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SUMMARY

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SECTIONS

ORIGINAL ARTICLES

REVIEW ARTICLES

Yellow fever

Febre amarela

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Yellow fever

Yellow fever is a potentially very serious disease, with high mortality, caused by a Flavivirus, which is a genus of the *Flaviviridae* family, inoculated in humans by arthropod vectors with two cycles of transmission: urban and sylvatic (jungle).[1] In Brazil, there is no record of urban transmission of the disease since 1942, but human cases in the sylvatic cycle do occur.[2]

Yellow fever is still relevant in countries on three continents: Africa, South America and Central America, with an annual estimate of 84,000 to 170,000 serious cases and about 29,000 to 60,000 deaths, according to the World Health Organization (WHO).

The peak transmission season in Brazil is between December and May, with epizootic cases having occurred in non-human primates in atypical periods in the last year.

Cases of sylvatic yellow fever were recorded in the states of Goiás, Mato Grosso do Sul, Pará, Tocantins, Distrito Federal, Minas Gerais and São Paulo. The Brazilian Ministry of Health issued an official document, Informative Note No. 02/2017, which regulated the expansion of the areas of obligatory vaccination coverage in the country, also vaccinating its inhabitants and visitors.

It should be noted that myths, misinformation and neglect about yellow fever have caused diffuse panic in several countries.[3]

Epidemiology

Two distinct cycles of disease transmission occur in endemic areas (sylvatic and urban), with symptoms indistinguishable from each other. In sylvatic-cycle infections, the vector species are necessarily of the *Haemagogus* and/or *Sabethes* genera, which normally inhabit the tree canopy, as well as the susceptible reservoir, non-human primates, and occasionally humans. In urban-cycle infections, the vector responsible for the transmission of the virus is of the *Aedes* genus, with *Aedes aegypti*, an insect extremely adapted to urban conditions, as the main disease transmitter. In this case, humans are the only susceptible reservoir. In terms of transmission potential, sylvatic-cycle vectors are more efficient than those of the urban cycle. This, combined with mass vaccination campaigns in the country, led to the last case of urban-cycle infection in Brazil occurring more than seven decades ago, precisely in 1942, in the state of Acre. It is worth remembering that epizootic (death of monkeys due to yellow fever) is a red flag to verify the circulation of yellow fever virus in the region, which, *per se*, can generate immediate action of the sanitary authorities to elaborate contingency plans for halting transmission to humans.

The sylvatic form of transmission does not occur so markedly in other endemic countries as it does in Brazil. This is particularly true for the African continent, where approximately a thousand cases occurred in 2016 with urban transmission, with the countries most affected, in descending order, being Angola, Democratic Republic of Congo, Ethiopia and Uganda (http://www.who.int/emergencies/yellow-fever/en/).

In Latin America, apart from Brazil, a recent outbreak struck Peru, which notified health authorities of more than twice as many cases of yellow fever as normal.

In Brazil, an increase in the number of cases of yellow fever started to be noticed at the end of 2016, extending from the summer of 2017 to the present, with more than 680 cases reported and a case fatality rate of 34% (monitoring of yellow fever cases and deaths – Report 37, Ministry of Health). As previously reported, all cases have sylvatic transmission. The most affected states are Minas Gerais and Espírito Santo, but São Paulo and Rio de Janeiro (which traditionally does not register cases of the disease) are also on the list.

The intensification of sylvatic transmission of yellow fever with increasing numbers of patients every 5 to 8 years is a known fact that can be explained by the increase

in the number of unvaccinated susceptible individuals who enter or live in endemic areas. This leads to the immediate call for action by health authorities in order to contain the disease's progress through blocking vaccination campaigns.

Immunization

In 1937, an attenuated virus vaccine, specific for yellow fever, was developed conferring lifelong immunity in up to 99% of those vaccinated. Max Theiler, of the Rockefeller Foundation, received in 1951 the Nobel Prize of Medicine for such discovery.[4] Since then, vaccination campaigns in endemic countries have been the central axis that has significantly reduced the number of cases in the world and in Brazil.[5]

Today, being vaccinated against yellow fever is a condition for entry into several countries due to the risk of contracting the disease at the destination or the possibility of introducing the virus into an epidemiologically compatible environment.

In Brazil, two vaccines are distributed against yellow fever, one produced by Biomanguinhos (Fiocruz) and another by the Sanofi-Pasteur laboratory. For the list of centers authorized to issue an official vaccination document, visit the website of the National Health Surveillance Agency, www.anvisa.gov.br. For the list of countries requiring vaccination against yellow fever as a condition for issuing the entry visa, visit the World Health Organization website, or the US Centers for Disease Control and Prevention, www.who.int and www.cdc.gov/travel, respectively. Travelers with contraindications to the vaccine and who are going to countries that require the International Certificate of Vaccination against Yellow Fever must present a medical statement attesting to the fact to one of the authorized centers for the emission of the vaccine exemption affidavit form, valid internationally.

The yellow fever vaccine consists of attenuated live virus, strain 17D, with two sub-strains: 17DD, used in Brazil, and 17D-204, used in other countries. Protection, which reaches levels above 95%, begins after the tenth day of application and probably extends for decades. Current evidence shows that the protection conferred by the vaccine is long-lasting, probably lifelong, and therefore there is no recommendation for revaccination, even if there is new displacement to endemic areas.[6] Contraindications include: children under 6 months of age; gestation; immunosuppression associated with disease or therapy (cancer, including lymphomas and leukemias, AIDS, systemic corticosteroid therapy, chemotherapy and radiotherapy), previous history of egg anaphylaxis, and allergic reaction to the previous dose of the vaccine. For those with contraindications, regions where the disease is endemic should be avoided; if the trip is essential, follow the methods of individual protection against mosquitoes.

Non-serious reactions are common, including pain at the site of application, fever, myalgia and headache, which generally appear after vaccination between the second and fifth day after receiving the dose. Serious adverse events such as yellow fever vaccine-associated viscerotropic and neurotropic disease, although infrequent, may arise. Cases similar to the disease, with visceral involvement, have been described since 1996, with an approximate incidence of 1 for every 40,000-50,000 doses in the United States, especially in individuals over 60 years of age, with mortality around 65%. Likewise, rare cases of encephalitis have been reported, with a higher frequency in children, particularly those under 6 months. Such occurrences are probably linked to individual immune responses and not to changes in the vaccine virus. The fact that four of the 62 cases of viscerotropism already reported in the world (up to the year 2016) were linked to diseases leading to thymus dysfunction or previous thymectomy corroborates this assumption.[7]

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Brachytherapy guideline in prostate cancer (high and low dose rate)

Diretriz de tratamento com braquiterapia em câncer de próstata (alta e baixa taxa de dose)

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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 procedures 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.

Grades of recommendation and levels of evidence

- **A:** Experimental or observational studies of higher consistency.
- **B:** Experimental or observational studies of lower consistency.
- **C:** Cases reports (non-controlled studies).
- **D:** Opinion without critical evaluation, based on consensus, physiological studies or animal models.

