of evacuations increased to 20, without mucus or blood. During this short period, the patient lost 24 kg.

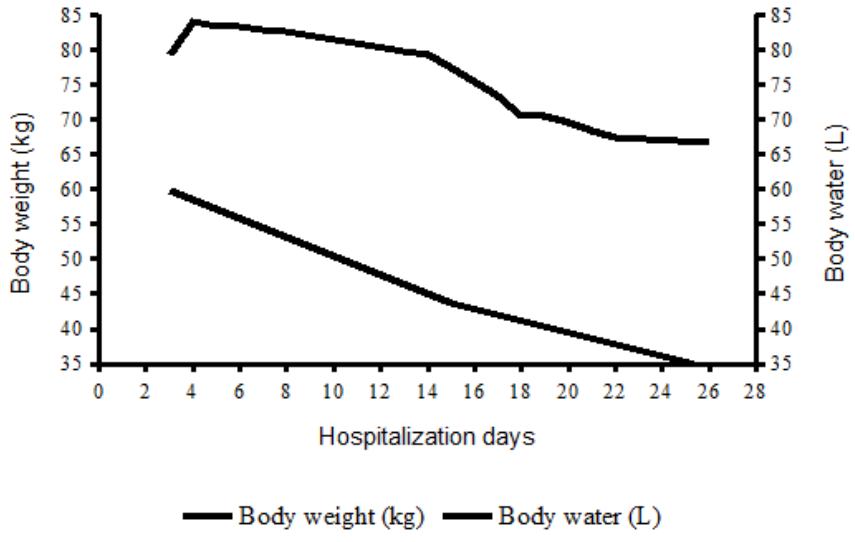
The patient was admitted due to a transient loss of consciousness. She complained of edema in the lower limbs, weakness, and dizziness, with worsening of symptoms two weeks before hospitalization. At admission, she was 84 kg and 162 cm tall, BMI of 32 kg/m^2. She had discolored mucous membranes, slow thinking, confused speech, and no fever. Physical examination showed brittle nails, alopecia, angular cheilitis, and tongue papillary atrophy. There were no cardiac abnormalities, but the lungs were congested. She presented severe peripheral edema and ascites. The dorsal surface of the right foot presented swelling and fluctuation, with spontaneous drainage of purulent material, without local blush or heat.

INVESTIGATIONS

The patient denied ingesting alcoholic beverages. Her usual diet showed high intakes of meat and soft drinks and low intake of fruit, vegetables, milk, cheese, and yogurt. She reported a hypercaloric (2600 kcal/day) and high-protein (97 g/day) diet, and the intake of calcium and vitamins B12, C, A, E, and acid folic were below the nutritional recommendations.[5] We measured the body weight regularly and analyzed the total body water by bioelectrical impedance (Biodynamics BIA 450 Analyzer, Biodynamics Corporation, Shoreline, WA, USA) (Figure 1).

The electroencephalogram (EEG) and cranial tomography were normal. The patient presented negative serology for HIV and typical serum values of hepatic transaminases, urea, and creatinine. There was a marked reduction in the serum values of hemoglobin, total protein, albumin, transferrin, and an increase in reactive C-protein (Table 1). The 24-hour urine collection did not show proteinuria. The lipid profile and the ultrasound of the liver were normal. Low blood levels of vitamin A, iron, copper, magnesium, phosphorus, and calcium were also detected. Simple chest radiography presented signs of pulmonary edema. The barium enema examination evidenced a

FIGURE 1. BODYWEIGHT AND BODY WATER EVOLUTION DURING ADMISSION, ANALYZED BY BIOELECTRICAL IMPEDANCE.



small gastric segment, reduced ileal and cecal loops compatible with short bowel syndrome, besides an enlargement of the lumen and increased intestinal villus in the distal portion (Figure 2). Exudate from the foot's lesion showed the growth of Streptococcus sp, sensitive to ciprofloxacin and clindamycin.

TREATMENT

The patient received an oral diet and intravenous antibiotics on the first day of inpatient care. The following morning, the patient became apathetic and unresponsive to verbal stimuli. The electroencephalogram and the computerized tomography brain were normal. At the time, the patient was given nil by mouth status and did not receive artificial nutrition, enterally or parenterally. The patient received intramuscular vitamin B12 and intravenous vitamin B1 and iron. There were partial improvements in memory and speech patterns.

On the third hospitalization day, the patient still presented anasarca and diarrhea. Intravenous albumin was not administered. Parenteral nutrition by central venous access was prescribed with 600 kcal per day, with a progressive increase to 1800 kcal and 1.5 g of protein/kg on the 12th day. The status of nil by mouth was maintained until the fifth day, when she started receiving liquid orally. Afterward, the diet was fractionated into six meals, fiber-restricted, without lactose and sucrose. The patient received red blood cell transfusions on the 10th day. We did not measure thiamine serum levels, but the patient received high quantities of thiamine (300 mg/day) for three days during the beginning of parenteral nutrition because of the high risk of the refeeding syndrome. Apart from the vitamin and mineral content in the parenteral nutrition, the patient received an extra amount of oral vitamin D (2000 IU/day), vitamin A (5000 IU/week), and calcium (500 mg, 12/12h) for 13 days. She received loperamide (2 mg/day) during all hospital stay. After 25 days of receiving parenteral nutrition, the therapy was suspended, and the patient was kept only on an oral diet.

OUTCOME AND FOLLOW-UP

During hospitalization, there was a progressive bodyweight reduction (18.4 kg), attributed to fluid loss (25.3 kg), with the anasarca elimination. The patient was discharged from the hospital with stable vital signs, and conscious. Her specialized oral diet with dietary recommendations included 1300 kcal and 62 g of protein/day, in addition to oral mineral and vitamin supplementation. Outpatient follow-up was weekly in the first month and monthly after that. Bodyweight remained relatively stabilized without any fluid retention, and there were improvements in the laboratory data (table 1).

DISCUSSION

This case report describes a patient who developed clinical and laboratory findings of vitamin and mineral deficiencies, severe protein malnutrition, and an infectious condition in the late postoperative of bariatric surgery. It is likely that the patient presented multiple subclinical nutritional deficiencies for an extended period, due to the poor quality of food intake and to

FIGURE 2. RADIOGRAPHIC IMAGE OF THE CHEST AND BARIUM ENEMA STUDY.

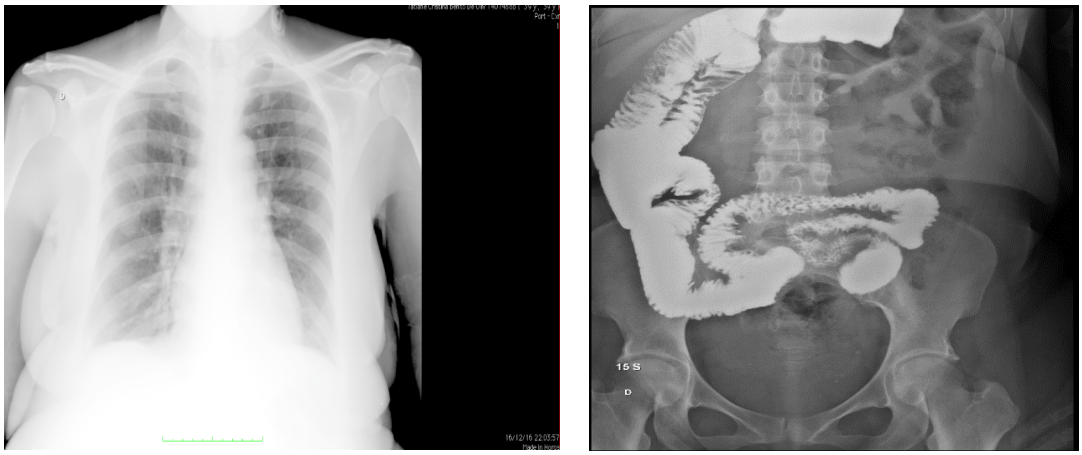


TABLE 1. HEMATOLOGIC AND BLOOD BIOCHEMICAL DATA AT THE MOMENT OF ADMISSION, DISCHARGE (28 DAYS AFTER ADMISSION), AND IN OUTPATIENT CARE (60 DAYS AFTER DISCHARGE).

	Hospitalization		Outpatient care	Reference values
	Admission	Discharge		
Blood fasting glucose (mg/dL)	95	100	-	70 - 100
Urea (mg/dL)	37	20	21	10 - 50
Creatinine (mg/dL)	0.73	0.74	0.64	0.7 - 1.6
Aspartate aminotransferase (U/L)	21	17.3	17.8	< 32
Alanine aminotransferase (U/L)	19	15	17.2	< 31
Alkaline phosphatase (U/L)	264	138	185	65 - 300
γ-glutamyl transferase (U/L)	55	71	25	11 - 50
C-reactive protein (mg/dL)	7.85	2.32	-	< 0.5
Ferritin (ng/mL)	-	123	70	6 - 159
Hemoglobin (g/dL)	5.9	8.7	11.7	12.4 - 16.1
Mean corpuscular volume (fL)	94	91	97	80.1 - 95.3
Leukocytes (103/µL)	7.3	4.6	5.4	4.05 - 11.8
Neutrophils (103/µL)	5.3	2.6	-	1.2 - 7.2
Lymphocytes (103/µL)	1.6	1.5	1.5	900 - 2900
Total protein (g/dL)	4.4	5.6	5.9	6.0 - 8.0
Albumin (g/dL)	1.5	2.8	3.4	3.5 - 4.8
Transferrin (mg/dL)	10.6	162	150	> 170
Vitamin B12 (pg/mL)	-	>1000	934	174 - 878
Folic acid (ng/mL)	-	9	14	> 5.9
Vitamin A (mg/L)	0.2	0.4	0.3	0.3 - 0.7
Vitamin E (mg/L)	5.0	6.2	4.7	5 - 20
Vitamin D (ng/mL)	-	-	14.8	20 - 50
Iron (µg/dL)	30	29	53	50 - 170
Copper (µg/dL)	39.4	-	79	85.0 - 155.0
Total calcium (mg/dL)	7.0	8.6	8.9	8.4 - 10.5

chronic diarrhea, secondary to an intestinal failure.[6]

Duodenal Switch reduces the absorption of approximately 25% of protein, and 72% of fat ingested.[7] Although there was no information about the second intestinal resection, most likely, this late procedure contributed to the aggravation of nutrient malabsorption. On the other hand, the barium enema showed that there were some adaptive changes to the remaining small intestine, as it occurs in the late postoperative in short bowel syndrome.[8]

The patient presented manifestations of protein malnutrition like alopecia, anemia, water and electrolytic imbalance, severe hypoalbuminemia and anasarca.[9] Hypoalbuminemia occurs in 13% of patients after two months and approximately 30% in ten years after bariatric surgery.[10] In this report, hypoalbuminemia can be partially ascribed to intestinal malabsorption, but not to low protein intake, liver disease, or renal protein loss. Protein malnutrition can lead to the atrophy of the intestinal villus and decrease in digestive enzymes, thus aggravating diarrhea. In its turn, diarrhea aggravates the hypoalbuminemia, thus creating a vicious cycle.

The patient presented infection by Streptococcus sp in a subcutaneous abscess in the foot, which may have possibly induced an acute phase response. This hypothesis is in agreement with the increase in reactive C-protein, the marked reduction in serum albumin, and fluid retention. In the acute phase response, there is an increase in protein catabolism, hepatic synthesis of acute-phase reactants at the expense of albumin, and capillary leakage of albumin into the interstitial space.[11] Patients with protein and micronutrient deficiencies present an elevated risk to develop infections due to the deterioration of immune response and the frailty of biological barriers.[12] In malnourished people, an infection can cause atypical neurologic manifestations, the absence of fever, and no alterations in white blood cell count or signs of inflammatory processes,[13] which is compatible with our findings.

Bariatric surgery leads to deficiencies of iron, vitamin B1, vitamin B12, folic acid, vitamin D, and calcium, preventable by a multi-professional approach during the postoperative period.[14] However, low adherence to postoperative follow-up often occurs, since patients feel well and pleased with the weight loss. Besides,

nutritional deficiencies can be asymptomatic, and when symptoms appear, the micro and macronutrient storages are already severely diminished.[15]

We did not measure vitamin B12 and folic acid before the patient received parenteral supplementation at our hospital. She presented vitamin A deficiency due to low vitamin absorption. The reduced absorption of this vitamin seems to be the cause of hypovitaminosis A. However, we cannot rule out the possibility of a systemic inflammatory response reducing the hepatic synthesis of transporting proteins for that vitamin,[16] which seems to be an epiphenomenon of acute-phase response.[17]

CONCLUSION

The patient who underwent bariatric surgery presented protein malnutrition that was aggravated by worsening diarrhea and the development of a subcutaneous abscess. In addition to the correct use of antibiotics, there was a need for parenteral nutrition until absorptive capacity was recovered. This report illustrates the need for specialized follow-up aimed at the prevention of nutritional complications in patients undergoing bariatric surgery. Besides, patients with multiple nutritional deficiencies may present atypical clinical manifestations of infection.

RESUMO

Este relato descreve a evolução pós-cirurgia bariátrica de uma paciente obesa que apresentou baixa adesão à dieta e suplementação de micronutrientes. Quatro anos após duas cirurgias bariátricas, a paciente foi internada por perda transitória de consciência, raciocínio lento, anasarca, hipoalbuminemia grave, além de deficiências vitamínicas e minerais. Apresentava abscesso subcutâneo no pé, mas não apresentava febre. Recebeu antibióticos, vitaminas A, D, B12, tiamina, cálcio e nutrição parenteral. Após a internação (28 dias) houve redução significativa do peso corporal, provavelmente devido ao desaparecimento clínico da anasarca. A nutrição parenteral foi suspensa após 25 dias e a dieta oral foi mantida fracionada. Após a internação (atendimento ambulatorial semanal) houve uma melhora gradativa dos dados laboratoriais, que estavam próximos dos valores de referência. Tal desfecho mostra a necessidade de cuidados especializados na prevenção e tratamento de complicações nutricionais após cirurgias bariátricas, bem como manifestações clínicas de infecção em pacientes previamente desnutridos.

PALAVRAS-CHAVE: *Obesidade. Cirurgia bariátrica. Deficiência de proteína. Deficiência de vitaminas. Deficiência de minerais.*

REFERENCES

1. Heymsfield SB, Wadden TA. Mechanisms, pathophysiology, and management of obesity. N Engl J Med. 2017;376(3):254-66.
2. Kreykes A, Choxi H, Rothberg A. Post-bariatric surgery patients: your role in their long-term care. J Fam Pract. 2017;66(6):356-63.
3. Pinhel MA, Nicoletti CF, Oliveira BA, Chaves RC, Parreiras LT, Sivieri T, et al. Weight loss and metabolic outcomes 12 months after roux-en-y gastric bypass in a population of southeastern Brazil. Nutr Hosp. 2015;32(3):1017-21.
4. Bennett JM, Mehta S, Rhodes M. Surgery for morbid obesity. Postgrad Med J. 2007;83(975):8-15.
5. Institute of Medicine (US) Subcommittee on Interpretation and Uses of Dietary Reference Intakes; Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. DRI dietary reference intakes: applications in dietary assessment. Washington: National Academies Press; 2000.
6. Nicoletti CF, Lima TP, Donadelli SP, Salgado Jr W, Marchini JS, Nonino CB. New look at nutritional care for obese patient candidates for bariatric surgery. Surg Obes Relat Dis. 2013;9(4):520-5.
7. Topart P, Becouarn G, Delarue J. Weight loss and nutritional outcomes 10 years after biliopancreatic diversion with duodenal switch. Obes Surg. 2017;27(7):1645-50.
8. Chagas-Neto FA, Barreto ARF, Muglia VF, Elias Junior J, Bellucci AD, Marchini JS, et al. Barium follow through in the assessment and follow-up of adult patients with short bowel syndrome. Radiol Bras. 2011;44(3):188-91.
9. Martins TC, Duarte TC, Mosca ER, Pinheiro CF, Marçola MA, De-Souza DA. Severe protein malnutrition in a morbidly obese patient after bariatric surgery. Nutrition. 2015;31(3):535-8.
10. Taton N, Borel AL, Chobert Bakouline M, Fauconnier J, Arvieux C, Reche F. Malnutrition after bariatric surgery. Minerva Chir. 2017;72(6):464-74.
11. Cunha DF, Monteiro JP, Santos VM, Oliveira FA, Cunha SFC. Hyponatremia in acute-phase response syndrome patients in general surgical wards. Am J Nephrol. 2000;20(1):37-41.
12. Scrimshaw NS, SanGiovanni JP. Synergism of nutrition, infection, and immunity: an overview. Am J Clin Nutr. 1997;66(2):464S-77.
13. Cunha SFC, Parada AO, Araújo GT. Abordagem clínica e nutricional do paciente subnutrido. In: Vannucchi H, Marchini JS, eds. Manual de procedimentos em nutrologia. Rio de Janeiro: Guanabara Koogan; 2009. p.329-42.
14. Donadelli SP, Junqueira-Franco MV, Mattos Donadelli CA, Salgado Jr W, Ceneviva R, Marchini JS, et al. Daily vitamin supplementation and hypovitaminosis after obesity surgery. Nutrition. 2012;28(4):391-6.
15. Santarpia L, Grandone I, Alfonsi L, Sodo M, Contaldo F, Pasanisi F. Long-term medical complications after malabsorptive procedures: effects of a late clinical nutritional intervention. Nutrition. 2014;30(11-12):1301-5.
16. Stephensen CB, Gildengorin G. Serum retinol, the acute phase response, and the apparent misclassification of vitamin A status in the third National Health and Nutrition Examination Survey. Am J Clin Nutr. 2000;72(5):1170-8.
17. Barbosa KC, Cunha DF, Jordão AA Jr, Weffort VR, Cunha SF. Transient decreased retinol serum levels in children with pneumonia and acute phase response. J Pediatr (Rio J). 2011;87(5):457-60.

ORIGINAL ARTICLE

Is Uric Acid elevation a random finding or a causative agent of diabetic nephropathy?

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SUMMARY

OBJECTIVE: *In this study, we aimed to analyze the relationship between serum uric acid (UA) and microalbuminuria as a marker of renal injury in type 2 diabetes mellitus.*

METHODS: *A total of 100 patients with type 2 diabetes mellitus were enrolled in the study. Participants were divided into two groups according to the urinary microalbumin/creatinine ratio: diabetic nephropathy and non-nephropathy group. UA and microalbuminuria were compared between the study groups.*

RESULTS: *Serum UA levels of diabetic nephropathy patients were significantly higher than those in the non-nephropathy group (UA in patients with diabetic nephropathy groups: 6.3 (1.82) mg/dl, UA in patients of the non-nephropathic group: 4.85 (1.92) mg/dl) (p<0.001). There was a correlation between microalbuminuria and UA (r=0.238). This correlation was statistically significant (p=0.017).*

CONCLUSION: *UA levels may be an important predictor of nephropathy in diabetic patients.*

KEYWORDS: *Uric acid. Diabetic nephropathies. Albuminuria.*

INTRODUCTION

Diabetic renal disease is the most common cause of end-stage renal failure.[1] Experimental and clinical studies indicate that inflammation is a cardinal pathogenetic mechanism in diabetic nephropathy.[2,3] The elements of the diabetic environment (immune complexes, hyperglycemia, and advanced glycation end-products) stimulate kidney cells, causing the extricate of chemokines and regulation of cell adhesion molecules.[4] These conditions facilitate renal infiltration by lymphocytes and monocytes in diabetic kidneys and the secretion of reactive oxygen products.[4]

During uric acid (UA) synthesis, oxidants are produced that can play a pivotal role in renal injury.[5] It has been reported that free oxygen radicals driven by UA have important effects in endothelial dysfunction by inducing inflammation, which leads to the development of diabetic nephropathy.[6] The relationship between UA and endothelial dysfunction, oxidative stress, nitric oxide activity, and smooth muscle cell proliferation has been reported.[7-9] Furthermore, it is still under investigation whether UA is an independent risk factor for macrovascular disease in diabetics.[10]

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Microalbuminuria in subjects with type 2 diabetes mellitus is known to be a marker of the last phase in which renal injury can be reversed. We concluded that UA might be an easy and beneficial index of oxidative stress, and elevated UA might be a marker of renal injury. We aimed to study the association between microalbuminuria and serum UA levels as a marker of renal injury in diabetic patients.

METHODS

Our study was performed retrospectively after approval by the Ethics Committee of the Abant İzzet Baysal University Medical Faculty (Ethical approval number: 2018/196). A total of 100 patients with type 2 diabetes mellitus (51 female, 49 male) who were admitted to the internal medicine clinic between December 2017 and April 2018 were included. Participants were divided into groups according to the urinary microalbumin/creatinine ratio: diabetic nephropathy and non-nephropathy. Patients' age, gender, waist circumference (WC), body mass index (BMI), duration of diabetes, systolic blood pressures (SBP), and diastolic blood pressures (DBP) were recorded. The UA, fasting blood glucose (FBG), total cholesterol, triglyceride, low-density protein (LDL), high-density lipoprotein (HDL), glycated hemoglobin (HbA1c), creatinine, urea, glomerular filtration rate (GFR), and microalbumin and creatinine in spot urine were recorded. Microalbuminuria was calculated using formula [spot urine microalbumin (gr/l)/spot urine creatinine g/dl)] x 100. None of the patients had a history of gout and thiazide diuretics, and allopurinol users, malignant conditions, congenital disorders associated with elevated UA were not included in the study.

Statistical analysis

Statistical data were analyzed using SPSS software (SPSS 15.0 for Windows, IBM, Chicago, IL, USA). Descriptive statistics were presented as Median (min-max) and Mean ± SD. Normal distribution of continuous variables was evaluated by Kolmogorov-Smirnov tests. Homogeneous variables were analyzed using the Student t-test, and non-homogenously variables were analyzed using the Mann-Whitney U-test. The receiver operating characteristic (ROC) curve analyses were performed to determine UA cut-off values, the area under the curve (AUC), sensitivity, and specificity to predict diabetic nephropathy. Correlation analysis was conducted by Pearson correlation test. P<0.05 was accepted as statistically significant.

RESULTS

A total of 100 subjects enrolled in the study. The nephropathy group had 36 (36%) patients, and the non-nephropathy 64 (64%). In the nephropathy group, 17 patients were women, and 19 were men. There were 34 women and 30 men in the non- nephropathy group (p=0.57). The mean age of the patients in the diabetic nephropathy and non-nephropathy groups were 62.4±7.6 and 59.3±9.3 years, respectively (p=0.1). There was no significant difference between WC, BMI, SBP, and DBP between the study groups (p>0.05 for all) (Table 1).

FBG was 225.5(62-466) mg/dl in the diabetic nephropathy group, and 135.5 (72-394) mg/dl in the non-nephropathy group (p<0.001). HbA1c was 9.6 (6.1-15.5) in the diabetic nephropathy group, and 6.9 (6.1-10.8) in the non-nephropathy group; there was a statistically significant difference between the groups

TABLE 1. DEMOGRAPHIC CHARACTERISTICS AND LABORATORY DATA OF THE STUDY GROUP.

	Diabetic nephropathy group	Diabetic non-nephropathy group	p
	Mean±StD		
Age (year)	62.4±7.6	59.3±9.3	0.1
Glomerular filtration rate (%)	73±20	83.8±15.5	0.003
	Median (min-max)		
Systolic Blood Pressure (mm/Hg)	130(120-180)	130 (100-180)	0.86
Diastolic Blood Pressure (mm/Hg)	80 (70-105)	80 (60-100)	0.41
Body Mass Index (kg/m2)	30.5(21.5-48.3)	30.3(22.3-49.3)	0.85
Waist Circumference (cm)	105.5(84-135)	103.5(77-144)	0.77
Uric acid (mg/dl)	6.3 (4.2-9.6)	4.9 (3-8.5)	<0.001
Hemoglobin A 1C (%)	9.6 (6.1-15.5)	6.9 (6.1-10.8)	<0.001
Fasting Blood Glucose (mg/dl)	225.5(62-466)	135.5 (72-394)	<0.001
Low-Density Lipoprotein (mg/dl)	106 (102-259)	118 (90-189)	0.81
Triglycerides (mg/dl)	150 (59-600)	145 (46-615)	0.42
High Dansity Lipoprotein (mg/dl)	41 (25-73)	46 (25-79)	0.011
Creatinine (mg/dl)	0.92 (0.65-1.2)	0.83(0.63-1.2)	0.009
Microalbuminuria (mg/gr)	48.2(531.4-624)	10.7(3-29)	<0.001

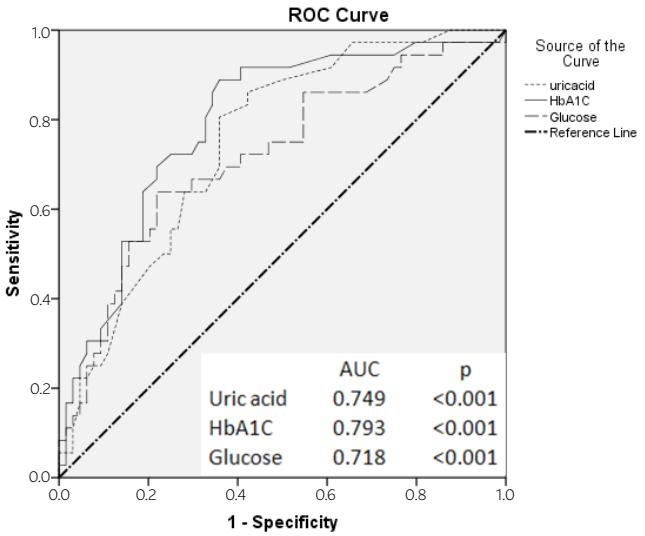
(p<0.001). Serum creatinine level was 0.92 (0.65-1.2) mg/dl in the diabetic nephropathy, and 0.83 (0.63-1.2) mg/dl in the non-nephropathy group (p=0.009); GFR was significantly higher in the diabetic nephropathy group (p=0.003) (Table 1). HDL-cholesterol was 41 (25-73) mg/dl in the nephropathy group, and 46 (25-79) mg/dl in the non-nephropathy group (p=0.003). There were no significant differences in triglyceride and LDL-cholesterol levels between the study groups (p>0.05 for all) (Table 1). The UA in patients in the diabetic nephropathy and non-nephropathic groups were 6.3 (4.2-9.6) mg/dl and 4.9 (3-8.5) mg/dl, respectively; this difference was statistically significant (p<0.001).

In the study group, the correlation between microalbuminuria and UA was examined. There was a correlation between microalbuminuria and UA (r=0.238). This correlation was statistically significant (p=0.017). A ROC curve analysis was used to determine HbA1C, FBG, and UA in detecting diabetic nephropathy. The best cut-off values for UA was 5.2 mg/dl (AUC=0.749, p<0.001)(Figure 1). According to ROC analysis, UA predicted diabetic nephropathy with 80.6% sensitivity and 64.1% specificity. ROC curve analysis of HbA1c and FBG are shown in figure 1.

DISCUSSION

This study showed a relationship between diabetic nephropathy and UA. It was found that the microalbuminuria levels were also increased due to the oxidants formed by the increased UA level. Thus, in type 2 diabetic patients, the increased UA levels may be a marker for early detection of diabetic nephropathies, such as microalbuminuria.

FIGURE 1. THE ROC CURVE OF URIC ACID, HBA1C, FASTING BLOOD GLUCOSE, HDL-CHOLESTEROL, AND EGFR FOR DIABETIC NEPHROPATHY.



Macrophages and T cells accumulate in the interstitium, in the initial phase of the diabetic nephropathy.[4] These T cells and macrophages secrete proinflammatory cytokines such as tumor necrosis factor-a (TNF-a) and interleukin-1 (IL-1). Advanced glycation end-products and hyperglycemia stimulate chemokine production in renal cells.[4] These increased chemokines then direct the migration of additional leukocytes to the kidneys and form an inflammatory cycle that causes renal damage. These events enable renal infiltration of lymphocytes and monocytes in the kidneys and facilitate the secretion of destructive molecules such as reactive oxygen products.[4]

UA is produced by xanthine oxidase and is a product of purine degradation.[11] While UA is synthesized, oxidants are produced that can cause renal dysfunction and cardiovascular disease.[5] It has been reported that UA plays an important role in endothelial dysfunction by inducing inflammation with these oxygen-radical products and may lead to the development of diabetic nephropathy.[6] Hyperuricemia-induced endothelial dysfunction has been suggested to reduce renal perfusion, along with glomerular hypertension and renal hypertrophy, by stimulating afferent vascular smooth muscle cell proliferation, which suggests that increased UA levels are a detrimental factor in the kidneys.[10,12,13]

One study found that serum UA correlated significantly with diabetic nephropathy and that the serum UA level was found to be an important cause of nephropathy in diabetic patients.[14] A study by Neupane et al.[15] reported that serum UA levels match urinary albumin excretion. Another study showed a positive correlation between microalbuminuria and serum UA. Therefore, it was concluded that serum UA and microalbuminuria levels are early diagnostic markers for cardiovascular and renal diseases. In addition, it has been reported that it is very useful to identify the prognosis of the disease in diabetic patients.[16] In our study, UA and microalbuminuria had weak correlations and were not statistically significant, whereas UA levels were statistically significantly higher in nephropathic patients.

Elevated serum UA is related to renal injury by glomerular hypertrophy and sclerosis,[1] but there are controversial reports on the association between UA levels and chronic renal disease in the literature.[17-19]

The independent role of mild hyperuricemia on the progression of renal disease is uncertain at present.[20] Severe elevated serum UA levels have been found to be an independent risk factor for renal damage.[21] Serum UA levels in our study were mildly elevated and associated with diabetic nephropathy.

Reactive oxygen radicals are formed during UA production, and it is reported that UA may be a simple and beneficial marker of increased oxidative stress.[22] With the use of allopurinol, UA levels were reduced, as were the harmful effects of UA.[23] With the decrease in UA levels, were also reported suppression of the aldosterone system of renin-angiotensin, increased nitric oxide, reduced oxidative stress, improved endothelial function, and decreased levels of markers for urinary inflammation.[23]

Hyperuricemia is an individual risk factor for the development of chronic renal disease in patients with Type 2 diabetes and normal renal function.[24] Most patients with type 2 diabetes mellitus have been found to have manifested renal glomerular and tubular damage even before the occurrence of microalbuminuria.[25] Prevention or reduction of hyperuricemia in diabetic patients may prevent nephropathy progression.

Limitations of the present study are its retrospective design and relatively small study population. However, increased UA levels in diabetic nephropathy are important results that may contribute to the current literature.

CONCLUSION

The effect of elevated UA levels on renal damage is evident. In conclusion, UA elevation is not a random finding; it is correlated with microalbumin levels in patients with diabetic nephropathy. UA levels may be an important predictor of nephropathy in diabetic patients.

RESUMO

OBJETIVO: *O objetivo deste estudo foi analisar a relação entre o ácido úrico sérico (AU) e a microalbuminúria como marcador de lesão renal no diabetes mellitus tipo 2.*

MÉTODOS: *Um total de 100 pacientes com diabetes mellitus tipo 2 foram inscritos no estudo. Os grupos de estudo foram divididos em dois, de acordo com a relação microalbumina/creatinina na urina: nefropatia diabética e grupo não nefropático. UA e microalbuminúria foram comparados entre os grupos de estudo.*

RESULTADOS: *Os níveis séricos de AU de pacientes com nefropatia diabética foram significativamente maiores do que o grupo sem nefropatia (AU em pacientes com grupos de nefropatia diabética: 6,3 (1,82) mg/dl, AU em pacientes com grupos não nefropáticos: 4,85 (1,92) mg/dl) (p<0,001). Houve correlação entre microalbuminúria e AU (r=0,238). Essa correlação foi estatisticamente significativa (p=0,017).*

CONCLUSÃO: *Os níveis de AU podem ser um importante preditor de nefropatia em pacientes diabéticos.*

PALAVRAS-CHAVE: *Ácido úrico. Nefropatias diabéticas. Albuminúria.*

REFERENCES

1. Latif H, Iqbal A, Rathore R, Butt NF. Correlation between serum uric acid level and microalbuminuria in type-2 diabetic nephropathy. Pak J Med Sci. 2017;33(6):1371-5.
2. Dalla Vestra M, Masiero A, Roiter AM, Saller A, Crepaldi G, Fioretto P. Is podocyte injury relevant in diabetic nephropathy? Studies in patients with type 2 diabetes. Diabetes. 2003;52(4):1031-5.
3. Chow FY, Nikolic-Paterson DJ, Ma FY, Ozols E, Rollins BJ, Tesch GH. Monocyte chemoattractant protein-1-induced tissue inflammation is critical for the development of renal injury but not type 2 diabetes in obese db/db mice. Diabetologia. 2007;50(2):471-80.
4. Lim AK, Tesch GH. Inflammation in diabetic nephropathy. Mediators inflamm. 2012;2012:146154.
5. Muiesan ML, Agabiti-Rosei C, Paini A, Salvetti M. Uric acid and cardiovascular disease: an update. Eur Cardiol. 2016;11(1):54-9.
6. Cai W, Duan XM, Liu Y, Yu J, Tang YL, Liu ZL, et al. Uric acid ınduces endothelial dysfunction by activating the HMGB1/RAGE signaling pathway. Biomed Res Int. 2017;2017:4391920.
7. Khosla UM, Zharikov S, Finch JL, Nakagawa, T, Roncal C, Mu W, et al. Hyperuricemia induces endothelial dysfunction. Kidney Int. 2005;67(5):1739-42.
8. Kanellis J, Kang DH. Uric acid as a mediator of endothelial dysfunction, inflammation, and vascular disease. Semin Nephrol. 2005;25(1):39-42.
9. Maxwell AJ, Bruinsma KA. Uric acid is closely linked to vascular nitric oxide activity: evidence for mechanism of association with cardiovascular disease. J Am Coll Cardiol. 2001;38(7):1850-8.
10. Yan D, Wang J, Jiang F, Zhang R, Wang T, Wang S, et al. A causal relationship between uric acid and diabetic macrovascular disease in Chinese type 2 diabetes patients: a Mendelian randomization analysis. Int J Cardiol. 2016;214:194-9.
11. Maiuolo J, Oppedisano F, Gratteri S, Muscoli C, Mollace V. Regulation of uric acid metabolism and excretion. Int J Cardiol. 2016;213:8-14.
12. Tseng CH. Correlation of uric acid and urinary albumin excretion rate in patients with type 2 diabetes mellitus in Taiwan. Kidney Int. 2005;68(2):796-801.
13. Fukui M, Tanaka M, Shiraishi E, Harusato I, Hosoda H, Asano M, et al. Serum uric acid is associated with microalbuminuria and subclinical atherosclerosis in men with type 2 diabetes mellitus. Metabolism. 2008;57(5):625-9.

14. Behradmanesh S, Horestani MK, Baradaran A, Nasri H. Association of serum uric acid with proteinuria in type 2 diabetic patients. J Res Med Sci. 2013;18(1):44-6.

15. Neupane S, Dubey RK, Gautam N, Agrawal KK, Jayan A, Shrestha S, et al. Association between serum uric acid, urinary albumin excretion, and glycated hemoglobin in type 2 diabetic patient. Niger Med J. 2016;57(2):119-23.

16. Suryawanshi K, Jagtap P, Belwalkar G, Dhonde S, Nagane N, Joshi V. To study serum uric acid and urine microalbumin in type-2 diabetes mellitus. Int J Med Sci. 2015;2(3):24-9.

17. Suzuki K, Konta T, Kudo K, Sato H, Ikeda A, Ichikawa K, et al. The association between serum uric acid and renal damage in a community-based population: the Takahata study. Clin Exp Nephrol. 2013;17(4):541-8.

18. Weiner DE, Tighiouart H, Elsayed EF, Griffith JL, Salem DN, Levey AS. Uric acid and incident kidney disease in the community. J Am Soc Nephrol. 2008;19(6):1204-11.

19. Oh CM, Park SK, Ryoo JH. Serum uric acid level is associated with the development of microalbuminuria in Korean men. Eur J Clin Invest. 2014;44(1):4-12.

20. Viazzi F, Leoncini G, Ratto E, Falqui V, Parodi A, Conti N, et al. Mild hyperuricemia and subclinical renal damage in untreated primary hypertension. Am J Hypertens. 2007;20(12):1276-82.

21. De Cosmo S, Viazzi F, Pacilli A, Giorda C. Ceriello A, Gentile S, et al; AMD-Annals Study Group. Serum uric acid and risk of CKD in type 2 diabetes. Clin J Am Soc Nephrol. 2015;10(11):1921-9.

22. Neves JA, Neves JA, Oliveira RCM. Biomarcadores de função endotelial em doenças cardiovasculares: hipertensão. J Vasc Bras. 2016;15(3):224-33.

23. Maahs DM, Caramori L, Cherney DZ, Galecki AT, Gao C, Jalal D, et al; PERL Consortium. Uric acid lowering to prevent kidney function loss in diabetes: the preventing early renal function loss (PERL) allopurinol study. Curr Diab Rep. 2013;13(4):550-9.

24. Zoppini G, Targher G, Chonchol M, Ortalda V, Abaterusso C, Pichiri I, et al. Serum uric acid levels and incident chronic kidney disease in patients with type 2 diabetes and preserved kidney function. Diabetes Care. 2012;35(1):99-104.

25. Zhang Y, Yang J, Zheng M, Wang Y, Ren H, Xu Y, et al. Clinical characteristics and predictive factors of subclinical diabetic nephropathy. Exp Clin Endocrinol Diabetes. 2015;123(2):132-8.

ORIGINAL ARTICLE

Bilevel positive airway pressure in two moments after bariatric surgery

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SUMMARY

OBJECTIVE: *To investigate the use of Bilevel Positive Airway Pressure (BiPAP) in morbidly obese individuals in two moments following bariatric surgery (Roux-en-Y gastric bypass): post-anesthetic recovery (PAR) and first postoperative day (1PO).*

DESIGN: *Randomized and blinded clinical trial.*

METHODS: *We studied 40 morbidly obese individuals aged between 25 and 55 years who underwent pulmonary function test and chest X-ray preoperatively, and on the day of discharge (2nd day after surgery). They were randomly allocated into two groups: PAR-G (BiPAP in PAR for one hour), and 1PO-G (BIPAP for one hour on the 1PO).*

RESULTS: *In the PAR-G and 1PO-G, respectively there were significant reductions in slow vital capacity (SVC) (p=0.0007 vs. p<0.0001), inspiratory reserve volume (IRV) (p=0.0016 vs. p=0.0026), and forced vital capacity (FVC) (p=0.0013 vs. p<0.0001) and expiratory reserve volume (ERV) was maintained only for the PAR-G (p=0.4446 vs. p=0.0191). Comparing the groups, the SVC (p=0.0027) and FVC (p=0.0028) showed a significant difference between the treatments, while the PAR-G showed smaller declines in these capacities. The prevalence of atelectasis was 10% for the PAR-G and 30% for the 1PO-G (p=0.0027).*

CONCLUSION: *Thus, the use of BiPAP in PAR can promote restoration of ERV and contribute to the reduction of atelectasis.*

KEYWORDS: *Pulmonary Atelectasis. Bariatric Surgery. Physical Therapy Modalities. Respiratory Function Tests. Continuous Positive Airway Pressure.*

INTRODUCTION

Recent research by the Brazilian Ministry of Health[1] shows that 54% of Brasil's population over the age of 18 is overweight. Among men, 57.3% are overweight, and among women, 51.2%. Recent estimates by the World Health Organization[2] show that in 2016, more than 1.9 billion adults (39%) were overweight and, of these, more than 650 million (13%) were obese.

Conservative treatment usually consists of diet, exercise, and use of medications; however, that is not effective for weight loss in cases of morbid obesity (body mass index (BMI) ≥ 40 kg/m²). In these cases, bariatric surgery is considered the most effective therapy for weight reduction with long-term maintenance, positive effects on most cardiovascular risk factors over a 10-year period, excellent effects on es-

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tablished type 2 diabetes, in addition to preventing the development of new cases of this disease[3,4].

In upper abdominal surgical procedures, mainly by laparotomy, obesity is a risk factor for intra- and postoperative complications when compared to non-obese individuals, due to previous changes in ventilatory function, arising from greater deposition of adipose tissue, especially in the abdominal area[5,6].

Along with these factors, general anesthesia contributes significantly to changes in respiratory mechanics, increasing the reduction in functional residual capacity (FRC) through the early closure of the small airways and thus reducing lung volumes and capacities[7,8]. Lung alterations during the surgical procedure can persist for days postoperatively, increasing the risk of respiratory complications, duration of hospital stay, morbidity and mortality, and costs to the health system[9,10].

Several studies have reported the benefits of pre- and postoperative chest physical therapy for the restoration of lung volumes and capacities and thoracic-abdominal mobility in obese patients[11,12]. The use of positive pressure devices not only contributes to the restoration of pulmonary function, but it is also important for the prevention of atelectasis in the post-operative period[13-15].

Therefore, the present research group has been studying effective ways to restore lung volumes and capacities and prevent atelectasis in the post-operative period following bariatric surgery. A preliminary study by Baltieri et al.[16] demonstrated beneficial effects of the application of bilevel positive airway pressure (BiPAP) on the restoration of expiratory reserve volume (ERV) and reduction in atelectasis immediately after extubation, while still in the post-anesthetic recovery (PAR) room. The objective of the present study was to compare the effects of BiPAP application on the immediate postoperative period, while still in the post-anesthetic recovery room, during the first postoperative day, concerning pulmonary function and the prevalence of atelectasis. Confirmation of these benefits can help reduce any restrictions to physiotherapy in the PAR room.

METHODS

Study design and ethical aspects

This was a randomized blind study approved by the Research Ethics Committee of the Methodist University of Piracicaba, São Paulo, Brasil (approval 89/12).

Sample Size calculation

The sample size calculation was based on a pilot study, considering the minimum significant difference (0,18L) and standard error (0,11L) of the differences between the preoperative and postoperative values for ERV. Using the Mann-Whitney test and adopting a statistical power of 80% and an alpha of 0.05, 20 volunteers were required per group. The sample size calculation was processed using BioEstat, version 5.3. According to Sood[17] obesity markedly reduces ERV; therefore, this volume is considered the outcome variable of this study.

Inclusion and exclusion criteria

People with body mass index (BMI) between 40 and 55 kg/m^2, age between 25 and 55 years, who underwent Roux-en-Y gastric bypass by laparotomy were included. People with abnormal preoperative pulmonary function and chest x-ray, smokers, obstructive sleep apnea syndrome, postoperative hemodynamic instability, with hospital stay greater than three days or postoperative complications were excluded.

Investigators

The study included three researchers: one responsible for the initial evaluation and inclusion of individuals, one blind to initial data of volunteers and responsible for randomization, and one responsible for treatment application. After the assessment of eligibility, block randomization was carried out using Microsoft Excel 2007® for allocation into the groups, and a sealed envelope was handed to the investigator responsible for treatment application.

Outcome measures

Pulmonary function test

For spirometry, a computerized ultrasonic spirometer (Cosmed®, PONY, Rome, Italy) was used and calibrated daily. For this procedure, the volunteers were asked to remain seated, with their feet on the floor, wearing a nasal clip.

They were also instructed with appropriate verbal commands to perform the maneuvers of slow vital capacity (SVC) and forced vital capacity (FVC), according to the guidelines of the American Thoracic Society (ATS) and European Respiratory Society (ERS)[18]. Each maneuver was repeated until three acceptable, and two reproducible curves were obtained, not exceeding more than eight attempts. The

extracted values of each curve were selected according to the recommendations of Pereira[19]. The evaluations were performed in the preoperative period, i.e., before the surgery, and on the day of discharge, i.e., on the second postoperative day.

Before starting the postoperative assessment, the pain was evaluated using the Visual Analogue Scale (VAS) with scores ranging from 0 to 10, according to Downie et al.[20]. When the pain was classified as ≤ 4, the evaluation was conducted normally, but when the pain was classified as > 4, analgesia was given, and the pain was classified again after 30 minutes[14].

Chest X-ray

A radiological examination of the thorax was performed at the time of hospital discharge, i.e., on the second postoperative day. The highest total lung capacity (TLC) achieved prior to exhalation was required for the X-rays. The analysis of the presence of atelectasis was based on the radiological report of posterior-anterior and lateral chest x-rays in inspiration issued by the hospital's radiologist, who was blinded to the treatments. The radiological reports that showed atelectasis, pulmonary hypoexpansion, or lung area(s) hypoexpansion(s), regardless of size and location, were recorded.

Experimental procedure

After hospitalization and preoperative evaluation, 43 individuals who fulfilled the inclusion criteria were evaluated and randomly allocated to one of the following groups:

PARG: After the surgical procedure, the individuals in this group were extubated and transferred to the PAR room, where they began treatment through the application of BiPAP by a facial interface for one hour, using the device BiPAP Synchrony II (Philips Respironics, Murrysville, PA, USA). After discharge from the PAR room on the same day of surgery, the individuals were transferred to the hospital room and given conventional chest physical therapy treatment.

1PO-G: The people in this group started the treatment on the first postoperative day (1PO), with two sessions of BiPAP application of 30 minutes each, totaling one hour of treatment on the 1PO.

Both groups received BiPAP with the following settings: positive inspiratory pressure was initially adjusted to 12 cmH2O and subsequently adjusted according to the patient's tolerance while maintaining a respiratory rate between 12 and 20 bpm and a tidal volume between 8 and 10 ml/kg of ideal weight (height^2 x ideal BMI). Positive expiratory pressure was set at 8 cmH2O.

All individuals in the study received conventional chest physical therapy (CCP) twice a day on immediate postoperative and twice on the 1PO. Sessions included diaphragmatic breathing exercises, deep inspirations, fractional inspirations, breathing exercises combined with upper limb movement and deambulation, prevention of deep vein thrombosis, and the use of incentive spirometry[11]. A set of 15 repetitions was performed for each exercise, with an average duration of 20 to 30 minutes per session.

All people underwent bariatric surgery performed by the same team, under general anesthesia (induction with sevoflurane and propofol and maintenance with remifentanil) and standardized mechanical ventilation with the Dräger Fabius GS ventilator, in volume control mode, with tidal volume of 6-8 mL/kg, PEEP of 5 cmH2O, and fraction of inspired oxygen between 0.4 and 0.6. A pre-operative assessment (on the same day of surgery) and a postoperative assessment (on the second day after surgery) were conducted by the same researcher, who was blind to the treatment groups.

The interventions were always performed by the same researcher, who was blind to the pre-and post-operative assessments.

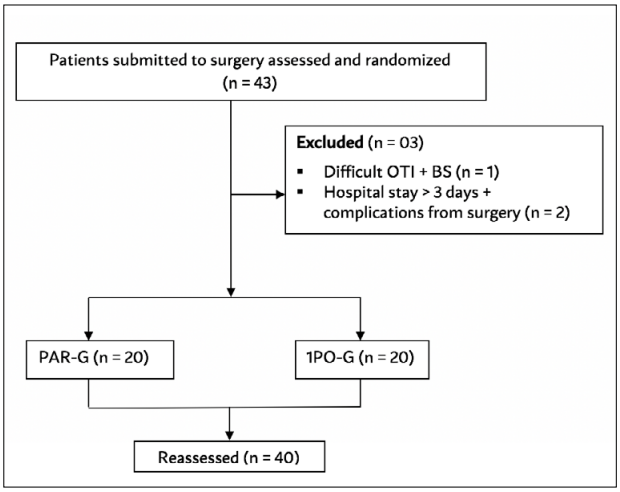
Data analysis

For the normally distributed data, we used the Shapiro-Wilk test to compare the spirometric variables, pre and postoperatively (intra-group analysis), the Student's t-test (parametric test) for paired samples, and the Wilcoxon (non-parametric test) for intergroup comparison. For the analysis, BioEstat version 5.3 was used. To compare the prevalence of atelectasis between the groups, the chi-squared (non-parametric test) was used. A significance level of 0.05 was adopted for all analyzes.

RESULTS

Patients who met the inclusion criteria (n=43) were randomized and allocated to both groups. Before the implementation of interventions, 03 volunteers were excluded (one due to the difficulty in intubation and presence of bronchospasm, generating a bias for respiratory intervention; two others due to surgical complications (fistula) and hospital stay

FIGURE 1. FLOWCHART OF THE VOLUNTEERS.



OTI = orotracheal intubation; BS = bronchospasm.

greater than 3 days), totaling 40 volunteers divided into 2 groups of 20 each (Figure 1).

Anthropometric and demographic characteristics

In Table 1, the age and anthropometric characteristics of the volunteers are presented for both groups. There was no significant difference between them (p>0.05).

Lung volumes and capacities

In table 2, the measures for the spirometric variables were shown, obtained in SVC and in FVC for each group as well as the evaluations before and after surgery and statistical results. The results of the statistical analysis of the values of the differences between the preoperative and postoperative periods of the two groups are also shown.

In the PAR-G, there was a significant reduction in SVC (p=0.0007), IRV (p=0.0016), and FVC (p=0.0013) postoperatively. For ERV (p=0.4446), there was no difference between the evaluation moments. As for the 1PO-G, there was significant reduction in all variables SVC (p< 0.0001), ERV (p=0.0191), VRI (p=0.0026), and FVC (p<0.0001). In the intergroup analysis, there were significant differences between the treatments for the variables SVC (p=0.0027) and FVC (p=0.0028), i.e. the PAR-G showed a smaller decrease in these capacities. As for ERV (p=0.1646) and IRV (p=0.3973), there was no significant difference between the groups.

Prevalence of atelectasis

Figure 2 shows the prevalence of atelectasis evaluated by chest X-rays taken on the day of discharge (2PO): 10% for the PAR-G and 30% for 1PO-G, with

TABLE 1. GENDER, AGE, AND ANTHROPOMETRIC CHARACTERISTICS OF 40 VOLUNTEERS ALLOCATED TO GROUPS.

	PAR-G	1PO-G
Gender (F)	20	20
Age (years)	42.04 + 6.15	40.14 + 6.85
Body mass (kg)	125.90 + 10.09	121.48 + 8.19
Height (cm)	158.12 + 0.10	161.60 + 1.10
BMI (kg/m²)	45.23 + 7,02	46.12 + 5.10
Ideal body mass (kg)[a]	59.68 + 5,70	62.08 + 6,90

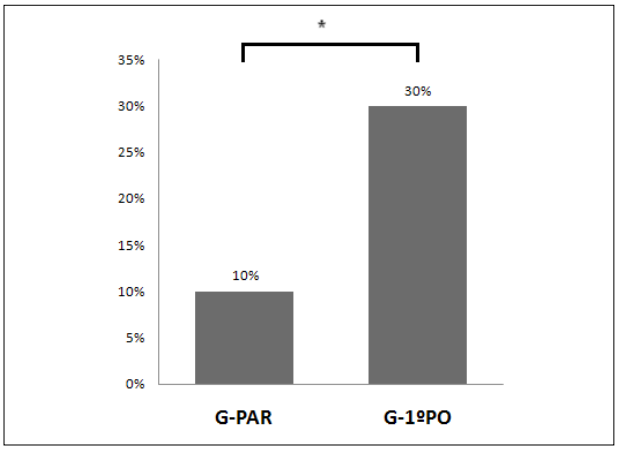
F = female; BMI = Body mass index; value based on the Metropolitan Life Foundation (1983). Values expressed as mean and standard deviation. No differences between the groups.

TABLE 2. MEASURES OF SPIROMETRIC VARIABLES, OBTAINED IN THE MANEUVERS FOR SLOW VITAL CAPACITY (SVC) AND FORCED VITAL CAPACITY (FVC) FOR EACH GROUP, PRE- AND POSTOPERATIVELY. STATISTICAL RESULTS OF INTRAGROUP AND INTERGROUP COMPARISONS.

		PAR-G (n = 20)			1PO-G (n = 20)			p-value (DIF)
		PRE	POST	DIF	PRE	POST	DIF	
SVC	M	3.34	2.78	0.56	3.63	2.47	1.16	0.0027#
(L)	SD	0.69	0.72	0.35	0.47	0.55	0.43	
p-value		0.0007*			< 0.0001*			
ERV	M	0.5	0.49	0.01	0.72	0.52	0.20	0.1646
(L)	SD	0.41	0.28	0.34	0.33	0.25	0.22	
p-value		0.4446			0.0191*			
IRV	M	2.17	1.57	0.6	2.18	1.37	0.81	0.3973
(L)	SD	0.61	0.62	0.43	0.55	0.39	0.62	
p-value		0.0016*			0.0026*			
FVC	M	3.54	2.95	0.59	3.61	2.49	1.12	0.0028#
(L)	SD	0.72	0.79	0.4	0.47	0.65	0.47	
p-value		0.0013*			< 0.0001*			

PAR-G = post-anesthetic recovery group; 1PO-G = first postoperative day group; n = volunteers allocated to each group; PRE = preoperative; POST = postoperative; SVC = slow vital capacity; L = liter; ERV: = expiratory reserve volume; IRV = inspiratory reserve volume; FVC = forced vital capacity; M = mean; SD = standard deviation; *significant difference between the pre- and postoperative periods; #: Significant difference between the values of the difference between the pre- and postoperative periods. Values expressed as mean and standard deviation.

FIGURE 2. PREVALENCE OF ATELECTASIS IN GROUPS.



* Significant difference between groups: p=0.0027

a significant difference between the proportions (p=0.0027).

DISCUSSION

In summary, in the PAR-G and 1PO-G, respectively there were significant reductions in SVC, IRV, and FVC but ERV was maintained only for the PAR-G. Comparing the groups, the SVC and FVC showed a significant difference between the treatments; the PAR-G showed smaller declines in these capacities. The prevalence of atelectasis was 10% for the PAR-G and 30% for the 1PO-G.

In this study, there was a decrease in the spirometric variables SVC, FVC, and IRV compared to preoperative values in both groups. In fact, after any surgical procedure, particularly those to the upper abdomen, a decrease in lung volume and capacity is expected, as well as increased respiratory muscle dysfunction[21] and impaired gas exchange.

Such postoperative conditions are generated due to compression of the lung parenchyma by cephalic diaphragm displacement, especially in the supine position, but also to the early collapse of the airways of the lung-dependent regions[17] and manipulation of the abdominal cavity. These factors reduce the FRC[22] and worsen the pain, the limitation in deep inspiration[23], and the precondition of alveolar hypoventilation. The increase in fat in the abdominal area and the changes in ventilatory mechanics[24] predispose the obese individual to respiratory complications when combined with general anesthesia, especially in the early hours during their stay in the recovery room[25].

According to Chau et al.[26], morbidly obese individuals present alveolar hypoventilation, which, combined with anesthetic procedures, influence the decrease in postoperative lung volumes and capacities. These findings are more detectable immediately after extubation.

However, there was a smaller decrease in SVC and FVC in the group that received positive pressure while still in the PAR, as well as, maintenance of ERV. Therefore, the use of BiPAP immediately after extubation, in the PAR, may have corrected the alveolar hypoventilation and expanded areas that collapsed during the surgical procedure. These results are evident in this study and reflect a lower prevalence of postoperative atelectasis in the PAR-G compared to the 1PO-G.

Eichenberger et al.[27] studied morbidly obese and eutrophic individuals evaluated using computed tomography of the thorax and analyzed the presence of atelectasis in three moments: before anesthetic induction for high abdominal surgery, immediately after extubation, and 24 hours after extubation. The authors observed that, among obese individuals, the rate of atelectasis was high even before the anesthesia. After extubation, both groups presented greater alveolar collapse. However, 24 hours later, eutrophic individuals presented a fall in atelectasis, while the obese ones showed an increased prevalence of atelectasis.

According to Melero et al.[28], the deterioration of lung function seems to be more evident within the first 24 hours, which can justify the use of positive pressure in this most critical period, as performed in the present study.

The chest X-ray of the present study was performed on the 2nd PO, in other words, 48 hours after the surgical procedure, demonstrating that the lung hypoexpansion of these people remains during this period and that, although subclinical, it may trigger other pulmonary complications and thus effective prophylactic measures should be studied.

Non-invasive positive pressure ventilation (NPPV) has been used successfully in postoperative patients after abdominal surgery[13,29] to reverse atelectasis, restore FRC, and prevent the collapse of the upper airways and lung complications[30].

The use of BiPAP, in particular, seems to be more effective when applied within the first 48 hours after extubation in morbidly obese people[31]. Prophylactic application of positive pressure after gastroplasty has shown improvement of gas exchange and lung function when compared to the use of oxygen therapy alone[32]. The results of Pessoa et al.[31] corroborate these studies by showing a comparison of the use of BiPAP with oxygen therapy in the recovery room. Although the authors did not demonstrate a significant difference in the prevalence of atelectasis, they found that the group that carried out NPPV evolved with better oxygenation, probably by increasing the FRC promoted by positive pressure without compromising the integrity of the gastrojejunal anastomosis.

In this study, the fact that the ERV did not change compared to preoperative values in the PAR-G shortly after extubation and that the 1PO-G had lower values demonstrates the need to reexpand collapsed alveolar units as early as possible. A study by Baltieri et al.[16] comparing the use of positive pressure in the

preoperative, intraoperative, and immediate postoperative phases demonstrated that it is always beneficial in restoring the ERV; however, when applied in the immediate postoperative, it decreases the prevalence of atelectasis.

Therefore, the use of BiPAP in the PAR demonstrates the best results in the restoration of lung volumes and capacity and the decrease in the prevalence of atelectasis. However, in most Brazilian institutions, the role of the physiotherapist in the ARRs is not part of the routine, and there are challenges in its insertion in the multi-professional team. Nevertheless, studies such as this reinforce the benefits of having the professional present in PAR to identify patients at risk for physical therapy treatment and thus contribute to better and faster healing of surgical patients.

STUDY LIMITATIONS

The small number of individuals evaluated and the lack of a control group. Most bariatric procedures are laparoscopically performed over recent years; this approach might have led to different results if the same interventions were performed.

CONCLUSION

The application of bilevel positive pressure in people with morbid obesity during post-anesthetic recovery, i.e., immediately after extubation, following bariatric surgery can bring more benefits in relation to the maintenance of volumes and lung capacity and decrease in the appearance of atelectasis than when applied on the first day after surgery. The application of BiPAP in the anesthetic recovery room can also be beneficial in the maintenance of the ERV, which is considered the most affected volume in morbid obesity.

Based on the results of this study, it can be concluded that, although there are difficulties in inserting the physical therapist in ARRs, this seems to be the most effective moment for the implementation of chest physical therapy, especially the application of bilevel positive pressure.

Institution

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RESUMO

OBJETIVO: *Investigar o uso da pressão positiva em dois níveis nas vias aéreas (BiPAP) em obesos mórbidos em dois momentos após a cirurgia bariátrica (bypass gástrico em Y-de-Roux): recuperação pós-anestésica (RPA) e primeiro dia de pós-operatório (1PO).*

DESENHO: *Ensaio clínico randomizado e cego.*

MÉTODO: *Foram estudados 40 obesos mórbidos, com idade entre 25 e 55 anos, submetidos à prova de função pulmonar e radiografia de tórax no pré-operatório e no dia da alta (segundo dia de pós-operatório). Eles foram alocados aleatoriamente em dois grupos: G-RPA (BiPAP na RPA por uma hora) e G-1PO (BiPAP por uma hora no 1PO).*

RESULTADOS: *No G-RPA e G-1PO, respectivamente, houve reduções significativas na capacidade vital lenta (CVL) (p=0,0007 vs p<0,0001), volume de reserva inspiratório (VRI) (p=0,0016 vs p=0,0026) e capacidade vital forçada (CVF) (p=0,0013 vs p<0,0001). O volume de reserva expiratório (VRE) foi mantido apenas para o G-RPA (p=0,4446 vs p=0,0191). Comparando os grupos, a CVL (p=0,0027) e a CVF (p=0,0028) apresentaram diferenças significativas entre os tratamentos e o G-RPA apresentou menores declínios nessas capacidades. A prevalência de atelectasia foi de 10% para o G-RPA e 30% para o 1PO-G (p=0,0027).*

CONCLUSÃO: *O uso de BiPAP na RPA pode promover uma restauração do VRE e contribuir para a redução de atelectasias.*

PALAVRAS-CHAVE: *Atelectasia pulmonar. Cirurgia bariátrica. Modalidades de fisioterapia. Testes de função respiratória. Pressão positiva contínua nas vias aéreas.*

REFERENCES

1. Brasil. Ministério da Saúde. Vigitel Brasil 2017. Vigilância de fatores de risco e proteção para doenças crônicas por inquérito telefônico. Brasília: Ministério da Saúde; 2018. [Accessed in 2019 Jan 19]. Available from: http://bvsms.saude.gov.br/bvs/publicacoes/vigitel_brasil_2017_vigilancia_fatores_riscos.pdf.
2. World Health Organization. Obesity and overweight [homepage on internet]. [Updated in 16 Feb 2018; acessed on 28 Jan 2019]. Available from: https://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight
3. Sjöström L. Bariatric surgery and reduction in morbidity and mortality: experiences from the SOS study. Int J Obes (Lond). 2008;32(Suppl 7):S93-7.
4. Pentin PL, Nashelsky J. What are the indications for bariatric surgery? J Fam Pract. 2005;54(7):633-4.
5. Chung F, Mezei G, Tong D. Pre-existing medical conditions as predictors of adverse events in day-case surgery. Br J Anaesth. 1999;83(2):262-70.
6. Livingston EH, Huerta S, Arthur D, Lee S, De Shields S, Heber D. Male gender is a predictor of morbidity and age a predictor of mortality for patients undergoing gastric bypass surgery. Ann Surg. 2002;236(5):576-82.
7. Nguyen NT, Lee SL, Goldman C, Fleming N, Arango A, McFall R, et al. Comparison of pulmonary function and postoperative pain after laparoscopic versus open gastric bypass: a randomized trial. J Am Coll Surg. 2001;192(4):469-76.
8. DeMaria EJ, Portenier D, Wolfe L. Obesity surgery mortality risk score: proposal for a clinically useful score to predict mortality risk in patients undergoing gastric bypass. Surg Obes Relat Dis. 2007;3(2):134-40.
9. Smetana GW, Lawrence VA, Cornell JE; American College of Physicians. Preoperative pulmonary risk stratification for noncardiothoracic surgery: systematic review for the American College of Physicians. Ann Intern Med. 2006;144(8):581-95.
10. Chawla G, Drummond GB. Fentanyl decreases end-expiratory lung volume in patients anaesthetized with sevoflurane. Br J Anaesth. 2008;100(3):411-4.
11. Forti E, Ike D, Barbalho-Moulim M, Rasera I Jr, Costa D. Effects of chest physiotherapy on the respiratory function of postoperative gastroplasty patients. Clinics. 2009;64(7):683-9.
12. Brigatto P, Carbinatto JC, Costa CM, Montebelo MI, Rasera-Júnior I, Pazzianotto-Forti EM. Application of positive airway pressure in restoring pulmonary function and thoracic mobility in the postoperative period of bariatric surgery: a randomized clinical trial. Braz J Phys Ther. 2014;18(6):553-62.
13. Jaber S, Delay JM, Chanques G, Sebbane M, Jacquet E, Souche B, et al. Outcomes of patients with acute respiratory failure after abdominal surgery treated with noninvasive positive pressure ventilation. Chest. 2005;128(4):2688-95.
14. Gaszynski T, Tokarz A, Piotrowski D, Machala W. Boussignac CPAP in the postoperative period in morbidly obese patients. Obes Surg. 2007;17(4):452-6.
15. Remístico PP, Araújo S, Figueiredo LC, Aquim EE, Gomes LM, Sombrio ML, et al. Impact of alveolar recruitment maneuver in the postoperative period of videolaparoscopic bariatric surgery. Rev Bras Anestesiol. 2011;61(2):163-8.
16. Baltieri L, Santos LA, Rasera I Jr, Montebelo MI, Pazzianotto-Forti EM. Use of positive pressure in the bariatric surgery and effects on pulmonary function and prevalence of atelectasis: randomized and blinded clinical trial. Arq Bras Cir Dig. 2014;27(Suppl 1):26-30.
17. Sood A. Altered resting and exercise respiratory physiology in obesity. Clin Chest Med. 2009;30(3):445-54.
18. ATS/ERS Task Force. Standardisation of lung function testing: standardisation of spirometry. Eur Respir J. 2005;26(2):319-38.
19. Pereira CAC. Directives for pulmonary function tests. J Pneumol. 2002;28(3):1-82.
20. Downie WW, Leatham PA, Rhind VM, Wright V, Branco JA, Anderson JA. Studies with pain rating scales. Anna Rheum Dis. 1978;37(4):378-81.
21. Barbalho-Moulim MC, Miguel GPS, Forti EM, Campos FA, Costa D. Effects of preoperative inspiratory muscle training in obese women undergoing open bariatric surgery: respiratory muscle strength, lung volumes, and diaphragmatic excursion. Clinics. 2011;66(10):1721-7.
22. Levi D, Goodman ER, Patel M, Savransky Y. Critical care of the obese and bariatric surgical patient. Crit Care Clin. 2003;19(1):11-32.
23. Smith MCL, Ellis ER. Is retained mucus a risk factor for the development of postoperative atelectasis and pneumonia? Implications for the physiotherapist. Physiother Theory Pract. 2000;16(2):69-80.
24. Zammit C, Liddicoat H, Moonsie I, Makker H. Obesity and respiratory diseases. Int J Gen Med. 2010;3:335-43.
25. Moore CE, Forrest M, Ammori B. Anaesthesia for obesity surgery. Anaesth Intensive Care Med. 2011;12(7):280-2.
26. Chau EH, Lam D, Wong J, Mokhlesi B, Chung F. Obesity hypoventilation syndrome: a review of epidemiology, pathophysiology, and perioperative considerations. Anesthesiology. 2012;117(1):188-205.
27. Eichenberger AS, Proietti S, Wicky S, Frascarolo P, Suter M, Spahn DR, et al. Morbid obesity and postoperative pulmonary atelectasis: an underestimated problem. Anesth Analg. 2002;95(6):1788-92.
28. Melero A, Vallés J, Vila P, Canet J, Vidal F. Anesthesia recovery, gas exchange and postoperative hepatic and renal function in patients with morbid obesity undergoing bariatric surgery: comparison of the effects of halothane, isoflurane and fentanyl. Rev Esp Anestesiol Reanim. 1993;40(5):268-72.
29. Conti G, Cavaliere F, Costa R, Craba A, Catarci S, Festa V, et al. Noninvasive positive-pressure ventilation with different interfaces in patients with respiratory failure after abdominal surgery: a matched-control study. Respir Care. 2007;52(11):1463-71.
30. Sungur M, Layon AJ, Gabrielli A. Treatment of postoperative respiratory insufficiency in the obese patient: who makes the call? Obes Surg. 2007;17(4):457-9.
31. Pessoa KC, Araújo GF, Pinheiro AN, Ramos MRS, Maia SC. Ventilação não invasiva no pós-operatório imediato de derivação gastrojejunal com bypass em Y de Roux. Rev Bras Fisioter. 2010;14(4):290-6.
32. Liesching T, Kwok H, Hill NS. Acute applications of noninvasive positive pressure ventilation. Chest. 2003;124(2):699-713.

ORIGINAL ARTICLE

Do-not-resuscitate and treatment limitation decisions – Six years of experience from a Portuguese General Intensive Care Unit

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SUMMARY

OBJECTIVE: *Treatment limitation, as well as do-not-resuscitate (DNR) directives, are difficult but important to improve patients' quality of life and minimize dysthanasia. We aimed to study the approach to withholding, withdrawal, and DNR decisions, patients' characteristics, and process documentation in a general Intensive Care Unit (ICU) in Portugal.*

METHODS: *A retrospective analysis of data regarding the limitation of treatment decisions collected from previously-designed forms and complemented by medical record consultation.*

RESULTS: *A total of 1602 patients were admitted to the ICU between 2011 and 2016. DNR decisions were documented in 127 cases (7.9%). Patients with treatment limitations were older and had higher Simplified Acute Physiology Score II. The most frequent diagnosis preceding these decisions was sepsis (52.0%, n = 66); the most common main reason for limiting treatment was a poor prognosis of acute illness. Of the patients to whom a DNR was implemented, 117 (92.1%) died in the ICU (40.1% of the total number of ICU deaths), and hospital mortality was 100%. Participants in these decisions, as well as types of treatment withdrawn and their respective timings, were not registered in medical records.*

CONCLUSION: *Treatment limitation and DNR decisions were relatively common, in line with other Southern European studies, but behind Northern European and North American centers. Patients with these limitations were older and more severely ill than patients without such decisions. Documentation of these processes should be clear and detailed, either in specific forms or computerized clinical records; there is room for improvement in this area.*

KEYWORDS: *Palliative care. Critical Care. Resuscitation Orders. Critical Illness. Withholding Treatment.*

INTRODUCTION

Beginning in the 1950s, the development of artificial life-sustaining and/or organ-substituting techniques has progressively changed the definition of "death" into a relatively predictable and partially controlled process. This kind of advanced medical care is most often provided in intensive care units (ICU)[1]. Nowadays, between 20 and 30% of the global population die in ICU[2,3]; approximately one-fifth of all deaths in the United States occurs in or soon after a stay in the ICU, and half of the patients who die in a hospital has been admitted to an ICU during the 3 days prior to death[4,5].

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Cardiopulmonary resuscitation (CPR) was approved for clinical use in 1974 by the American Heart Association; however, not initiating or suspending this intervention was also considered ethically appropriate when the perceived benefit was supplanted by the potential harm[4]. Since then, society has begun to reach a consensus that medical treatments, in general, may be withheld or withdrawn in certain situations[6,7], even in an ICU setting. Indeed, the primary goal of intensive care is not to avoid death "at any cost", but rather returning critically ill patients to a quality of life they would find acceptable[1,6].

In order to avoid unnecessary treatments, a number of directives have been developed over time to establish adequate goals of care for terminal patients. The strict definition of "do-not-resuscitate" (DNR) means only "in the event of cardiac arrest, do not provide CPR", rather than an immediate cessation of all treatments. "Withholding therapy" is defined as a decision not to start or increase a life-sustaining intervention. In turn, "withdrawing therapy" is defined as a decision to actively stop a life-sustaining intervention that is presently being administered[8]. When appropriate, a more in-depth three-level hierarchy for classifying decisions of limitation can also be used ("stop", "do not increase", and "do not start"), and more than one can be applied to the same patient simultaneously or sequentially[1]. Although, from an ethical standpoint, these decisions to withhold or withdraw treatment are labeled as equivalents, many clinicians still distinguish between the two, with the former being perceived as more "passive"[1]. More recently, to avoid the negative connotation sometimes attributed to these terms, a shift towards adopting "decisions to limit treatment" as a new, more encompassing terminology has been suggested[2].

Even though ICU clinicians are frequently confronted with this kind of decisions, there is little documented evidence about how to approach these situations in practice. As an example, clinicians will sometimes decide to withdraw one life-sustaining therapy while continuing others; this method of incremental treatment withdrawal, also known as "stuttering withdrawal", has been considered by some authors as a marker of suboptimal quality of care[5].

The goal of this study was to evaluate the approach to DNR status and treatment limitation – both withholding and withdrawal (WhWd) - in a general ICU in Portugal, in regard to the incidence of these decisions, patients' demographic and clinical characteristics, and how these processes were documented.

METHODS

In this study, we included all consecutive patients entering the mixed/general ICU at Hospital de Egas Moniz in Lisbon, Portugal, from 1 January 2011 to 31 December 2016.

During the ICU stay, patients were scored according to the Simplified Acute Physiology Score II (SAPS II) and grouped into disease categories based on the Acute Physiology, Age, and Chronic Health Evaluation (APACHE) III prognostic system: cardiovascular, respiratory, gastrointestinal, neurological, sepsis, trauma, metabolic, and other. Additionally, information on age, gender, type of admission, length of ICU stay, and outcome were recorded in a local database.

We retrospectively reviewed the computerized medical records of all patients who died during the ICU stay, as well as that of all patients who had DNR decisions implemented or treatment withheld or withdrawn. These patients were identified by consultation of a specific form, already employed in our ICU prior to the beginning of this study, where it is possible to record each DNR, withholding and withdrawal decision independently, as well as their respective dates, but which was not designed for this single purpose – the HELICS-ICU protocol9. Reasons for withholding or withdrawing treatment and the type of treatment withheld or withdrawn were, when obtainable from medical records, registered using a form developed for this purpose. When such information was unavailable, it was classified as "not documented in clinical records".

A descriptive analysis of the data was performed with categorical variables presented as proportions/percentages and continuous variables as median, mean, and standard deviation where applicable. Data analysis was conducted using Statistical Package for Social Sciences - SPSS® version 20.0 for Windows®.

RESULTS

Over a period of six years, from a total of 1603 admissions, treatment limitation decisions were formally documented in 127 cases, corresponding to 7.9% of all ICU patients in this time frame. Eighty patients were male (63.0%). The median age of DNR/WhWd patients was 71

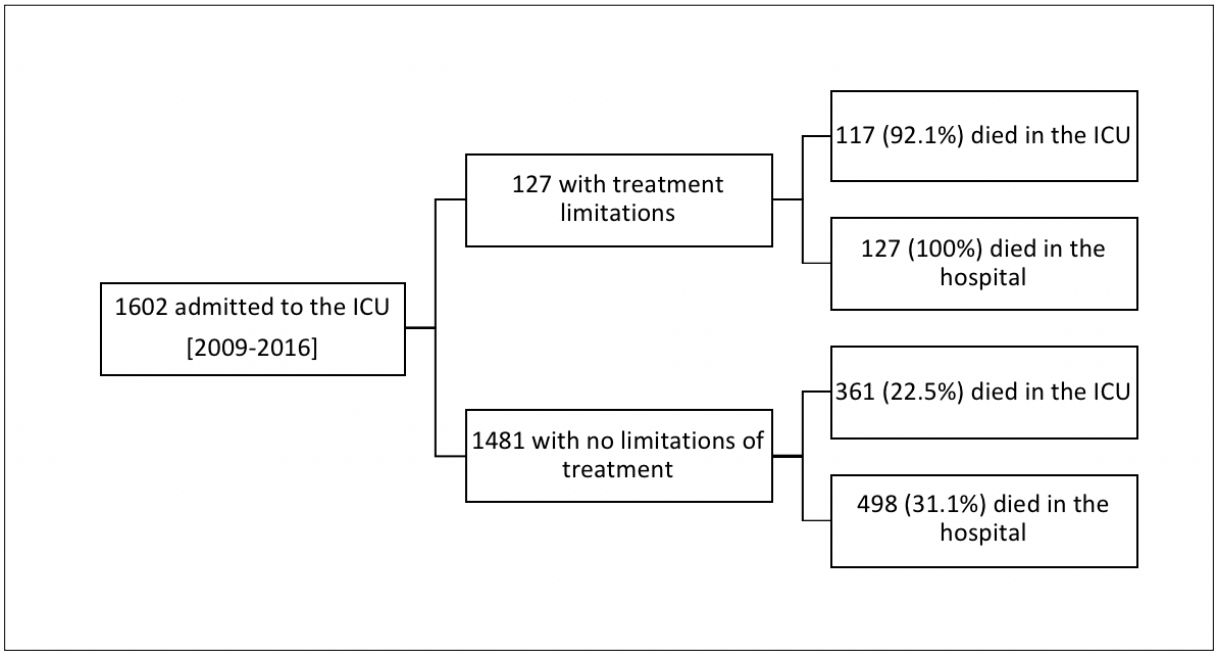
years (50; 82), while the median of all patients admitted to the ICU was 65 years (45; 80). The median SAPS II score for DNR/WhWd patients was 59 (30; 79), whereas the overall median SAPS II during the same period was 39 (22; 52). During the study period, the global median ICU stay was 10 days (4; 14) whereas the median length of stay for DNR/WhWd patients was 12 days (5; 20). Of the patients in whom DNR/WhWd was implemented, 117 (92.1%) died in the ICU, corresponding to 40.1% of the total number of ICU deaths during this period (n = 316), while the in-hospital mortality for these patients was 100%. [Figure 1]

The most frequent diagnoses which preceded DNR/WhWd decisions were sepsis (52.0%, n = 66), neurological event (16.5%, n = 21), and respiratory failure (10.2%, n = 13). [Table 1] Upon ICU admission, 78.0% of patients were considered medical patients (n = 99) and 22.0% were considered surgical patients (n = 28) of which 6 were initially scheduled elective procedures (27.3%) and 22 were unscheduled urgent interventions (72.8%).

DNR status was formally documented in the HELICS-ICU form in 90.6% (n = 115) of patients with either decision to withhold or withdraw treatment during their stay in the ICU. There was no difference in limitations between males and females. The decision to withhold treatment was formally documented in 63.0% (n = 80), while treatment withdrawal was established in 32.3% (n = 41); in the remaining cases (n = 6, 7.7%) DNR orders were formally admitted, but neither decisions to withhold or withdraw treatments were put in place. In 41.5% (n = 17) of cases where treatment withdrawal was documented, this decision was made at a later moment from documentation of DNR status. The justification of DNR/WhWd directives was not featured on the HELICS-ICU template for formal documentation of these decisions, and was instead obtained from clinical records when available; of those where predictors could be ascertained from the medical notes (68.5%, n = 87), the most frequently identified causes of DNR/WhWd decisions were poor prognosis of acute illness due to non-responsiveness to medical therapy in 93.1% of cases (n = 81) and limited subsequent relational quality of life in 49.4% (n = 43) [Table 2]. Factors such as patient's age, patient's request, religious beliefs, or family's request were never documented to have been the cause for treatment limitation decision. Although the general incidence of withdrawal decisions was lower than withholding for all kinds of admissions, there was no statistically significant difference between the type of treatment limitation and whether patients were transferred as medical, unscheduled surgical or scheduled surgical admissions [Table 3].

Prognostic and therapeutic limitation discussion with family members was not routinely registered in clinical records, and as such, the frequency of these family conferences could not be accurately estimated. Similarly, the healthcare-providing participants in the decision process were not routinely documented in medical records.