Objectives and description of evidence collection method

Through the elaboration of seven relevant clinical questions related to the proposed theme, we sought to present the main evidences regarding safety, toxicity and effectiveness of the presented radiotherapy (RT) techniques. The study population consisted of male patients of all ages with early primary prostate cancer and candidates for treatment with curative intent. For this, a systematic review of the literature was carried out in primary scientific databases (MEDLINE – PubMed; Embase – Elsevier; LILACS – BIREME; Cochrane Library – Record of Controlled Trials). All articles available through February 22, 2015 were considered. The search strategy used in MEDLINE searches is described in Appendix 1. The articles were selected based on critical evaluation, seeking the best evidence available. The recommendations were elaborated from discussions held with a drafting group composed of four members of the Brazilian Society of Radiotherapy. The guideline was reviewed by an independent group, which specializes in evidence-based clinical guidelines. After completion, the guideline was released for public consultation for 15 days; the suggestions obtained were forwarded to the authors for evaluation and possible insertion in the final text.

Introduction

Prostate cancer is the most common cancer in men and its incidence has been increasing in recent decades. The main reasons for this are increased life expectancy, marked presence of the Western lifestyle (sedentary lifestyle and high-calorie diet) and the development of more accurate diagnostic methods.

Around the world, in 2008, 903,000 new cases of prostate cancer were estimated with 258,000 deaths attributed to the disease, making it the second most commonly diagnosed neoplasm in men.[1] Although globally it accounts for 9.7% of tumors in man, this distribution differs between developed and developing countries, reaching 15.3% in the former and only 4.3% in the latter.[2]

In 2014, in the United States, 233,000 new cases were diagnosed with about 29,500 deaths related to prostate cancer.[3]

In Brazil, in 2014, there were 68,800 new cases of prostate cancer. This figure corresponds to a risk of 62 new cases per 100,000 men.[4]

The discovery of prostate-specific antigen (PSA) three decades ago revolutionized the diagnosis and treatment of prostate cancer. Increased early detection was observed, mostly in asymptomatic individuals.[5]

The initial clinical diagnostic evaluation aims to determine the precise extent of the disease, which has prognostic implications, and indicates the most appropriate treatment. In addition to TNM staging,[6] which includes digital rectal examination, the most important factors to be analyzed for therapeutic decision are: histological grade of the tumor according to Gleason score, PSA level, age and the presence of comorbidities.[7-9] Thus, patients are grouped by prognosis according to the following variables:

- Low risk: PSA ≤ 10 ng/mL plus Gleason ≤ 6 and stage T ≤ 2a disease.
- Moderate risk: one of the criteria above is not met.
- High risk: two of the criteria above not met, or Gleason > 7 or T > 2b or PSA > 20 ng/mL.

In early tumors, radical locoregional treatment can alter the natural course of the disease by decreasing local progression, distant metastasis and death from prostate cancer.[10-12]

The ideal therapy for localized prostate cancer is still the subject of controversy. The long natural history of early and low-risk tumors means that not all patients need treatment if their life expectancy is less than 10 years (active surveillance[13]).

Several treatment alternatives may be employed in initial management as monotherapy or combination therapy, such as radical prostatectomy, external beam RT and brachytherapy (BT). However, there is still no direct comparison between the three modalities based on randomized clinical trials.

BT has been used in prostate cancer since the last century. However, in the 1980s, there were incorporations to the historically described technique that made it more systematized, such as the use of real-time images to guide the placement of isotopes, computerized planning and, lastly, the transperineal approach – less invasive and less toxic.

In fact, in comparison to other modalities, BT became attractive for some reasons: a supposed lower invasiveness and toxicity compared to surgery and even to external irradiation; it allows the patient to return to normal activities faster; and, finally, it is a treatment that generates less cost.[14]

Below, practical questions to be answered in this guideline will be presented. BT (also called an implant) can be divided into two modalities:

- High-dose rate brachytherapy (HDR-BT): use of iridium-192 as a high activity source, controlled by a remote system that connects several needles placed strategically in the prostate and is later removed from the patient (temporary implantation).
- Low dose-rate brachytherapy (LDR-BT): insertion of seeds of iodine-125 (I-125) or palladium-103 (Pd-103) into needles that will be strategically implanted into the prostate and will remain in position allowing the release of the irradiating dose (seed implantation).

The modalities are similar in terms of complexity, and usually follow the steps below:

- Pre-implantation preparation (low-residue diet, intestinal preparation, pre-anesthetic visit, etc.).
- Anesthesia.
- Preplanning (placing the patient in a position favorable to implantation and acquisition of ultrasound images to determine the strategy of insertion of the radioactive material), also called volume study.
- Medical and physical planning.
- The implantation itself: refers to the insertion of the BT needles, guided by a template (installed in a device called stepper unit or attached to the patient's perineum using sutures and stitches on the skin) and ultrasound (fluoroscopy can also be used, if available).
- Cystoscopy for urinary tract inventory, if available.
- Post-implant dosimetry (CT scan to check the position of the radioactive material) – performed only in low dose-rate BT.

	High dose-rate	**Low dose-rate**
Implant type	Temporary	Permanent
Anesthesia	Yes	Yes
Pre-planning	Yes	Yes
Outpatient	Yes	No
Number of procedures	More than one	One
Conference in real time	Yes	Yes
Post-implant dosimetry	No	Yes
Pre-procedure preparation	Yes	Yes

Is low dose-rate brachytherapy an equally effective option as monotherapy?

For low-risk patients, there are two randomized studies comparing BT and surgery as monotherapy of patients with localized tumors.

A North American and Canadian multicenter study[15] included 263 patients with localized prostate tumors and compared radical prostatectomy with LDR-BT (144 Gy). At 5.3 years of median follow-up, PSA levels reached by the two groups were 0.05 ng/mL and 0.05 ng/mL, demonstrating equivalent biochemical control **(A)**.

A similar study performed by Italian centers[16] included 200 patients with low risk tumors and median age of 65 years. After 5 years of follow-up, 174 of them could be analyzed. Biochemical failure-free survival rates were at 91% in the surgery group and 91.7% in the LDR-BT group, which did not reach statistical significance **(A)**.

Comparison between BT monotherapy and external beam RT is the object of some observational studies. An American series of case reports[17] included 282 patients with low-risk tumors (137 treated with BT and 145 treated with external beam RT). After 5 years of follow-up, there were 8% of relapses in each group (p=0.09), with a similar toxicity profile **(C)**.

A medical literature review study[18] selected only articles involving all therapeutic modalities for localized and stratified prostate cancer, per risk group, that had at least 100 patients and 5 years of follow-up between 2000 and 2010. Out of 18,000 selected studies, 848 met the above criteria. Of these, 3% involved high-intensity focused ultrasound (HiFU), 5% involved robotic prostatectomy, 9% involved open radical prostatectomy, 15% involved proton external beam RT, 16% involved cryotherapy, 18% involved photon external beam RT and 31% involved BT (both modalities). Over 50,000 patients were assessed. In the comparison of outcomes (mainly PSA progression-free survival), BT presented results similar to those of surgery and external beam RT for low- and moderate-risk patients, but not for high-risk patients who benefited from combination therapies **(B)**.

The American Society of Brachytherapy[19] and the American Urological Association[20] indicate that the best candidates to undergo prostate BT are patients at low risk for the disease **(B)**. Remarks should be made for patients with moderate-risk prostate cancer, since within this group there are individuals with a favorable prognosis and who could possibly be treated with BT as well. Patients with low disease volume characteristics (total biopsy tissue invaded by tumor < 50%), predominant Gleason 3 pattern (3+4 and not 4+3) and absence of perineural invasion would be the candidates to receive monotherapy with BT.