FIGURE 1. FLOW CHART OF PATIENTS ADMITTED TO THE INTENSIVE CARE UNIT (ICU) AND THEIR OUTCOME



Of the studied population, 90.6% (n = 115) of patients received mechanical ventilation, for a mean duration of 12.0 days (±12.5); 45.7% (n = 58) of these patients received dialysis at some point prior to therapeutic limitation decisions, for a mean duration of 10.0 days (±9.6); and 19.7% (n = 25) received parenteral nutrition for a mean duration of 14.6 days (±11.7). The template form for therapeutic limitation decisions did not specify which treatment options were withdrawn or withheld, nor was this information consistently featured on clinical records. Likewise, we could not determine the number of patients in whom withdrawal of all life-sustaining treatments happened on the same day, or incrementally across several days.

TABLE 1. DIAGNOSES AT THE TIME OF WITHDRAWAL OR WITHHOLDING LIFE-SUSTAINING TREATMENT

	n	%
Sepsis	66	52.0
Neurological	21	16.5
Respiratory failure	13	10.2
Malignant disease	9	7.1
Cardiovascular failure	9	7.1
Multiple-organ failure	4	3.1
Renal failure	3	2.4
Gastrointestinal	1	0.8
Hepatic failure	1	0.8

TABLE 2. PREDICTORS OF TREATMENT LIMITATION DECISIONS AS DOCUMENTED IN MEDICAL RECORDS

	Withholding N = 52	Withdrawal N = 35	Total N = 87	p
Non-response to treatment	49 (56.3)	32 (36.8)	81 (93.1)	<0.01
Limited subsequent relational QOL	26 (29.9)	17 (19.5)	43 (49.4)	<0.01
Limited autonomy before admission	25 (28.7)	14 (16.1)	39 (44.8)	<0.01
Absence of curative therapy	9 (10.3)	8 (9.2)	17 (19.5)	<0.01
End-stage incurable severe disease	8 (9.2)	5 (5.7)	13 (14.9)	<0.01

Values are represented as number (%). QOL = quality of life.

TABLE 3. INCIDENCE OF TREATMENT LIMITATION DECISIONS BASED ON TYPE OF PATIENT ADMISSION

	Scheduled Surgical N = 6	Unscheduled Surgical N = 22	Medical N = 99
Treatment withheld	3 (50.0)	12 (54.5)	72 (56.7)
Treatment withdrawn	1 (16.7)	9 (40.9)	31 (31.3)

Values are represented as number (%).

DISCUSSION

Although this study confirms that limitation of treatment has become a relatively common practice in ICUs, it also serves to underline the geographical differences which have already been noted in several other publications. There is a well-documented geographical pattern to the disparity in ICU end-of-life practices in the Northern Europe when compared with Southern, having a significantly higher rate of withholding and withdrawal of life-sustaining therapies.[2] Similar disparities in treatment limitation approaches have also been described between continents like North America and Europe when compared to Asia, particularly of Middle Eastern countries[10-12]. Case-mix, religion, culture, jurisdictional law, individual physician, and institutional characteristics are known to be contributing factors[2].

To demonstrate, whereas in this study treatment limitation decisions were made in just under 8% of around 1600 admitted patients, similar works from Northern Europe report a considerably higher prevalence of DNR decisions – for example, Hoel et al.[13] have registered a number of DNR decisions close to 25% in 1200 patients in their single-center study in a Norwegian ICU. Likewise, a single-center study by Yazigi et al.[12], conducted in a Lebanese ICU, has reported an incidence of treatment limitation directives in under 10% of 446 patients.

Factors associated with limitation of treatment were older age, higher estimated mortality, and higher SAPS II score (despite this severity score not having been designed to estimate individual risk). Regarding patients' age, although it was never cited as being a factor in the decision to limit treatment, limitations were more frequent in older patients. This suggests age was indeed part of the clinical assessment that led to these decisions, as has also been proposed by other studies[14].

A considerably higher number of patients had treatments withheld rather than withdrawn, which falls in line with other works published in Southern Europe, in comparison with Northern European publications which show a more even ratio of treatment withholding versus withdrawing[11,15-17].

Similarly to other works, no patient survived the hospital stay after having any treatment withdrawn, and close to half of the patients who died had undergone treatment limitation decisions[1,1317]. The median length of ICU stay was higher in this sample than in other single-center studies – while DNR patients

specifically had a median length of stay comparable to that reported in other Southern European countries (10 to 12 days), the global length of stay was considerably lower in several other centres[1,12,13,17]. This difference could perhaps be explained by the lack of an Intermediate Care Unit in our Hospital, which could serve as a bridge of increased vigilance between a patient's ICU discharge and return to a conventional infirmary; it could also perhaps be attributed to the time gap between patient admission and the implementation of DNR/WhWd decisions, or the stuttering withdrawal that was documented in over 40% of cases. ICU lengths of stay – as well as use of invasive procedures - have consistently been demonstrated to decrease when palliative interventions are adopted in a timely manner, which can result in a long-term decrease in hospital costs[18].

Regarding the documentation of DNR decisions, there is a wide range of approaches described in several different studies, highlighting a lack of standardized procedures for this process. In the case of our ICU, a specific document – the HELICS-ICU form - had already been created, prior to the design of this study, which allowed for the registration of DNR, withholding, and withdrawal decisions and their dates, although it was not specifically designed for this purpose. However, this form may be considered lacking in some aspects, such as the motive(s) behind these decisions, the intervening parties and which specific treatment measures have been withdrawn for each patient. Similarly, clinical records were scarce in regard to this type of information, most often simply bringing to attention that the patient had treatment limitations in place. Furthermore, it should be noted that, in almost 10% of patients with documented treatment limitations (WhWd) in this form, the accompanying DNR decision was not formally registered at the same time; this can perhaps be attributed to oversights when filling out the HELICS-ICU form, which should ideally be minimized. When compared to other published works, it becomes clear that there is room for improvement, but also that this problem persists in several other ICUs: several Asian centers go into detail regarding the intervening elements in the decision process, demonstrating a considerable input from family members or surrogates while very little contribution from nursing staff[11,12]; a French study by Lesieur et al.[1] developed a specific form to document reasons for WhWd decisions, participants, and type of treatment withheld. Esteban et al.[17] also recorded specific data pertaining to the participants in the decision and the type of discussion with family members or surrogates. It should be noted, however, that these two studies were prospective in nature, which made it possible to optimize the data collection process. The retrospective study by Hoel et al.[13] also brought to attention the lack of detail in clinical records, which made it difficult to ascertain data like patient and relatives' input in the decisions, as well as the accurate timing of the formal written directives in relation to the medical staff's actual moment of treatment limitation decision.

Our study has some limitations. As a retrospective study, it heavily relied on computerized clinical records for data collection, which were naturally liable to variable interpretations. At the same time, this highlights there is room for improvement regarding the level of detail in the process of documentation of treatment limitation decisions. Also, this study was carried out in a single general ICU, which may not allow for the generalization of results to other institutions with different settings and populations.

CONCLUSION

Steady advances are being made regarding decisions to limit life-sustaining treatments in the ICU. Nowadays, there is a clear notion that timely implementation of a palliative-focused approach, when adequate, improves patient quality of life while simultaneously resulting in lower ICU lengths of stay. However, in comparison to regions such as North America or Northern Europe, there is still room for improvement in our practice on several fronts, among which: more detailed documentation of these decisions, focus on a shared-decision model (medical and nursing staff as well as family members) and commitment to the treatment limitations decided upon, so as to avoid stuttering withdrawal and prolonged continuation of potentially futile measures.

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RESUMO

OBJETIVO: *Decisões de limitação terapêutica (DLT) e de não reanimação (DNR) são difíceis, mas importantes, visando melhorar a qualidade de vida dos doentes e minimizar distanásia. O objetivo deste estudo foi avaliar a abordagem das DNR e DLT, as características dos doentes e a documentação dessas decisões numa Unidade de Cuidados Intensivos Polivalente (Ucip) em Portugal.*

MÉTODOS: *Análise retrospectiva dos dados referentes a DLT e DNR, recolhidos a partir de formulários previamente elaborados e complementados por consulta de processo clínico.*

RESULTADOS: *Um total de 1.602 doentes foi internado na Ucip entre 2011 e 2016. DNR foi documentada em 127 casos (7,9%). Doentes com DLT eram mais velhos e tinham um Simplified Acute Physiology Score II mais elevado. O diagnóstico mais frequente que precedeu essas decisões foi sepse (52,0%, n=66); A razão mais comum para limitar o tratamento foi mau prognóstico da doença aguda. Dos doentes nos quais a DNR foi implementada, 117 (92,1%) morreram na Ucip (40,1% do total de óbitos na Ucip) e a mortalidade hospitalar foi de 100%. Os intervenientes nessas decisões, bem como os tipos de tratamento retirados, não foram rotineiramente registrados.*

CONCLUSÃO: *As DLT e DNR foram relativamente comuns, em consonância com outros estudos do sul da Europa, mas atrás dos centros do norte da Europa e da América do Norte. Os doentes com essas limitações eram mais velhos e mais gravemente doentes. A documentação dessas decisões deve ser clara e detalhada, seja em formulários específicos, seja em registros clínicos informatizados. Há espaço para melhorias nessa área.*

PALAVRAS-CHAVE: *Cuidados paliativos. Cuidados críticos. Ordens quanto à conduta (ética médica). Estado terminal. Suspensão de tratamento.*

REFERENCES

1. Lesieur O, Leloup M, Gonzalez F, Mamzer MF; EPILAT study group. Withholding or withdrawal of treatment under French rules : a study performed in 43 intensive care units. Ann Intensive Care. 2015;5(1):56.
2. Connolly C, Miskolci O, Phelan D, Buggy DJ. End-of-life in the ICU: moving from "withdrawal of care" to a palliative care, patient-centred approach. Br J Anaesth. 2016;117(2):143-5.
3. Coelho CBT, Yankaskas JR. New concepts in palliative care in the intensive care unit. Rev Bras Ter Intensiva. 2017;29(2):222-30.
4. Fuchs L, Anstey M, Feng M, Toledano R, Kogan S, Howell MD, et al. Quantifying the mortality impact of do-not-resuscitate orders in the ICU. Crit Care Med. 2017;45(6):1019-27.
5. Gerstel E, Engelberg RA, Koepsell T, Curtis JR. Duration of withdrawal of life support in the intensive care unit and association with family satisfaction. Am J Respir Crit Care Med. 2008;178(8):798-804.
6. Halcomb E, Daly J, Jackson D, Davidson P. An insight into Australian nurses' experience of withdrawal/withholding of treatment in the ICU. Intensive Crit Care Nurs. 2004;20(4):214-22.
7. Patel SS, Holley JL. Withholding and withdrawing dialysis in the intensive care unit: benefits derived from consulting the Renal Physicians Association/American Society of Nephrology Clinical Practice Guideline, shared decision-making in the appropriate initiation of and withdrawal from dialysis. Clin J Am Soc Nephrol. 2008;3(2):587-93.
8. Jensen HI, Ammentorp J, Ording H. Guidelines for withholding and withdrawing therapy in the ICU: impact on decision-making process and interdisciplinary collaboration. Hear Lung Vessel. 2013;5(3):158-67.
9. Suetens C, Morales I, Savey A, Palomar M, Hiesmayr M, Lepape A, et al. European surveillance of ICU-acquired infections (HELICS-ICU): methods and main results. J Hosp Infect. 2007;65:171-3.
10. Phua J, Joynt GM, Nishimura M, Deng Y, Myatra SN, Chan YH, et al; ACME Study Investigators and the Asian Critical Care Clinical Trials Group. Withholding and withdrawal of life-sustaining treatments in intensive care units in Asia. JAMA Intern Med. 2015;175(3):363-71.
11. Zhao Q, Zhang X, Fang Y, Gong J, Gu B, Ma G. Current situation and associated factors of withdrawing or withholding life support to patients in an intensive care unit of cancer center in China. PLoS One. 2014;9(5):e98545.
12. Yazigi A, Riachi M, Dabbar G. Withholding and withdrawal of life-sustaining treatment in a Lebanese intensive care unit: a prospective observational study. Intensive Care Med. 2005;31(4):562-7.
13. Hoel H, Skjaker SA, Haagensen R, Stavem K. Decisions to withhold or withdraw life-sustaining treatment in a Norwegian intensive care unit. Acta Anaesthesiol Scand. 2014;58(3):329-36.
14. Brandberg C, Blomqvist H, Jirwe M. What is the importance of age on treatment of the elderly in the intensive care unit? Acta Anaesthesiol Scand. 2013;57(6):698-703.
15. Hemphill JC 3rd. Do-not-resuscitate orders, unintended consequences, and the ripple effect. Crit Care. 2007;11(2):121.
16. Vincent JL. Withdrawing may be preferable to withholding. Crit Care. 2005;9(3):226-9.
17. Esteban A, Gordo F, Solsona JF, Alía I, Caballero J, Bouza C, et al. Withdrawing and withholding life support in the intensive care unit: a Spanish prospective multi-centre observational study. Intensive Care Med. 2001;27(11):1744-9.
18. Aslakson R, Cheng J, Vollenweider D, Galusca D, Smith TJ, Pronovost PJ. Evidence-based palliative care in the intensive care unit: a systematic review of interventions. J Palliat Med. 2014;17(2):219-35.

ORIGINAL ARTICLE

Preoperative anxiety induces chronic postoperative pain by activating astrocytes in the anterior cingulate cortex region

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SUMMARY

OBJECTIVE: *The study aims to explore the relationship between preoperative anxiety and chronic postoperative pain.*

METHODS: *A total of forty rats were divided into four groups, control, single-prolonged stress alone, Hysterectomy alone, and SPS+ Hysterectomy. The paw withdrawal mechanical thresholds (PWMT) were examined. qRT-PCR and western blotting assay were performed to detect the GFAP expression in astrocytes isolated from the anterior cingulate cortex (ACC) region. In addition, the long-term potentiation (LTP) in ACC was examined.*

RESULTS: *Rats in the SPS group or the Hysterectomy alone group had no significant effect on chronic pain formation, but SPS can significantly induce chronic pain after surgery. Astrocytes were still active, and the LTP was significantly increased three days after modeling in the SPS+Hysterectomy group.*

CONCLUSIONS: *anxiety can induce chronic pain by activating astrocytes in the ACC region.*

KEYWORDS: *Ansiedade. Pain, postoperative. Astrocytes.*

INTRODUCTION

Clinical investigations have demonstrated that patients with serious anxiety have lower pain threshold and higher sensitivity to pain. In particular, patients who experience serious preoperative anxiety may suffer from postoperative chronic pain [1,2]. The mechanism proposed is that anxiety could increase the central sensitivity of pain by regulating corticotropin-releasing and inflammatory factors such as IL-1, IL-6, IL-10, TNF-α, and noradrenaline, which would increase the pain feeling. [3-5] Anxiety can also have a negative impact on the treatment of painful diseases by enhancing the pain feeling. It is important to use psychological intervention to relief patients' pain and improve their quality of life. However, those treatments sometimes may not be effective. Therefore, it is urgent that we find how to mediate pain enhanced by anxiety.

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Neuroactive substances and receptors are abundant on the neurons in the posterior horn of the spinal cord [6,7]. Previous studies have generally believed that only neurons and their associated neurotransmitters played important roles in pain development, while the role of glial cells was ignored. Recently, studies have found that glial cells in the nervous system play various roles. Glial cells are essential in the propagation of pain signaling, especially in the course of neuropathic pain [8]. It was found that the expression of glial fibrillary acidic protein (GFAP) was significantly increased in a rat model of hyperalgesia, suggesting that glial cells may be activated and participate in the process of pain sensitization. Some studies have also found that the activation of glial cells (including astrocytes and microglia) were usually observed during many chronic pain model [9-12].

Astrocytes are particularly sensitive to the changes in the microenvironment around the neurons, and once receiving the signal, activated astrocytes can release massive proinflammatory and neuroactive substances [13]. The anterior cingulate cortex (ACC) region is a cortical area in the brain that contributes to the regulations of pain and emotions and contains abundant astrocytes. [14,15] Some studies using neuroimaging techniques demonstrated increased activity in the ACC during chronic pain formation [16,17]. In addition, it has been reported that astrocytes in the ACC can be activated in the inflammatory pain model [18]. As such, we wondered if preoperative anxiety can activate astrocytes in the ACC, subsequently leading to the formation of postoperative chronic pain.

This study investigated the effects of preoperative anxiety on postoperative chronic pain by stablishing a preoperative anxiety model. By using techniques such as ACC slice, western blot, RT-PCR, immunohistochemistry, immunofluorescence, and electrophysiology, the alterations of astrocyte activation and LTP in the mice model were evaluated. As a result, we found that preoperative anxiety can induce postoperative chronic pain by activating astrocytes in the ACC region.

METHODS

Animals

Adult female Sprague-Dawley rats (210–260 g) were purchased from the Shanghai Laboratory Animal Center, Chinese Academy of Sciences. Rats were housed six per cage in a controlled environment, fed a standard rodent food, and allowed distilled water ad libitum. Rats were given at least one week to adapt to the new environment before any manipulation. Forty rats were randomly assigned to four groups (n=10): control group; SPS group (Single-prolonged stress model); Hysterectomy only group; SPS + Hysterectomy group.

Isolation of astrocytes in the rat ACC region.

Five rats in each group were euthanized around three weeks after modeling for astrocytes isolation by placing rats in a CO2 enriched tank. The ACC region in the brain was dissected, and the meninges were removed. The rodent brain was kept in Hank's Balanced Salt Solution (HBSS) containing 0.05% trypsin and 0.005% DNase at 4 ^{o}C during the dissection process. The tissue was then triturated for around 5 min by pipetting up and down using a Pasteur pipette. Triturated tissue was then centrifuged at 400 g for 5 min at 22 ^{o}C. After removing the supernatant, the pellet was resuspended into the HBSS containing 34 U papain/ml, 0.02% cysteine and 0.005% DNase and triturated for further 5 min. Followed by another centrifugation at 400 g for 5 min at 22 ^{o}C, the cells were resuspended in HBSS containing 0.005% DNase and put on ice for 30 min. The supernatant was collected and centrifuged again for another 10 min at 400 g.

Single-prolonged stress (SPS) modeling

When establishing the SPS model, rats were first restrained for 2 hr by placing them in plastic bags individually and immobilizing tightly. Several holes were made on the bag to make sure rats could breathe freely. After restraint, the rats were placed in a swimming pool (24 cm in diameter, 50 cm in height) at 24°C and forced to swim for 20 min. After 15-min rest, the rats in all groups were anesthetized with isoflurane. Thereafter, the rats were placed in a ventilated place until they waked up naturally and returned to the cage.

Hysterectomy

24 hr after the SPS modeling, the rats in the Hysterectomy and SPS+Hysterectomy groups underwent a hysterectomy. The rats were anesthetized with isoflurane and then fixed onto a warm pad. The fur over the surgery area was shaved, and the skin was sterilized with ethanol. The skin was cut with approximately 1.5cm length at 0.5 cm above the

midline of the pubic symphysis. The abdominal muscles and peritoneum were bluntly separated. Thereafter, the uterus was exposed by pushing intestine upwards. The lower edge of the cervix was severed, and the cut was sealed with wire. Then, cellulite was removed, and pink or yellow-red ovary was exposed. The fallopian tube, peri-uterine, and fascia were ligatured, and the uterus was removed. At last, the surgical wound was double sutured. A sham operation was performed in the control and SPS groups, and the wounds were sutured only at 0.5 cm above the midline of the pubic symphysis

Animal behavioral test

The mechanical paw withdrawal threshold (PWMT) was performed by Von Frey cilia at 0d (before modeling) every three days after the operation. It was examined by Chaplan's "Up-and-Down" method. Briefly, the rats were placed on a metal frame with a clear plexiglass box cover. The bottom of the box was an empty metal grid. The Electronic von Frey monofilament was used to stimulate the pain at the inner and outer sides of the rat's paw with a series of upward forces. We gradually increased the force until the appearance of a sharp retraction of the hind paw. The number on the electronic display was recorded as a mechanical pain threshold. The threshold was measured three times for each rat with a 5-10 min break interval. The average of three measurements was taken for statistical analysis

qRT-PCR

One-Step SYBR ® PrimeScript ™ (Takara, Japan) qPCR Kit and 7300 real-time fluorescence quantitative PCR instrument were used to detect GFAP, per kit instructions. The reaction conditions were as follows: 95°C for 30 s; 95°C for 5 s; 60°C for 30 s, 40 cycles. The relative mRNA expression level of GFAP was calculated by the $2^{-\Delta\Delta Ct}$ method. β-actin was used as an internal reference, and the primer sequences were:

GFAP forward-5'-GTACCAGGACCTGCTCAAT-3', reverse-5'-CAACTATCCTGCTTCTGCTC-3';

Actin forward-5'-AGAGCTACGAGCTGCCT-GAC-3', reverse-5'-AGCACTGTGTTGGCGTACAG-3'.

Western Blot

Isolated astrocytes from the rats were spin down, and 200 μl of protein lysis buffer was added into each tube and mixed well. After 30 min of lysis, the cell lysates were transferred into another EP tube and centrifuged at 12000 r/min for 10 min. The protein concentration was measured by BCA assay. The SDS buffer was added into cell lysates followed by 10 min protein denature. SDS-PAGE gel electrophoresis was performed, and the wet transfer method was applied to transfer the protein gel onto a PVDF membrane. After 5% skimmed milk powder block at room temperature for 2h, samples were incubated with primary antibody anti-GFAP at 4ºC overnight (ab7260, Abcam, USA, dilution ratio of 1: 10000). On the next day, samples were incubated with corresponding secondary antibodies at room temperature for 1h (Abcam, USA) Membranes were washed three times by TBST containing ECL chemiluminescence solution (Promega). Gel imaging equipment was used for band observation. The results were presented as the ratio of the optical density of the target strip to internal GAPDH.

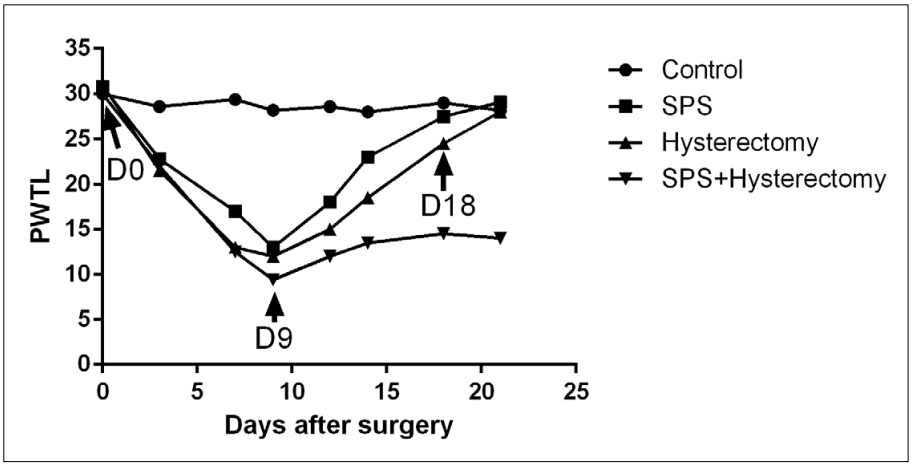


FIGURE1. POSTOPERATIVE CHRONIC PAIN FORMATION INDUCED BY SPS+HYSTERECTOMY.

The PWMT of rats in each group were examined before modeling and every three days after it for three weeks. n=10

LTP measurement

Experiments were performed in a recording chamber on the stage of an Axioskop 2FS microscope with infrared DIC optics for visualizing whole-cell patch-clamp recordings. Excitatory postsynaptic currents (EPSCs) were recorded from layer II-III neurons using an Axon 200B amplifier (Axon Instruments, CA) and stimulations were delivered using a bipolar tungsten stimulating electrode placed in layer V of the ACC. The EPSCs were induced by repetitive stimulations at 0.02 Hz, and the neurons were voltage-clamped at -70 mV. LTP was induced within 10 min after obtaining stable EPSCs to prevent the washout effect. The protocol involved paired presynaptic 80 pulses at 2 Hz with postsynaptic depolarization at +30 mV (referred to as pairing training). The neurons were then voltage-clamped at -30 mV and EPSCs were evoked at 0.05 Hz. The access resistance was 15-30 MΩ and was monitored throughout the experiment. Data were discarded if access resistance changed by more than 15% during an experiment.

Statistical analysis

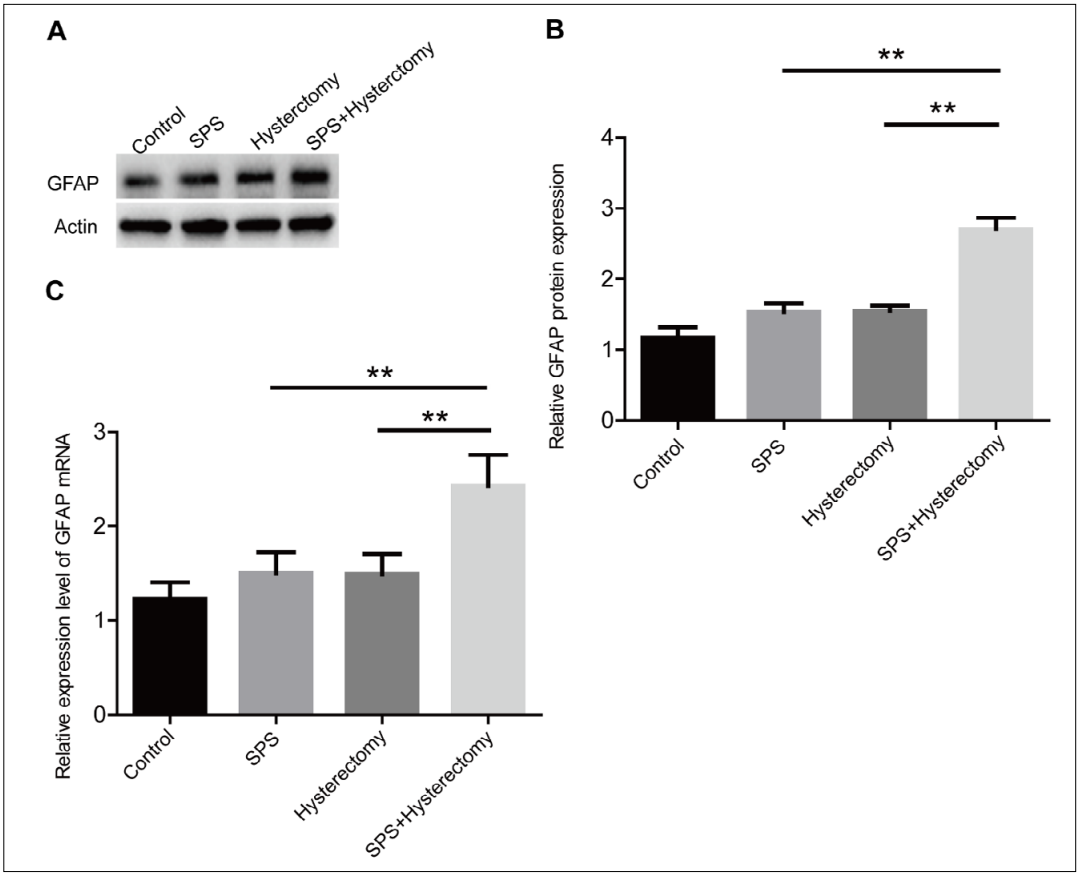
All the statistical analysis was performed using SPSS 17.0(SPSS, USA) and Prism 6 software. Statistical analysis for all the raw data was then performed using student t-test.

RESULTS

PWMT test after modeling

The mechanical PWMT in each group were tested before modeling and every three days after it. As shown in figure 1, these two thresholds in the SPS, Hysterectomy, and SPS+Hysterectomy groups were decreased significantly within the first week compared to day0 or the control group. Notably, there was no obvious variation of PWMT between different model groups for the first week, suggesting the modeling did not have an impact on the acute pain. However, around one week later, the thresholds of the SPS and Hysterectomy alone groups started to increase and recovered back to the control level at

FIGURE2. ASTROCYTES WERE STILL ACTIVE 3 WEEKS AFTER MODELING IN THE SPS+HYSTERECTOMY GROUP.



Astrocytes were isolated from the ACC region, and the expression of GFAP was detected by Westernport. B. The protein expression level was normalized to actin. C. The expression of GFAP was further confirmed by RT-PCR. n=5. *P < 0.05; **P < 0.01. Data are presented as mean ± SEM.

around day18. However, rats in the SPS+Hysterectomy did not present any improvement on pain thresholds at all after one week and showed a noticeable difference compared to the SPS or Hysterectomy groups after day10. This indicates chronic pain was formed in the SPS+Hysterectomy group but not in the SPS or Hysterectomy groups.

GFAP expression in astrocytes isolated from the ACC region

To investigate the reason why chronic pain was formed in the SPS+Hysterectomy group but not in the others, the astrocytes from the ACC region were isolated from the rats in each group three weeks after the modeling. Since GFAP is the activation marker of astrocytes, its expression level was detected in these cells. The western blot results presented that GAFP in the SPS+Hysterectomy group had higher expression than in the other groups. The GAFP expressions in the SPS and Hysterectomy alone groups did not show a significant difference compared to the control, suggesting astrocytes were only activated in the SPS+Hysterectomy group three weeks after the modeling. This observation was further confirmed by RT-PCR(Figure 2).

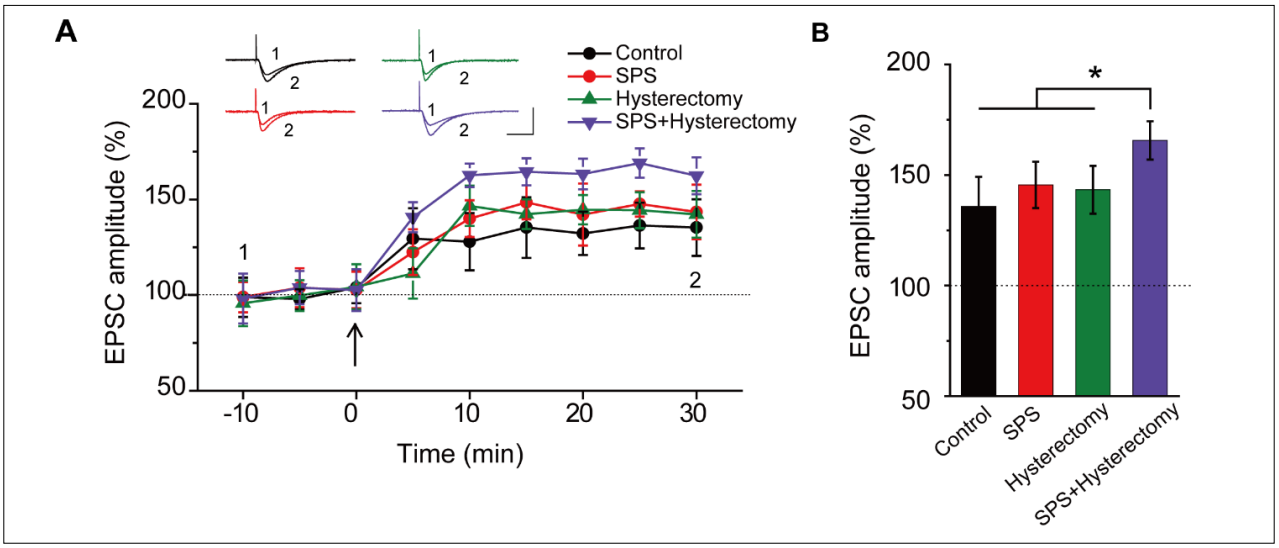
LTP change after modeling

To determine the LTP in different rat models, the ACC region were isolated and sliced for electrophysiological test three weeks after the modeling. As a result, the EPSC amplitude in the ACC region of the SPS+Hysterectomy group was significantly enhanced after induction compared to the control (Figure 3), SPS, and Hysterectomy groups. This suggests the LTP in the SPS+Hysterectomy group was enhanced, and chronic pain occurred after the modeling. Though a small enhancement of LTP in the SPS and Hysterectomy rats was recorded, the difference is not significant compared to the control group. We did not observe any noticeable change of LTP in the ACC region between the SPS and Hysterectomy groups. This indicates that SPS+Hysterectomy affects synaptic potentiation in the ACC.

DISCUSSION

Negative emotions, including anxiety, fear, and depression, are common among patients before operation. Preoperative anxiety, in particular, is very common and apparent. This kind of anxiety can reduce postoperative pain tolerance, diminish treatment efficiency, and damage patients' physical and mental health. It has been reported that patients who undergo operations tend to stimulate pain after the surgery, especially those receiving local anesthesia such as peripheral nerve block anesthesia, epidural anesthesia, and spinal anesthesia. The body damage caused by the surgery and postoperative pain they experience are usually overestimated, resulting in unnecessary anxi-

FIGURE3. LTP WAS ENHANCED IN THE ACC OF SPS+HYSTERECTOMY RATS.



A. Synaptic potentiation of EPSCs in the ACC of SPS+Hysterectomy rats was compared with that in the control, SPS, and Hysterectomy rats. B. Summarized data of EPSC amplitude 30 min after LTP induction in the ACC slices of rats. n=5. *P < 0.05. Data are presented as mean ± SEM.

ety in patients [19]. Clinical approaches, such as non-steroidal anti-inflammatory drugs and non-medical intervention, have been applied to relieve postoperative pain [20] while the treatment outcomes are not ideal in many cases. Despite an increased focus and development of new standards for pain management, many patients still experience intense pain after surgery. Additional efforts are required to improve patients' postoperative pain relief [21].

The ACC region is important to control the nociceptive emotion. Some studies suggest that astrocytes in this region may be activated and participate in the process of pain sensitization. Therefore, we wondered if the activation of astrocytes could be the link between preoperative anxiety and postoperative pain. In this project, we used a SPS model to investigate the relationship between preoperative anxiety and postoperative pain. SPS is a frequently used rat model of posttraumatic stress disorder (PTSD) that involves exposure to several successive stressors. [22] Hereby, we report a novel animal model which combines SPS and hysterectomy to study the formation of postoperative pain induced by preoperative anxiety. In addition, we found that chronic pain was formed in the SPS+Hysterectomy group but not in the SPS or Hysterectomy groups, indicating preoperative anxiety did enhance the postoperative pain. Moreover, astrocytes were found to be still activated, and increased LTP was also observed in the ACC region of rats in the SPS+Hysterectomy group three weeks after modeling, suggesting the formation of chronic pain.

CONCLUSION

Anxiety can induce chronic pain by activating astrocytes in the ACC region.

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study, in collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Author Contributions

Conceptualization, H.C. .and T.L.M..; methodology, D.Y.L..; software D.Y.L..; validation, S.H.X..; formal analysis, D.Y.L.; investigation, T.P.; resources, H.C.; data curation, Y.B.Y.; writing—original draft preparation, D.Y.L.; writing—review and editing, T.L.M..; visualization, T.L.M..; supervision, T.L.M..; project administration, H.C.; funding acquisition, T.L.M.

RESUMO

OBJETIVO: *O objetivo deste estudo é explorar a relação entre a ansiedade no pré-operatório e a dor crônica no pós-operatório.*

MÉTODOS: *Um total de 40 ratos foram divididos em quatro grupos: controle, estresse prolongado (SPS), histerectomia e SPS + histerectomia. Os limiares de retirada da pata em resposta a estímulo mecânico (PWMT) foram examinados. Ensaios qRT-PCR e imunoenzimáticos (western blotting) foram realizados para detectar a expressão de GFAP em astrócitos isolados da região do córtex cingulado anterior (CCA). Além disso, a potenciação de longa duração (LTP) no CCA também foi examinada.*

RESULTADOS: *Os ratos no grupo de estresse prolongado e no grupo de histerectomia não apresentaram nenhum efeito significativo na formação de dor crônica. Porém, o estresse prolongado foi capaz de induzir dor crônica significativamente após a cirurgia. Três dias após o modelo, o grupo de SPS + histerectomia ainda apresentava astrócitos ativos e LTP significativamente maior.*

CONCLUSÃO: *A ansiedade pode provocar dor crônica através da ativação de astrócitos na região do CCA.*

PALAVRAS-CHAVE: *Ansiedade. Pós-operatória, dor. Astrócitos.*

REFERENCES

1. Tanasale B, Kits J, Kluin PM, Trip A, Kluin-Nelemans HC. Pain and anxiety during bone marrow biopsy. Pain Manag Nurs. 2013;14(4):310-7.
2. Johanes C, Monoarfa RA, Ismail RI, Umbas R. Anxiety level of early- and late-stage prostate cancer patients. Prostate Int. 2013;1(4):177-82.
3. Phyomaung PP, Dubowitz J, Cicuttini FM, Fernando S, Wluka AE, Raaijmaakers P, et al. Are depression, anxiety and poor mental health risk factors for knee pain? A systematic review. BMC Musculoskelet Disord. 2014;15:10.
4. Merritt C. Fear and loathing in the ER: managing procedural pain and anxiety in the Pediatric Emergency Department. R I Med J (2013). 2014;97(1):31-4.

5. Dogan MC, Yazicioglu I, Antmen B. Anxiety and pain during dental treatment among children with haemophilia. Eur J Paediatr Dent. 2013;14(4):284-8.

6. González S, Labombarda F, Gonzalez Deniselle MC, Saravia FE, Roig P, De Nicola AF. Glucocorticoid effects on Fos immunoreactivity and NADPH-diaphorase histochemical staining following spinal cord injury. Brain Res. 2001;912(2):144-53.

7. Fürst S. Transmitters involved in antinociception in the spinal cord. Brain Res Bull. 1999;48(2):129-41.

8. Tsuda M, Inoue K, Salter MW. Neuropathic pain and spinal microglia: a big problem from molecules in „small" glia. Trends Neurosci. 2005;28(2):101-7.

9. Hashizume H, DeLeo JA, Colburn RW, Weinstein JN. Spinal glial activation and cytokine expression after lumbar root injury in the rat. Spine (Phila Pa 1976). 2000;25(10):1206-17.

10. Scheggi S, Mangiavacchi S, Masi F, Gambarana C, Tagliamonte A, De Montis MG. Dizocilpine infusion has a different effect in the development of morphine and cocaine sensitization: behavioral and neurochemical aspects. Neuroscience. 2002;109(2):267-74.

11. Mika J, Wawrzczak-Bargiela A, Osikowicz M, Makuch W, Przewlocka B. Attenuation of morphine tolerance by minocycline and pentoxifylline in naive and neuropathic mice. Brain Behav Immun. 2009;23(1):75-84.

12. Maolood N, Meister B. Protein components of the blood-brain barrier (BBB) in the brainstem area postrema-nucleus tractus solitarius region. J Chem Neuroanat. 2009;37(3):182-95.

13. Allen NJ, Barres BA. Signaling between glia and neurons: focus on synaptic plasticity. Curr Opin Neurobiol. 2005;15(5):542-8.