Is high dose-rate brachytherapy an equally effective option as monotherapy?

There is no formal comparison in clinical studies between HDR-BT and other modalities.

A US Phase 2 study[21] involved 110 patients with low- and moderate-risk tumors for treatment with HDR-BT as monotherapy (three dose types were used: 34 Gy in four fractions, 36 Gy in four fractions and 31,5 Gy in three fractions with intervals of 6 hours between them). Hormone replacement therapy was allowed. Acute toxicities observed were relatively high, but there was no biochemical recurrence after 30 months of median follow-up **(C)**.

A single-center retrospective study[22] included 77 patients treated with HDR-BT as monotherapy (three implants with a dose of 15 Gy each every 3 weeks). Hormone replacement was allowed for patients with high-risk tumors. At a median follow-up of 57 months, overall survival, biochemical control and local control were 98.7%, 96.7% and 96.9%, respectively **(C)**.

A single-center retrospective study[23] involved 351 patients also treated with HDR-BT as monotherapy (four fractions of 9.5 Gy with a 14-day interval between them), but only patients with low-risk tumors were included and hormone replacement was not allowed. At a 5-year follow-up, biochemical control and metastasis-free survival were respectively 99% and 98% **(C)**.

The American Society of Brachytherapy specifically recommends for the indication of HDR-BT[24] that the procedure be performed only in low- or moderate-risk patients as monotherapy, on an investigational basis **(B)**.

Is low dose-rate brachytherapy an equally effective option as boost after external beam RT?

There is some evidence based on observational series and randomized studies, but without a direct comparison.

The RTOG 0019[25] is a phase 2 study that included 138 patients predominantly at moderate risk for treatment with external beam RT (45 Gy prescription dose) targeting the prostate and seminal vesicles, followed by a LDR-BT boost (108 Gy prescription dose). After 48 months of median follow-up, the observed rate of biochemical failure was 14% **(B)**.

A single center observational study[26] showed the follow-up of 223 patients with T1 and T3 stages treated with external beam RT (45 Gy) followed by LDR-BT (I-125 or Pd-103). After 15 years of follow-up, PSA failure-free survival was 74% for the entire sample. Classified according to risk groups, patients with low, moderate and high risk presented 85.8%, 80.3% and 67.8%, respectively (p=0.002) **(C)**.

Is high dose-rate brachytherapy an equally effective option as boost after external beam RT?

Some studies have analyzed the strategy of irradiation dose escalation with HDR-BT after external beam RT in patients preferably at moderate risk.

A randomized study from the UK analyzed 220 patients (T1 to T3 without metastases, PSA < 50 ng/mL) treated with external beam RT alone (55 Gy in 20 fractions) versus external beam RT (35.7 Gy in 13 fractions) followed by HDR-BT boost (two implants with 24h interval and 8.5 Gy prescribed dose per implant). Mean PSA failure-free survival was 4.3 versus 5.1 years (p=0.03). Acute rectal toxicity was favorably attributed to the HDR-BT group: lower rate of grade II proctitis (14% versus 5%, p=0.025). Other toxicity indicators were similar **(A)**.

A prospective US multicenter study[27] analyzed 207 patients (T2b, Gleason ≥ 7, PSA ≥ 10 ng/mL) treated with external beam RT (46 Gy) and HDR-BT, using two implants (first and third weeks of external beam RT) with doses between 5.5 and 11 Gy per implant. After a median follow-up of 4.7 years, biochemical control was at 74% for

the whole sample. Actuarial rates of biochemical control after 5 years were at 85% for patients with one prognostic factor, 75% for two factors and 50% for three factors (p=0.001). Global, cause-specific and disease-free survival rates were respectively at 92%, 98% and 68%. Complication rates grade 3 or above totaled 8% for genitourinary and 1% for rectal, with an impotence rate of 51% **(B)**.

Is low dose-rate brachytherapy less toxic than the other therapeutic options for prostate cancer?

The expected toxicities for BT are suitably comparable to the toxicity obtained with external beam RT. The expected toxicity pattern (rectal and urinary toxicity) differs greatly from that observed with surgical options (infection, abscess, lymphocele, surgical death), and therefore we do not see a reason for analysis. The comparison of quality of life will be approached in another question.

A multicenter US and Canadian randomized study[15] comparing a surgical approach and BT analyzed toxicity in 263 patients using standardized scores. At 5.2 years of median follow-up, there were no differences in gastrointestinal and hormonal toxicity between the two groups. However, the BT group had lower rates of urinary (91.8% versus 88.1%, p=0.02) and sexual (52.5% versus 39.2%, p=0.001) toxicity **(A)**.

An Italian randomized study[16] with 200 patients compared toxicities between patients undergoing surgery or LDR-BT. Urinary incontinence rates were 18.4% versus zero, in favor of patients undergoing LDR-BT. The rates of urethral stenosis were 6.5% versus 2%, also in favor of BT, whereas the latter group presented 10% urinary retention at 12 months of follow-up versus zero in the surgery group. Rectal toxicity was observed only in the BT group (4%). Erectile dysfunction was assessed based on the International Index of Erectile Function (IIEF) Questionnaire and rates were similar between groups (62% versus 60% of patients with preserved function). After 5 years of follow-up, no toxicity rate was significantly different between the two groups **(A)**.

An American retrospective population-based study that included 60,134 patients from the SEER (Epidemiology Department) database treated with BT (both modalities), external beam RT and BT (both modalities) plus external beam RT, and 25,904 patients undergoing observation alone in order to examine the matter of genitourinary toxicity.[28] The results showed that genitourinary toxicity grades 2 to 4 accumulated in 10 years was 27.8%, 23.5% and 20.1% for BT plus external beam RT, BT alone, and external beam RT alone, respectively, while patients without active treatment had 19.9% of toxicity, which can be considered as baseline level **(C)**.

Two other retrospective series reported toxicity data with sufficient follow-up time for comparison. A US study analyzed patients treated with 81-Gy intensity-modulated radiotherapy (IMRT) and showed a similar rate to those reported in BT studies (18% toxicity with a grade greater than or equal to 2 in 10 years)[29] **(C)**.

Another American study retrospectively analyzed 1903 consecutive patients undergoing three modern techniques of RT – BT alone (HDR-BT or LDR-BT using Pd-103), external beam RT plus image-guided radiotherapy (IGRT), or the combination of both.[30] Acute grade 2 or greater urinary and intestinal toxicity was lower in the group treated with BT alone. Late toxicity was worse when the modalities were associated compared to each one performed alone **(C)**.

Is high dose-rate brachytherapy less toxic than the other therapeutic options for prostate cancer?

A study cited in the previous question retrospectively examined 1,903 patients undergoing three modern RT techniques including HDR-BT or LDR-BT using Pd-103 and IGRT.[26] The reported genitourinary toxicity rates were 28%, 22% and 21% in patients submitted to BT plus external beam RT, BT alone and external beam RT alone, respectively. Patients kept on observation had 19.9% of grade 2 or higher toxicity. In the same study, a lower rate of rectal bleeding was demonstrated with BT alone compared to the combination of external beam RT and BT, or external beam RT alone, with respective rates at 0.9%, 7% and 16% **(C)**.