14. Vogt BA. Pain and emotion interactions in subregions of the cingulate gyrus. Nat Rev Neurosci. 2005;6(7):533-44.

15. Xie YF, Huo FQ, Tang JS. Cerebral cortex modulation of pain. Acta Pharmacol Sin. 2009;30(1):31-41.

16. Hsieh JC, Belfrage M, Stone-Elander S, Hansson P, Ingvar M. Central representation of chronic ongoing neuropathic pain studied by positron emission tomography. Pain. 1995;63(2):225-36.

17. Tseng MT, Chiang MC, Chao CC, Tseng WY, Hsieh ST. fMRI evidence of degeneration-induced neuropathic pain in diabetes: enhanced limbic and striatal activations. Hum Brain Mapp. 2013;34(10):2733-46.

18. Chen FL, Dong YL, Zhang ZJ, Cao DL, Xu J, Hui J, et al. Activation of astrocytes in the anterior cingulate cortex contributes to the affective component of pain in an inflammatory pain model. Brain Res Bull. 2012;87(1):60-6.

19. Gerbershagen HJ, Aduckathil S, van Wijck AJ, Peelen LM, Kalkman CJ, Meissner W. Pain intensity on the first day after surgery: a prospective cohort study comparing 179 surgical procedures. Anesthesiology. 2013;118(4):934-44.

20. Møiniche S, Kehlet H, Dahl JB. A qualitative and quantitative systematic review of preemptive analgesia for postoperative pain relief: the role of timing of analgesia. Anesthesiology. 2002;96(3):725-41.

21. Apfelbaum JL, Chen C, Mehta SS, Gan TJ. Postoperative pain experience: results from a national survey suggest postoperative pain continues to be undermanaged. Anesth Analg. 2003;97(2):534-40.

22. Souza RR, Noble LJ, McIntyre CK. Using the single prolonged stress model to examine the pathophysiology of PTSD. Front Pharmacol. 2017;8:615.

COMMENTARY

Comments: "Preoperative anxiety induces chronic postoperative pain by activating astrocytes in the anterior cingulate cortex region"

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Dear Editor,

It was with great interest that we read the study by Daming Gu[1] and colleagues in which they demonstrated that anxiety can induce chronic pain by activating astrocytes in the anterior cingulate cortex region. The authors believe that the activation of astrocytes can induce chronic pain. In our opinion, more experiments are necessary to reach any conclusion.

To begin with, the authors should confirm the success of the mouse model of anxiety since no related experiment was performed to evaluate the anxiety model prior to the operation. In order to examine the anxiety model, the authors could use the elevated plus maze and open field tests[2]. Additionally, the operation skill also has great influence on chronic pain, and the authors should address this issue.

There are many risk factors for chronic pain, such as inflammation, which plays an important role in regulating this type of pain. Thus, we wonder why the authors only focused on the activation of astrocytes. In conclusion, more experiments should be done to exclude the role of corticotropin-releasing factors, inflammation factors, and noradrenalin[3].

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REFERENCES

1. Damin Gu, Minmin Zhou, Chao Han, Daoyun Lei, Songhui Xie, Yanbo Yuan, Tieliang Ma - Preoperative anxiety induces chronic postoperative pain by activating astrocytes in anterior cingulate cortex region. *Rev Assoc Med Bras* 2019; 65(9)1174-1180
2. Wei, D. Zhao, N. Xie, L. Huang, B. Zhuang, Z. Tang, Y., *et al.* Electroacupuncture and Moxibustion Improved Anxiety Behavior in DSS-Induced Colitis Mice. *Gastroenterol Res Pract* 2019;2019: 2345890.
3. Phyomaung, P. P. Dubowitz, J. Cicuttini, F. M. Fernando, S. Wluka, A. E. Raaijmaakers, P., *et al.* Are depression, anxiety and poor mental health risk factors for knee pain? A systematic review. *BMC Musculoskelet Disord* 2014;15: 10.

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ORIGINAL ARTICLE

Platelet-to-lymphocyte ratio (PLR) and Plateletcrit (PCT) in young patients with morbid obesity

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SUMMARY

OBJECTIVE: *To compare the complete blood counts, namely the plateletcrit (PCT) and Platelet-To-Lymphocyte Ratio (PLR) of healthy subjects and those with morbid obesity in the young population.*

METHODS: *We included 45 patients with morbid obesity (body mass index -BMI - greater than or equal to 45 kg/m2) and 45 healthy subjects (BMI less than or equal to 25 kg/m2) in our study. Blood samples were obtained from the participants following a 12-hour fasting period. Then we evaluated the levels of hemoglobin (Hb), hematocrit (HCT), red cell distribution width (RDW), mean platelet volume (MPV), white blood cell (WBC), PLR, platelet counts, and PCT in the complete blood count.*

RESULTS: *The morbid obesity group had significantly higher platelet counts and PCT values (p<0.001), and PLR values (p=0.033). The value of WBC was also higher in the obese group (p=0.001). MPV was lower in the obesity group but not statistically significant (p=0.815). No significant difference was found between hemoglobin and hematocrit values in these groups; but RDW valuewere higher and statistically significant in the obese group (p=0.001).*

CONCLUSION: *PLR or PCT may be more useful as a marker in determining an increased thrombotic state and inflammatory response in morbid obesity.*

KEYWORDS: *Obesity, Morbid. Blood Platelets. Lymphocytes.*

INTRODUCTION

Obesity is one of the most common health conditions and its incidence has recently increased, almost escalating to a real epidemic. [1] It is a risk factor for cardiovascular diseases, including angina pectoris, hypertension, congestive heart failure, myocardial infarction, and atrial fibrillation. [2]

Obesity is defined as an excessive or unhealthy buildup of fat and is most likely to have an adverse effect on health. According to the classification by the World Health Organization (WHO) for overweight and obesity based on body mass index (BMI), obesity is defined as a BMI greater than or equal to 30.0 kg/m2, and it is classified as morbid when the BMI is greater than or equal to 40 kg/m2. [3]

Hypertrophy and hyperplasia of fatty tissue give rise to hypoxia in adipocytes, thus increasing the lev-

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el of stress in cells. Consequently, local pro-inflammatory substances are released, leading to inflammation. [4]Inflammation accelerates the development of atherosclerosis and may also induce atherosclerotic plaque rupture and thrombosis. [5]

Complete Blood Count (CBC) is an inexpensive yet simple and easy test to perform. Researchers have studied the effect of platelet indices including platelet count, platelet distribution width (PDW), plateletcrit (PCT), and mean platelet volume (MPV) on the diagnosis, treatment, and follow-up of various conditions. Additionally, a CBC test can be used to evaluate white blood cell (WBC), red cell distribution (RDW), neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR), all of which may give insight into an inflammation.[6] PLR is calculated by dividing the platelet count by the lymphocytes.

Platelets have an important effect on inflammation, thrombosis, and atherogenesis.[7] Previous studies have demonstrated increased platelet counts in cardiovascular diseases and vascular complications. [8]Likewise, as an indicator of platelet activation, mean platelet volume (MPV) has been shown to increase in acute myocardial infarction. [9]

Platelet lymphocyte ratio (PLR) has been identified as a biomarker of inflammation and proved to be significant in prognosis.[10] Some studies have shown a relationship between poor prognosis and low lymphocyte count and high platelet count in acute coronary syndrome. Additionally, PLR's effect on mortality it has been shown to be independent of platelet or lymphocyte counts. [11]

Similarly, a correlation has been found between PLR and adverse outcomes in various cardiac pathologies. [12]

Some of the results were not consistent with recent studies on platelet counts and MPV for individuals with obesity. The calculation of PCT, which gives better information on total platelet mass, is done according to the following formula: PCT = Platelet count x MPV / 10,000. By comparing BMI with whole blood parameters in their study, Furuncuoğlu et al. [13] demonstrated that MPV was not statistically significant; however, they found a statistically significant positive correlation between BMI and PCT. To this respect, PCT and PLR values can provide us with more accurate insight into inflammation as well as increased thrombogenic activities. [14]

In this paper, we aim to compare the complete blood counts, namely the PCT and PLR values, of healthy subjects with those of morbid obese individuals in the young population.

METHODS

Study population

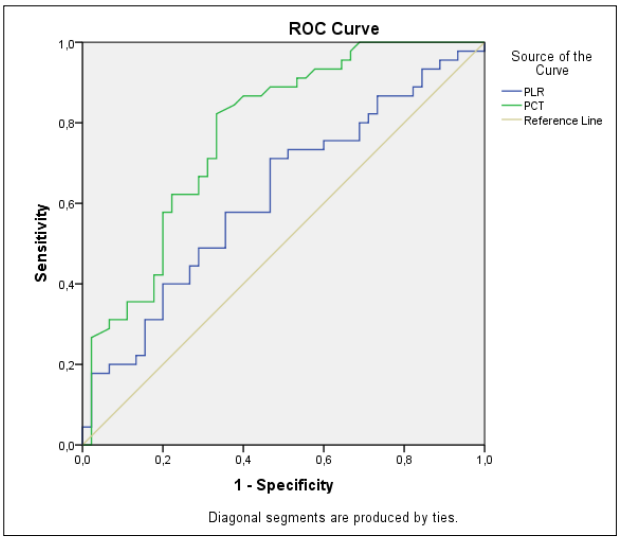
The present cross-sectional study was conducted at Bolu Abant Izzet Baysal University Hospital between March 2018 and October 2018. The local Ethics Committee approved the study protocol. Following the exclusion procedure, we included 45 patients with morbid obesity (BMI greater than or equal to 45 kg/m2) and 45 healthy subjects (BMI value less than or equal to 25 kg/m2) in our study. The mean ages were 33 ± 7 and 33 ± 5 years for the obesity group and the healthy group, respectively. We did not include patients older than 45 years because of the high likelihood of unknown atherosclerosis and comorbidities, both of which could have affected the parameters of complete blood count. Exclusion criteria also included chronic diseases such as chronic renal failure, hypo/hyperthyroidism, coronary artery disease, any hematological abnormality, and medication such as antiplatelet agent and steroid use due to their ability to change the results of a complete blood count. In addition, pregnant women, and patients with anemia and vitamin deficiency (i.e., vitamins D and B12) were excluded from the study. Blood samples were obtained from the participants following a 12-hour fasting period. We then evaluated the levels of hemoglobin (Hb), hematocrit (HCT), PDW, RDW, MPV, WBC, PLR, platelet counts, and plateletcrit (PCT) in the complete blood count.

Statistical analysis

We carried out analyses using SPSS 18.0 Statistical Package Software for Windows Operating System (SPSS Inc, Chicago, Illinois, USA). Quantitative and qualitative variables were expressed as mean ± standard deviation (SD), and numbers and percentages, respectively. In order to assess the differences between these groups, we used the Student t-test for normally distributed variables, the Mann-Whitney's U-test for variables without normal distribution, and the Chi-square test for qualitative variables. The correlations of MPV, WBC, PCT, RDW, PLR, and PLT were assessed using the Pearson correlation analyses. We used multivariate linear regression to analyze the value of different baseline characteristics as independent predictors of morbid obesity. ROC

(receiver operating characteristic) curves were used to evaluate the diagnostic ability of PCT and PLR to detect morbid obesity. All results at $p \leq 0.05$ were considered statistically significant.

FIGURE 1. ROC CURVE ANALYSIS OF PCT AND PLR FOR PREDICTION OF MORBID OBESITY. AT THE CUT-OFF VALUE OF > 0.203 , SENSITIVITY AND SPECIFICITY OF PCT WERE 80% AND 67%, RESPECTIVELY (AUC = 0.775, 95% CI,0.678-0.871). AT THE CUT-OFF VALUE OF > 108 MMHG, SENSITIVITY AND SPECIFICITY OF PLR WERE 68% AND 54%, RESPECTIVELY (AUC = 0.620, 95% CI,0.504-0.736). AUC: AREA UNDER THE CURVE, CI: CONFIDENCE INTERVAL, PCT: PLATELETCRIT, PLR: PLATELET-TO-LYMPHOCYTE RATIO



RESULTS

There was no significant difference between study patients and the control group regarding the frequencies of diabetes mellitus, hypertension, smoking, and hyperlipidemia (Table 1). The morbid obesity group had significantly higher platelet counts, PCT values ($p<0.001$), and PLR values ($p=0.033$) (Table 2). ROC curve analysis was performed to evaluate PCT and PLR in order to predict morbid obesity. At the cut-off value of > 0,203 , the sensitivity and specificity of PCT were 80% and 67%, respectively. At the cut-off value of > 108 mmHg, the sensitivity and specificity of PLR were 68% and 54%, respectively (Figure 1).

No significant difference was found between hemoglobin and hematocrit values in these groups, but RDW values were higher in the obese group and were statistically significant ($p=0.001$). Likewise, the value of WBC was higher in the obese group ($p=0.001$). PCT, PLR, RDW, and WBC distribution in the obesity and healthy groups are shown in Figure-2. MPV was lower in the obesity group but not statistically significant ($p=0.815$).

DISCUSSION

In this study, we compared CBC parameters between morbidly obese patients and healthy subjects

FIGURE 2. PCT, PLR, RDW AND WBC DISTRIBUITION OF HEALTHY AND OBESITY GROUP

PCT: Plateletcrit, PLR: Platelet to lymphocyte ratio, RDW: Red cell distribution width, WBC: White blood cell count

TABLE 1. GENERAL CHARACTERISTICS OF THE STUDY GROUPS

Baseline characteristics	Healthy group(n=45)	Obesity group=45	p
Age (mean ±SD) (years))	33±5	33±7	0.421
Male/female	29/16	34/11	0.25
Hypertension(%)	4(9%)	5(11%)	0.235
Smoking	11(24%)	9(20%)	0.162
Diabetes mellitus	3(7%)	4(9%)	0.173
Hyperlipidemia	2(4%)	3(6%)	0.317
BMI	19.4±1.5	46.0±6.5	0.000

BMI: body mass index, SD: standard deviation

TABLE 2. LABORATORY DATA OF THE STUDY COHORT

	Healthy group(n=45)	Obesity group =45	p
Creatinine (mg/dl)	0.75±0.168	0.7±0.108	0.178
Fasting plasma glucose (mg/dl)	88±11	91 ±12	0.235
LDL-cholesterol (mg/dl)	88.44±34	114,35±44	0.001
HDL-cholesterol (mg/dl)	58±15	45±9	0.000
Triglyceride (mg/dl)	74±32	125±59	0.000
Total cholesterol (mg/dl)	160±41	185±47	0.002
Hemoglobin (gr/dl)	13.6±1.9	13.2±1.8	0.141
Hematocrit (%)	40.41±5.3	40.55±4.9	0.945
MPW(fl)	8.4±1.52	8.38±1.45	0.815
Platelet counts (k/mm3)	235±74	298±74	0.000
PDW	17±1.5	17.6±1.3	0.127
RDW	15.4±2.3	16.3±1.6	0.001
PCT	0.192±0.056	0.244±0.051	0.000
PLR	118±44	141±56	0.033
NLR	2.1±0.8	2.5±1.2	0.132
WBC (x10 3µl)	7.2±1.89	8.3±1.97	0.001

HDL: high-density lipoprotein, LDL: low-density lipoprotein, MPV: mean platelet volume, NLR: neutrophil-to-lymphocyte ratio, PCT: Plateletcrit, PDW: platelet distribution width, PLR: platelet-to-lymphocyte ratio RDW: Red cell distribution width, platelet distribution width, WBC: white blood cell

in the young population. According to our results; platelet counts, PCT, and PLR values were significantly higher in the morbid obesity group. We found that RDW values were significantly higher in the obesity group, and there was no significant difference between hemoglobin and hematocrit values in these groups.

As a chronic inflammation, obesity is associated with an increased atherothrombotic process.[15,16] A positive correlation between cardiovascular disease and inflammatory markers has been shown in recent epidemiological studies. [5]

Previous studies have shown that RDW can be used in the prognosis of cardiovascular diseases and heart failure.[17] Increased RDW is an important predictor of mortality and morbidity in atherosclerotic disease and heart failure, regardless of hemoglobin level. [18] Vayá et al.[19] have shown that RDW values were significantly higher in the obesity group; however, hemoglobin was lower in morbidly obese patients. Therefore, they concluded that increased levels of RDW were due to other causes rather than inflammation.

In the obesity group of our study, platelet counts were significantly higher compared to healthy individuals ($p<0.001$), but MPV values were not different in both groups. In another study, MPV values were significantly higher in the obesity group than those in the non-obese group; however, there was no significant difference in platelet counts between these groups. [20] Contrarily, Farhangi et al.[21] reported that the difference for MCV values between the obesity and the healthy groups was not significant, and they found the platelet count to be significantly higher

in the obesity group (p=0.047). Another study has shown that MPV values decrease significantly after weight loss. [22]

In various studies, researchers investigated the correlation between MPV and platelet count along with weight loss after bariatric surgery. They have found platelet counts to decrease significantly (p=0.0015), but there was no significant decrease in MPV (p=0.34) [23] Kutluturk and Ozsoy[24] reported a significant decrease in platelet counts and a significant increase in MPV after sleeve gastrectomy.

The study results above are different and contradictory regarding MPV and platelet counts. In our opinion, PCT values may provide us with more accurate insight into platelet mass and their functions. PLR may also give more important information about an increased inflammatory status compared to platelet or lymphocyte count alone.

We reported that PLR was significantly higher in the obese group (p= 0,033). In several previous clinical trials in which PLR was compared in obese individuals, no statistically significant difference was found between PLR and BMI.[13] Recent studies have demonstrated that plateletcrit can provide more detailed and accurate information about platelet activation. Han et al.[25] reported a positive relationship between PCT values, platelet counts, and body fat; however, they found no significant correlations between the other platelet indices including PDW, MPV, and body fat mass in their study. Likewise, Furuncuglu et al.[13] found a statistically significant positive relationship between PCT and BMI.

We examined PCT values from whole blood count and found that they were significantly higher in the obesity group (p < 0.001). Our results are also consistent with those of other recent studies. To the best of our knowledge, this is thus far the only study in the literature conducted on young morbid obese patients and the first clinical study demonstrating that PLR is significantly higher in patients with morbid obesity.

CONCLUSION

PLR or PCT may be more useful as a marker in determining an increased thrombotic state and inflammatory response in morbidly obese patients. We need to support our findings with larger, prospective, and randomized studies.

Authors' contributions

Concept, study design, and project management were done by Dr. Erdal; statistics and writing were done by Dr. Inanir.

RESUMO

OBJETIVO: *Comparar as contagens sanguíneas completas, nomeadamente o plateletcrit (PCT) e a razão plaquetas/linfócitos (PPL) de indivíduos saudáveis com aqueles que têm obesidade mórbida na população jovem.*

MÉTODOS: *Incluímos 45 pacientes com obesidade mórbida (índice de massa corporal superior a 45 kg/m2) e 45 indivíduos saudáveis (índice de massa corporal inferior a 25 kg/m2) em nosso estudo. Foram obtidas amostras de sangue dos participantes após um período de jejum de 12 horas. Depois, avaliamos os níveis de hemoglobina, hematócrito, largura de distribuição dos glóbulos vermelhos, volume médio de plaquetas, glóbulos brancos, razão plaquetas/linfócitos, contagem de plaquetas e plateletcrit no hemograma completo.*

RESULTADOS: *O grupo de obesidade mórbida teve contagens plaquetárias e valores plateletcrit significativamente mais elevados (p<0, 001), e valores razão plaquetas/linfócitos (p=0, 033). O valor dos glóbulos brancos também foi maior no grupo obeso (p=0, 001). O volume médio dos plateletes foi inferior no grupo da obesidade, mas não estatisticamente significativo (p=0, 815). Não foi encontrada diferença significativa entre os valores de hemoglobina e hematócrito nesses grupos, mas os valores da largura de distribuição dos glóbulos vermelhos foram mais elevados no grupo obeso e estatisticamente significativos (p=0, 001).*

CONCLUSÃO: *Relação plaquetas-linfócitos e valores de plateletcrit podem ser mais úteis como marcadores na determinação de um estado trombótico aumentado e da resposta inflamatória na obesidade mórbida.*

PALAVRAS-CHAVE: *Obesidade mórbida. Plaquetas. Linfócitos.*

REFERENCES

1. Khan SS, Ning H, Wilkins JT, Allen N, Carnethon M, Berry JD, et al. Association of body mass index with lifetime risk of cardiovascular disease and compression of morbidity. JAMA Cardiol. 2018;3(4):280-7.
2. Pi-Sunyer X. The medical risks of obesity. Postgrad Med. 2009;121(6):21-33.
3. National Institutes of Health. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults-the evidence report. Obes Res. 1998;6(Suppl 2):51S-209S.
4. Cousin B, Munoz O, André M, Fontanilles A, Dani C, Cousin J, et al. A role for preadipocytes as macrophage-like cells. FASEB J. 1999;13(2):305-12.
5. Sabatine MS, Morrow DA, Cannon CP, Murphy SA, Demopoulos LA,

DiBattiste PM, et al. Relationship between baseline white blood cell count and degree of coronary artery disease and mortality in patients with acute coronary syndromes: a TACTICS-TIMI 18 (Treat Angina with Aggrastat and determine Cost of Therapy with an Invasive or Conservative Strategy-Thrombolysis in Myocardial Infarction 18 trial) substudy. J Am Coll Cardiol. 2002;40(10):1761-8.

6. Sahin F, Yildiz P. Serum platelet, MPV, PCT and PDW values, neutrophil to lymphocyte and platelet to lymphocyte ratios in lung cancer diagnosis. Eur Respir J. 2015;46(suppl 59):PA4279.
7. Gawaz M, Langer H, May AE. Platelets in inflammation and atherogenesis. J Clin Invest. 2005;115(12):3378-84.
8. Schneider DJ. Factors contributing to increased platelet reactivity in people with diabetes. Diabetes Care. 2009;32(4):525-7.
9. Park Y, Schoene N, Harris W. Mean platelet volume as an indicator of platelet activation: methodological issues. Platelets. 2002;13(5-6):301-6.
10. Gunaldi M, Goksu S, Erdem D, Gunduz S, Okuturlar Y, Tiken E, et al. Prognostic impact of platelet/lymphocyte and neutrophil/lymphocyte ratios in patients with gastric cancer: a multicenter study. Int J Clin Exp Med. 2015;8(4):5937-42.
11. Kurtul A, Murat SN, Yarlioglues M, Duran M, Ergun G, Acikgoz SK, et al. Association of platelet-to-lymphocyte ratio with severity and complexity of coronary artery disease in patients with acute coronary syndromes. Am J Cardiol. 2014;114(7):972-8.
12. Akboga MK, Canpolat U, Balci KG, Akyel A, Sen F, Yayla C, et al. Increased platelet to lymphocyte ratio is related to slow coronary flow. Angiology. 2016;67(1):21-6.
13. Furuncuoğlu Y, Tulgar S, Dogan AN, Cakar S, Tulgar YK, Cakiroglu B. How obesity affects the neutrophil/lymphocyte and platelet/lymphocyte ratio, systemic immune-inflammatory index and platelet indices: a retrospective study. Eur Rev Med Pharmacol Sci. 2016;20(7):1300-6.
14. Yayla Ç, Akboğa MK, Canpolat U, Akyel A, Yayla KG, Doğan M, et al. Platelet to lymphocyte ratio can be a predictor of infarct-related artery patency in patients with ST-segment elevation myocardial infarction. Angiology. 2015;66(9):831-6.
15. Eckel RH, Krauss RM. American Heart Association call to action: obesity as a major risk factor for coronary heart disease. AHA Nutrition Committee. Circulation. 1998;97(21):2099-100.
16. Li N, Hu H, Lindqvist M, Wikström-Jonsson E, Goodall AH, Hjemdahl P. Platelet-leukocyte cross talk in whole blood. Arterioscler Thromb Vasc Biol. 2000;20(12):2702-8.
17. Gürel OM, Demircelik MB, Bilgic MA, Yilmaz H, Yilmaz OC, Cakmak M, et al. Association between red blood cell distribution width and coronary artery calcification in patients undergoing 64-multidetector computed tomography. Korean Circ J. 2015;45(5):372-7.
18. van Kimmenade RR, Mohammed AA, Uthamalingam S, van der Meer P, Felker GM, Januzzi Jr JL. Red blood cell distribution width and 1[0]year mortality in acute heart failure. Eur J Heart Fail. 2010;12(2):129-36.
19. Vayá A, Alis R, Hernandez-Mijares A, Solá E, Cámara R, Rivera L, et al. Red blood cell distribution width is not related with inflammatory parameters in morbidly obese patients. Clin Biochem. 2014;47(6):464-6.
20. Coban E, Ozdogan M, Yazicioglu G, Akcit F. The mean platelet volume in patients with obesity. Int J Clin Pract. 2005;59(8):981-2.
21. Farhangi MA, Keshavarz SA, Eshraghian M, Ostadrahimi A, Saboor-Yaraghi AA. White blood cell count in women: relation to inflammatory biomarkers, haematological profiles, visceral adiposity, and other cardiovascular risk factors. J Health Popul Nutr. 2013;31(1):58-64.
22. Toplak H, Wascher TC. Influence of weight reduction on platelet volume: different effects of a hypocaloric diet and a very low calorie diet. Eur J Clin Invest. 1994;24(11):778-80.
23. Raoux L, Moszkowicz D, Vychnevskaia K, Poghosyan T, Beauchet A, Clauser S, et al. Effect of bariatric surgery-induced weight loss on platelet count and mean platelet volume: a 12-month follow-up study. Obes Surg. 2017;27(2):387-93.
24. Kutluturk F, Ozsoy Z. Effect of sleeve gastrectomy on platelet counts and mean platelet volumes. Obes Surg. 2018;28(10):3159-64.
25. Han S, Gan D, Wang G, Ru Y, Huang C, Lin J, et al. Associations of platelet indices with body fat mass and fat distribution. Obesity. 2018;26(10):1637-43.

ORIGINAL ARTICLE

Modulatory effects of neuropeptides on pentylenetetrazol-induced epileptic seizures and neuroinflammation in rats

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SUMMARY

OBJECTIVE: *We aimed to explore the effects of neuropeptides ghrelin, obestatin, and vasoactive intestinal peptide (VIP) on seizures and plasma concentrations of neuroinflammation biomarkers including calcitonin gene-related peptide (CGRP), substance-P (SP), and interleukin-1 beta (IL-1β) in pentylenetetrazol-induced seizures in rats.*

METHODS: *Ghrelin (80 µg/kg), obestatin (1 µg/kg), VIP (25 ng/kg) or saline were administered to rats intraperitoneally 30 min before pentylenetetrazole (PTZ, 50 mg/kg) injections. Stages of epileptic seizures were evaluated by Racine's scale, and plasma CGRP, SP, and IL-1β concentrations were measured using ELISA.*

RESULTS: *Both obestatin and VIP shortened onset-time of generalized tonic-clonic seizure, respectively, moreover VIP also shortened the onset-time of first myoclonic-jerk induced by PTZ. While PTZ increased plasma CGRP, SP and IL-1β concentrations, ghrelin reduced the increases evoked by PTZ. While VIP further increased PTZ-evoked CGRP levels, it diminished IL-1β concentrations. However, obestatin did not change CGRP, SP, and IL-1β concentrations.*

CONCLUSION: *Our results suggest that ghrelin acts as an anticonvulsant, obestatin acts as a proconvulsant, and VIP has dual action on epilepsy. Receptors of those neuropeptides may be promising targets for epilepsy treatment.*

KEYWORDS: *Epilepsy. Neuroinflammation. Ghrelin. Vasoactive intestinal peptide. Obestatin.*

INTRODUCTION

There are plenty of data suggesting that neuroinflammation is involved in the pathophysiology of epilepsy.[1] It has been reported that neuroinflammation leads to excessive neuronal discharges in the seizure.[2] Although neuroinflammation is considered to play a key role in the pathophysiology of epilepsy, neuroinflammatory processes underlying epileptogenesis are still unclear. An experimental study with transgenic mice overexpressing pro-inflammatory cytokines, such as TNF-α and IL-6, in astrocytes reported that age-dependent reduced seizure threshold and spontaneous seizures developed in these mice.[3] In addition, it was reported that inhibition of IL-1β signaling resulted in the inhibition of seizure generalization and increased the seizure threshold in the surrogate kindling model of epileptogenesis in rats.[4]

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It is well established that the calcitonin gene-related peptide (CGRP) and substance P (SP) released from trigeminal sensory fibers lead to neurogenic inflammation by inducing vasodilatation and plasma protein extravasation in the meninges.[5] Therefore IL-1β, CGRP, and SP are biomarkers of neuroinflammation in the brain and meninges.

Neuropeptides are regulators of a wide range of physiological processes in the nervous system.

Ghrelin, obestatin and vasoactive intestinal peptide (VIP) are neuropeptides synthesized in both the gastrointestinal system and the central and peripheral nervous systems. These neuropeptides have key roles in a lot of physiological and pathophysiological events because their receptors are widely distributed through the nervous and gastrointestinal systems.

Although there are some studies on the effects of ghrelin, obestatin, and VIP on epileptic seizures, their effects on the neuroinflammation underlying pathogenesis of epilepsy remain unclear. Therefore, in the present study, we investigated the effects of neuropeptides ghrelin, obestatin, and VIP on seizures and plasma concentrations of neuroinflammation biomarkers, including CGRP, SP, and IL-1beta in pentylenetetrazol-induced seizures in rats.

METHODS

Experimental Animals

Thirty-five Wistar male rats (200-250 g) were used in the study. The animals were fed standard pellets ad libitum and tap water and were housed in cages under standard conditions, including a 12 hour light/dark cycle at 22 ± 2 °C. The experimental processes were approved by the Animal Experiment Local Ethics Committee of the University (license number 2018/04).

Materials

Pentylenetetrazole, ghrelin, obestatin, and vasoactive intestinal peptide were purchased from Sigma-Aldrich (Schnelldorf, Germany). CGRP, SP, and IL-1 beta ELISA kits were purchased from ELABscience (Wuhan, P.R. China), ketasol (10 %) was purchased from Richter Pharma (Wels, Austria).

Experimental groups and procedures

Thirty-five rats were randomly divided into five groups, with seven rats in each group (n=7). All injections were carried out intraperitoneally. Rats in the control group were administered 0.2 ml normal saline and received another 0.2 ml normal saline dose 30 min after the first injection. Rats in the PTZ group were administered 0.2 ml normal saline and received 50 mg/kg pentylenetetrazole 30 min after the normal saline injection. Rats in the Ghrelin+PTZ group were administered 80 µg/kg ghrelin[6] and received 50 mg/kg pentylenetetrazole 30 min after the ghrelin injection. Rats in the Obestatin+PTZ group were administered 1 µg/kg obestatin[7] and received 50 mg/kg pentylenetetrazole 30 min after the obestatin injection. Rats in the VIP+PTZ group were administered 25 ng/kg vasoactive intestinal peptide[8] and received 50 mg/kg pentylenetetrazole 30 min after vasoactive intestinal peptide injection.

Induction and scoring of epileptic seizures

In order to induce a seizure, rats were administered PTZ with a single dose of 50 mg/kg as stated previously.[6] After PTZ injections, the rats were placed in plexiglass cages (40 cm X 40 cm X 30 cm) and their behavior was videotaped for 30 min. The intensity of seizures was assessed using Racine's scoring (0-5) as follows[9]: stage 0, no response; stage 1, facial movements with vellication of ears and whiskers; stage 2, myoclonic jerks without rearing; stage 3, clonus of one forelimb; stage 4, rearing with bilateral forelimb clonus; stage 5, generalized tonic-clonic seizures.

Blood sample collection from animals

Blood samples were taken from the right ventricle 24 h after the PTZ injections and coagulated at room temperature for 30 min. Then, blood samples were centrifuged at 3000 rpm for 15 min at 4 °C, and supernatants were kept at -80°C until assayed for CGRP, SP, and IL-1β immunoreactivities.

Determination of plasma CGRP, SP and IL-1 beta concentrations

CGRP, SP, and VIP contents in plasma samples were measured using the ELISA method with detection kits (ELABscience, Wuhan, P.R. China). The detection limit for CGRP is ~ 9 pg/ml; for SP is ~ 47 pg/ml; and for IL-1β is ~ 19 pg/ml. The assay procedures were performed following the manufacturer's instructions and in duplicates. After samples or CGRP, IL-1β, SP standards were added and the 96-well plates incubated according to the instructions, the optical densities for CGRP, SP, and IL-1β were measured at

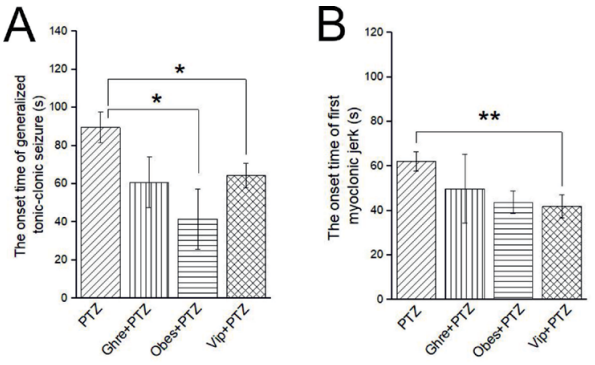

FIGURE 1. EFFECTS OF GHRELIN, OBESTATIN, AND VASOACTIVE INTESTINAL PEPTIDE ON THE ONSET TIME OF GENERALIZED TONIC-CLONIC SEIZURE (A) AND ONSET TIME OF FIRST MYOCLONIC JERK (B) IN PTZ-INDUCED EPILEPTIC RATS. *P < 0.05, **P < 0.01

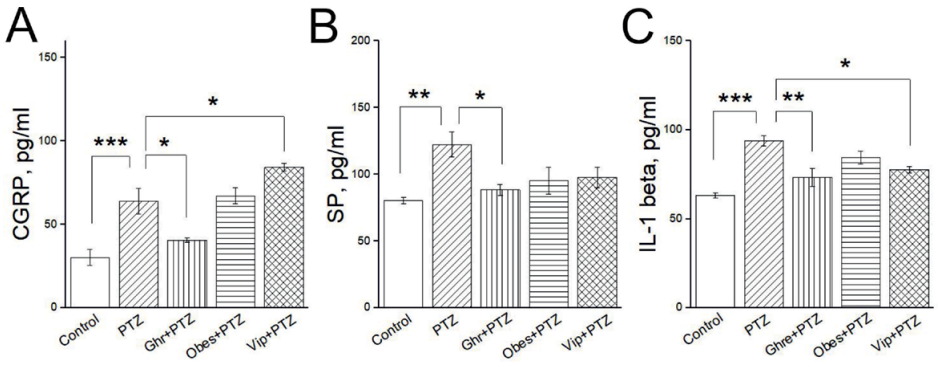

FIGURE 2. EFFECTS OF GHRELIN, OBESTATIN, AND VASOACTIVE INTESTINAL PEPTIDE ON THE PLASMA CGRP (A), SP (B) AND IL-1 BETA (C) LEVELS IN PTZ-INDUCED EPILEPTIC RATS. *P < 0.05, **P < 0.01, ***P < 0.001.

450 nm using a microplate reader (Epoch BioTek Instruments, Inc. Highland Park, Winooski, VT, USA).

STATISTICAL ANALYSIS

The data were given as mean ± standard error of the mean. Statistical analysis was performed using SPSS for Windows (version 17.0, SPSS Inc., Chicago, IL, USA). Data obtained from the experimental groups were analyzed by one-way analysis of variance followed by Tukey's multiple comparisons test. *A value of p<0.05* was considered statistically significant.

RESULTS

The effects of ghrelin, obestatin, and VIP on the duration of the characteristic behavioral changes in PTZ-induced epileptic rats

The obestatin treatment shortened the onset-time of the generalized tonic-clonic seizure (P=0.037, Fig. 1A) compared to the PTZ group. Moreover, VIP shortened the onset-time of the generalized tonic-clonic seizure (P=0.039, Fig. 1A) and first myoclonic-jerk (P= 0.009, Fig. 1B), respectively. On the other hand, ghrelin changed neither the generalized tonic-clonic seizure (P=0.075, Fig. 1A) nor the first myoclonic-jerk (P=0.791, Fig. 1B) compared to the PTZ group.

The effects of ghrelin, obestatin, and VIP on plasma CGRP, SP, and IL-1 beta concentrations in PTZ induced-epileptic rats

The pentylenetetrazol treatment led to significant increases in the plasma CGRP (P=0.001, Fig. 2A), SP (P=0.003, Fig. 2B), and IL-1 beta (P=0.001, Fig. 2C) concentrations compared to the normal saline-treated control group. On the other hand, ghrelin pretreatment decreased PTZ-induced plasma CGRP (P=0.013, Fig. 2A), SP (P=0.02, Fig. 2B), and IL-1 beta (P= 0.001, Fig. 2C) concentrations (PTZ group versus ghrelin+PTZ group).

Obestatin pretreatment did not show any effect on the PTZ-induced plasma CGRP, SP and IL-1 beta concentrations (P=0.98 for CGRP, Fig. 2A; P=0.09 for SP, Fig. 2B; and P=0.283 for IL-1 beta Fig. 2C). While VIP pretreatment further increased PTZ-induced plasma CGRP concentration (P=0.038, Fig. 2A), it decreased PTZ-induced plasma IL-1 beta concentration (P=0.011, Fig. 2C, PTZ group versus VIP+PTZ group). However, VIP pretreatment did not show any effect on the PTZ-induced plasma SP concentration (P=0.147, Fig. 2B).

DISCUSSION

Existing antiepileptic drugs (AEDs) target ion channels or ionotropic receptors of excitatory or in-

hibitory neurotransmitters. Approximately one-third of patients with epilepsy do not correctly respond to any of these AEDs.[10] Therefore, neuropeptide-mediated modulation of synaptic transmission may represent a novel treatment approach for patients suffering from different types of epilepsy. Pursuant to this hypothesis, we explored the effects of neuropeptides ghrelin, obestatin, and VIP on PTZ-evoked epileptic seizures and neuroinflammation markers including IL-1β, CGRP, and SP.

In the present study, ghrelin attenuated plasma CGRP, SP, and IL-1β concentrations induced by PTZ. This result is important because CGRP, SP, and IL-1β are key biomarkers of neuroinflammation. Moreover, inflammatory processes in the brain have been implicated in the pathophysiology of seizures and epilepsy.[11] An inflammation without infection in the central nervous system is called sterile inflammation characterized by the release of vasoactive neuropeptides, including CGRP and SP, from sensory nerves.[5] SP has a great role in inflammatory processes. Moreover, cytokines such as IL-1β are also released by the glia and neurons in the CNS in addition to immune cells and endothelial cells during neuro-inflammation.[12]

Most clinical studies have reported that plasma IL-1β levels were higher in children with febrile seizures than controls.[13] Furthermore, it is well established that IL-1β has a key role in exacerbating seizures in animal models of epilepsy.[14] On the other hand, in the present study, ghrelin did not show any significant effect on the PTZ-induced generalized tonic-clonic seizure or first myoclonic-jerk. In a previous study, it was reported that ghrelin repressed the onset time of PTZ-induced seizures in rats.[6] Although consensus claims that ghrelin has an anticonvulsant action, some studies report that ghrelin did not have any effect on the seizures. One of those studies reported that ghrelin fails to inhibit seizures induced by kainic acid or pilocarpine.[15] Like those studies, we also did not find any effect of ghrelin on the characteristic behavioral changes of epilepsy. However, we first demonstrate the modulating effect of ghrelin on PTZ-induced plasma CGRP, SP, and IL-1β levels. These results suggest that ghrelin may alleviate seizures by decreasing the the neuroinflammation underlying pathophysiology of epilepsy.

Unlike ghrelin, there are some studies about the effects of obestatin on epilepsy. In a clinical study, it was found that plasma concentrations of obestatin were higher in patients with primary generalized and partial epilepsy than in controls.[16] In line with this study, we found that obestatin shortened the onset-time of generalized tonic-clonic seizure while it did not affect the onset-time of first myoclonic-jerk in PTZ-induced seizures in rats. Therefore, our results suggest that obestatin has a proconvulsant effect on the behavioral characteristics of PTZ-induced seizure. Contrarily, a recent study reported that obestatin alleviated the intensity of PTZ-induced seizures and decreased neuronal damage by delimitating oxidative damage in rats.[7] This difference may be due to the measurement of different parameters, and, therefore, more clinical and experimental studies are needed to explain the possible mechanisms of these differences. Interestingly, in the present study, the obestatin pretreatment did not change plasma levels of biomarkers associated with neuroinflammation including CGRP, SP and IL-1 beta. Based on these results, we can speculate that obestatin might not exhibit its proconvulsant action through neuroinflammatory mechanisms.

VIP is a neuropeptide that has anti-inflammatory and neuroprotective effects. However, VIP can play a key role in seizure disorders because it demonstrates excitatory effects on synaptic transmission in different brain regions, such as the hippocampus. It has been demonstrated that there was a short-term decrease in brain VIP concentrations after PTZ-induced seizures in rats.[17] Conversely, a clinical study reported that VIP concentrations were higher in the serum and cerebrospinal fluid of children with seizure disorders than in controls.[18] In the present study, we found that VIP shortened both onset-time of generalized tonic-clonic seizure and first myoclonic-jerk. Moreover, VIP pretreatment further enhanced plasma CGRP levels induced by PTZ. These findings indicate that VIP exhibits a proconvulsant effect on the behavioral characteristics of seizure and also exacerbates neuroinflammation induced by PTZ. However, in the current study, interestingly, VIP alleviated PTZ-induced plasma IL-1β levels. Therefore we can speculate that VIP exacerbates seizures by shortening the onset-time of generalized tonic-clonic seizure and first myoclonic-jerk and also by increasing CGRP levels. VIP also endeavors to protect neuroinflammation by reducing IL-1β levels. These results indicate that VIP may have a dual role in the generation of seizures.

As a whole, our results suggest that ghrelin has

an anticonvulsant action, obestatin has a proconvulsant action, and VIP has dual action on epilepsy. Receptors of neuropeptides ghrelin, obestatin, and VIP may be favorable targets for epilepsy treatment. However, studies with specific agonists and antagonists of these receptors are needed.

Acknowledgments

We declare there was no conflict of interest in this paper.

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Contributions of authors

Erkan Kilinc: Hypothesis, conception, design of research, statistical data analysis, manuscript preparation, final approval

Handan Gunes: Implementation of experiments, data acquisition, manuscript preparation, production of graphics, final approval.

RESUMO

OBJETIVO: *Nosso objetivo foi explorar os efeitos dos neuropeptídeos grelina, obestatina e peptídeo intestinal vasoativo (VIP) nas convulsões e concentrações plasmáticas de biomarcadores neuroinflamatórios, incluindo peptídeo relacionado ao gene da calcitonina (CGRP), substância-P (SP) e interleucina-1 beta (IL-1β) em convulsões induzidas por pentilenotetrazol em ratos.*

MÉTODOS: *Grelina (80 µg/kg), obestatina (1 µg/kg), VIP (25 ng/kg) ou solução salina foram administrados a ratos intraperitonealmente 30 minutos antes de injeções de pentilenotetrazol (PTZ, 50 mg/kg). Os estágios das crises epilépticas foram avaliados pela escala de Racine e as concentrações plasmáticas de CGRP, SP e IL-1β foram medidas usando Elisa.*

RESULTADOS: *Tanto a obestatina quanto o VIP encurtaram o tempo de início da crise tônico-clônica generalizada, respectivamente. Além disso, o VIP também encurtou o tempo de início do primeiro impulso mioclônico induzido por PTZ. Enquanto o PTZ aumentou as concentrações plasmáticas de CGRP, SP e IL-1β, a grelina reduziu os aumentos evocados por PTZ. Enquanto o VIP aumenta ainda mais os níveis de CGRP evocados por PTZ, diminui as concentrações de IL-1β. No entanto, a obestatina não alterou as concentrações de CGRP, SP e IL-1β.*

CONCLUSÃO: *Nossos resultados sugerem que a grelina tem anticonvulsivante, a obestatina tem proconvulsivante e o VIP tem ação dupla na epilepsia. Receptores desses neuropeptídeos podem ser alvos promissores para o tratamento da epilepsia.*

PALAVRAS-CHAVE: *Epilepsia. Encefalite. Grelina. Peptídeo intestinal vasoativo. Ratos.*

REFERENCES

1. Vezzani A, French J, Bartfai T, Baram TZ. The role of inflammation in epilepsy. Nat Rev Neurol. 2011;7(1):31-40.
2. Granata T, Cross H, Theodore W, Avanzini G. Immune-mediated epilepsies. Epilepsia. 2011;52(Suppl 3):5-11.
3. Probert L, Akassoglou K, Kassiotis G, Pasparakis M, Alexopoulou L, Kollias G. TNF-alpha transgenic and knockout models of CNS inflammation and degeneration. J Neuroimmunol. 1997;72(2):137-41.
4. Auvin S, Shin D, Mazarati A, Sankar R. Inflammation induced by LPS enhances epileptogenesis in immature rat and may be partially reversed by IL1RA. Epilepsia 2010;51(Suppl 3):34-8.
5. Ramachandran R. Neurogenic inflammation and its role in migraine. Semin Immunopathol. 2018;40(3):301-14.
6. Obay BD, Tasdemir E, Tümer C, Bilgin HM, Sermet A. Antiepileptic effects of ghrelin on pentylenetetrazole-induced seizures in rats. Peptides. 2007;28(6):1214-9.
7. Koyuncuoğlu T, Vızdıklar C, Üren D, Yılmaz H, Yıldırım Ç, Atal SS, et al. Obestatin improves oxidative brain damage and memory dysfunction in rats induced with an epileptic seizure. Peptides. 2017;90:37-47.
8. Kilinc E, Firat T, Tore F, Kiyan A, Kukner A, Tunçel N. Vasoactive intestinal peptide modulates c-Fos activity in the trigeminal nucleus and dura mater mast cells in sympathectomized rats. J Neurosci Res. 2015;93(4):644-50.
9. Racine RJ, Gartner JG, Burnham WM. Epileptiform activity and neural plasticity in limbic structures. Brain Res. 1972;47(1):262-8.
10. Perucca E, French J, Bialer M. Development of new antiepileptic drugs: challenges, incentives, and recent advances. Lancet Neurol. 2007;6(9):793-804.
11. Riazi K, Galic MA, Pittman QJ. Contributions of peripheral inflammation to seizure susceptibility: cytokines and brain excitability. Epilepsy Res. 2010;89(1):34-42.
12. Bartfai T, Schultzberg M. Cytokines in neuronal cell types. Neurochem Int. 1993;22(5):435-44.
13. Li G, Bauer S, Nowak M, Norwood B, Tackenberg B, Rosenow F, et al. Cytokines and epilepsy. Seizure. 2011;20(3):249-56.
14. Vezzani A, Moneta D, Conti M, Richichi C, Ravizza T, De Luigi A, et al. Powerful anticonvulsant action of IL-1 receptor antagonist on intracerebral injection and astrocytic overexpression in mice. Proc Nat Acad Sci USA. 2000;97(21):11534-9.
15. Biagini G, Torsello A, Marinelli C, Gualtieri F, Vezzali R, Coco S, et al. Beneficial effects of desacyl-ghrelin, hexarelin and EP-80317 in models of status epilepticus. Eur J Pharmacol. 2011;670(1):130-6.
16. Dag E, Aydin S, Ozkan Y, Erman F, Dagli AF, Gurger M. Alteration in chromogranin A, obestatin and total ghrelin levels of saliva and serum in epilepsy cases. Peptides. 2010;31(5):932-7.
17. Romualdi P, Lesa G, Donatini A, Balboni G, Tomatis R, Ferri S. Alterations in vasoactive intestinal polypeptide-related peptides after pentylenetetrazole-induced seizures in rat brain. Eur J Pharmacol. 1992;229(2-3):149-53.
18. Ko FJ, Chiang CH, Liu WJ, Chiang W. Somatostatin, substance P, prolactin and vasoactive intestinal peptide levels in serum and cerebrospinal fluid of children with seizure disorders. Gaoxiong Yi Xue Ke Xue Za Zhi. 1991;7(8):391-7.

ORIGINAL ARTICLE

Investigation of the protective effect of enoxaparin and ticagrelor pretreatment against ischemia-reperfusion injury in rat lung tissue

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SUMMARY

OBJECTIVES: *This study was conducted to reveal the possible protective effects of ticagrelor and enoxaparin pretreatment against ischemia-reperfusion (IR)-induced injury on the lung tissue of a rat model.*

METHODS: *Wistar albino rats were randomly divided into 4 groups as follows: group-1 (control-sham), group-2 (control-saline+IR), group-3 (ticagrelor+IR), group-4 (enoxaparin+IR). Before the ischemic period, saline, ticagrelor, and enoxaparin were administered to the 2nd-4th groups, respectively. In these groups, IR injury was induced by clamping the aorta infrarenally for 2 h, followed by 4 h of reperfusion except group-1. After the rats were euthanized, the lungs were processed for histological examinations. Paraffin sections were stained with Haematoxylin&Eosin (H&E) for light microscopic observation. Apoptosis was evaluated by caspase-3 immunoreactivity. Data were statistically analyzed using the SPSS software.*

RESULTS: *In the lung sections stained with H&E, a normal histological structure was observed in group-1, whereas disorganized epithelial cells, hemorrhage, and inflammatory cell infiltration were seen in the alveolar wall in group-2. The histologic structure of the treatment groups was better than that of group-2. Caspase-3(+) apoptotic cells were noticeable in sections of group-2 and were lower in the treatment groups. In group-4, caspase-3 immunostaining was lower than in group-3. In group-2, apoptotic cells were significantly higher than in the other groups ($p<0.001$).*

CONCLUSION: *Based on the histological results, we suggested that both therapies ameliorated the detrimental effects of IR. Caspase-3 immunohistochemistry results also revealed that pre-treatment with enoxaparin gave better results in an IR-induced rat injury model. In further studies, other parameters such as ROS and inflammatory gene expressions should be evaluated for accurate results.*

KEYWORDS: *ischemia-reperfusion, rat lung tissue, caspase-3, apoptosis.*

INTRODUCTION

Aortic surgery with clamping of the thoracic or thoracoabdominal aorta causes rapid and significant physiologic changes that can result in major complications in several organ systems. Cross-clamping can also contribute to pulmonary complications, although the mechanisms for this effect are complex and not fully understood[1]. The cross-clamping of the aorta is called 'ischemia', and the declamping after completion of the anastomosis is 'reperfusion'. Reperfusion causes both local and systemic damage,

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particularly through inflammatory mediators, and often by a rapid release of O_2-free radicals from polymorphonuclear leukocytes[2,3]. These products can cause terrible complications during reperfusion and even death due to systemic inflammatory response and multiple organ failure[4].

Distant organ damage and reperfusion after ischemia are mainly seen in the lung, kidney, and heart. Pathophysiological manifestations of aortic clamping resulting in hemodynamic compromisation were summarized by Katseni et al.[5], and Kalogeris et al.[6] reported the mechanisms contributing to tissue injury in ischemia/reperfusion (IR) as follows. Cellular hypoxia secondary to ischemia results in decreased ATP production, disrupts ion pump function, further promoted by a shift to anaerobic glycolysis for energy production. Activation and upregulated expression of enzymes capable of producing reactive oxygen species (ROS) and electron transport chain (ETC) dysfunction are also initiated during ischemia. These events set the stage for a burst of ROS generation when molecular O2 is reintroduced to ischemic tissues when the blood supply is re-established. Phagocytic Nox2 activation results in the respiratory burst of superoxide production, which further intensifies the massive oxidative stress that directly damages virtually every biomolecule found in cells and induces the programmed cell death responses, apoptosis and necroptosis. Postischemic ROS generation also activates matrix metalloproteinases (MMPs) and other proteases that act to cleave proteins and receptors, thereby impairing their function. The net impact of these ROS-dependent events is the opening of mitochondrial permeability transition pores (MPTPs), which contributes to swelling and lysis of cells. Increases in leukocyte stiffness induced by hypoxia and acidosis during ischemia lead to the impaction of these cells in capillaries, an effect that is exacerbated by ROS-dependent endothelial cell swelling which in turn reduces their diameter when the blood supply is re-established. So, a nutritive perfusion impairment becomes prominent during reperfusion, despite the repair of the precipitating ischemic event. In direct contrast to these catastrophic effects of ROS generation secondary to events occurring in ischemia and early reperfusion, oxidant production also occurs at later stages of reperfusion as tissue repair is initiated. However, ROS production occurs at lower levels that allow oxidant species to serve as signaling molecules that participate in transcriptional activation of growth factors and promote cell proliferation, differentiation, and migration. The net effect of these processes is tissue and vascular remodeling, including angiogenesis. While some of these repair processes help to restore organ function, others such as tissue fibrosis contribute overtime to eventual organ failure. The mechanisms emphasize the concept that ROS generation plays key roles in all three phases of IR injury and cell death[6].

Ticagrelor is a direct-acting antagonist of P2Y12, a purinergic receptor of ADP expressed by thrombocytes. P2Y12 plays important roles in hemostasis and thrombosis[7,8]. It is essential for ADP-induced thrombocyte aggregation and its defects result in bleeding[9,10]. Ticagrelor is, therefore, a widely used drug for the prevention of cardiovascular events and stroke[11]. It is reported to inhibit cellular uptake of adenosine, a purine nucleoside produced by metabolism of ADP[12,13]. Adenosine levels in plasma increase after inflammation, injury, or IR[14]. As ticagrelor inhibits the cellular uptake of adenosine, the level of endogenous adenosine concentration increases, resulting in the reduction of inflammatory markers[15].

There are several commercially available anticoagulants that interfere with different stages of blood coagulation[16]. Heparins are widely used, including enoxaparin, a low-molecular-weight heparin, for the treatment of ischemia and infarction[17]. Enoxaparin inhibits the conversion of prothrombin to thrombin and reduces the conversion of fibrinogen to fibrin, preventing clot formation. It also reduces coagulation factors and inactivates factor X[16]. Enoxaparin is used to treat or prevent a type of blood clot called deep vein thrombosis (DVT), which can lead to blood clots in the lungs (pulmonary embolism). A DVT can occur after certain types of surgery, or in people who are bed-ridden due to a prolonged illness. Enoxaparin is also used to prevent blood vessel complications in people with certain types of angina (chest pain) or heart attack[18].

As mentioned above, IR causes fibrosis, and enoxaparin has anti-fibrotic effects on animal fibrosis models shown in previous studies[19]. Therefore, we aimed to evaluate the effect of ticagrelor and enoxaparin pre-treatment, in rats, for the prevention of abdominal aorta IR-induced lung injury. To detect these alterations, we examined the histologic sections of all lungs belonging to all groups in addition to caspase-3 immunoreactivity by light microscope.

METHODS

Experimental animals

Kocaeli University Animal Experiments Local Ethics Committee (KOÜ HADYEK:KOÜ HADYEK:1/9-2016) approved this study, and the experiments complied with the established guidelines for animal care. Thirty-six mature male Wistar albino rats weighing 350-400 g were randomly divided into four groups as follows: group 1 (sham-control), group 2 (control-saline+IR), group 3 (ticagrelor+IR), group 4 (enoxaparin+IR). The rats were initially anesthetized with intraperitoneal ketamine hydrochloride (Ketalar; Pfizer, Istanbul, Turkey), 100 mg/kg body weight. The animals were given 0.1 ml/kg normal saline in group 2, and a single dose of 25 mg/kg ticagrelor (Brilinta-Astra-Zeneca, Södertalje, Sweden) in group 3 , orally, via gastric gavage while a single dose of enoxaparin (0.75 mg/kg) was administered via subcutaneous injection just before the ischemic period. Except for group-1, IR injury was induced by clamping the aorta infrarenally for 2 h, followed by 4 h of reperfusion. Cessation of arterial flow was confirmed by the absence of an audible continuous-wave Doppler signal. The rats were euthanized with a lethal injection of sodium thiopental (Pentothal Sodium, Abbot, Italy) after 4 h of reperfusion. Then, the lungs of the rats were removed through midline sternotomy and washed with 0.9% saline solution for tissue processing.

Hematoxylin and Eosin (H&E) Staining

The lungs were removed and fixed with %10 neutral buffered formalin solution and, after tissue processing, embedded into paraffin for histological examinations for all rats. Paraffin sections (4 µm) were prepared and then stained with Hematoxylin and Eosin (H&E) for routine histologic examination. Lung damage was evaluated by histological changes[20]. Tissue injury of the lungs was assessed according to the structural integrity of the alveolar wall, disorganized epithelial cells, alveolar hemorrhage, inflammatory cell infiltration, and alveolar wall thickening.

Caspase-3 immunohistochemical (IHC) staining and analysis

Apoptotic cells were detected by caspase-3 immunoreactivity. After deparaffinization and rehydration, all sections were incubated in 0.1 mol/L sodium citrate buffer (pH 6.0) in a microwave oven (medium-low temperature) for 5-7 min. Endogenous peroxidase was blocked by 3% H_2O_2 in PBS 15 min and again washed three times in PBS. Sections were incubated in a blocking serum for 15 mins at room temperature to block non-specific binding. For immunostaining, a primary anti-Caspase-3 monoclonal antibody (74T2, Life Technologies) at a dilution of 1.100 overnight was applied at room temperature in a humidified chamber. Sections were washed three times in PBS and incubated with the biotinylated secondary antibodies (ab80437, Abcam) for 20 mins at room temperature. After three washes with PBS, the sections were incubated with peroxidase-labeled streptavidin for 15 min. The peroxidase activity was visualized with DAB for Caspase-3. All incubations were performed in a humidified chamber at room temperature using PBS for washes between incubation steps. The sections were counterstained with Mayer's hematoxylin and mounted with entellan on glass slides. All samples were treated following exactly the same protocols. Two independent observers, who were blinded to this study, evaluated the staining semi-quantitatively. Apoptosis was evaluated based on spread and intensity of caspase-3 (+) immunoreactivity in ten random fields in each section (no expression (-), very weak (1+), moderate (2+), strong (3+) to very strong (4+) expression[21]. All slides were examined under a light microscope, and photomicrographs were taken with a Leica camera.

Statistical analysis

Caspase-3 scoring was analyzed by SPSS 21.0 statistical software and presented as mean ± standard deviation (SD). The comparison of the mean among groups was made using ANOVA. $P< 0.05$ was considered to indicate statistically significant differences.

RESULTS

H&E staining slides showed that lung tissues had a normal histological arrangement in group-1. The alveolar wall had normal structural integrity; the alveolar epithelial cells had an orderly manner with clear boundaries. There was no alveolar hemorrhage, inflammatory cell infiltration, and thickening of the alveolar wall (Figure 1A, B).

Histological alterations and lung injury such as intra-alveolar hemorrhage thickened alveolar wall, edematous septum, and inflammatory cell infiltration (mononuclear/neutrophilic cells) moderately occupying the interalveolar wall were seen in group-2.

The structural integrity of the alveolar wall was damaged, and the alveolar epithelial cells were disorganized; leucocytes and cell shed debris were present in the alveoli (Figure 1C, D).

In group-3 and group-4, the alveolar wall had a structural integrity and the alveolar epithelial cells were arranged in an orderly manner; there was no alveolar bleeding, and only occasional inflammatory cell infiltration was seen (Figure 1E-H). Lung sections of pretreatment groups with ticagrelor and enoxaparin were better than group 2. Apoptotic cells were noticeable in the sections of group 2, and these cells were lower in the pretreatment groups (Figure 2). In group 2, apoptotic cells were significantly higher than sham-control and pretreatment groups ($p< 0.01$; Figure 3). In Enoxaparin+ IR group, caspase-3 immunostaining was lower than Ticagrelor+IR group. These results indicated that lung injury score was significantly increased by IR, whereas ticagrelor and enoxaparin treatment significantly decreased lung injury score (Figure 3).

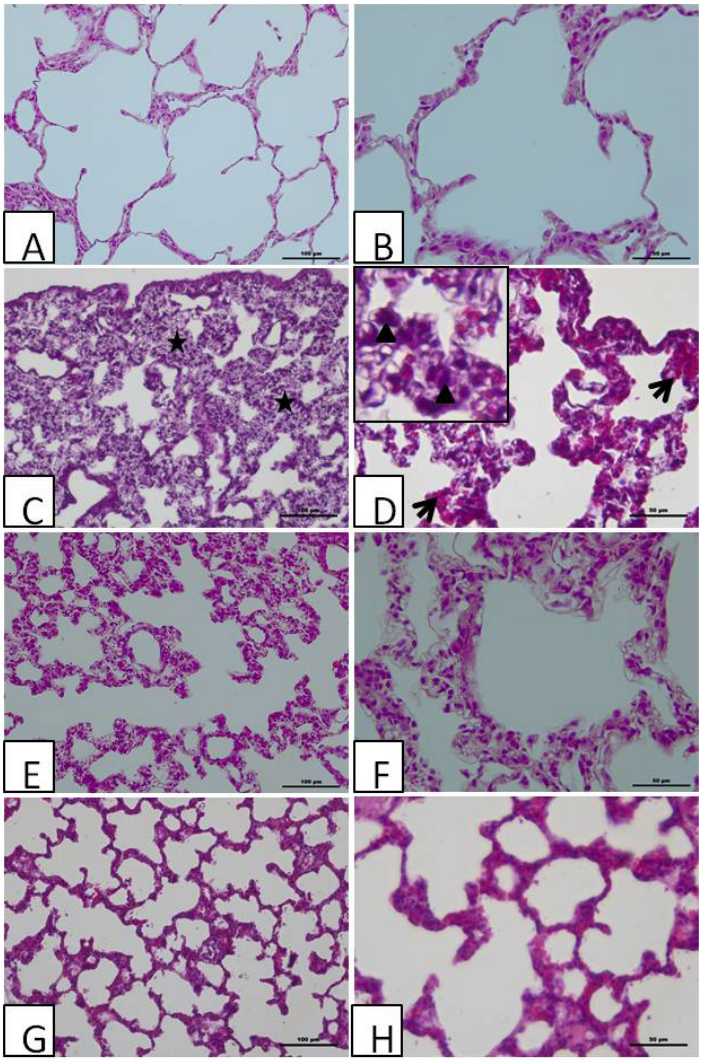

FIGURE 1. PHOTOMICROGRAPHS OF LUNG TISSUE. Sham control (A, B); Control-Saline+IR (C, D); Ticagrelor+IR (E, F) and Enoxaparin+IR (G, H). Note the presence of intra-alveolar hemorrhage (arrows) and mononüclear/neutrophilic cell infiltration (arroheads), thickened alveolar wall and edematous septum (asterix) in the Control-saline+IR group. Treatment groups (E-H) exhibit structural integrity and arranged alveolar epithelium. H&E. Pictures from left column is 200X, right colum is 400X magnification.

DISCUSSION

Tang et al.[22] have stated that infrarenal aortic cross-clamping is a standard procedure during infrarenal vascular operations. It often causes IR injury to lower limbs, resulting in systemic inflammatory response and damage to remote organs, particularly lungs.

Acute lung injury as a remote sequela of severe lower torso IR has been demonstrated experimentally in a process involving leukosequestration and generation of the arachidonate derivatives thromboxane and leukotriene B4. The lung injury was characterized by progressive hypoxemia, pulmonary hypertension, decreased lung compliance, and non-hydrostatic pulmonary edema, consistent with adult respiratory distress syndrome. Their report has reinforced the concept that humoral mediators generated at reflow might induce end-organ injury at a site remote from the focus of IR, and that the lung was a target organ[23].

The clamp-induced increase in cardiac afterload raises mean arterial pressure, causing shifts in blood volume and increasing myocardial O_2 demand, which can lead to left ventricular decompensation and failure. Visceral IR injury is a significant adverse effect of aortic cross-clamping, both in and of themselves, and because visceral ischemia can promote systemic coagulopathy. Shunting and left heart bypass can be used to minimize the duration of visceral ischemia, while serial abdominal examinations and blood gas monitoring should be used postoperatively to detect any visceral ischemic injury[1].

If we look at the operation at the cellular level; ischemia is a state where inadequate or interrupted blood flow leads to intracellular O_2 depletion and a subsequent decrease in oxidative phosphorylation and ATP depletion; this situation leads to a loss of cell membrane integrity, intracellular swelling and

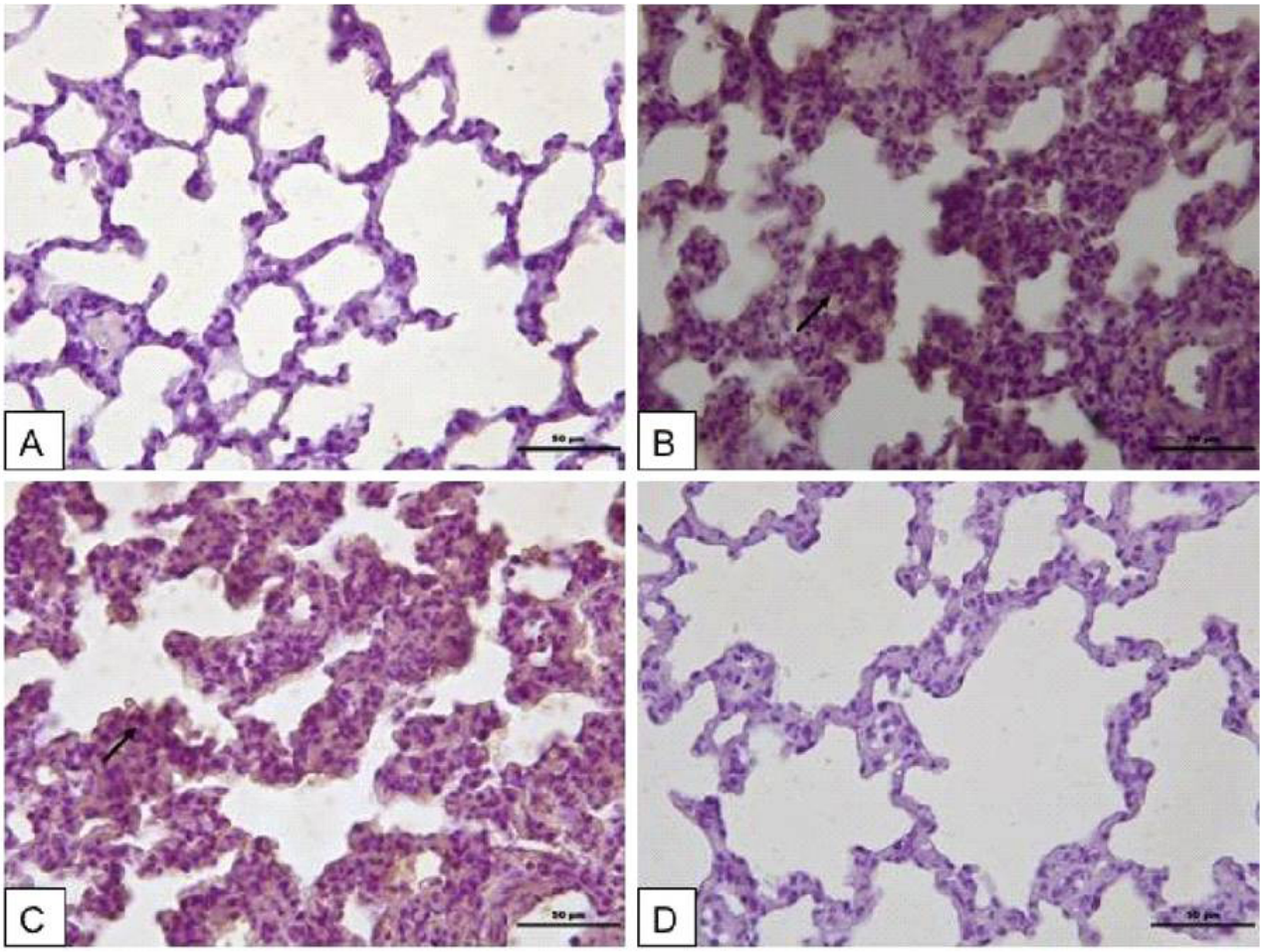

FIGURE 2. PHOTOMICROGRAPHS OF LUNG TISSUE APPLIED TO CASPASE-3 IMMUNOHISTOCHEMISTRY.
Sham-control (A); Control-Saline + IR (B); Ticagrelor+IR (C) and Enoxaaparin+IR (D) groups. Arrows indicate the caspase-3 immunoreactivity of apoptotic cells. 400X.

derangement in cellular Ca^{++} homeostasis. Increased intracellular Ca^{++} activates cytosolic phospholipases and proteases, leading to cell membrane disruption and activation of apoptotic and necrotic pathways[24]. While ischemia primes the cells for damage, the actual injury usually manifests after the restoration of blood flow and tissue oxygenation. The key mechanism of tissue injury is the intense and excessive inflammatory response to reperfusion. In general, ROS generation and complement activation occur early during reperfusion[24]. It has been stated that lung injury was characterized in histological sections with diffuse lung inflammation, alveolar-capillary destruction, and alveolar flooding, resulting in respiratory failure[25,26]. In our histological study, we found lung injury such as intra-alveolar hemorrhage, inflammatory cell infiltration in the contol-saline+IR group. In the sections of this group, the alveolar wall was damaged, and alveolar epithelial cells were disorganized; leucocytes, cell shed debris, and apoptotic cells were present in many alveoli. In the study of Ibrahim et al.[27], lungs showed normal histological structures of the bronchioles, air alveoli, and blood vessels in both groups with no histopathological alterations after injection of control and Enoxaparin. They have not observed emboli or tissue infarction, macro- or microscopically in any of the samples. In our study, the alveolar wall and epithelial cells had more structural integrity, and there was no alveolar bleeding in the pretreatment groups of Ticagrelor and Enoxaparin. Lung sections of these groups were better than those of the control-saline+IR group. Inflammatory cells and apoptotic cells were lower in the pretreatment groups of Ticagrelor and Enoxaparin.

Lu et al.[28] have stated that low-molecular-weight heparin prevented cecal ligation and puncture (CLP)-induced acute lung injury in rats by anti-inflammatory coagulation. They have found that histology

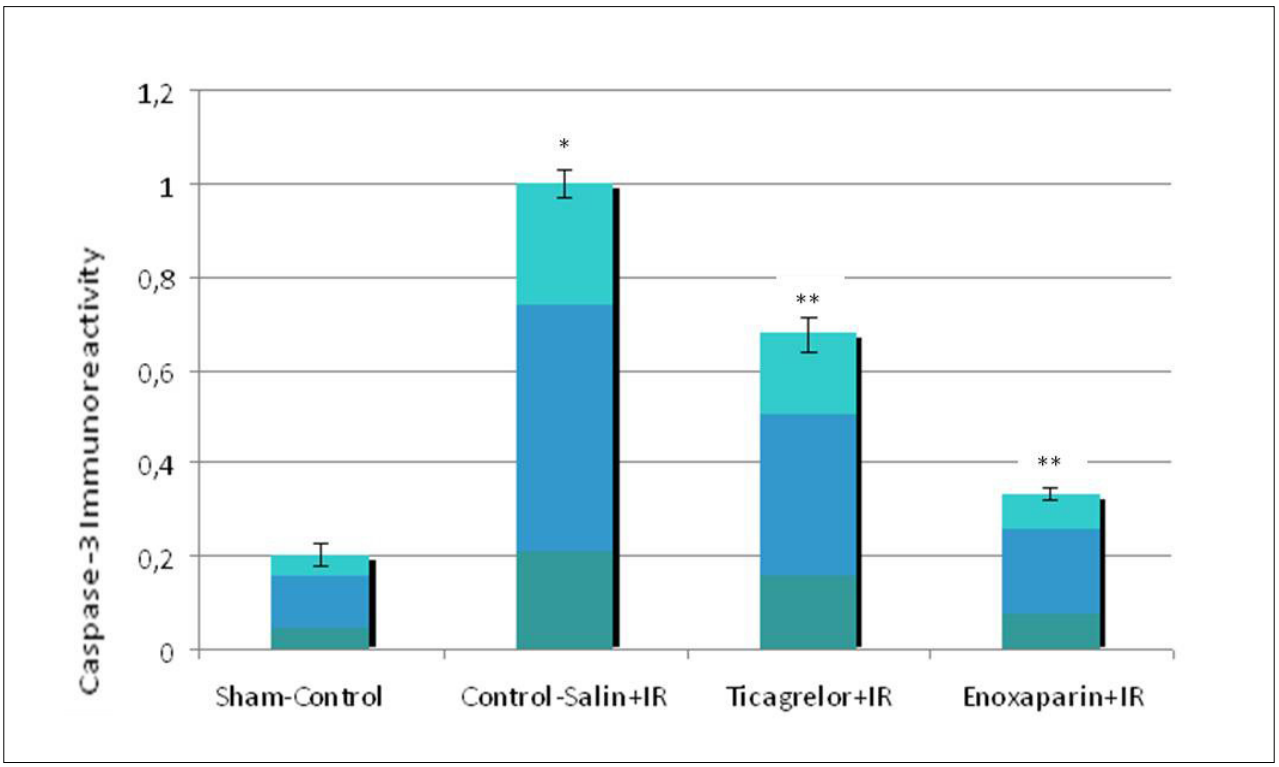

FIGURE 3. SEMIQUANTITATIVE IMMUNOLABELLING SCORE OF THE CASPASE-3 POSITIVE CELLS IN THE SHAM-CONTROL, CONTROL-SALIN+IR, TICAGRELOR+IR AND ENOXAPARIN+IR GROUPS. *P<0.01 COMPARED TO SHAM-CONTROL. ** P<0.01 COMPARED TO CONTROL-SALIN+IR.

scores, based on the number of areas with congestion, edema, Inflammation, and hemorrhaging, were all significantly higher after the administration of CLP than in the control group, and all of the scores were lower in the pretreated group. The findings of these researchers are similar to those of our study because enoxaparin had protective effects on the lung[28].

Findik et al.[29] studied the effect of a ticagrelor pretreatment on the prevention of lung injury induced by abdominal aorta IR. They observed obvious changes (atelectasis, thickening of the alveolar interwall, infiltration of inflammatory cells) in the saline-IR group compared to the sham group. Their findings showed that histological changes decreased in the group treated with 25 mg of ticagrelor compared with the saline IR and other treatment groups[29]. Therefore, we also studied the dose of 25 mg/kg ticagrelor and compared it with enoxaparin. Some investigators stated that heparin presented several biological activities such as anti-inflammatory action, immunological modulation, and activation of vascular endothelial growth factors, fibroblasts growth factors, and epidermal growth associated to heparin, all vital for healing[30-33]. In this study, light microscopic findings of the sections stained with H&E suggested that the pretreatment with enoxaparin and ticagrelor reduces the damage to the lungs in an IR rat model. Caspase-3 immunohistochemistry technique showed that the pre-treatment with enoxaparin was healthier than those of the other groups.

CONCLUSIONS

Lung sections of the ticagrelor and enoxaparin pretreatment groups were histologically similar to the healthy control group stained with H&E. Our light microscopic findings showed that pretreatment with enoxaparin and ticagrelor reduced lung damage in the IR rat model. Pretreatment with enoxaparin, in lung tissue sections, produced better results by Caspase-3 immunocytochemistry technique. In further studies, other parameters such as ROS and inflammatory gene expressions should be evaluated for accurate results.