Does low or high dose rate brachytherapy affect quality of life less?

Although there is no consensus on how to evaluate the various domains that impact quality of life after the various treatments of prostate cancer, there is a difference in the results of each domain depending on the therapeutic modalities.[31]

Three prospective studies directly compared quality of life according to therapeutic modality, specifically LDR-BT and surgery.

The first, Canadian, prospectively evaluated 190 patients undergoing radical prostatectomy or BT in a partially randomized phase III design.[15] The evaluations were done with an instrument based on 50 items reported by the patients (EPIC HRQOL). Questions regarding urinary incontinence, urinary control and the degree of urinary

loss showed a statistically significant difference in favor of BT (p<0.001). Meanwhile, none of the questions regarding irritative or obstructive symptoms that are usually concerns in BT-treated patients showed a significant difference. In the sexual domain, questions about the ability to have an erection (p=0.001), quality of erections (p=0.001), frequency of erections (p=0.003), waking with morning erection (p=0.002) and ability to have a satisfactory sexual function (p=0.003) all favored BT. BT was statistically superior in the urinary, sexual and patient satisfaction domains. There was no difference in the other domains. Specifically in relation to urinary incontinence, more than 80% of patients treated with BT reported having zero incidence of urinary incontinence, whereas less than 60% of those undergoing surgery did the same **(A)**.

The second, an American study, involved 1,201 patients and 625 female partners prospectively evaluated in a non-randomized study interviewed by telephone before and 2, 6, 12 and 24 months after radical prostatectomy, prostate external beam RT, or LDR-BT.[24] The interview was started before the use of androgen blockade, if any. Reduction of erectile function was reported by the partner in 44% of cases treated with radical prostatectomy, 22% of those treated with external beam RT, and 13% of those treated with LDR-BT. Analyzing the quality of life charts, specifically regarding sexual function and urinary incontinence, the steepest decline in rates in the surgery group compared to the baseline assessment is clear. Such decline is not relevant in the group treated with BT. It is difficult to compare the modalities, however, since the author does not report a statistical comparison between them. This study demonstrated that changes caused by LDR-BT are lighter in some domains and more relevant in other ones **(B)**.

The third study, Italian, was randomized and included 200 participants in the analysis of quality of life scores (EORTC-QLQ-C30/PR25) between surgical patients and others submitted to LDR-BT. There were no significant differences in the domains peculiar to this evaluation tool (physical, emotional, cognitive and social functions, global health, fatigue, nausea/vomiting, pain, dyspnea, insomnia, lack of appetite, constipation, diarrhea, financial problems, urinary, intestinal and sexual symptoms) **(A)**.

Specifically for patients undergoing HDR-BT, a single arm observational series[32] analyzed 51 patients using three scores, analyzed at 2 and 4 weeks, and also at 3, 9, and 12 months. The Functional Assessment of Cancer Therapy--Prostate (FACT-P) questionnaire did not show significant variation in all domains (physical, social, family, emotional and functional well-being). The IIEF index did not show significant variation, either. The International Prostate Symptom Score (IPSS), in turn, showed a significant increase at weeks 2 and 4, but recovery was seen at 3 months **(C)**.

Appendix

Search strategies for MEDLINE

(Prostate Neoplasms [Mesh] OR Prostate Neoplasm OR Neoplasm, Prostate OR Neoplasms, Prostate OR Tumors, Prostate OR Prostate Tumors OR Prostate Tumor OR Tumor, Prostate OR Prostatic Carcinoma, Human OR Carcinoma, Human Prostatic OR Carcinomas, Human Prostatic OR Human Prostatic Carcinomas OR Prostatic Carcinomas, Human OR Human Prostatic Carcinoma OR Prostatic Neoplasms, Human OR Human Prostatic Neoplasm OR Human Prostatic Neoplasms OR Neoplasm, Human Prostatic OR Neoplasms, Human Prostatic OR Prostatic Neoplasm, Human OR Prostate Cancer OR Cancer, Prostate OR Cancer of the Prostate OR Cancer of Prostate) AND (Brachytherapy [MeSH] OR Radioisotopes [MeSH] OR Radiotherapy [MeSH] OR Radioisotopes [MeSH] AND Therapeutics [MeSH] OR Iodine [MeSH] OR Palladium [MeSH] OR Interstitial [MeSH] OR Permanent [MeSH] OR Implant [MeSH])

Conflict of interest

The authors declare no conflict of interest.

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Is dexmedetomidine the gold standard for pediatric procedural sedation and anxiolysis?

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Pediatric procedural sedation is a growing issue in the emergency setting, and finding the right drug to perform safe and effective sedation is still a challenge. I would like to discuss the article "Double-blind randomized controlled trial of intranasal dexmedetomidine versus intranasal midazolam as anxiolysis prior to pediatric laceration repair in the emergency department," by Neville et al.,[1] which is currently in press in the Academic Emergency Medicine Journal. The authors randomized 38 children to receive either intranasal dexmedetomidine (DEX) or intranasal midazolam before laceration repairs, and chose as primary outcome the anxiety score at the time of patient positioning for the repair. The proportion of patients who were classified as not anxious at the position for procedure was significantly higher in the dexmedetomidine group (70%) versus the midazolam group (11%). Authors concluded that intranasal DEX is an alternative with good results for anxiolysis prior to painful procedures in children compared to midazolam.

DEX is a highly selective α2 adrenergic agonist that offers some unique and unmatched sedation characteristics.[2] Without pediatric labeling, DEX has been studied for pediatric sedation and anxiolysis, intravenously or using other administration routes, such as intranasal (IN). In contrast to all other sedatives, DEX produces a sleep somnolence state which closely resembles that of non-REM sleep on electroencephalogram.[3] DEX maintains spontaneous ventilation, has minimal respiratory effects and preserves upper airway tone, making it an attractive choice for pediatric procedural sedation and anxiolysis.

The majority of pediatric sedation literature on DEX described its application for non-painful radiological imaging studies such as MRI, computerized tomography scans, and nuclear medicine studies. A few studies addressed this sedative for anxiolytic purposes. Some authors studied DEX alone or carried out clinical trials comparing it with other drugs. Recently, Sidhu et al.[4] studied 105 ASA 1-2 surgical patients comparing IN DEX with IN clonidine. Using an initial dose of 2 mcg/kg of IN DEX, satisfactory anxiolysis was achieved in 88.5% of these patients and in 60% of the clonidine patients, with significantly less rescue analgesia requirements in the DEX group. Another recent and very interesting study was conducted by Yao et al.[5] with 90 children receiving 1-2 mcg/kg of IN DEX prior to laryngeal mask insertion, which concluded that patients receiving 2 mcg/kg had significant lower alveolar concentrations of sevoflurane prior to the procedure and less emergency delirium after it.

The two studies above described were the only ones focused on DEX premedication in children, prior to two meta-analyses[6,7] published in 2014 that verified the efficacy and safety of premedication with DEX in children, alone or associated with midazolam. Together, the authors pooled 24 randomized controlled trials and concluded that DEX is superior to midazolam premedication because it resulted in enhanced preoperative sedation and decreased postoperative pain. In addition, DEX premedication provided clinical benefits that included reduced requirements for rescue analgesia and reduced agitation or delirium and shivering during the postoperative period.