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Author contributions

Conception and design: OF; Analysis and interpretation: MYY, YY, OF
Data collection: SFR, KK, MYY;
Writing the article: OF, MYY, OF, SFR;
Critical revision of the article: OF, MY;
Final approval of the article: OF;
Statistical analysis: KK, SFR;
Obtained funding: OF
Overall responsibility: OF, MYY, YY, ATK

RESUMO

OBJETIVOS: *Este estudo foi realizado para revelar os possíveis efeitos protetores do ticagrelor e do pré-tratamento da enoxaparina no tecido pulmonar contra o modelo de lesão induzida por isquemia-reperfusão (IR).*

MÉTODOS: *Ratos albinos Wistar foram randomizados e divididos em quatro grupos: grupo 1 (controle-sham), grupo 2 (controle-salina + IR), grupo 3 (ticagrelor + IR), grupo 4 (enoxaparina + IR). Antes do período isquêmico, salina, ticagrelor e enoxaparina foram administrados nos grupos 2-4, respectivamente. Nesses grupos, a lesão de IR foi induzida pelo clampeamento da aorta na região da infrarrenal por duas horas, seguida por quatro horas de reperfusão, exceto no grupo 1. Após a sacrificação, os pulmões foram processados para exames histológicos. Secções de parafina foram coradas com hematoxilina e eosina (H&E) para observação microscópica de luz. A apoptose foi avaliada pela imunorreatividade da caspase-3. Os dados foram analisados estatisticamente pelo programa SPSS.*

RESULTADOS: *Nas secções pulmonares coradas com H&E, estrutura histológica normal foi observada no grupo 1, enquanto células epiteliais desorganizadas, hemorragia e infiltração de células inflamatórias foram observadas na parede alveolar no grupo 2. A estrutura histológica dos grupos de tratamento foi melhor que o grupo 2. Células apoptóticas caspase-3 (+) foram notadas em secções do grupo 2, e essas células foram mais baixas nos grupos de tratamento. No grupo 4, a imunocoloração com caspase-3 foi menor que no grupo 3. No grupo 2, as células apoptóticas foram significativamente maiores que nos outros grupos (p<0,001).*

CONCLUSÃO: *Com base nos resultados histológicos, sugerimos que ambas as terapias atenuaram os efeitos prejudiciais da RI. Resultados de imuno-histoquímica com caspase-3 também revelaram que o pré-tratamento com enoxaparina proporcionou melhores resultados no modelo de lesão induzida por IR. Em estudos posteriores, outros parâmetros, como ROS e expressões gênicas inflamatórias, devem ser avaliados quanto a resultados precisos.*

PALAVRAS-CHAVE: *Pulmão. Enoxaparin. Ticagrelor. Traumatismo por reperfusão. Ratos Wistar.*

REFERENCES

1. Cuzick LM, Lopez AR, Cooper JR. Pathophysiology of aortic cross-clamping. In: Chiesa R, Melissano G, Zangrillo A, eds. Thoraco-abdominal aorta: surgical and anesthetic management. Milão: Springer-Verlag; 2011. pp.65-72.
2. Klausner JM, Paterson IS, Valeri CR, Shepro D, Hechtman HB. Limb ischemia-induced increase in permeability is mediated by leukocytes and leukotrienes. Ann Surg. 1988;208(6):755-60.
3. Klausner JM, Paterson IS, Kozbik L, Valeri R, Shepro D, Hetchtman HB. Oxygen free radicals mediate ischemia-induced lung injury. Surgery. 1989;105(2 Pt 1):192-9.
4. Gyurkovics E, Aranyi P, Stagni R, Onody P, Ferreira G, Lotz G, et al. Postconditioning of the lower limb: protection against the reperfusion syndrome. J Surg Res. 2011;169(1):139-47.
5. Katseni K, Chalkias A, Kotsis T, Dafnios N, Arapoglou V, Kaparos G, et al. The effect of perioperative ischemia and reperfusion on multiorgan dysfunction following abdominal aortic aneurysm repair. Biomed Res Int. 2015;2015:598980.
6. Kalogeris T, Bao Y, Korthuis RJ. Mitochondrial reactive oxygen species: a double edged sword in ischemia/reperfusion vs preconditioning. Redox Biol. 2014;2:702-14.
7. Yassin MM, Harkin DW, Barros D'Sá AA, Halliday MI, Rowlands BJ. Lower limb ischemia-reperfusion injury triggers a systemic inflammatory response and multiple organ dysfunction. World J Surg. 2002;26(1):115-21.
8. Carvalho AC, Guillaumon AT, Cintra EA, Figueiredo LC, Moreira MM, Araújo S. Plasmatic vasopressin in patients undergoing conventional infra-renal abdominal aorta aneurysm repair. Rev Bras Cir Cardiovasc. 2011;26(3):404-12.
9. Cattaneo M. The platelet $P2Y_{12}$ receptor for adenosine diphosphate: congenital and drug-induced defects. Blood. 2011;117(7):2102-12.
10. Catteneo M, Schulz R, Nylander S. Adenosine-mediated effects of ticagrelor: evidence and potential clinical relevance. J Am Coll Cardiol. 2014;63(23):2503-9.
11. Wallentin L, Becker RC, Budaj A, Cannon CP, Emanuelsson H, Held C, et al; PLATO Investigators. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. N Engl J Med. 2009;361(11):1045-57.
12. Armstrong A, Summers C, Ewart L, Nylander S, Sidaway JE, van Giezen JJ. Characterization of the adenosine pharmacology of ticagrelor reveals therapeutically relevant inhibition of equilibrative nucleoside transporter. J Cardiovasc Pharmacol Ther. 2014;19(2):209-19.
13. Van Giezen JJ, Sidaway J, Glaves P, Kirk I, Björkman JA. Ticagrelor inhibits adenosine uptake in vitro and enhances adenosine-mediated hyperemia responses in a canine model. J Cardiovasc Pharmacol Ther. 2012;17(2):164-72.
14. Headrick JP, Lasley RD. Adenosine receptors and reperfusion injury of the heart. Handb Exp Pharmacol. 2009;(193):189-214.
15. Csóka B, Németh ZH, Rosenberger P, Eltzschig HK, Spolarics Z, Pacher P, et al. A2B adenosine receptors protect against sepsis-induced mortality by dampening excessive inflammation. J Immunol. 2010;185(1):542-50.
16. Harter K, Levine M, Henderson SO. Anticoagulation drug therapy: a review. West J Emerg Med. 2015;16(1):11-7.
17. Silvain J, Beygui F, Barthélémy O, Pollack C Jr, Cohen M, Zeymer U, et al. Efficacy and safety of enoxaparin versus unfractionated heparin during percutaneous coronary intervention: systematic review and meta-analysis. BMJ. 2012;344:e553.
18. E-MedicineHealth. Enoxaparin (Lovenox). [cited 2019 Feb 5]. Available from: https://www.emedicinehealth.com/drug-enoxaparin/article_em.htm.
19. Li CJ, Yang ZH, Shi XL, Liu DL. Effects of aspirin and enoxaparin in a rat model of liver fibrosis. World J Gastroenterol. 2017;23(35):6412-9.
20. Szapiel SV, Elson NA, Fulmer JD, Hunninghake GW, Crystal RG. Bleomycin-induced interstitial pulmonary diseases in the nude, athymic mouse. Am Rev Resp Dis. 1979;120(4):893-9.
21. Huang JS, Yang CM, Wang JS, Liou HH, Hsieh IC, Li GC, et al. Caspase-3 expression in tumorigenesis and prognosis of buccal mucosa squamous cell carcinoma. Oncotarget. 2017;8(48):84237-47.

22. Tang B, Ma L, Yao X, Tan G, Han P, Yu T, et al. Hydrogen sulfide ameliorates acute lung injury induced by infrarenal aortic cross-clamping by inhibiting inflammation and angiopoietin 2 release. J Vasc Surg. 2017;65(2):501-8.

23. Fantini GA, Conte MS. Pulmonary failure following lower torso ischemia: clinical evidence for a remote effect of reperfusion injury. Am Surg. 1995;61(4):316-9.

24. Nieuwenhuijs VB, De Bruijn MT, Padbury RT, Barritt GJ. Hepatic ischemia-reperfusion injury: roles of Ca2+ and other intracellular mediators of impaired bile flow and hepatocyte damage. Dig Dis Sci. 2006;51(6):1087-102.

25. An J, Park SH, Ko IG, Jin JJ, Hwang L, Ji ES, et al. Polydeoxyribonucleotide ameliorates lipopolysaccharide-induced lung injury by inhibiting apoptotic cell death in rats. Int J Mol Sci.2017;18(9). pii: E1847.

26. Matute-Bello G, Frevert CW, Martin TR. Animal models of acute lung injury. Am J Physiol Lung Cell Mol Physiol. 2008;295(3):L379-99.

27. Ibrahim SS, Osman R, Mortada ND, Geneidy AS, Awad GA. Passive targeting and lung tolerability of enoxaparin microspheres for a sustained antithrombotic activity in rats. Drug Deliv. 2017;24(1):243-51.

28. Lu X, Zhao L, Xu YH. Low molecular weight heparin prevents CLP-induced acute lung injury in rats by anti-inflammatory coagulation. Bosn J Basic Med Sci. 2013;13(1):50-6.

29. Findik O, Kunt AT, Yazir Y, Yardimoğlu M, Yilmaz SG, Aydin U, et al. Ticagrelor attenuates apoptosis of lung and myocardial cells induced by abdominal aorta ischemia/reperfusion. In Vivo. 2016;30(3):243-9.

30. Levine A, Kenet G, Bruck R, Avni Y, Avinoach I, Aeed H, et al. Effect of heparin on tissue binding activity of fibroblast growth factor and heparin-binding epidermal growth factor in experimental colitis in rats. Pediatr Res. 2002;5(51):635-40.

31. Street JT, McGrath M, O'Regan K, Wakai A, McGuinness A, Redmond HP. Thromboprophylaxis using a low molecular weight heparin delays fracture repair. Clin Orthop Relat Res. 2000;(381):278-89.

32. Michell NP, Lalor P, Langman MJ. Heparin therapy for ulcerative colitis? Effects and mechanisms. Eur J Gastroenterol Hepatol. 2001;13(4):449-56.

33. Galvan L. Effects of heparin on wound healing. J Wound Ostomy Continence Nurs. 1996;23(4):224-6.

ORIGINAL ARTICLE

TEP versus Lichtenstein, which one to choose? A retrospective cohort study

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SUMMARY

OBJECTIVES: *Inguinal hernioplasty techniques have been improved since the first hernioplasty. Tension-free techniques that apply synthetic mesh materials, as in the Lichtenstein approach, are the gold standard. Laparoscopic hernioplasty is the strongest alternative to Lichtenstein. The superiority of laparoscopic hernioplasty over Lichtenstein is a major topic of debate. In this study, we aimed to find a conclusion to this debate by comparing our totally extraperitoneal (TEP) experiences with Lichtenstein experiences.*

METHODS: *Patients who underwent inguinal hernioplasty at the Gulhane Training and Research Hospital from 2013 to 2018 were included in this retrospective cohort study. The sample included 96 TEP and 90 Lichtenstein patients for a total of 186 patients. The variables assessed were hospitalization duration, postoperative early visual analog scale score, chronic pain, paresthesia, recurrence, and early postoperative complications. Data were collected from patient records and via telephone questionnaire if needed. Data analysis was done by SPSS v20, using chi-square, Fisher's exact, and Mann-Whitney U tests.*

RESULTS: *Male/female ratios were similar between the TEP and Lichtenstein groups. There was no difference in mean age between groups (p=0.1). The hospital stay was shorter (p=0.0001), and early postoperative visual analog scale score was lower in the TEP group (p=0.003). Chronic pain, paresthesia, recurrence, and early postoperative complications (hematoma, seroma, wound infection) were similar.*

CONCLUSIONS: *TEP is superior to Lichtenstein with shorter hospitalization duration and lower rates of early postoperative pain. No difference between the two techniques was found for chronic pain. We believe that laparoscopic hernioplasty approach may be the best alternative technique for inguinal hernia repair.*

KEYWORDS: *Herniorrhaphy. Laparoscopy. Hernia, Inguinal/surgery.*

INTRODUCTION

Inguinal hernia repair is one of the most common surgical procedures performed by general surgeons. The technique used for inguinal hernias has changed since the beginning of hernia repair, with the first hernia terminology defined in 1552 B.C. in Erb's papyrus[1]. Many techniques have since been described for inguinal hernia repair, and many modifications have been applied to these techniques. After the invention of biocompatible synthetic meshes, new techniques such as Lichtenstein, offering tension-free hernioplasty with low rates of recurrence and high postoperative quality of life, became the

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gold standard for inguinal hernioplasties. However, a low recurrence rate and high postoperative quality of life are not the only parameters in achieving excellent inguinal hernia repair. Length of hospital stay, postoperative complication rate, and cost are also important in determining the perfect hernioplasty technique.

Lichtenstein was the tension-free gold standard technique for hernia repair but regressed from being a favorite when minimally invasive approaches in surgery came into fashion. Laparoscopic hernioplasty techniques such as totally extra-peritoneal (TEP) and trans-abdominal pre-peritoneal (TAPP) are accepted by many surgeons and have begun to be applied in many centers. As a result of recent developments in hernia repair, the current major debate about inguinal hernioplasty is which technique is more feasible, a tension-free open conventional technique or a laparoscopic technique, although both are accepted as standard methods of inguinal hernia repair[2]. The conventional technique currently insists on a tension-free approach, and the most commonly applied is Lichtenstein. TAPP and TEP are tension free also because no repair is applied to the hernia defect; instead, a mesh is laid over the defect and fixed to the fascia in current laparoscopic techniques (Figure 1-3).

Conventional tension-free repair (Lichtenstein) was first described in 1989 for anterior mesh hernioplasty[3]. Laparoscopic techniques, however, are used for posterior hernioplasty, which is built upon the Stoppa et al.[4] approach with the development of laparoscopic technologies. Studies have shown that the major advantage of laparoscopic repair over conventional repair is its shorter recovery times and hospital length of stay[5,6].

Laparoscopic approaches also have the advantage of better cosmetic results with a 3-trocar approach to the abdomen, for an 11 to 25 mm incision instead of the average 50 to 80 mm incision. Differences between the two techniques in terms of other parameters such as relapses, postoperative visual analog scale (VAS) scores, and complication rates are still being debated; no consensus has been reached because the experience of the surgeon and the surgical techniques used can affect the balance[7-9]. Thus, in our study, we compared the TEP technique, which is used as a laparoscopic technique, in our center with the Lichtenstein repair. Operations were performed by two experienced hernia surgeons with standardized techniques, and results were compared for the parameters that are currently debated.

METHODS

Patients who underwent hernia repair by two surgeons at the Gulhane Training and Research Hospital between January 2013 and April 2018 were included in the study. Both surgeons applied polypropylene-based meshes and fixated them with laparoscopic fixators as tacks, instead of intracorporal saturation. Data of the patients were collected retrospectively from hospital records, and postoperative long-term follow-up data were collected by telephone questionnaire. Our study is a retrospective cohort study that included follow-up results of 96 TEP and 90 Lichtenstein procedures, for a total of 186 patients who underwent hernioplasty. The mean follow-up duration was 21.93 months. Demographic data such as age and sex and data related to the type of the hernia, postoperative VAS score, conversion to the conventional technique, early postoperative complications, hospitalization duration, and type of surgery were obtained from hospital records. Long term follow-up parameters, including chronic pain, paresthesia, and relapse, were collected by telephone questionnaire and by radiological test results if needed. All data obtained were noted on patient sheets and were organized in Microsoft Excel 2010 (Redmond, Washington, U.S.). The independent variable in this study was the type of surgery (TEP or Lichtenstein), and the dependent variables were postoperative hospitalization duration, VAS score, chronical pain, paresthesia, relapse, and early postoperative complications. Data analysis was done using SPSS v.20 (New York, U.S.). Chi-square, Fisher's exact, and Mann-Whitney U tests were used for statistical evaluation.

RESULTS

Our study compared only newly diagnosed inguinal hernias repaired with the TEP or Lichtenstein technique. No recurrent hernias were evaluated in our study. The 186 patients in the study had inguinal hernioplasty for direct, indirect, or combined inguinal hernia. None of the patients had femoral hernias. A total of 96 (51.6%) patients were operated with the TEP technique and 90 (48.4%) with the Lichtenstein conventional technique. None of the TEP operations were converted to Lichtenstein, so there was no con-

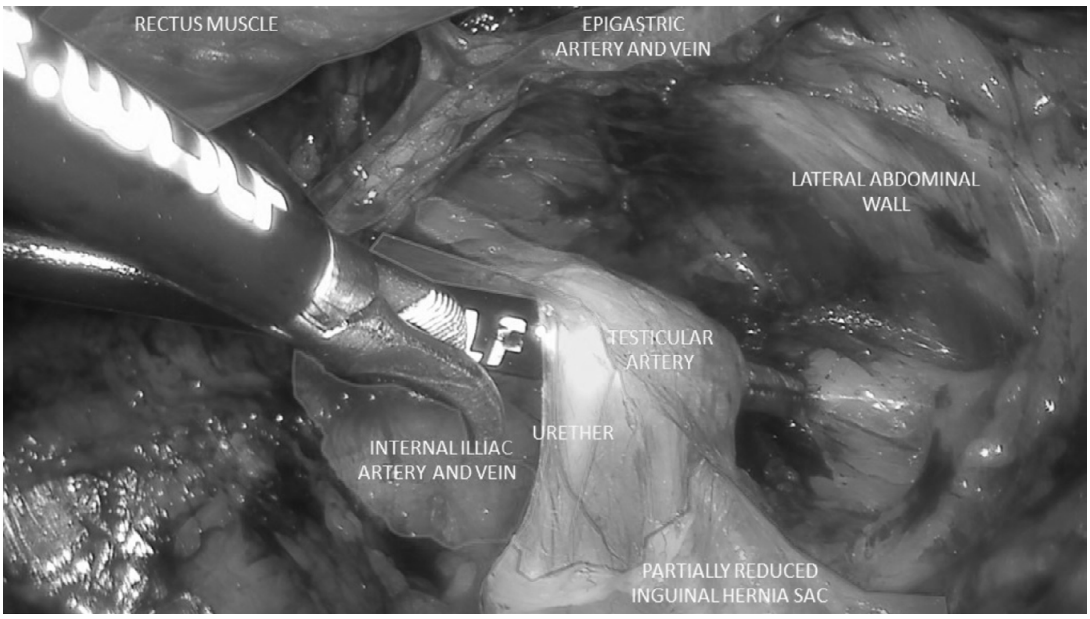

FIGURE 1

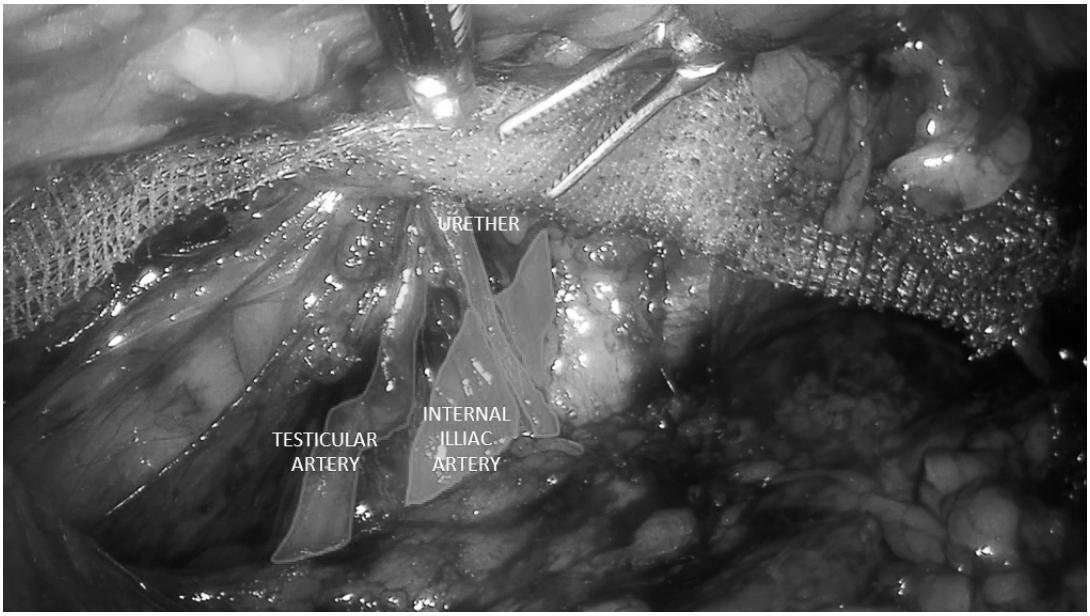

FIGURE 2

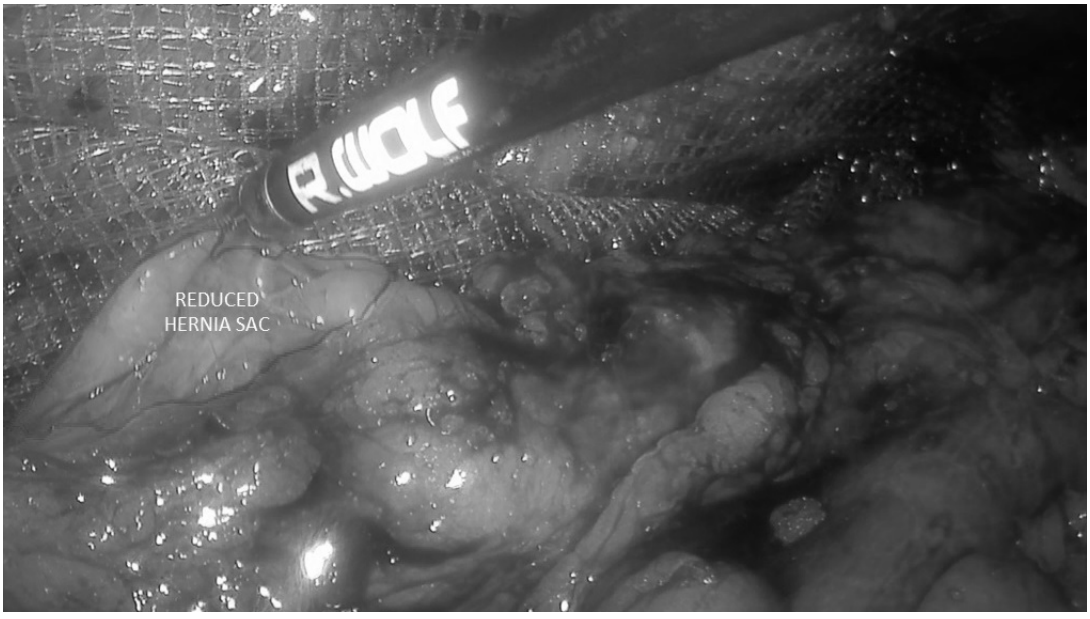

FIGURE 3

version to open hernioplasty. The mean follow-up duration was 21.93 (3-66) months. Ten (10.4%) patients from the TEP group and 9 (10.0%) from the Lichtenstein group, totaling 19 (10.2%) patients, underwent surgery for bilateral inguinal hernia. These patients were considered to have one hernia, and relapses or complications that occurred on one side or both sides were considered a relapse or complication.

The gender of the patients was analyzed; 171 (91.9%) were male, and 15 (8.1%) female. We found no statistically significant difference between the two groups in terms of gender (X2=.881, p=0.348).

The mean age of the patients was 48.7 (18.0-83.7) years. The mean age was 46.7 (18.0-80.3) years for the TEP group and 50.8 (19.9-83.7) for the Lichtenstein group. There was no statistical difference between the groups (p=0.1). Mean length of hospital stay was 2.0 days (1-7): 1.6 (1-5) days for the TEP group and 2.4 (1-7) for the Lichtenstein group. The difference between the two groups was statistically significant (p=0.0001). Patients were evaluated on the postoperative day 1 for the VAS score. The mean VAS score was 1.9 (1-5) for the TEP group and 2.3 (1-5) for the Lichtenstein group. The difference between the two groups was statistically significant (p=0.003) (Table 1).

Patients were evaluated for chronic pain, paresthesia, and relapse; 24 (12.9%) patients reported postoperative chronic pain of different severities, 9 (9.3%) from the TEP group and 15 (16.6%) from the Lichtenstein group. There was no statistically significant difference between groups (x2=2.198, p=0.138). The two groups were evaluated for postoperative long-term paresthesia. A total of 19 (10.2%) patients had paresthesia, 6 (6.3%) from the TEP group, and 13 (14.4%) from the Lichtenstein group. There was no significant difference between the two groups (x2=3.40, p=0.065).

Total relapses were seen in 8 (4.30%) patients, 3 (3.13%) from the TEP group, and 5 (5.56%) from the Lichtenstein group. Relapses were confirmed with ultrasound, and there was no significant difference between the groups (Fisher exact test, p=0.48). Relapses were re-operated according to patient's request by the same surgeon with the open technique.

Postoperative early complications (hematoma, seroma, and wound infection) were evaluated (Table 2). There were no significant differences between the two groups according to total postoperative complications (x2=0.334, p=0.563).

DISCUSSION

Inguinal hernia is one of the most common ailments treated by general surgeons. For this reason, determining the best hernioplasty technique affects many people. Recent consensus for inguinal hernioplasty is on a tension-free approach due to its low recurrence rates and better postoperative quality of life[1]. Liechtenstein has been the gold standard for conventional tension-free hernioplasties for more than three decades[11,12]. Because a minimally invasive surgical approach is the currently preferred approach for many operations, TEP is debated as the new gold standard for hernioplasty if expertise is available[2]. As a result, comparing the conventional Liechtenstein technique with minimally invasive TEP is a vital topic.

A total of 186 patients, 96 with TEP and 90 with Liechtenstein, were compared for age and sex, and no statistically significant difference was found (p=0.1, p=0.34 respectively). The two groups were also similar in terms of the rate of bilateral hernioplasties (10%). As a result of these similarities, the two groups were suitable for comparison of the remaining parameters.

The two groups were compared for hospitalization duration, which was statistically significantly shorter in the TEP group than in the Lichtenstein group (p=0.0001). Short hospitalization duration may be an indicator of lower complication rates and enhanced recovery. Even though TEP is accepted as a higher-cost operation than Lichtenstein[13], enhanced recovery after TEP may decrease its cost. Further cost analysis should be done to reach a conclusion[1].

According to our results, postoperative early VAS scores were lower in the TEP group (p=0.003) (Table 1), as in the study by O'Reilly et al.[1] This is also the probable reason for the statistically significant lower length of hospital stay in the TEP group. However,

TABLE 1. DISTRIBUTION OF AGE, LENGTH OF STAY, AND POSTOPERATIVE VAS SCORES OF THE PATIENTS.

	TEP Mean (min-max)	Lichtenstein Mean (min-max)	Total Mean (min-max)	p value
Age (years)	46.7 (18-80)	50.8 (19-83)	48.7 (18-83)	p=0.1
Length of stay (days)	1.60 (1-5)	2.44 (1-7)	2.01 (1-7)	p=0.0001
Postoperative VAS score	1.93 (1-5)	2,30 (1-5)	2,10 (1-5)	p=0.003

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the main debate in comparing open hernioplasty to laparoscopic hernioplasty is not early postoperative pain but chronic postoperative pain, which is one of the major complications of inguinal hernioplasty. Our study found lower chronic postoperative pain in the TEP group (9.3%) than in the Lichtenstein group (16.6%), as in many studies, but there was no statistically significant difference between the groups.

Manangi et al.[1] studied the reasons for postoperative pain after inguinal hernioplasty and found that preoperative pain is statistically strongly significant for the development of postoperative pain, which probably is related to the patient's pain threshold level. The relationship of preserving or scarifying the regional sensory nerves at the operation site is a matter of debate for postoperative chronic pain. Some studies conclude that preserving or scarifying nerves increases chronic postoperative pain; for this reason, prophylactic neurectomy and pragmatic neurectomy are being debated, without any high-quality evidence[17-19]. This is a debate for neuropathic pain. The incorporation of nerves with staplers, sutures, or mesh is another major cause of neuropathic postoperative chronic pain. Ilioinguinal, iliohypogastric, genitofemoral, and lateral femoral cutaneous nerves are commonly involved. Somatic pain can also be the reason for postoperative pain. Reaction to the prosthetic mesh material, osteitis pubis, and tendon-muscle injuries during surgery may be the reason why somatic pain is more common than neuropathic pain[2].

TEP has an advantage over Lichtenstein because it interferes less with the chronic pain etiologies discussed above. Because TEP is a posterior hernioplasty, the surgeon is unlikely to dissect regional sensory nerves. As a note, surgeons must be careful of the pain triangle during TEP. Also, staplers and sutures applied for mesh fixation in TEP can barely incorporate the nerves. Furthermore, because no primary repair to the hernia defect is applied through muscles and tendons, the incidence of somatic pain also decreases. All these factors also decrease the rate of early postoperative pain. However, we could not find a statistically significant difference between postoperative chronic pain with open and laparoscopic inguinal hernioplasty.

Laparoscopic posterior hernioplasty has the advantage of less muscle and tendon injury during dissection and lower incorporation of regional sensory nerves, which may result in less postoperative paresthesia. As our clinic routine, we give importance to preserving the local sensory nerves during Lichtenstein hernioplasty. We have a lower rate of postoperative paresthesia with TEP (6.3%) than with Lichtenstein (14.4%), but there was no statistically significant difference between the two groups (p=0.065). Douek et al.[21] studied paresthesia in open vs. laparoscopic inguinal hernioplasty and found that 12 of 242 patients had paresthesia five years after hernioplasty, which decreases the quality of life of the patients. All patients with paresthesia underwent open hernioplasty, and they concluded that the laparoscopic approach is strongly related to lower rates of postoperative paresthesia. Our study confirms the findings of Douek et al.[21], but unfortunately, we could not prove this statistically. Postoperative recurrence rates of both groups were in acceptable ranges, and there was no statistically significant difference between them (p=0.48) (Table 2). One of the largest systemic reviews on the topic by McCormack et al.[22], with 7161 patients from 41 studies in 2003, found no statistically significant difference for postoperative hernia recurrence between open and laparoscopic hernioplasty approaches (p=0.16), as in this study. The laparoscopic approach had the same or lower recurrence rates compared with the Lichtenstein technique, which endorses the laparoscopic technique for becoming the gold

TABLE 2. POSTOPERATIVE COMPLICATIONS PER HERNIOPLASTY TECHNIQUE.

Type of complication	TEP n (%)	Lichtenstein n (%)	Total n (%)	p-value
Chronical pain	9 (%9.3)	15 (%16.6)	24 (%12.9)	p=0.138
Long term paresthesia	6 (%6.25)	13 (%14.44)	19 (%10.21)	p=0.065
Relapse hernia	3 (%3.13)	5 (%5.56)	8 (%4.3)	p=0.48
Postoperative early complications (Hematoma, seroma, wound infection)	11 (%11.4)	8 (%8.8)	19 (%10.2)	p=0.563

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standard in inguinal hernioplasty. However, in 2012, O'Reilly et al.[1] found that the recurrence rate of TAPP is equal to that of the open technique and is statistically higher than the other two techniques. The common attitude about recurrence rates of hernioplasty according to current data is that they are accepted as equal for laparoscopic and open hernioplasty techniques.

The TEP and Lichtenstein groups were compared for early postoperative complications such as hematoma, seroma, and operation site infections, and there was no statistically significant difference between the two groups (p=0.563) (Table 2). Koning et al.[23] studied severe adverse effects of hernioplasty with the conventional and laparoscopic techniques; their systematic review showed that there is no significant difference between them in terms of severe adverse effects.

CONCLUSION

In conclusion, chronic pain, long-term paresthesia, relapses, and early postoperative complications were not different between the groups. The major differences between the groups were early postoperative VAS scores and length of stay, which were lower in the TEP group. These results may be the reason why surgeons prefer a minimally invasive hernioplasty approach instead of the conventional technique and why the minimally invasive approach should become the gold standard hernioplasty technique.

Authors Contribution

Yasar Subutay Peker was responsible for data analysis and writing the manuscript, and Murat Urkan was responsible for data collection and final revision of the manuscript.

RESUMO

OBJETIVOS: *As técnicas de hernioplastia inguinal foram melhoradas desde a primeira hernioplastia. Técnicas livres de tensão que aplicam materiais de malha sintética, como na abordagem de Lichtenstein, são o padrão ouro. A hernioplastia laparoscópica é a alternativa mais forte ao Lichtenstein. A superioridade da hernioplastia laparoscópica sobre o Lichtenstein é um dos principais temas debatidos. Neste estudo, procuramos encontrar uma conclusão para esse debate comparando nossas experiências totalmente extraperitoneais (TEP) com as experiências de Lichtenstein.*

MÉTODOS: *Pacientes submetidos à hernioplastia inguinal no Gulhane Training and Research Hospital de 2013 a 2018 foram incluídos neste estudo de coorte retrospectivo. A amostra incluiu 96 pacientes TEP e 90 pacientes Lichtenstein para um total de 186 pacientes. As variáveis avaliadas foram tempo de internação, escore da escala analógica visual precoce no pós-operatório, dor crônica, parestesia, recidiva e complicações pós-operatórias precoces. Os dados foram coletados dos prontuários e do questionário por telefone, se necessário. A análise dos dados foi realizada pelo SPSS v20, utilizando os testes qui-quadrado, exato de Fisher e U de Mann-Whitney.*

RESULTADOS: *As razões homem/mulher foram semelhantes entre os grupos TEP e Lichtenstein. Não houve diferença na média de idade entre os grupos (p=0,1). A permanência hospitalar foi menor (p=0,1) e a escala visual analógica precoce foi menor no grupo TEP (p=0,003). Dor crônica, parestesia, recorrência e complicações pós-operatórias imediatas (hematoma, seroma, infecção da ferida) foram semelhantes.*

CONCLUSÕES: *O TEP é superior ao Lichtenstein, com menor tempo de internação e menores taxas de dor pós-operatória precoce. Nenhuma diferença entre as duas técnicas foi encontrada para dor crônica. Acreditamos que a abordagem de hernioplastia laparoscópica pode ser a melhor técnica alternativa para correção de hérnia inguinal.*

PALAVRAS-CHAVE: *Herniorrafia. Laparoscopia. Hérnia inguinal/cirurgia.*

REFERENCES

1. Skandalakis LJ. Skandalakis surgical anatomy: the embryologic and anatomic basis of modern surgery. Athens: Paschalidis Periklis Ch Ltd Medical Publications; 2008.
2. Simons MP, Aufenacker T, Bay-Nielsen M, Bouillot JL, Campanelli G, Conze J, et al. European Hernia Society guidelines on the treatment of inguinal hernia in adult patients. Hernia. 2009;13(4):343-403.
3. Lichtenstein IL, Shulman AG, Amid PK, Montllor MM. The tension-free hernioplasty. Am J Surg. 1989;157(2):188-93.
4. Stoppa R, Petit J, Henry X. Unsutured Dacron prosthesis in groin hernias. Int Surg. 1975;60(8):411-2.
5. Wake BL, McCormack K, Fraser C, Vale L, Perez J, Grant AM. Transabdominal pre-peritoneal (TAPP) vs totally extraperitoneal (TEP) laparoscopic techniques for inguinal hernia repair. Cochrane Database Syst Rev. 2005;(1):CD004703.
6. Myers E, Browne KM, Kavanagh DO, Hurley M. Laparoscopic (TEP) versus Lichtenstein inguinal hernia repair: a comparison of quality-of-life outcomes. World J Surg. 2010;34(12):3059-64.
7. Schmedt CG, Sauerland S, Bittner R. Comparison of endoscopic procedures vs Lichtenstein and other open mesh techniques for inguinal hernia repair: a meta-analysis of randomized controlled trials. Surg Endosc. 2005;19(2):188-99.

8. Eklund AS, Montgomery AK, Rasmussen IC, Sandbue RP, Bergkvist LA, Rudberg CR. Low recurrence rate after laparoscopic (TEP) and open (Lichtenstein) inguinal hernia repair: a randomized, multicenter trial with 5-year follow-up. Ann Surg. 2009;249(1):33-8.

9. Butters M, Redecke J, Köninger J. Long-term results of a randomized clinical trial of Shouldice, Lichtenstein and transabdominal preperitoneal hernia repairs. Br J Surg. 2007;94(5):562-5.

10. HerniaSurge Group. International guidelines for groin hernia management. Hernia. 2018;22(1):1-165.

11. Bay-Nielsen M, Kehlet H, Strand L, Malmstrøm J, Andersen FH, Wara P, et al. Quality assessment of 26,304 herniorrhaphies in Denmark: a prospective nationwide study. Lancet. 2001;358(9288):1124-8.

12. Bay-Nielsen M, Thomsen H, Andersen FH, Bendix JH, Sørensen OK, Skovgaard N, et al. Convalescence after inguinal herniorrhaphy. Brit J Surg. 2004;91:362-7.

13. Ielpo B, Nuñez-Alfonsel J, Duran H, Diaz E, Fabra I, Caruso R, et al. Cost-effectiveness of randomized study of laparoscopic versus open bilateral inguinal hernia repair. Ann Surg. 2018;268(5):725-30.

14. Roos MM. Improving TEP inguinal hernia repair [Dissertation]. Utrecht: Utrecht University; 2018.

15. O'Reilly EA, Burke JP, O'Connell PR. A meta-analysis of surgical morbidity and recurrence after laparoscopic and open repair of primary unilateral inguinal hernia. Ann Surg. 2012;255(5):846-53.

16. Manangi M, Shivashankar S, Vijayakumar A. Chronic pain after inguinal hernia repair. Int Sch Res Notices. 2014;2014:839681.

17. Smeds S, Löfström L, Eriksson O. Influence of nerve identification and the resection of nerves 'at risk' on postoperative pain in open inguinal hernia repair. Hernia. 2010;14(3):265-70.

18. Wijsmuller AR, van Veen RN, Bosch JL, Lange JFM, Kleinrensink GJ, Jeekel J, et al. Nerve management during open hernia repair - reply. Brit J Surg. 2007;94(1):17-22.

19. Alfieri S, Rotondi F, Di Giorgio A, Funagalli U, Salzano A, Di Miceli D, et al. Influence of preservation versus division of ilioinguinal, iliohypogastric, and genital nerves during open mesh herniorrhaphy: prospective multicentric study of chronic pain. Ann Surg. 2006;243(4):553-8.

20. Mazin JB. Causes of postoperative pain following inguinal hernia repair: what the literature shows. [cited 2019 Mar 5]. Available from: https://www.practicalpainmanagement.com/causes-postoperative-pain-following-inguinal-hernia-repair-what-literature-shows

21. Douek M, Smith G, Oshowo A, Stoker DL, Wellwood JM. Prospective randomised controlled trial of laparoscopic versus open inguinal hernia mesh repair: five year follow up. BMJ. 2003;326(7397):1012-3.

22. McCormack K, Scott NW, Go PM, Ross S, Grant AM; EU Hernia Trialists Collaboration. Laparoscopic techniques versus open techniques for inguinal hernia repair. Cochrane Database Syst Rev. 2003:CD001785.

23. Koning GG, Wetterslev J, Van Laarhoven CJ, Keus F. The totally extraperitoneal method versus Lichtenstein's technique for inguinal hernia repair: a systematic review with meta-analyses and trial sequential analyses of randomized clinical trials. PloS One. 2013;8(1):e52599.

Comments: "TEP versus Lichtenstein, Which One to Choose? A Retrospective Cohort Study"

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Minimally invasive approaches are the trending topic concerning surgery. They provide better postoperative patient satisfaction and lower hospitalization duration, resulting in lower costs. There are now minimally invasive approaches even for major surgeries such as hepatectomies and colectomies, so it is the time to revise the guidelines for inguinal hernioplasty surgical approaches. There is no doubt regarding the superiority of tension-free techniques for inguinal hernioplasty, but with the development of new surgical technologies, laparoscopic tension-free hernioplasties are the main rivals of conventional open tension-free hernioplasties.

The article "TEP versus Lichtenstein, Which One to Choose? A Retrospective Cohort Study" weighs in on laparoscopic inguinal hernioplasties by comparing it with conventional techniques1. This article tries to fill the gap in the literature around the superiority of laparoscopic inguinal hernioplasty. Future studies stemming from this one are likely to give surgeons enough confidence to update the current guidelines, which some have already started to change. Thus, I hope clinicians who read this article will highly benefit from the new perspective it presents to readers.

REFERENCE

1. Urkan M, Subutay PY. TEP versus Lichtenstein, which one to choose? A retrospective cohort study. *Rev Assoc Med Bras* 2019; 65(9)1201-1207

ORIGINAL ARTICLE

Adolescent pregnancy trends in the last decade

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SUMMARY

INTRODUCTION: *Teenage pregnancy is a universal phenomenon, with higher prevalence in developing countries. Although there has been a reduction in Brasil since the year 2000, the age-specific fertility rate for this age group remains high.*

OBJECTIVE: *To evaluate the frequency of adolescence pregnancy in in Brasil from 2006 to 2015 and its association with the Human Development Index (HDI).*

METHODS: *A descriptive epidemiological study, conducted by searching the database of the Department of Informatics of the Unified Health System (DATASUS), using information from the Information System on Live Births (SINASC) for the five Brazilian regions.*

RESULTS: *There was a reduction in the percentage of live births (LB) from adolescent mothers (10 to 19 years old) in Brasil by 13.0% over the last ten years. This decline was observed in all Brazilian regions among mothers aged 15 to 19 years. The number of LB increased by 5.0% among mothers aged 10 to 14 years in the North and decreased in the other regions, with higher rates in the South (18.0%). The specific fertility rate for the 15-19-year-old group decreased from 70.9/1,000 to 61.8/1,000 in the period. The proportion of LB is inversely associated with the HDI, except in the Northeast (the lowest HDI in the country), where there was a significant reduction (18.0%) among mothers aged 15-19 and 2% among those aged 10-14 years.*

CONCLUSION: *Teenage pregnancy in Brasil is in slow decline, especially among mothers aged 10-14 years and is inversely associated with the HDI, except in the Northeast.*

KEYWORDS: *Pregnancy in Adolescence. Prevalence. Epidemiology. Adolescent.*

INTRODUCTION

Worldwide, approximately 16 million girls aged between 15 and 19 years and 2 million girls younger than 15 years have children each year, with a higher frequency of live births (LB) from adolescent mothers in developing countries. Over half of the women in Africa and around one third in Latin America and the Caribbean will give birth before they are 20 years old.[1,2] In Brasil, approximately one in every five Brazilian women has their first child before the age of

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20, a proportion that has remained the same in the past ten years, despite the drop in the percentage of LB from adolescent mothers between 2000-2011. This reduction was noticed in all Brazilian regions for women aged between 15 and 19 years, but numbers increased in the North and Northeast region for ages between 10-14 years.[3]

According to the 2018 Report of the Pan American Health Organization/World Health Organization (PAHO/WHO), the United Nations Children's Fund (Unicef) and the United Nations Population Fund (UNFPA), the global rate of teenage pregnancy remains high, estimated at 46 births per 1,000 girls, while in Latin America and the Caribbean the rate remains the second-highest in the world, estimated at 66.5 births/1,000 women aged between 15 and 19 years, behind only Sub-Saharan Africa. The Brazilian rate is estimated at 68.4 births/1,000 adolescents.[4]

Poverty and low formal education levels, which are intrinsically intertwined, constitute the backdrop for of the countries where early pregnancy rates remain high, unlike what is observed in most developed countries.[4,5] Therefore, this should be treated as a public health problem, especially since it affects populations of developing countries. However, in certain cases, this may be the result of a decision by the teenager or of their own local culture, especially in countries of South Asia and Sub-Saharan regions.[5]

The objective of this paper is to determine the frequency of adolescence pregnancy in both age groups (10-14 and 15-19 years), in all five regions of Brasil, and investigate its association with the Human Development Index (HDI) of each region.

METHODS

This is a descriptive study based on data from the Information System on Live Births (SINASC) of the Single Health System Department of Informatics (DATASUS), a system managed by the Secretariat of Health Surveillance, along with state and municipal health secretariats. These institutions collect the Declarations of Live Births (DLB) from health services and notary offices (for home births) and input the data into the SINASC.[6]

The completion of a DLB is mandatory for the civil registry of a newborn. To prepare this study, we used the following variables: birth according to the place of residence of the mother, birth according to the region of Brasil, year of birth, and mother's age.[6]

The study included all women who had an LB in the years 2006 to 2015 in Brasil. We sought data on the total number of LB per region, as well as in the age ranges of 10-14 and 15-19 years, to calculate the percentage of LB from adolescent mothers. We excluded from the total of LB those whose mother's age was not reported (1,048 LB between 2006-2011 and 282 LB between 2012-2015). We also analyzed the association between the frequency of adolescence pregnancy and the Human Development Index (HDI) of each region, which is a summarized measurement of progress in the long term, using three basic dimensions: income, education, and health.[7]

Since the census by the Brazilian Institute of Geography and Statistics (IBGE) provides the HDI per state/municipality, we calculated the average HDI of each state weighted by the population to obtain the HDI of each region. We used as a reference the HDI of the IBGE census (2010) because it is the most recent data available.[8] Relative and absolute frequencies of the number of LB according to the mother's age and year of occurrence were calculated. Increases or reductions in the percentages from 2006-2015 were calculated using the formula:

[(% of LB from 2015 – % of LB from 2006) / % of LB from 2006] x 100

The age-specific fertility rate (ASFR) represents the average number of children born alive a woman of a specific age and of a specific area has had in the year considered.[9] The rate may be presented per group of 1,000 women for each age group. The ASFR was calculated by dividing the total number of LB from mothers aged between 10-14 years and 15-19 years by the total resident population of adolescents of this age, multiplied by 1000.[9,10]

Since the database used is of public domain, it was not necessary to submit the project for approval by our institution's Research Ethics Committee.

RESULTS

The percentage of LB from adolescents between 2006-2010 increased from 21.5% to 19.3% (a reduction of 12.7%), with a slight increment between 2010-2014 (an increase of 1.8%) and a drop in 2015 (a reduction of 2.7%). Considering the period studied, there was a decrease from 21.5% (2006) to 18.1% (2015), driven by the proportion of mothers aged between 15 and 19 years. The reduction of births among adolescent mothers in Brasil totaled 13.5% in ten years (Table 1). The ASFR

TABLE 1. DISTRIBUTION OF LIVE BIRTHS (LB) ACCORDING TO THE MOTHER'S AGE AND PERCENTAGE VARIATION IN THE RATE OF ADOLESCENT PREGNANCY (AP) FROM 2006 TO 2015.

Year	10 to 14 years	15 to 19 years	Total of LB from adolescents	Total of LB	Freq % AP
2006	27,610	605,270	632,880	2,944,928	21.5
2007	27,963	582,409	610,372	2,891,328	21.1
2008	28,678	570,560	599,238	2,934,828	20.4
2009	27,807	546,959	574,766	2,881,581	19.9
2010	27,049	525,581	552,630	2,861,868	19.3
2011	27,785	533,103	560,888	2,913,160	19.3
2012	28,236	531,909	560,145	2,905,789	19.3
2013	27,989	532,002	559,991	2,904,027	19.3
2014	28,244	534,364	562,608	2,979,259	18.9
2015	26,700	520,864	547,564	3,017,668	18.1
Total	278,061	5,483,021	5,761,082	29,234,436	

Source: MS/SVS/Dasis - Information System on Live Births - SINASC.

TABLE 2. AGE-SPECIFIC FERTILITY RATE PER AGE GROUP (10-14 AND 15-19 YEARS) TOTAL LB FROM MOTHERS AGED BETWEEN 10-14 YEARS AND 15-19 YEARS/TOTAL POPULATION RESIDENT ADOLESCENTS, FROM THESE GROUPS, MULTIPLIED BY 1,000.

Year	10 to 14 years	Adolescent population	ASFR/1,000 adol	15 to 19 years	Adolescent population	ASFR/1,000 adol
2006	27,610	8,462,615	3.26	605,270	8,537,516	70.90
2007	27,963	8,455,516	3.31	582,409	8,501,358	68.51
2008	28,678	8,451,680	3.39	570,560	8,482,441	67.26
2009	27,807	8,449,676	3.29	546,959	8,469,621	64.58
2010	27,049	8,444,955	3.20	525,581	8,456,048	62.15
2011	27,785	8,453,733	3.29	533,103	8,445,364	63.12
2012	28,236	8,441,389	3.34	531,909	8,438,804	63.03
2013	27,989	8,407,297	3.33	532,002	8,435,542	63.07
2014	28,244	8,351,178	3.38	534,364	8,434,160	63.36
2015	26,700	8,276,054	3.23	520,864	8,430,077	61.79
Total	278,061	84,194,093	3.30	5,483,021	84,630,931	64.79

Source: IBGE/Directorate of Research. Coordination of Population and Social Indicators. Management of Studies and Analyses of Demographic Dynamics. Projection of the population of Brasil and Federated Units per age and gender for 2000-2030.

for the age group between 15-19 years had a reduction from 70.9/1,000 in 2006 to 61.8% in 2015 (Table 2). The reduction in the number of LB from mothers aged between 15-19 years was 14.0%, while among those aged between 10-14 years, it was only 3% (Table 3).

After analyzing the regions of the country separately regarding these ten years, we found that the number of LB from mothers aged between 10 and 14 years increased in the Northern Region (5.0%), while in other Brazilian regions, it decreased (2.0% in the Northeast; 8.0% in the Central-West; 3.0% in the Southeast; and 18.0% in the South). The number of LB among mothers aged between 15-19 years decreased in all Brazilian regions (9.0% in the North; 18.0% in the Northeast; 11.0% in the Central-West; 12.0% in the Southeast, and 14.0% in the South) (Table 3).

After analyzing the last HDI record available, we found that the regions that have the highest HDI are the Southeast, South, and Central-West, with HDIs between 0.75 to 0.76, while the North and Northeast have HDIs between 0.65 and 0.66. The regions that have the highest HDI in the country were the ones with the lowest percentage of LB from adolescent mothers, while the regions with the lowest HDI had the highest percentages of LB from adolescent mothers. The Northeast had the lowest percentage of reduc-

TABLE 3. DISTRIBUTION OF LIVE BIRTHS ACCORDING TO THE MOTHER'S AGE AND PERCENTAGE VARIATION IN THE RATE OF ADOLESCENT PREGNANCY (AP) FROM 2006 TO 2015.

Age of mother	Region	2006	2010	2014	2015	Variation 2006-2010	Variation 2010-2014	Variation 2014-2015	10 years
10 to 14 years	Southeast	7,288	7,028	7,700	7,081	-4%	10%	-8%	-3%
	Central-West	2,232	2,100	2,311	2,050	-6%	10%	-11%	-8%
	Northeast	10,287	10,292	10,176	10,064	0	-1%	-1%	-2%
	Norte	4,773	4,864	5,190	5,014	2%	7%	-3%	5%
	South	3,030	2,765	2,867	2,491	-9%	4%	-13%	-18%
	Brasil	27,610	27049	28244	26,700	-2%	4%	-5%	-3%
15 to 19 years	Southeast	196,111	172,266	177,945	172,251	-12%	3%	-3%	-12%
	Central-West	46,284	40,525	43,185	41,319	-12%	7%	-4%	-11%
	Northeast	208,291	174,929	171,784	170,122	-16%	-2%	-1%	-18%
	Norte	84,474	75,829	79,190	77,098	-10%	4%	-3%	-9%
	South	70,110	62,032	62,260	60,074	-12%	0	-4%	-14%
	Brasil	605,270	525,581	534,364	520,864	-13.2%	1.7%	-2.5%	-14%
Total Brasil(10-19 years)		632,880	552,630	562,608	547,564	-12.7%	1.8%	-2.7%	-13.5%

Source: MS/SVS/Dasis - Information System on Live Births - SINASC

tion in the age group between 10-14 years, while in the North there was an increase in the percentage of LB from adolescents aged between 10-14 years (Figure 1).

DISCUSSION

The present study shows a tendency of reduction of teenage pregnancy over the decade studied. Its prevalence decreased between 2006-2010 and remained stable until 2014. A new reduction in 2015 may have been driven by the expansion of the Family Health Program and tan increased access to contraceptive methods.[11] Another explanation may be related to the country's demographic transition, with the reduction of the adolescent population and increase of the population over 60 years old or older.[12]

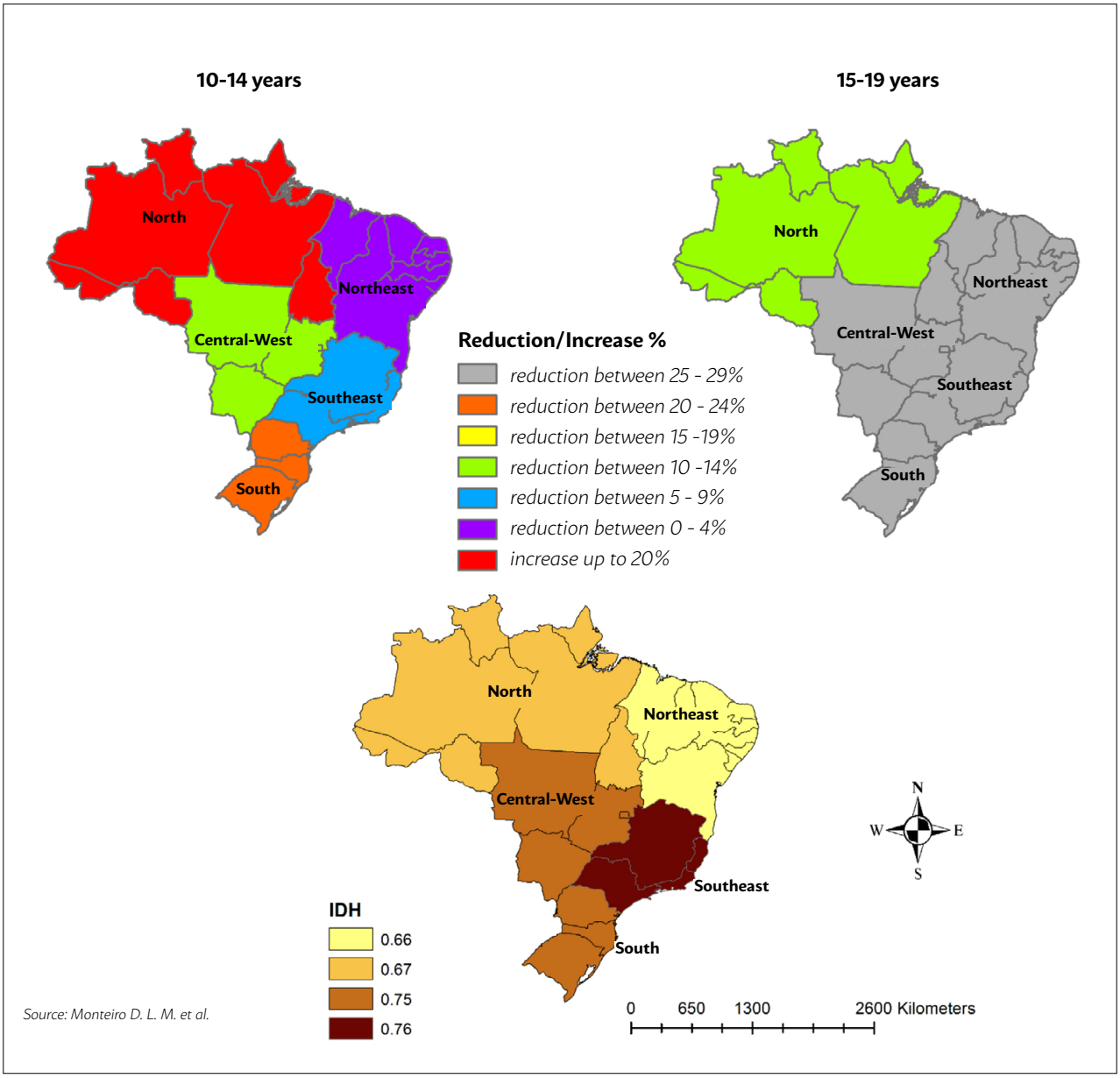
The study found a decrease of LB from adolescent mothers in Brasil caused by a reduction in the number of LB from mothers aged between 15 and 19 years old. However, it also found a slight increase in births in the age group younger than 15 years old, over the period studied.

According to the IBGE, Brasil had a sharp drop in the total number of live births between 2000-2001, 2005-2006, 2008-2009, and 2015-2016. Between 2009 and 2013, births remained at the same level, with an increase of 2.5% and 1.5%, on average, in 2014 and 2015, respectively. The behavior of the total number of LB followed a reduction trend similar to that of the group of adolescent mothers, except in 2015. The North and Northeast had the greatest drop in the Total Fertility Rate (TFR) between 2000-2015. The drop was caused by the reduction of the TEF among women aged between 15-29 years old. It is estimated that the average TFR of the Northern region reached, in 2015, 2.1 children per woman, which corresponds to the limit that ensures the population replacement level. This same figure was reached in the Northeast in 2004, and at the beginning of the 2000s in other regions. In the Northeast, there was aging in the fertility pattern because, in recent years, it has become evident the increased participation in fertility by women aged 30-34 years and a reduction by the age group between 15-24 years in the total fecundity. The Southeast and South regions had fewer variations in the TFR, with slight drops or increases over the period, characterizing a postponement of pregnancy from 15-24 years to 30-39 years.[10]

Although the Brazilian TFR is already low, teenage pregnancy is still quite high. In Brasil, in 2015, the TFR was 1.72 children per woman, placing the country at the 158th position among countries with the highest fertility rate.[9] In the United States of America (USA), in 2015, the fertility rate was 62.5/1,000 women aged between 15 and 44 years, and the TFR was 1.84 births/1,000 women.[2,4]

This study shows that, in Brasil, in 2015, there were 547,564 LB from adolescent mothers. In the US, in the same year, the overall rate of LB reached a historic low of 22.3 births for every 1,000 adolescents aged between 15-19 years old, a reduction of more than 60% since 1991, totaling 229,715 LB from adoles-

FIGURE. RATIO BETWEEN THE PERCENTAGE OF LIVE BIRTHS AND MOTHERS IN THE AGE GROUP OF 10-14 YEARS AND 15-19 YEARS BETWEEN 2006-2015, PER MACROREGION



cent mothers.[2,4] According to the WHO, in Brasil, that figure is 68.4/1,000 adolescents aged between 15-19 years old, higher than the Latin American average. Venezuela occupies the first position, with 80.9/1,000, followed by Ecuador with 77.3/1,000, Bolivia with 72.6/1,000, and then the United States.[4,13] France has the lowest rates, with seven pregnancies per thousand teenagers.[4] The results of this study indicate that, in Brasil, the ASFR for the age group between 15-19 years old dropped to 61.8/1,000 teenagers in 2015. The global rate of births among adolescents decreased from 65 births per 1,000 in 1990 to 47 in 2015.[14] In Brasil, it remains high even with the reduction of births among adolescents aged between 15 and 19 years. What is most worrying is the stabilization tendency among the age group between 10-14 years old.[3,6]

The data presented represent only the total number of births among adolescent mothers, not the totality of teenage pregnancy cases, since it is not possible to quantify the number of abortions and stillbirths, which is a limitation of the study. Another limitation was the proportion of mothers whose age was not recorded because it could include adolescents. Therefore, the data may be underestimated and might not reflect the actual frequency of teenage pregnancy in Brasil. However, the reduction in the number of mothers whose age was not reported indicates an improvement in the quality of data collection by SINASC.[12]

Despite the reduction in fertility rates in Latin America and the Caribbean in recent years, among ad-

olescents, that drop has been minimal over the last 30 years, and a tendency of increase has remained among women younger than 15 years old. West Africa has the highest rate of teenage pregnancy in the world, with a birth rate of 115 births per 1,000 adolescents.[1,15]

In Brasil, over the past ten years, the fertility of adolescents aged between 15 and 19 years dropped about 18.6%.[16] Nevertheless, the participation of this group in total fertility remained high[16]. In Rio Grande do Sul, in 1999, the fertility rate was 20.2% and 17.4% in 2008, with a reduction of 50 thousand births over this period.[17] However, this is not the reality of the entire country, considering the results of this study in relation to mothers aged between 10-14 years.

A previous study by our research group has confirmed the decrease in the percentage of live births from mothers aged between 10-19 years old in Brasil, from 23.5% in 2000 to 19.3% in 2011, and the reduction of the number of mothers whose aged is ignored, especially after 2005. The reduction in the number of LB was observed in all Brazilian macroregions among mothers aged between 15-19 years old, but there was an increase among mothers younger than 15 years old in the North and Northeast regions (12.5% and 13.4%, respectively).[3] The present study shows that in the North, the situation is now changing since there was a slight reduction in the rate of births from younger mothers. This is the first study to show this important result. The literature, when describing data on teenage pregnancy, most often refers to the age range between 15-19 years old. The relationship between adolescence pregnancy and social, educational, economic, and cultural factors indicate a decision to postpone the age of the pregnancy. Income inequality, underemployment, and low levels of formal education contribute to the increase in its incidence.[18] This study confirms that Brazilian regions with higher HDI (South, Southeast, and Central-West) have lower rates of LB from adolescent mothers, which could be considered as a possible marker of development.[3] The exception was the Northeast, where births from women aged 15-19 years had a greater reduction than expected since this is the region with the lowest HDI. Duarte et al.[18] compared adolescents who lived in four areas with different degrees of social exclusion in Santo André (SP). Formal education had a statistically significant relationship with poorer areas, which accounted for a higher number of adolescents with less schooling. In addition, 76.8% of babies with low birth weight and a higher rate of fertility were found in poorer areas of the city.[18]

Pregnancy can take different meanings from the teenager's perspective. Therefore, it is important to emphasize that intentional pregnancy at a young age can be seen as a life project by the adolescent. Pregnancy at a young age may represent a search for autonomy and responsibility, as well as a source of satisfaction and a new identity with the role of a mother. Pregnancy can be seen as a way to mark their space in the family and be acknowledged by friends and family.[19] When there is support by the family and partner, proper prenatal care, and continuation of the studies, a planned pregnancy at a young age can be a positive event.

Thus, although the statistics show a slight decline in their frequency, it is important to highlight the strategies for addressing the problem, so that the adolescence pregnancy can be a decision and not the consequence of the lack of public policies targeted at adolescents.

RESUMO

INTRODUÇÃO: *A gravidez na adolescência é fenômeno universal, com maior prevalência nos países em desenvolvimento. Embora venha apresentando redução desde 2000 no Brasil, a taxa de fecundidade específica para essa faixa etária permanece elevada.*

OBJETIVO: *Avaliar a frequência da gravidez na adolescência no Brasil, no período de 2006 a 2015, e a associação com o Índice de Desenvolvimento Humano (IDH).*

MÉTODO: *Estudo epidemiológico, descritivo, realizado por busca no banco de dados no Departamento de Informática do Sistema Único de Saúde (Datasus), utilizando informações do Sistema de Informação sobre Nascidos Vivos (Sinasc) sobre as cinco regiões brasileiras.*

RESULTADOS: *Ocorreu queda do percentual de nascidos vivos (NV) de mães adolescentes (10 a 19 anos) no Brasil de 13,5% nos últimos dez anos. Essa redução foi notada em todas as regiões brasileiras, entre mães de 15 e 19 anos. O número de NV aumentou 5,0% entre aquelas de 10 a 14 anos na Região Norte e foi reduzido nas demais regiões, sendo maior no Sul (18,0%). A taxa de fecundidade específica de 15-19 anos diminiu de 70,9/1.000 para 61,8/1.000 no período. A proporção de NV se associa inversamente ao IDH, exceto no Nordeste, onde ocorreu importante redução (18,0%) entre as mães de 15-19 anos e de 2% entre 10-14 anos.*

CONCLUSÃO: *A gravidez na adolescência no Brasil encontra-se em lento declínio, especialmente entre 10-14 anos, e está inversamente associada ao IDH, exceto no Nordeste.*

PALAVRAS-CHAVE: *Gravidez na adolescência. Prevalência. Epidemiologia. Adolescente.*

REFERENCES

1. World Health Organization (WHO). Adolescent pregnancy. [cited 2018 Feb 23]. Available from: https://www.who.int/news-room/fact-sheets/detail/adolescent-pregnancy
2. Martin JA, Hamilton BE, Ventura SJ, Osterman MJK, Kirmeyer S, Mathews TJ, et al. Births: final data for 2015. Nat Vital Stat Rep. 2017;66(1):1. [cited 2018 May 30]. Available from: https://www.cdc.gov/nchs/data/nvsr/nvsr66/nvsr66_01.pdf
3. Vaz RF, Monteiro DLM, Rodrigues NCP. Tendências da gravidez na adolescência no Brasil, 2000-2011. Rev Assoc Med Bras. 2016;62(4):330-5.
4. ONUBR Nações Unidas no Brasil. Taxa de gravidez adolescente no Brasil está acima da média latino-americana e caribenha. [cited 2018 Ago 11]. Available from: https://nacoesunidas.org/taxa-de-gravidez-adolescente-no-brasil-esta-acima-da-media-latino-americana-e-caribenha/
5. Pinto e Silva JL, Surita FGC. Gravidez na adolescência: situação atual. Rev Bras Ginecol Obstet. 2012;34(8):347-50.
6. Ministério da Saúde. Departamento de Informática do SUS (DATASUS). Informações de Saúde (Tabnet) – Estatísticas vitais. [cited 2018 Mar 20]. Available from: http://www2.datasus.gov.br/DATASUS/index.php?area=0205&id=6936
7. Programa das Nações Unidas para o Desenvolvimento (PNUD). PNUD Brasil. Desenvolvimento humano e IDH. [cited 2018 Nov 21]. Available from: http://www.br.undp.org/content/brazil/pt/home/idh0.html
8. Instituto Brasileiro de Geografia e Estatística (IBGE). Censo demográfico 2010. [cited 2018 Nov 21]. Available from: https://ww2.ibge.gov.br/home/estatistica/populacao/ censo2010/
9. Instituto Brasileiro de Geografia e Estatística (IBGE). Brasil em síntese. [cited 2018 Dec 10]. Available from: https://brasilemsintese.ibge.gov.br/populacao/taxas-de-fecundidade-total.html
10. Instituto Brasileiro de Geografia e Estatística (IBGE). Projeções da população. Brasil e Unidades da Federação. Revisão 2018. 2a. ed. [cited 2019 Apr 20]. Available from: https://loja.ibge.gov.br/projecoes-da-populac-o-brasil-e-unidades-da-federac-o-revis-o-2018.html
11. Ministério da Saúde. Portal Brasil. [cited 2018 Dez 18]. Available from: http://www.brasil.gov.br/noticias/saude/2017/05/numero-de-adolescentes-gravidas-cai-17-no-brasil
12. Vasconcelos AMN, Gomes MMF. Transição demográfica: a experiência brasileira. Epidemiol Serv Saúde. 2012;21(4):539-48.
13. Pan American Health Organization (PAHO), World Health Organization (WHO), United Nations Population Fund (UNFPA), United Nations Children's Fund (UNICEF). Accelerating progress toward the reduction of adolescent pregnancy in Latin America and the Caribbean. In: Washington, D.C., USA, 2016. [cited 2018 Sep 20]. Available from: http://iris.paho.org/xmlui/ bitstream/handle/123456789/34493/9789275119761eng.pdf?sequence=1&isAllowed=y&ua=1
14. UN DESA, Population Division. World population prospects: the 2017 revision, DVD edition. New York: UN DESA; 2017.UNDESA, Population Division. World population prospects, the 2015 revision (DVD edition). New York: UNDESA, Population Division; 2015.
15. UNFPA. Maternidade precoce: enfrentando o desafio da gravidez na adolescência UNFPA: situação da população mundial; 2013. [cited 2018 Sep 23]. Available from: http://www.unfpa. org.br/Arquivos/swop2013.pdf
16. Instituto Brasileiro de Geografia e Estatística (IBGE). Síntese de indicadores sociais, 2015. [cited 2018 Nov 23]. Available from: https://ww2.ibge.gov.br/home/estatistica/populacao/ condicaodevida/indicadoresminimos/sinteseindicsociais2015/default.shtm
17. Cesar JA, Mendoza-Sassi RA, Gonzalez-Chica DA, Mano PS, Goulart-Filha SM. Características sociodemográficas e de assistência à gestação e ao parto no extremo sul do Brasil. Cad Saúde Pública. 2011;27(5):985-94.
18. Duarte CM, Nascimento VB, Akerman M. Gravidez na adolescência e exclusão social: análise de disparidades intra-urbanas. Rev Panam Salud Publica. 2006;19(4):236-43.
19. Santos BR, Magalhães DR, Mora GG, Cunha A. Gravidez na adolescência no Brasil: vozes de meninas e de especialistas. Brasília: Instituto dos Direitos da Criança e do Adolescente (INDICA); 2017. [cited 2019 Jan 15]. Available from: http://unfpa.org.br/Arquivos/br_ gravidez_ adolescencia_2017.pdf.

REVIEW ARTICLE

Treatment of iron overload syndrome: a general review

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SUMMARY

INTRODUCTION: *Iron overload is a broad syndrome with a large spectrum of causative etiologies that lead to iron deposition. When iron exceeds defenses, it causes oxidative damage and tissular disfunction. Treatment may prevent organ dysfunction, leading to greater life expectancy.*

METHODS: *Literature from the last five years was reviewed through the use of the PubMed database in search of treatment strategies.*

DISCUSSION: *Different pharmacological and non-pharmacological strategies are available for the treatment of iron overload and must be used according to etiology and patient compliance. Therapeutic phlebotomy is the basis for the treatment of hereditary hemochromatosis. Transfusional overload patients and those who cannot tolerate phlebotomy need iron chelators.*

CONCLUSION: *Advances in the understanding of iron overload have lead to great advances in therapies and new pharmacological targets. Research has lead to better compliance with the use of oral chelators and less toxic drugs.*

KEYWORDS: *Iron Overload. Iron Chelating Agents. Phlebotomy. Hemochromatosis.*

INTRODUCTION

Iron is essential for the transport of oxygen and to various metabolic processes[1-3]. This participation occurs through its potential in accepting and donating electrons, alternating between its ferrous (Fe^{2+}) and ferric (Fe^{3+}) forms[2].

This oxirreductive capacity may also cause tissue damage. Ferrous iron interacts with hydrogen peroxide, generating hydroxyl radicals (OH•), causing lipid peroxidation and damage to cellular organelles and DNA[4]. Due to this potential toxicity, iron homeostasis is strictly controlled. Since the human organism cannot eliminate iron, the main point of control in iron homeostasis is its absorption.

Dietary iron exists in two forms. Protein-bound iron, also known as organic or heme iron, which accounts for 10% of ingested iron, is absorbed directly.

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Non-protein-bound iron, also known as non-heme or inorganic iron, which accounts for 90% of the ingested iron, requires a broad metabolic mechanism for its absorption and transport[2].

The discovery of hepcidin was one of the great steps in understanding iron metabolism and hereditary hemochromatosis (HH)[5]. Hepcidin inhibits iron absorption, as well as its mobilization from tissues[5,6]. Most types of HH are based on pathophysiological changes in hepcidin synthesis.

Another way iron accumulation occurs is through either iron compounds for pharmaceutical use or repetitive transfusions, a common event in the management of patients with chronic anemia. This mechanism contributes to the mortality of individuals with hemoglobinopathies[1].

Iron accumulates in the liver, endocrine glands, heart, and reticuloendothelial cells. When iron exceeds the binding capacity of apoferritin and apotransferrin, non-transferrin bound iron (NTBI) arises. A specific fraction of NTBI, called labile iron pool (LPI), has oxidative potential and is capable of cellular damage.

METHODS

We searched the PubMed Central database using the keywords: "Hemochromatosis" AND "Therapy" OR "Treatment" and "Iron Chelators". The results were filtered for the last five years and evaluated by title. We analyzed 51 articles for evaluation based on their abstracts, of which 25 were selected for the final version.

Discussion (Table 1)

The objective of treatment in iron overload is the rapid reduction of levels of NTBI and LPI, thereby reducing iron-mediated tissue lesions. Aggressive treatment before the onset of target organ damage is the key to the management of these patients, making it possible to have a normal life expectancy[7,8].

Non-pharmacological measures

Phlebotomies

Therapeutic phlebotomies have been performed from the earliest stages of medicine. With the development of modern therapies, there has been a great decline in its use, but it remains one of the main forms of treatment in patients with HH[5,9].

The biological rationale for the use of therapeutic phlebotomy is to force the uptake of iron by the erythroid precursors in the bone marrow, necessary to replace the erythroid mass lost through bleeding[1] (Figure 1).

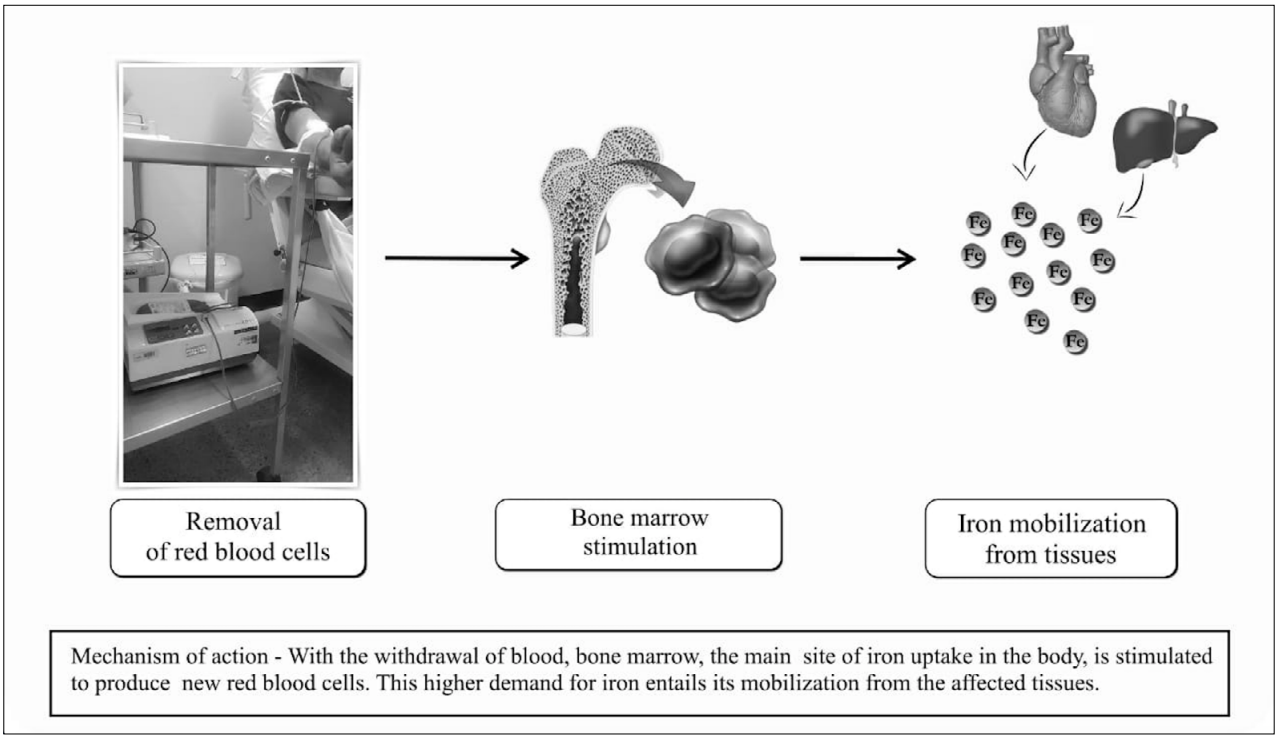
The introduction of phlebotomies as part of the therapy of HH patients in the 1950s increased survival and decreased disease progression when initiated in patients with clinical manifestations of iron overload. When initiated in patients in preclinical stages of the disease, it promotes survival equal to that of the healthy population.

The appropriate timing to start phlebotomies in

TABLE 1. TREATMENTS FOR IRON OVERLOAD SYNDROME

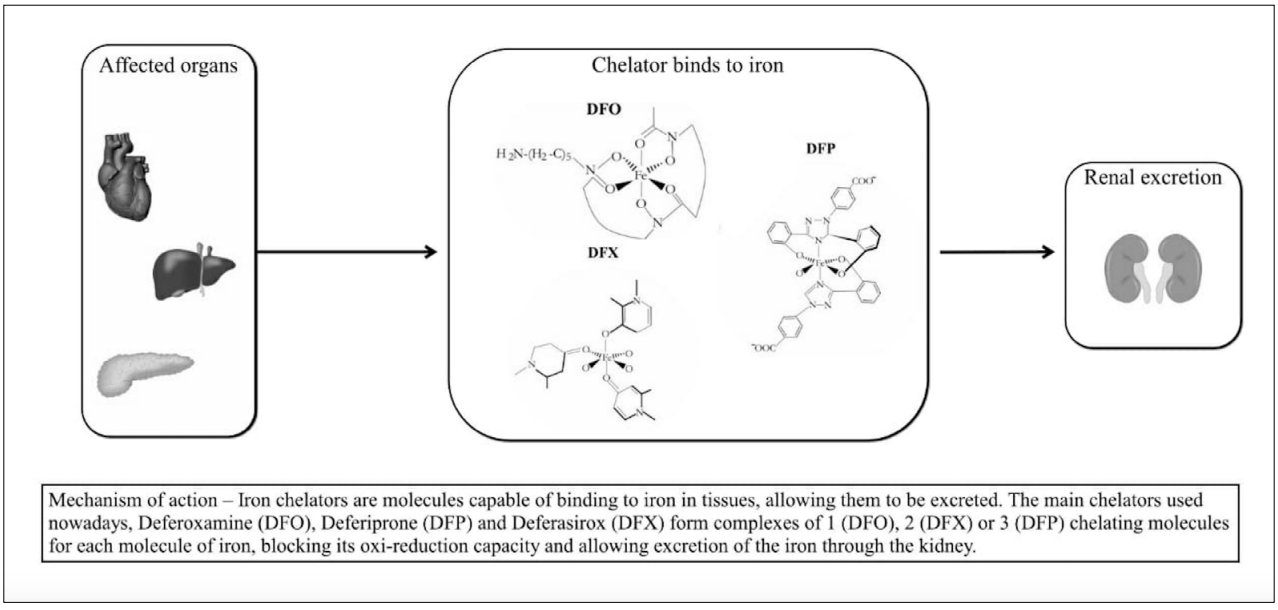
Treatment	Main Indication	Mechanism of Action	Comments	References
Therapeutic Phlebotomy	Hereditary hemochromatosis	Increased Iron Mobilization to Bone Marrow	Choice treatment for Hereditary Hemochromatosis Low cost, Safe and Effective	1, 3 - 6, 10, 11
Erythrocytoapheresis	Hereditary hemochromatosis	Increased Iron Mobilization to Bone Marrow	Indicated in patients with severe heart disease High cost (2-3x phlebotomy costs)	11, 12, 13
Desferrioxamine	Secondary hemosiderosis	Iron binding and elimination	Earliest chelator Parenteral use with continuous infusion	15 - 21
Deferiprone	Secondary hemosiderosis	Iron binding and elimination	Oral use - 3 daily dose regimen Option in heart iron overload and mitochondrial iron overload, as Friedreich's Ataxia or	15 – 24
Deferasirox	Secondary hemosiderosis	Iron binding and elimination	Newest chelator, main chelator agent in Brasil Oral use -Single daily dose regimen	15, 18 - 21, 23 -29
Deferitrin	Secondary hemosiderosis	Iron binding and elimination	Clinical investigations suspended after nephrotoxicity in Phase-1 trials	15, 16
Deferitazole	Secondary hemosiderosis	Iron binding and elimination	Phase-2 and -3 trials are being performed	15. 16
Mini-hepcidins	Hereditary hemochromatosis	Decreases iron absorption	Active fraction of the hormone hepcidin. Pre-clinical studies	4, 7, 30, 31

FIGURE 1. THERAPEUTIC PHLEBOTOMY



Mechanism of action - With the withdrawal of blood, bone marrow, the main site of iron uptake in the body, is stimulated to produce new red blood cells. This higher demand for iron entails its mobilization from the affected tissues.

FIGURE 2. IRON CHELATORS



Mechanism of action – Iron chelators are molecules capable of binding to iron in tissues, allowing them to be excreted. The main chelators used nowadays, Deferoxamine (DFO), Deferiprone (DFP) and Deferasirox (DFX) form complexes of 1 (DFO), 2 (DFX) or 3 (DFP) chelating molecules for each molecule of iron, blocking its oxi-reduction capacity and allowing excretion of the iron through the kidney.

patients diagnosed with HH is not consensually defined among the different Hepatology and Hematology societies. The American Society of Hematology (ASH) proposes to start phlebotomies when ferritin levels are above 300 µg/L in men or 200 µg/L in women of childbearing potential. American (AASLD) and European (EASL) hepatology societies recommend starting as soon as ferritin levels are above the upper limits of normality, regardless of symptoms or clinical manifestations[4]. The Mi-iron study on the use of phlebotomies in patients with mild iron overload (between the upper limit of normalcy and 1000 µg /L) is currently underway[4].