Our group has previously studied IN DEX and midazolam for pediatric procedural sedation, and we felt that the quality of anxiolysis and sedation provided by IN DEX[8,9] was far superior. However, we selected two prospective cohorts and our primary outcomes were time to sedation and rates of failed sedation. As we didn't

randomize the patients, comparison between the drugs is flawed, but no failed sedations occurred in the DEX patients, and no adverse events with clinical relevance were observed. Parent satisfaction, although not directly measured by Neville et al.,[1] was also greater with IN DEX.

One concern with the study conducted by Neville et al.[1] was that the anxiety score at positioning for procedure of the patients receiving DEX was 9.2 points lower than that of the patients receiving midazolam, according to the modified Yale Preoperative Anxiety Scale, which the authors used as a reference. Although other baseline characteristics between the two groups (DEX and midazolam) were similar, these random findings can cause a potential bias to the final conclusions. Despite probably being a better sedative than midazolam, one could conclude that DEX performed better in these patients because of their baseline anxiety conditions.

I believe that larger studies with IN DEX as premedication are needed in order to find the better option for pediatric anxiolysis. The article from Neville et al. substantiates the indication of IN DEX as a sedative of choice, with minimal adverse events and good parent and staff satisfaction.

Conflict of interest

The author declares no conflict of interest.

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Facial paralysis due to Ramsay Hunt syndrome – A rare condition

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Summary

Ramsay Hunt syndrome (or herpes zoster oticus) is a rare complication of herpes zoster in which reactivation of latent varicella zoster virus infection in the geniculate ganglion occurs. Usually, there are auricular vesicles and symptoms and signs such otalgia and peripheral facial paralysis. In addition, rarely, a rash around the mouth can be seen. Immunodeficient patients are more susceptible to this condition. Diagnosis is essentially based on symptoms. We report the case of a diabetic female patient who sought the emergency department with a complaint of this rare entity.

Keywords: facial paralysis, herpes zoster oticus.

A 68 year-old female patient with history of type II diabetes sought our emergency service (ER) with a clinical complaint of difficulty to blink and left-sided hearing loss associated with labial deviation to the right. The symptoms had begun eleven days before her admission. On neurological examination, we noted left facial paralysis House-Brackman grade IV associated with vesicles and crusted lesions on the left auricle (Figure 1). She complained of hearing loss on the same side. No other abnormalities were found in the general examination.

This clinical picture is therefore very suggestive of a rare condition known as Ramsay Hunt syndrome (herpes zoster oticus). It was first described by James Ramsay Hunt in 1907[1,2] and its occurrence is due to varicella zoster virus (VZV) reactivation in sensory root ganglia. The geniculate ganglion is located on the facial nerve in the depths of the internal auditory meatus at the entrance to the bony fallopian canal.[3] The infection involves facial and vestibulocochlear nerves, causing peripheral facial paralysis, otalgia and sensorineural hearing loss. Vestibular symptoms are rare.[2]

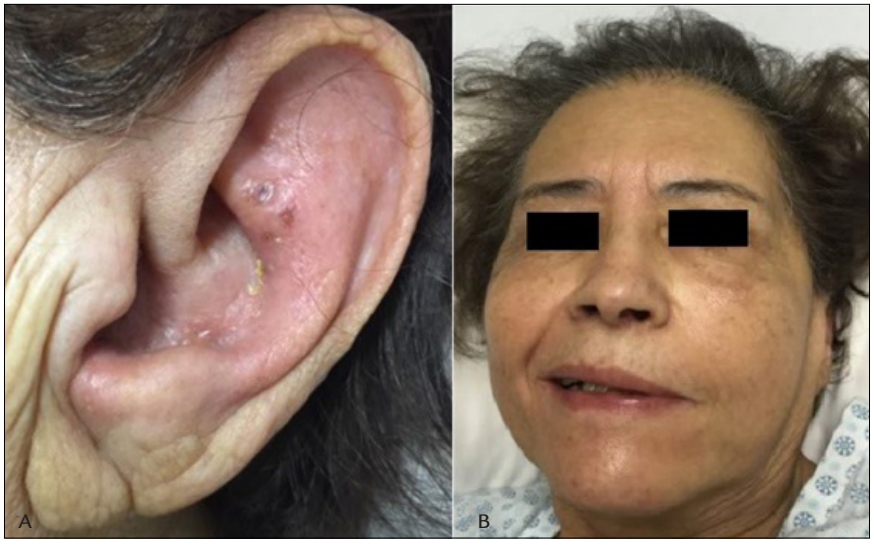

FIGURE 1 A. Auricular vesicles at the left pinna. B. Left peripheral facial paralysis House-Brackman IV, 149 x 91 mm (96 x 96 DPI).

Diagnosis is performed based on signs and symptoms. Examination of cerebrospinal fluid and gadolinium-enhanced MRIs have proved no diagnostic or prognostic value,[4] although a positive polymerase chain reaction (PCR) test for VZV is used for confirmation.[5] Proper physical examination and history is essential because in many cases, when facial paralysis is noted at the ER, the first hypothesis is that of stroke, which leads to misdiagnosis and wrong treatment and can cause sequelae, such as permanent hearing loss.

After diagnosis, the treatment includes steroids and antiviral drugs during the acute fase.[4,5] Usually, prednisone (duration and dose varies a lot but usually is 1 mg/kg/day for 5-7 day) is combined with intravenous or oral acyclovir (or similar drugs) concomitantly. It is essential to refer patients to rehabilitation after the acute phase for motor physiotherapy, biofeedback and massage therapy.[2,4] Our patient received seven days of prednisone and acyclovir, and a request for physioterapy as soon as the pain improved. She progressed with complete resolution of the facial paralysis 30 days after onset of treatment and no hearing sequelae was observed.

Resumo

Paralisia facial secundária à síndrome de Ramsay Hunt – Uma condição rara

A síndrome de Ramsay Hunt (ou zóster auricular) é uma complicação rara do herpes-zóster em que ocorre reativação de uma infecção latente pelo vírus varicela-zóster no gânglio geniculado. Geralmente, estão presentes vesículas auriculares e sintomas como otalgia e paralisia facial periférica. Além disso, mais raramente pode haver *rash* ao redor da boca. Pacientes com imunodeficiência apresentam maior susceptibilidade para essa condição. O diagnóstico é essencialmente pelo quadro clínico. É apresentado o caso de uma paciente diabética que compareceu ao setor de emergência com essa manifestação rara.

Palavras-chave: paralisia facial, herpes-zóster da orelha externa.

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Chronic recurrent multifocal osteomyelitis exhibiting predominance of periosteal reaction

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Summary

Chronic recurrent multifocal osteomyelitis is an idiopathic nonpyogenic autoinflammatory bone disorder involving multiple sites, with clinical progression persisting for more than 6 months and which may have episodes of remission and exacerbation in the long term. It represents up to 2-5% of the cases of osteomyelitis, with an approximate incidence of up to 4/1,000,000 individuals, and average age of disease onset estimated between 8-11 years, predominantly in females. The legs are the most affected, with a predilection for metaphyseal regions along the growth plate. We describe the case of a female patient, aged 2 years and 5 months, with involvement of the left ulna, right jaw and left tibia, showing a predominance of periosteal reaction as main finding.