The amount of blood withdrawn at each session is around 7 ml/kg body weight (maximum of 400-550 ml of blood). Sessions usually occur weekly or biweekly during the initial phase of treatment, and hemoglobin levels should be assessed before each session to avoid anemia[4]. Withdrawal of approximately 0.5 mg of iron for every 1 ml of blood withdrawn is

estimated, which would roughly correspond to a decrease in serum ferritin by 30 µg/L at each session[3].

Another controversial point is the target for ferritin in the first stage of the treatment. AASLD recommends reducing ferritin levels to a range between 50 and 100 µg/L. EASL recommends more liberal levels in the initial phase, aiming for a ferritin level below 300 µg/L[4]. Some authors recommend bringing ferritin closer to the levels of iron deficiency, below 50µg/L[9]. Ferritin levels must be monitored every three months[4,9].

In the maintenance stage, the frequency of phlebotomies is reduced to every four to six months, depending on the trends of ferritin levels. The aim at this stage is to keep ferritin levels between 50 and 100 µg/L[4,9].

The most common adverse events during phlebotomies are local manifestations such as pain or bruising at the puncture site. Hemodynamic changes due to phlebotomy are usually mild and may present as fatigue or syncope, but it can be a limiting factor, especially in those with cardiopathies. The consumption of large volumes of water on the day of phlebotomies is recommended as a way of reducing hemodynamic manifestations[4].

Erythrocytoapheresis

Erythrocytoapheresis is a technique that selectively removes erythrocytes, returning blood components such as leukocytes, platelets, and plasma. This selective withdrawal of red blood cells allows for a greater withdrawal of iron, as well as decreases the hemodynamic events related to phlebotomies, being more indicated in patients with severe heart disease[10,11].

In two randomized controlled trials[10,11], erythrocytapheresis was found to be a viable alternative to phlebotomy. Because a increased erythrocyte mass is withdrawn in each session, normalization of ferritin was faster and with fewer sessions, but final ferritin levels were similar between the groups.

Among the adverse events related to erythrocytapheresis, most were related to the use of citrate as an anticoagulant. By promoting calcium chelation, citrate may lead to nausea, paresthesias, or cramps[10,11]. These events were rare, between 0.4 and 8%[10,11].

A major limiting factor to erythrocytapheresis are the costs involved, two[10] to three[11] times more expensive than those of phlebotomy, and the need for specific equipment and trained technicians. The use of erythrocytapheresis in selected patients may be useful in cases of severe heart disease.

Dietary changes

Daily iron intake is around 15-25 mg/day. Some studies have sought to identify whether dietary iron restriction or measures that change iron bioavailability, such as consumption of non-citrus fruits, tea, or proton pump inhibitors, could contribute to the treatment of patients with iron overload.

Moretti et al.[12] found a small effect on iron absorption and serum ferritin levels with dietary interventions, but the impact these changes may have on the absorption of other nutrients need to be better evaluated. There are currently no consensual indications of routine iron restriction or measures that reduce the bioavailability of iron.

It is recommended to restrict the consumption of seafood due to the increased risk of *Vibrio vulnificus* and *Yersinia enterocolitica infections*[2,5]. Alcohol consumption is also not recommended because of the potential hepatic injury and interference with hepcidin secretion[4,5]. Iron and Vitamin C supplementation are formally contraindicated[5,7].

Pharmacological measures

Therapeutic phlebotomy, although an extremely useful measure in patients with hemochromatosis, cannot be performed in patients with chronic anemia. In these patients, iron withdrawal should be performed by substances that bind to iron in tissues and allow their excretion, namely iron chelators (Figure 2).

Three chelating substances are available for clinical use: deferoxamine (DFO), deferiprone (DFP), and deferasirox (DFX). We will review the characteristics of each of these substances in detail below.

Desferrioxamine

Deferoxamine (DFO) is a compound discovered in 1960 produced by *Streptomyces pilosus* and used since the 1970s in cases of iron overload. Its large molecule is highly hydrophilic and gives six fixing points (hexadentate ligand) to iron, in order to allow the formation of complexes in a 1:1 ratio[13,14].

This molecular structure hinders oral absorption; it needs to be administered parenterally, most commonly subcutaneous. Once in circulation, much of the DFO enters hepatocytes. Once inside the hepatocytes, complexes are formed, protecting the iron

from potential endogenous reducers, avoiding its toxicity[14]. The complex is then eliminated through urinary and fecal routes.

Because of its very short elimination half-life, around 10 to 30 minutes, continuous drug infusion is necessary[13-15]. This contributes to a decrease in patients' adherence to therapy.

The starting dose recommended is 20 to 40 mg/kg, infused over 8 to 24 hours subcutaneously or intravenously, 5 to 7 times a week[14-16]. Higher doses, up to 60 mg/kg, may be used in exceptional situations but should be avoided, especially in children because of the toxicity[16,17].

Most reported adverse events are related to local reactions at infusion sites[14-17]. Ocular and ototoxicity are also described and must be monitored at least once a year. Iron mobilization may provide iron to siderophore pathogens such as *Yersinia enterocolitica* and *Vibrio vulnificus*, increasing the risk of infections[17].

Deferiprone

Deferiprone (DFP) was the first chelating agent with good oral absorption in clinical practice. It was synthesized in 1982 and initially approved for use in 1995 in India and in 2000 in Europe. The USFDA approved its use only in 2011 as rescue therapy in patients with thalassemia who had an insufficient response to the available treatments[13,18].

The molecular structure of deferiprone has two attachment points for iron (bidentate ligand), allowing the formation of complexes at a 3:1 ratio. When the iron concentration exceeds that of DFP, 2:1 positively charged complexes are formed which are unable to fully protect the iron from endogenous reductants[13].

Its lipophilic molecule is the smallest among the three chelators, allowing for good oral bioavailability and better penetration in several tissues and in specific structures within the cell, such as the mitochondria. DFP may be a therapeutic option in other conditions related to iron metabolism, such as Friedreich's ataxia[18].

The main route of drug metabolism is through glucuronidation of the hydroxyl radical[13]. The compound is soluble and excreted in the urine. Its half-life is around 3 to 4 hours, and multiple daily doses are needed[14,18].

The initial dose recommended is 75 to 100 mg/kg a day, divided into three daily doses[14,18]. It has efficacy equivalent to DFO[18] and in severely loaded patients, especially those with severe cardiac siderosis, DFP can be associated with DFO, showing a synergistic effect[18,19]. There is no definitive data for the use of this drug in the pediatric population under 6 years of age[15-17].

The worse adverse event related to DFP is neutropenia[13-17], rarely culminating in agranulocytosis, which limits its routine clinical use. A weekly evaluation of leukocyte counts is recommended in patients taking deferiprone. Other side effects are arthralgia, hepatotoxicity, and gastrointestinal discomfort. There are isolated reports of drug-induced lupus and heart failure.

Deferasirox

Deferasirox (DFX) is the newest molecule among those clinically available. It was synthesized in 2002 by Novartis in a prospection program that included more than 700 molecules[13]. It was approved for clinical use in 2005 and 2006 by the USFDA and the European Union regulatory agency and rapidly became one of the major iron-chelating agents in clinical practice[19-21].

The molecular structure of DFX provides three points of attachment to iron (tridentate ligand), which allows the formation of complexes in a 2:1 ratio. At physiological pH conditions, the complexes formed are negatively charged, which makes them chemically incapable of reacting with the endogenous reducing agents[13].

Its oral bioavailability is around 70%. Another pharmacokinetic characteristic is its prolonged half-life, around 8 to 16 hours. This feature allows the use of DFX in a single daily dose[20,21].

The main route of excretion of the drug is fecal, with more than 60% of the drug eliminated in its natural form. It also can be metabolized by liver enzymes and eliminated by the renal route. This metabolic pathway accounts for approximately 8% of drug elimination. A small part is metabolized by the cytochrome p450 system[21].

The starting dose recommended of DFX is 20 mg/kg a day and can be increased up to 40 mg/kg a day. In patients with moderate hepatic impairment (Child-Pugh score B) a 50% reduction in the initial dose and closer monitoring for adverse events is recommended. DFX is not recommended for patients with severe hepatic impairment (Child-Pugh score C). There are few studies in patients with severe renal impairment, and there is no specific recommendation for dose ad-

justment in this population, but caution is advised in dialytic patients[21]. Studies in children demonstrate the safety of this drug, and it can also be used in patients older than 2 years old[15,16].

Several studies have demonstrated good efficacy in the reduction of iron deposits in several tissues[22-24]. In case of severe heart siderosis, the association with DFP has shown a synergistic effect [18,22-24].

Most adverse events related to DFX are of gastrointestinal origin[15-17,21], symptoms such as nausea, vomiting, diarrhea, and abdominal pain are common but usually mild and transient. In some cases, they can be a limiting factor to the escalation of the dose. A consideration to be made is the presence of lactose in the tablet composition, which may be the cause of the symptoms in intolerant patients.

Some of the serious adverse events related to DFX are hepatotoxicity and nephrotoxicity. These events usually manifest as mild changes in laboratory tests, with no clinical manifestations, but monthly monitoring of creatinine levels and liver enzymes is necessary.

Future perspectives

New chelators

Several new compounds are under study as new promises for clinical use as iron chelators. Some compounds studied at the moment are of the 3-hydroxy-4-pyridinone family. Several changes in the molecular structure have demonstrated compounds with better iron-binding capacity than Deferiprone in preclinical testing. Phase-1 clinical trials with some of these compounds are ongoing[13].

Another molecule with good in vitro potential for iron chelation was Deferitrin, but investigations were suspended after three cases of nephrotoxicity in a phase 1 study[13,14]. Several changes in the structure of the molecule are being evaluated in preclinical studies in an attempt to reduce this toxicity.

Phase-2 and -3 studies are being performed on the compound Deferitazole[13,14]. This compound is a derivative of desferythycin, a natural siderophore extracted from *Streptomyces antibioticus*, which has demonstrated long half-life, allowing daily dosing, and low toxicity.

Mini-hepcidins

Due to its unique chemical structure with four disulfide bridges, hepcidin is extremely difficult to synthesize. Studies with computational modeling and amino acid substitution were able to synthesize compounds from hepcidin fragments with biological activity, the mini-hepcidins[6].

Studies in murine models[25] have shown favorable results in decreasing iron absorption, demonstrating a potential use of these drugs in preclinical states or in conjunction with iron chelation or therapeutic phlebotomies.

Final considerations

Research with iron chelators has made possible a better adherence, with the discovery of oral substances with a good profile of side effects. With the advancement of research, we expect the emergence of more potent and less toxic drugs.

Recent advances in the understanding of iron overload syndrome have led to major advances in the therapy of these patients, with promises of addressing new pharmacological targets with new Hepcidin agonists.

CONFLITS OF INTEREST

The Authors declare no conflicts of interest that may have influenced this work.

Authors' Contributions:

All authors have reviewed and approved the final text of the article and are responsible for its content.

RESUMO

INTRODUÇÃO: *A síndrome de sobrecarga de ferro engloba um grande espectro de etiologias que levam a um aumento da quantidade de ferro nos tecidos. Esse ferro excede a capacidade de proteção dos tecidos, levando a dano oxidativo e lesão tissular. Tratamento pode prevenir esse dano, levando à melhor sobrevida.*

METODOLOGIA: *A literatura dos últimos cinco anos foi revisada por meio de pesquisa na base de dados PubMed buscando identificar estratégias de tratamento.*

DISCUSSÃO: *Medidas farmacológicas e não farmacológicas estão disponíveis para o tratamento da síndrome de sobrecarga de ferro e devem ser utilizadas de acordo com a etiologia e a aceitação do paciente. A flebotomia terapêutica é base do tratamento dos pacientes com hemocromatose hereditária. Pacientes com sobrecarga transfusional ou aqueles que não toleram flebotomias devem utilizar quelantes de ferro.*

CONSIDERAÇÕES FINAIS: *Avanços no entendimento da síndrome de sobrecarga de ferro têm levado a grandes progressos na terapêutica, com promessas de abordagem de novos alvos farmacológicos. A evolução da pesquisa tem possibilitado melhor aderência com o uso de quelantes orais e com possibilidade de drogas menos tóxicas.*

PALAVRAS-CHAVE: *Sobrecarga de ferro. Quelantes de ferro. Flebotomia. Hemocromatose.*

REFERENCES

1. Brissot P. Optimizing the diagnosis and the treatment of iron overload diseases. Expert Rev Gastroenterol Hepatol. 2016;10(3):359-70.
2. Thachill J, Solberg LA, Khan Jr MJ, McCrae KR. Iron metabolism, iron overload and the porphyrias. In: ASH-SAP: American Society of Hematology self-assessment program. Washington: American Society of Hematology; 2013.
3. Crownover BK, Covey CJ. Hereditary hemochromatosis. Am Fam Physician. 2013;87(3):183-90.
4. Powell LW, Seckington RC, Deugnier Y. Haemochromatosis. Lancet. 2016;388(10045):706-16.
5. Barton JC. Hemochromatosis and iron overload: from bench to clinic. Am J Med Sci. 2013;346(5):403-12.
6. Chua K, Fung E, Micewicz ED, Ganz T, Nemeth E, Ruchala P. Small cyclic agonists of iron regulatory hormone hepcidin. Bioorg Med Chem Lett. 2015;25(21):4961-9.
7. Salgia RJ, Brown K. Diagnosis and management of hereditary hemochromatosis. Clin Liver Dis. 2015;19(1):187-98.
8. Mohamed M, Phillips J. Hereditary haemochromatosis. BMJ. 2016;353:i3128.
9. Assi TB, Baz E. Current applications of therapeutic phlebotomy. Blood Transfus. 2014;12(Suppl 1):s75-83.
10. Rombout-Sestrienkova E, Winkens B, Essers BA, Nieman FH, Noord PA, Janssen MC, et al. Erythrocytapheresis versus phlebotomy in the maintenance treatment of HFE hemochromatosis patients: results from a randomized crossover trial. Transfusion. 2016;56(1):261-70.
11. Sundic T, Hervig T, Hannisdal S, Assmus J, Ulvik RJ, Olaussen RW. Erythrocytapheresis compared with whole blood phlebotomy for the treatment of hereditary haemochromatosis. Blood Transfus. 2014;12(Suppl 1):s84-9.
12. Moretti D, van Doorn GM, Swinkels DW, Melse-Boonstra A. Relevance of dietary iron intake and bioavailability in the management of HFE hemochromatosis: a systematic review. Am J Clin Nutr. 2013;98(2):468-79.
13. Nurchi VM, Crisponi G, Lachowicz JI, Medici S, Peana M, Zoroddu MA. Chemical features of in use and in progress chelators for iron overload. J Trace Elem Med Biol. 2016;38:10-8.
14. Sheth S. Iron chelation: an update. Curr Opin Hematol. 2014;21(3):179-85.
15. Botzenhardt S, Li N, Chan EW, Sing CW, Wong IC, Neubert A. Safety profiles of iron chelators in young patients with haemoglobinopathies. Eur J Haematol. 2017;98(3):198-217.
16. Kontoghiorghes CN, Kontoghiorghe GJ. Efficacy and safety of iron-chelation therapy with deferoxamine, deferiprone, and deferasirox for the treatment of iron-loaded patients with non-transfusion-dependent thalassemia syndromes. Drug Des Devel Ther. 2016;10:465-81.
17. Saliba AN, El Rassi F, Taher AT. Clinical monitoring and management of complications related to chelation therapy in patients with β-thalassemia. Expert Rev Hematol. 2016;9(2):151-68.
18. Belmont A, Kwiatkowski JL. Deferiprone for the treatment of transfusional iron overload in thalassemia. Expert Rev Hematol. 2017;10(6):493-503.
19. Wongjaikam S, Kumfu S, Chattipakorn SC, Fucharoen S, Chattipakorn N. Current and future treatment strategies for iron overload cardiomyopathy. Eur J Pharmacol. 2015;765:86-93.
20. Allegra S, De Francia S, Cusato J, Pirro E, Massano D, Piga A, et al. Deferasirox pharmacokinetic and toxicity correlation in β-thalassaemia major treatment. J Pharm Pharmacol. 2016;68(11):1417-21.
21. Tanaka C. Clinical pharmacology of deferasirox. Clin Pharmacokinet. 2014;53(8):679-94.
22. Totadri S, Bansal D, Bhatia P, Attri SV, Trehan A, Marwaha RK. The deferiprone and deferasirox combination is efficacious in iron overloaded patients with β-thalassemia major: a prospective, single center, open-label study. Pediatr Blood Cancer. 2015;62(9):1592-6.
23. Piga A, Longo F, Origa R, Roggero S, Pinna F, Zappu A, et al. Deferasirox for cardiac siderosis in β-thalassaemia major: a multicentre, open label, prospective study. Br J Haematol. 2014;167(3):423-6.
24. Vlachaki E, Agapidou A, Spanos G, Klonizakis P, Vetsiou E, Mavroudi M, et al. Five years of deferasirox therapy for cardiac iron in β-thalassemia major. Hemoglobin. 2015;39(5):299-304.
25. Ramos E, Ruchala P, Goodnough JB, Kautz L, Preza GC, Nemeth E, et al. Minihepcidins prevent iron overload in a hepcidin-deficient mouse model of severe hemochromatosis. Blood. 2012;120(18):3829-36.

REVIEW ARTICLE

Use of dupilumab on the treatment of moderate-to-severe asthma: a systematic review

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SUMMARY

OBJECTIVE: *The objective of this article was to conduct a systematic review of the treatment of moderate-to-severe asthma by administrating Dupilumab.*

METHODS: *A search on the online databases EBSCO, Scielo, PubMed, Medline Bireme, Lilacs, and The New England Journal of Medicine was conducted, publications from 2010 to 2018 were selected. The inclusion criteria were articles which contained control groups, tested the validity of Dupilumab, and verified the response of patients through controlled tests. For the search of such articles, the following keywords were used: "Dupilumab", "asthma", "Bronchial Asthma" AND "Asthma, Bronchial" AND their correspondent in Portuguese "asma", "Asma brônquica" and "Asma brônquica". The exclusion criteria were literature reviews, news, articles without control groups, articles on different subjects, Dupilumab studies on other diseases, articles concerning asthma without the use of Dupilumab, and repeated articles on the databases were discarded.*

RESULTS: *The literature considers that the medication shows a good response for the treatment of moderate-to-severe asthma and assists in the improvement of lung function, aside from resulting in few side effects. It presents good efficacy, safety, and tolerance by patients.*

CONCLUSIONS: *Dupilumab is promising for the treatment of asthma, whereas conventional therapy is deemed to be insufficient. More additional studies are needed to confirm the long-term safety and effectiveness.*

KEYWORDS: *Dupilumab. Asthma. Bronchial diseases. Anti-Asthmatic Agents.*

INTRODUCTION

The objective of this review is to evaluate the results from efficacy tests for a new medication available in the market that promises to improve the life quality of patients with moderate-to-severe asthmatic disease, as an alternative to conventional treatment. The medication under discussion is Dupilumab, already in use in the United States and European Union and approved by ANVISA (National Health Surveillance Agency) in December of 2017 for use in the Brazilian territory. For this article, clinical tests on the use of Dupilumab in comparison with control groups (placebo) for the treatment of asthma were selected.

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METHODS

This article is a literature review based on the search for the keywords "Dupilumab" and "asthma", and their Portuguese equivalent "asma" on the online databases EBSCO, Scielo (Scientific Electronic Library Online), PubMed (US National Library of Medicine), Medline Bireme (Medical Literature Analysis and Retrieval System Online), Lilacs (Literatura Latino-Americana de Ciências da Saúde), and The New England Journal of Medicine.

The inclusion criteria were articles published from 2010 to 2018 written in English, Spanish or Portuguese, containing control groups and patients with moderate-to-severe asthma under treatment with Dupilumab. Given the diversity of studies found, some exclusion criteria were necessary to narrow down the results. The first exclusion criteria were studies on asthma without the use of Dupilumab, studies about Dupilumab usage on other diseases, literature reviews, news, case reports, and repeated articles. As a second exclusion criterion, articles without a control group of patients with moderate-to-severe asthma under Dupilumab treatment were discarded.

A total of 165 articles were found in the online databases. After reading the titles and abstracts, we excluded articles that were repeated on the search databases, articles that did not concern the study topic, news, literature reviews, articles about Dupilumab usage on other diseases, and articles with asthmatic patients who did not use Dupilumab (first exclusion criteria). We found that 159 articles did not correspond to the current study purpose; thus, only six articles remained to be fully read. The second exclusion criterion was the lack of control groups on the treatment of moderate-to-severe asthma with Dupilumab; this excluded one more article, with only five articles remaining that included all the criteria mentioned above. These five articles were used for the final text (Table 1).

On the EBSCO platform, 20 articles were found; none of these were selected for the study after the exclusion criteria mentioned above were applied. Among these studies, 14 were news, three literature reviews, one focused on patients with sinusitis and atopic nasal polyps under treatment with Dupilumab, one was about Dupilumab as treatment for atopic dermatitis and, finally, one was on another subject. On the Lilacs database, only 1 article was found and not excluded based on the first criterion; however, it did not include a control group and was excluded. On The New England Journal of Medicine, 13 studies were found; of these, three were selected based on the inclusion criteria; one article was a duplicate, two were literature reviews, three were news, two were studies regarding atopic dermatitis, and two were articles without the use of Dupilumab; these were, therefore, excluded. On Medline, 64 articles were found; of these, 21 were news, 20 literature reviews, seven studies did not focus on Dupilumab use, six were about nasal polyps, rhinitis and sinusitis, six were about atopic dermatitis, two articles were duplicates; only one was selected based on the inclusion criteria of the research. On the Pubmed platform 67 studies were found, and only one met the inclusion criteria; 22 were literature reviews, 16 were news, 12 were articles about the use of Dupilumab in atopic dermatitis, six were about other diseases, four were duplicates, three did not include the use of Dupilumab, one was a case report and, finally, two were about unrelated subjects to this research. On the Scielo database, no article was found with the keywords (Figure 1).

The study included five articles containing control groups and patients with moderate-to-severe asthma in treatment with Dupilumab (Table 2).

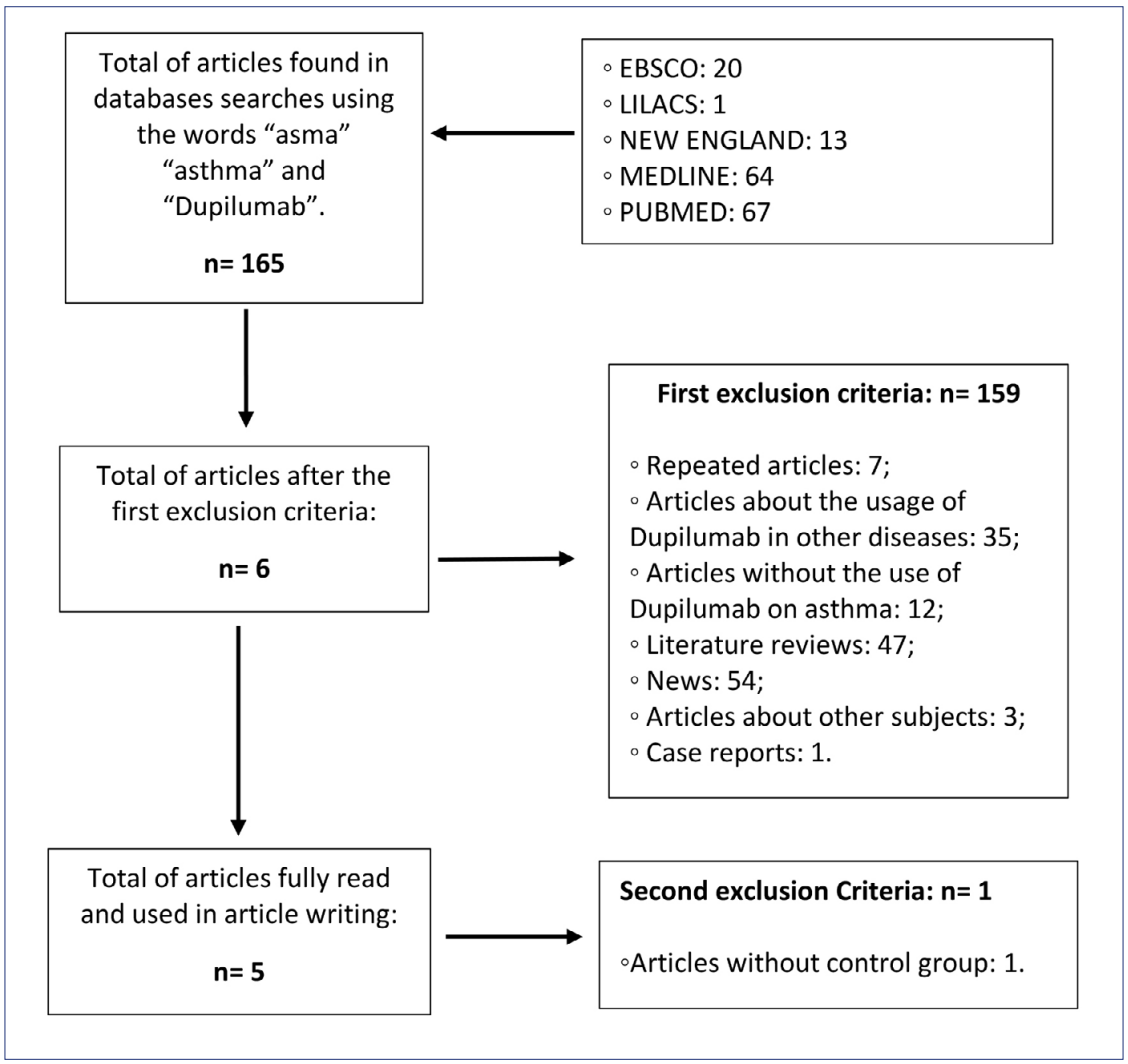
DISCUSSION

According to the World Health Organization (WHO)[6], asthma is a chronic inflammatory disease of the airways, which is characterized by a recurrent crisis of shortness of breath and wheezing. During an asthma attack, the bronchi are swollen and narrow the airways, making the intake and outlet of air from the lungs difficult. The usual symptomatology entails insomnia problems, daytime fatigue, a negative impact on academic and professional performance, and

TABLE 1. SEARCH RESULTS OF THE DATABASES AND SELECTION OF THE ARTICLES.

Database	First Criterion		Second Criterion	
	Total	Included	Total	Included
EBSCO	20	0	0	0
SCIELO	0	0	0	0
MEDLINE	64	1	1	1
LILACS	1	1	1	0
PUBMED	67	1	1	1
NEW ENGLAND J	13	3	3	3
Total	165	6	6	5

FIGURE 1. FLOWCHART OF ARTICLE SELECTION.



a decrease of physical activity standards; problems that reflect directly on the personal quality of life.

Per WHO estimates, all around the world, 235 millions of people have asthma. It is the most common chronic disease among children. However, it has low mortality rates compared to other respiratory diseases, such as chronic obstructive pulmonary disease (COPD)[6].

The causes of asthma are not completely understood, but it is evident that there is an association between genetic predisposition, environmental exposure to inhaled substances and allergenic substances, such as dust mites, animal hair, pollen, chemical irritants, and air pollution, which may result in the irritation of the respiratory tract mucous membranes or cause allergic reactions. Other triggers to the disease are meteorological changes, extreme emotional reactions, and physical exercises, as well as certain drugs like aspirin, other non-steroid anti-inflammatory drugs, and beta-blockers[6].

Most asthma patients have the disease at a low to medium degree and use inhaled corticosteroids with or without the association of bronchodilators or beta-agonists drugs, through inhalation or intravenous administration. However, for severe asthma cases, conventional therapy is often insufficient, leading to several hospitalizations and low life quality, besides being related to secondary inflammatory diseases with an unbalance of interleukins and immune cells. In those cases, frequently, magnesium sulfate or ipratropium bromide are used, along with gas mixtures of oxygen and helium, methylxanthines and non-invasive ventilatory support[7].

Recently, new drugs are being introduced in the industry for the treatment of moderate-to-severe asthma, the so-called monoclonal antibodies, biological drugs synthesized from living organisms that act specifically at the therapeutic targets of the disease's physiopathology. The first biological drug for the treatment of moderate-to-severe asthma was

TABLE 2. SUMMARY OF CHARACTERISTICS OF SELECTED ARTICLES.

Reference	Date of publication	Number of individuals and age group	Medication dosage	Outcome
Busse et al.[1]	May, 2018	1902 patients aged ≥12 years were randomized in a 2: 2: 2: 1 ratio. three groups. Duration of 52 weeks	Group 1: 634 patients. 300 mg of Dupilumab every two weeks. Group 2: 364 patients, 200 mg of Dupilumab every two weeks. Group 3: 317 patients, placebo.	Dupilumab had an excellent response to moderate to severe uncontrolled asthma, provides improvement in respiratory function and control of asthma. Severe exercise reduction and improved quality of life
Castro et al.[2]	June, 2018	1902 patients aged ≥12 years with uncontrolled asthma divided in 2: 2: 1 ratio. Three groups. Duration of 52 weeks.	Group 1: 300 mg of Dupimulab every two weeks. Group 2: 200 mg of Dupimulab every two weeks. Group 3: Placebo.	Patients who received Dupimulab had a significant improvement in lung function, asthma control, and minor exacerbations of severe asthma compared to the placebo group.
Rabe et al.[3]	June, 2018	210 patients aged ≥ 12 years. Two groups. Duration of 24 weeks.	Group 1: 200 mg of Dupimulab every two weeks. Group 2: Placebo.	The use of Dupimulab decreases the exacerbation of severe asthma and improved FEV1.
Wenzel et al.[4]	April, 2016	769 patients aged≥ 18 years, of whom 611 were in the Dupimulab and 158 in the placebo group. Five groups with a ratio of 1: 1: 1: 1: 1. Duration of 24 + 16 weeks.	Group 1: 200 mg of Dupimulab every 4 weeks. Group 2: Dupimulab 300 mg every 4 weeks. Group 3: 200 mg of Dupimulab every 2 weeks. Group 4: 300 mg of Dupimulab every 2 weeks. Group 5: Placebo.	Groups using Dupilumab, except for 200 mg every 4 weeks, showed a significant improvement in FEV1, reduced asthma exacerbation and eosenophilia. The best results were found in the 300 mg Dupilumab group every 2 weeks.
Wenzel et al.[5]	June, 2013	104 patients, divided into two, each with 52 patients. One group received Dupilumab and the other placebo. Duration of 12 weeks.	Group 1: 300 mg Dupilumab weekly. Group 2: Placebo.	The use of Dupilumab reduced asthma exacerbations and levels of inflammatory markers associated with Th2, in addition to showing improvement in lung function.

Subtitle: "≥" = Bigger or equal. Th2= T-helper-2-cell. FEV1= forced expiratory volume in 1 s.

the monoclonal antibody known as Omalizumab approved in 2003 by the United States' *Food and Drug Administration* (FDA), whose use was released to patients aged 12 years old or older. This medication targets the Fc fraction of free IgE antibodies and lymphocytes membranes. Such treatment was approved in 2005 by the *European Medicines Agency* (EMA) to individuals with uncontrolled severe allergic asthma, aged between 6 and 12 years and older than 12 years. Since then, several other monoclonal antibodies that target the IL-5 were produced in order to treat asthma. On the current scenario, researchers study monoclonal antibodies targeting the interleukins IL-13 and IL-4, in addition to Th2 cells[8].

This was the scenario when Dupilumab was released, a fully human monoclonal antibody that acts against the alpha-receptor of the interleukin 4 (IL-4) by inhibiting the IL-4/IL-13 signaling, used as a subcutaneous injection. On the treatment of patients with asthma, it is used instead of glucocorticoids or corticosteroids to obtain the most beneficial results at the disease control and treatment[9].

In a study performed with previously treated asthmatic patients, the concomitant use of Dupilumab reduced the glucocorticoids use on 70,1%, compared with the placebo group (retrenchment of 41,9%); the results were very promising. At the same case, 80% of the patients had a reduction of glucocorticoids dose of at least 50%, 69% had a dose adjustment to less than 5 mg a day, and 48% had the use of glucocorticoids completely discontinued. The rates of severe asthma exacerbation were 59% lower than in the placebo group, apart from an increase in forced expiratory volume in the first second (FEV1), which indicates improvement of lung function[3].

On uncontrolled asthma, the complementary use of Dupilumab every two weeks reduced twice as many episodes of exacerbated asthma attacks compared to placebo. FEV1 demonstrated a significant increase over time with the use of Dupilumab, but

4,1% of the patients exhibited eosinophilia after the treatment initiation[2].

Researchers performed a study with patients that had moderate-to-severe asthma and a high eosinophil count. A weekly dose of Dupilumab or placebo was administered. Patients were also instructed to discontinue conventional treatment during the study. The use of Dupilumab reduced asthmatic exacerbations by 87% compared to the placebo, in addition to improving most levels of pulmonary function. The research also revealed a reduction in biomarkers associated with inflammation by Th2 cells. In the same study, the safety and tolerability of the drug were evaluated. Concerning safety, Dupilumab was similar to the placebo and, in terms of effectiveness in the treatment of eosinophilic asthma, proved its effectiveness. Of the adverse reactions, the most common were nausea, headache, injection site reactions, and nasopharyngitis, all more frequent with the drug than with the placebo. Transient elevation of eosinophils in the peripheral blood of individuals also occurred, such as a rebound effect in response to IL-5 and blockade of IL-13 and IL-4. One patient developed hypereosinophilic syndrome; the treatment was discontinued, and corticotherapy was administered with immediate improvement[5].

In a second study with adults using corticosteroid therapy, the use of Dupilumab demonstrated significant increases in FEV1. The general population, as well as the group with eosinophil count below 300µL, presented similar results. As in the previous study, rates of asthmatic exacerbation were significantly reduced[4].

In a study that used dose variation to perform an analysis, the administration of 300 and 200 mg every two weeks presented better effectiveness when compared to the dose administered every four weeks. Both doses were well tolerated when compared to the placebo. In addition, the fact that Dupilumab was effective in patients who had an eosinophil count <300 and >300 cells/µL at the start of the study was of particular interest since other drugs are more effective in patients with high eosinophil counts[4].

CONCLUSIONS

It can be noticed that all the tests with control groups conducted up to now had similar results, showing that Dupilumab, a biological drug inhibitor of the interleukins IL-4 and IL-13 targeting Th2 cells, is promising for the treatment of asthma in moderate-to-severe cases when the conventional therapy proves to be insufficient because, aside from assisting with the symptoms control and reducing the exacerbation rate, it improves the patient pulmonary function. Its effects overcome the risks of collateral effects, such as peripheral eosinophilia and reactions at the injection site.

At the end of this review, we also concluded that additional more extensive trials are necessary to confirm the long-term effectiveness and safety of Dupilumab and evaluate the eosinophilic elevations that occurred in patients with asthma and serum elevated basal eosinophilia.

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Place of study: Hospital Servidor Público Estadual São Paulo (HSPE).

Author's contribuitions

Cíntia Bassani - Leonardo Saraiva - Article writing and methodology

Larissa Rossi; Kaian Siveris; Rafaella Lorenzon Sferelli; Luciana Kase Tanno - methodology.

RESUMO

OBJETIVO: *Este artigo teve como objetivo fazer uma revisão sistemática sobre o tratamento da asma moderada a grave, administrando Dupilumabe.*

MÉTODOS: *Foi realizada uma busca nas plataformas on-line Ebsco, SciELO, PubMed, Medline Bireme, Lilacs e New England Journal of Medicine. Foram selecionadas publicações de 2010 a 2018 referentes a artigos que continham grupos controle, que testaram a validade de Dupilumabe e verificaram a resposta dos pacientes por meio de testes controlados. Para a busca desses artigos, foram utilizadas as seguintes palavras-chave: "Dupilumab", "asthma", "Bronchial Asthma" and "Asthma, Bronchial". E o correspondente em português: "asma", "Asma brônquica" and "Asma brônquica". Os critérios de exclusão, revisões de literatura, notícias, artigos sem grupos de controle, artigos sobre diferentes assuntos, estudos de Dupilumabe sobre outras doenças, artigos sobre asma sem uso de Dupilumabe e artigos repetidos em plataformas de busca foram descartados.*

RESULTADOS: *A literatura aponta que a medicação apresenta boa resposta no tratamento da asma moderada a grave e auxilia na melhora da função pulmonar, além de resultar em poucos efeitos colaterais. Apresenta boa eficácia, segurança e tolerância pelos pacientes.*

CONCLUSÕES: *Dupilumabe é promissor para o tratamento da asma em que a terapia convencional se revela insuficiente. Maiores estudos adicionais são necessários para confirmar a segurança e a eficácia em longo prazo.*

PALAVRAS-CHAVE: *Dupilumabe. Asma. Broncopatias. Antiasmáticos.*

REFERENCES

1. Busse WW, Maspero JF, Rabe KF, Papi A, Wenzel SE, Ford LB, et al. Liberty Asthma QUEST: phase 3 randomized, double-blind, placebo-controlled, parallel-group study to evaluate dupilumab efficacy/safety in patients with uncontrolled, moderate-to-severe asthma. Adv Ther. 2018;35(5):737-48.
2. Castro M, Corren J, Pavord ID, Maspero J, Wenzel S, Rabe KF, et al. Dupilumab efficacy and safety in moderate-to-severe uncontrolled asthma. N Engl J Med. 2018;378(26):2486-96.
3. Rabe KF, Nair P, Brusselle G, Maspero JF, Castro M, Sher L, et al. Efficacy and safety of dupilumab in glucocorticoid-dependent severe asthma. N Engl J Med. 2018;378(26):2475-85.
4. Wenzel S, Castro M, Corren J, Maspero J, Wang L, Zhang B. et al. Dupilumab efficacy and safety in adults with uncontrolled persistent asthma despite use of medium-to-high-dose inhaled corticosteroids plus a long-acting β2 agonist: a randomised double-blind placebo-controlled pivotal phase 2b dose-ranging trial. Lancet. 2016;388(10039):31-44.
5. Wenzel S, Ford L, Pearlman D, Spector S, Sher L, Skobieranda F. et al. Dupilumab in persistent asthma with elevated eosinophil levels. N Engl J Med. 2013;368(26):2455-66.
6. World Health Organization. Chronic respiratory diseases. 2018. [cited 2018 June 5]. Disponível em: http://www.who.int/respiratory/asthma/en/.
7. Regateiro F, Moura AL, Faria E. Novos biológicos para o tratamento da asma. Rev Port Imunoalergologia. 2017;25(2):99-113.
8. Shein SL, Speicher RH, Proença-Filho JO, Gaston B, Rotta AT. Tratamento atual de crianças com asma crítica e quase fatal. Rev Bras Ter Intensiva. 2016;28(2):167-78.
9. European Medicines Agency. Dupixent, INN, Dupilumab 2017. [cited 2018 June 5]. Disponível em: https://ec.europa.eu/health/documents/community-register/2017/20170926138667/a nx_138667_pt.pdf