Keywords: osteomyelitis, chronic, multifocal, recurrent, periosteal.

Case report

A female patient aged 2 years and 5 months, being investigated due to pain and swelling in the left forearm for one month. Her parents denied episodes of fever, trauma, and other comorbidities. On physical examination, the child presented normal weight, normal skin color, she was well-hydrated, acyanotic, breathing normally and in good general conditions. Blood counts analyzed in the previous month revealed mild leukocytosis and normocytic normochromic anemia. Levels of C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were slightly elevated.

Computed tomography (CT) of the left forearm without intravenous contrast revealed a periosteal reaction and a small area of loss of cortical compaction, especially in the ulna (Figure 1A).

A biopsy of the left ulna was performed with results describing signs suggestive of chronic osteomyelitis. Culture of the biopsy material did not show growth of microorganisms.

Empirical antibiotic therapy was used starting one month after diagnosis, but there was no change in clinical status. A new biopsy was performed in the third month, showing the same findings associated with negative blood culture. Radiographs of the left forearm from the second to the fifth month showed progression of the multilamellar periosteal reaction to a solid type (Figure 1B).

Chest radiography, echocardiography, levels of TSH, T4, IgG, IgA, IgM and complement components were all normal. A hypothesis of chronic recurrent multifocal osteomyelitis (CRMO) was suggested and thus treated with ibuprofen combined with methotrexate, later replaced with sulfasalazine.

Between the eighth and ninth month, pain and swelling appeared in the right mandibular region and in the left leg. A facial CT scan showed mainly periosteal reaction at the right mandibular angle with mild bone sclerosis (Figure 2). CT scan of the left leg also characterized a periosteal reaction in the tibial diaphysis with a small area of cortical alteration (Figure 3), similar to that found in the ulna.

After 6 months of therapy, there was clinical and general laboratory improvement, with left forearm radiography in the 11th month showing a regression of the periosteal reaction, despite persistence of cortical thickening.

Discussion

CRMO is an idiopathic nonpyogenic autoinflammatory bone disorder involving multiple sites, with clinical progression persisting for more than six months and which

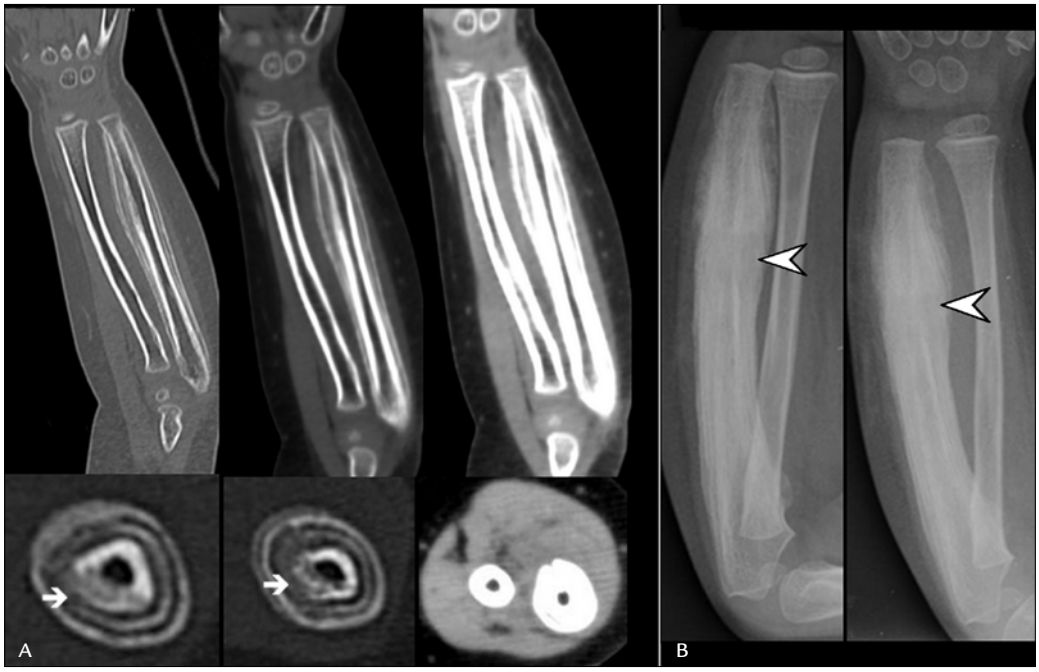

FIGURE 1 A. CT scan of the left forearm without administration of intravenous contrast medium showing intense metaphyseal and predominantly diaphyseal multilamellar periosteal reaction in the ulna and small area of cortical bone irregularity with loss of compaction (white arrows). B. Radiography of the left forearm after biopsies showing the periosteal reaction in the ulna and the point of collection of bone material (arrowheads).

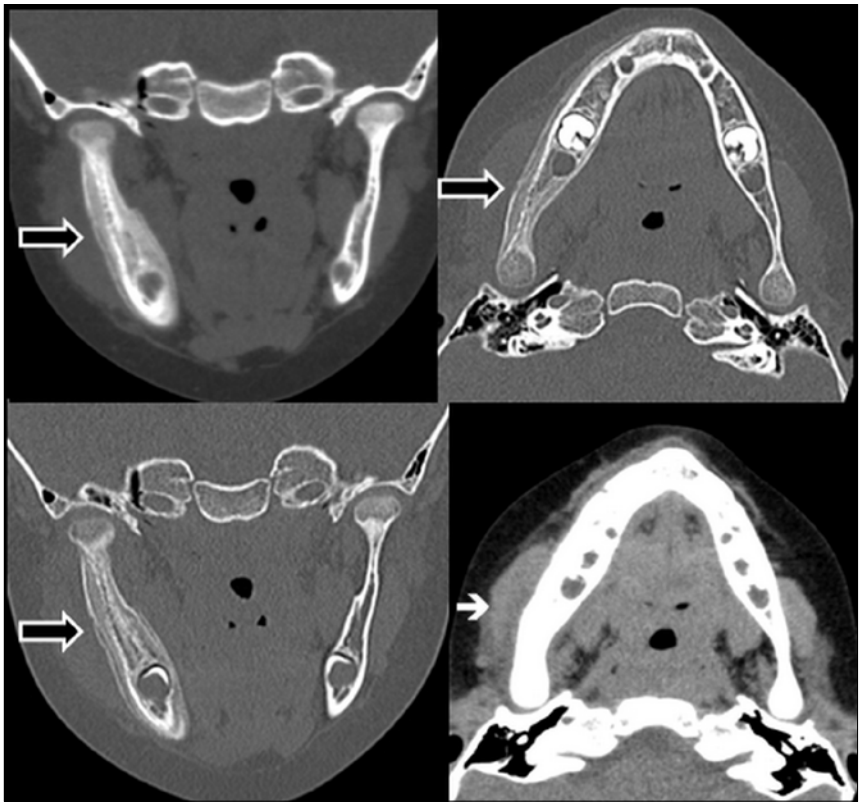

FIGURE 2 CT scan of the face mainly characterized by periosteal reaction in the right mandibular angle with tenuous bone sclerosis (black arrow), in addition to a discrete volumetric increase and densification of adjacent soft tissues, suggesting edema (white arrow).

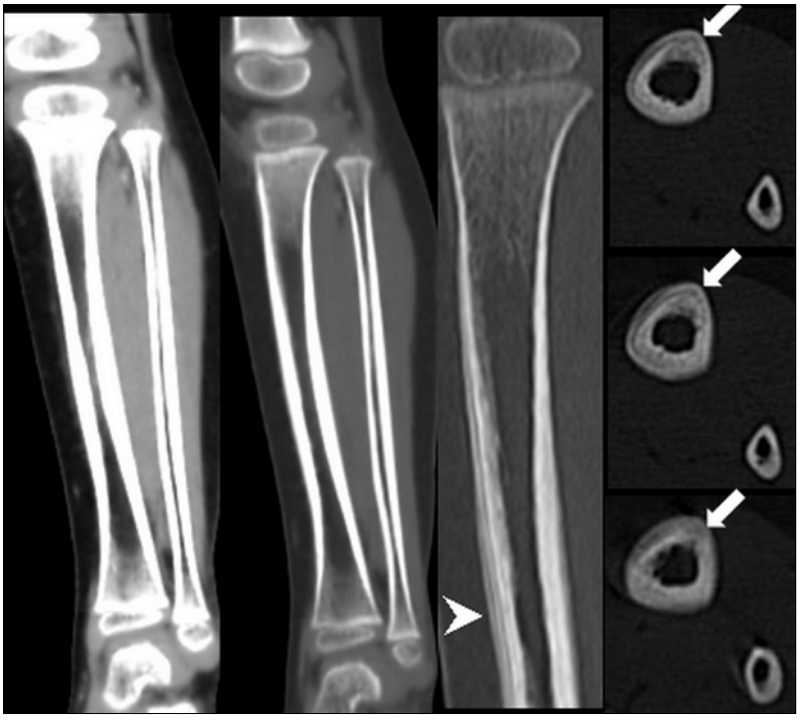

FIGURE 3 CT scan of the left leg demonstrating a periosteal reaction in the tibial diaphysis (arrowhead and white arrows) with a small area of cortical alteration similar to that found in the ulna (white arrows).

may have episodes of remission and exacerbation in the long term.[1] Some studies suggest that CRMO and other diseases such as SAPHO syndrome and Garré's osteomyelitis are variants of the same pathology, with their respective sites, characteristics and prevalent age groups.[1-3]

It represents up to 2-5% of the cases of osteomyelitis,[2] with an approximate incidence of up to 4/1,000,000 individuals,[4] and average age of disease onset estimated between 8-11 years, predominantly in females (64-83%).[1,4-6]

Signs and symptoms include local inflammation with pain and edema, episodes of mild fever, which do not profoundly affect the general condition of the patient, and common asymptomatic lesions (17-56%).[1] Positive personal or family history of psoriasis, palmoplantar pustules, chronic inflammatory bowel disease or other autoimmune diseases are also common (up to 50% of cases).[7-9]

In active disease, laboratory tests show a mild to moderate increase in ESR and CRP, with normal blood count or, more rarely, mild leukocytosis and anemia. Typically, the levels of complement components (C3 and C4), IgA and IgM do not change.[1,5,7,8,10] HLA-27 is positive in less than 30% of the patients.[1,9,11]

Performing a biopsy is important to rule out other diagnostic hypotheses. Histological findings are nonspecific and often similar to those found in septic osteomyelitis, but with no evidence of infection by any microorganism.[1,2-10]

Imaging tests such as radiography, CT, magnetic resonance imaging (MRI) and scintigraphy are some of the diagnostic tools. The latter two are most commonly used to rule out other diseases and to detect asymptomatic sites employing whole body screening techniques.[1,4-6,12]

Long and tubular bones are preferentially affected, especially the lower limbs.[1,4-6] In a study by von Kalle et al., the frequencies found for lesions by bone segment were: meta-epiphyseal (31%), metaphyseal (30%), epiphyseal (19%), meta-diaphyseal (10%), diaphyseal (1.8%), apophyseal (1.8%), and epimetadiaphyseal (1.4%).[4] According to a study by Fritz et al., diaphyseal involvement can reach 14%.[6] A predilection for regions in the metaphysis next to the growth plate is noted, and there is symmetry in at least 25% of the lesions.[1,4-6] The most common articular findings are joint effusion and synovitis, most often in the knees and ankles, often bilaterally, sometimes with affections in the sacroiliac joints.[6]

Analyzing the absolute number of lesions reported, the most frequently affected sites include tibia (15-31%), spine (7-30%), femur (11-23%), tarsal and metatarsal bones (8-19%), fibula (2-14%), hip (5-12%), clavicle (5-7%), radio (4-5%) and ulna (3-4%), and ribs (1-4%).[1,4-6]

In general, imaging tests show small lytic or sclerotic lesions, or a combination of both. Mixed lesions are usually characterized by lytic, circumscribed areas with sur-

rounding sclerosis. There is also the possibility of progression with locoregional cortical hyperostosis, in addition to simple, solid or multilamellar laminar periosteal reaction (7-48%), which may be the most obvious alteration in long and diaphyseal bones. Edema in bones and surrounding soft parts is usually present.[1,4-6]

Jansson et al. proposed the diagnosis of CRMO based on criteria, being positive if two major or one major and three minor criteria are met,[1,2,7,8] as follows:

- Major criteria – osteolytic/sclerotic lesions evident in imaging tests; multifocal bone lesions; presence of palmoplantar pustulosis or psoriasis; sterile bone biopsies with signs of inflammation and/or sclerosis.[1,2,7,8]
- Minor – normal blood count and leukogram; good general health; discrete/moderate elevation of ESR and CRP; disease course greater than 6 months; hyperostosis; association with other autoimmune diseases besides psoriasis and palmoplantar pustulosis; first or second degree relative with autoimmune or autoimmune disease.[1,2,7,8]

The most widely used treatment with the best results is nonsteroidal anti-inflammatory drugs (NSAIDs). Other therapeutic possibilities include the use of steroids, methotrexate, sulfasalazine, bisphosphonates, infliximab, colchicine, hyperbaric oxygen therapy, alpha and gamma interferons.[1,2,4-11]

CRMO is not usually lethal, but at least 20% of cases develop with sequelae. Chronic pain, pathological fractures, growth deformities, focal increase of bone volume, collapse in varying degrees of vertebral bodies with repercussion in the spinal canal are some of the possible complications, the latter being more serious.[1,4-6]

Resumo

Osteomielite crônica multifocal recorrente exibindo predomínio de reação periosteal

Osteomielite crônica multifocal recorrente é uma desordem autoinflamatória óssea idiopática não piogênica, envolvendo vários sítios e com evolução clínica persistindo por mais de 6 meses, podendo apresentar episódios de remissão e exacerbação em longo prazo. Representa de 2 a 5% das osteomielites, com incidência aproximada de até 4/1.000.000, com idade média de apresentação estimada entre 8 e 11 anos, predominando no gênero feminino. Os membros inferiores são os mais afetados, com predileção para regiões metafisárias junto à fise. Descrevemos um caso da doença em uma menina de 2 anos e 5 meses de idade, com acometimento de ulna esquerda, mandíbula à direita e tíbia esquerda, exibindo predomínio de reação periosteal como achado principal.

Palavras-chave: osteomielite, crônica, multifocal, recorrente, periosteal.

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Alternative option for osteogenesis imperfecta and trigeminal neuralgia

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Summary

Osteogenesis imperfecta (OI) is a bone disorder that can lead to skull base deformities such as basilar invagination, which can cause compression of cranial nerves, including the trigeminal nerve. Trigeminal neuralgia in such cases remains a challenge, given distorted anatomy and deformities. We present an alternative option, consisting in cannulation of the foramen ovale and classical percutaneous treatment. Percutaneous balloon microcompression was performed in a 28 year-old woman with OI and severe trigeminal neuralgia using computed tomography (CT) and radiographic-guided cannulation of the Gasserian ganglion without neuronavigation or stereotactic devices. The patient developed hypoesthesia on the left V1, V2 and V3 segments with good pain control. This alternative technique with a CT-guided puncture, using angiosuite without the need of any Mayfield clamp, neuronavigation systems, frame or frameless stereotactic devices can be a useful, safe and efficient alternative for patients with trigeminal neuralgia with other bone deforming diseases that severely affect the skull base.

Keywords: trigeminal neuralgia, pain, osteogenesis imperfect, percutaneous balloon compression.

Introduction

Osteogenesis imperfecta (OI), an inherited bone disorder, may produce severe disability and altered bone development, leading to multiple fractures after minimal or no trauma, thus inducing deformity.[1-3]

Often called "brittle bone disease," OI leads to various phenotypes. Mild forms can be premature or postmenopausal osteoporosis, and severe forms can lead to death in the perinatal period.[1,4]

Basilar invagination, a clinical manifestation, may cause cranial nerve compression due to odontoid process protrusion through foramen magnum into the intracranial cavity.[5,6]

The trigeminal nerve may be involved, resulting in neuralgic pain, often refractory to drug treatment. Other possible pathogeneses include arachnoid adhesions and increased vascularization in the foramen magnum area.[7-9]

Interventional treatment in such cases is difficult, mostly due to difficult access and bone fragility, besides cranial base anatomy distortion.[10]

In the past, foramen ovale cannulation was considered impossible for Gasser ganglion microcompression with conventional radiographic or tomographic-guided percutaneous approach without stereotactic or neuronavigation devices.[11]

Case report

Female patient, 28 years old, presenting OI type III, complaining of progressively worsening shooting pain with onset three years before, affecting her lower left jaw (V3 segment). Pain usually worsened while chewing, swallowing or talking and was refractory to multiple drug treatments, including carbamazepine at maximum dosage (1,200 mg daily) and pregabalin.

On physical examination, she showed multiple bone deformities, typical blue sclera, low height, marked thoracolumbar kyphoscoliosis and bilateral hearing loss, marked frontal bossing, no cranial nerve deficits, normal facial cutaneous sensation, and intact corneal reflexes.

Magnetic resonance imaging (MRI) revealed basal angle (Welcker) enlargement and clivus almost parallel to the palate, denoting platybasia. Remarkable basilar invagination was present, with odontoid process projecting 17 mm and 27 mm above the Chamberlain's line and the McGregor's line, respectively. Significant dorsal insinuation of odontoid process was found occluding pre-pontine subarachnoid space and compressing the pontine-medullary junction (Figure 1). No mass lesions or contrast enhancement over the fifth cranial nerves were seen.

Cranial-CT scan showed brachycephalic skull, with thin and irregular skull cap. Both foramina ovale showed irregular shape and reduced diameter.

The procedure was performed using angiography suite, allowing easier visualization of the foramen ovale. Under light sedation and with the patient in supine position, with slight neck extension, the X-ray intensifier was positioned on submental view to obtain the images followed by 3D CT-scan in the angiography suite. A #15 gauge cannula was inserted and positioned at the foramen ovale prior to a #4 Fogarty embolectomy catheter. The X-ray intensifier was displaced to lateral view and inflation was performed (1.0 mL of radiopaque dye). A pear-shaped image was obtained and the patient developed transitory bradycardia response. Position was confirmed with 3D CT-scan (Figure 2), balloon was deflated, process was repeated twice for 3 inflations (20 seconds/each, total 60 seconds) (Figure 3). No neuronavigation system, Mayfield clamps, frames or stereotactic devices were used.

Post-procedure, the patient reported painless hypoesthesia on left V1, V2 and V3 segments. She was discharged pain-free on the same day, with instructions to maintain the medication dosage until her first medical appointment. After one year of follow-up, the patient no longer complained of pain and ceased taking all medication.

Discussion

OI can be characterized by bone fragility secondary to reduced bone mass.[2,3] Fortunately, this clinically and genetically heterogeneous group of heritable disorders is

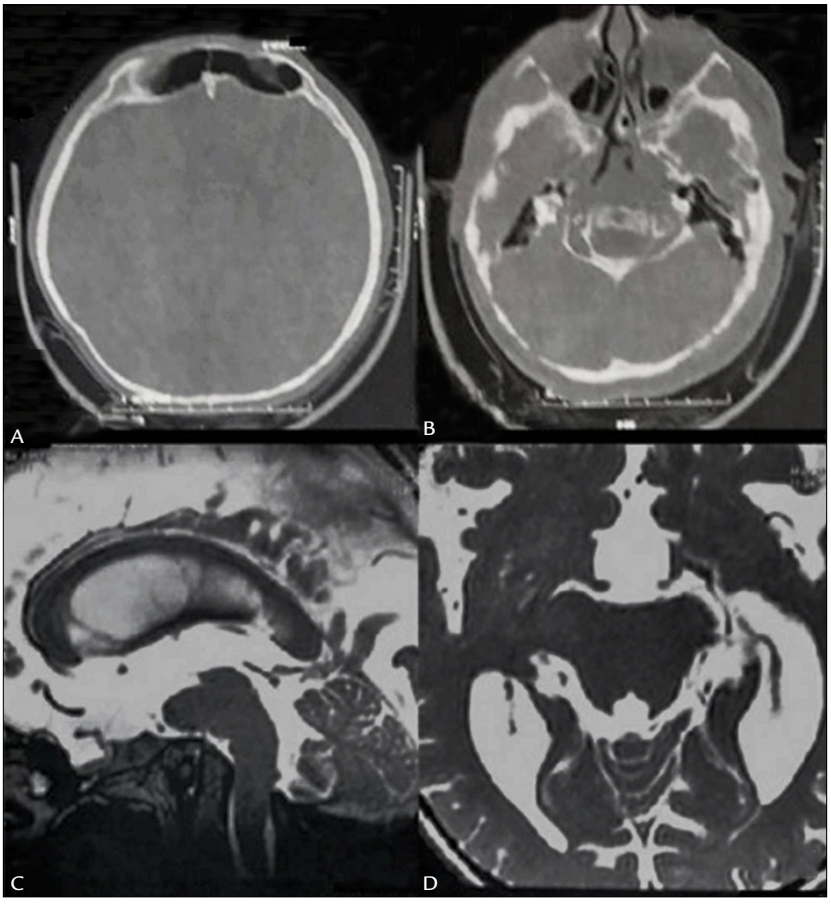

FIGURE 1 A. Wormian bones in a brachycephalic skull. B. Skull base abnormality. C. Basilar invagination obliterating the prepontine subarachnoid space, with compression of the brainstem. D. FIESTA image depicting the cisternal portion of the left trigeminal nerve.

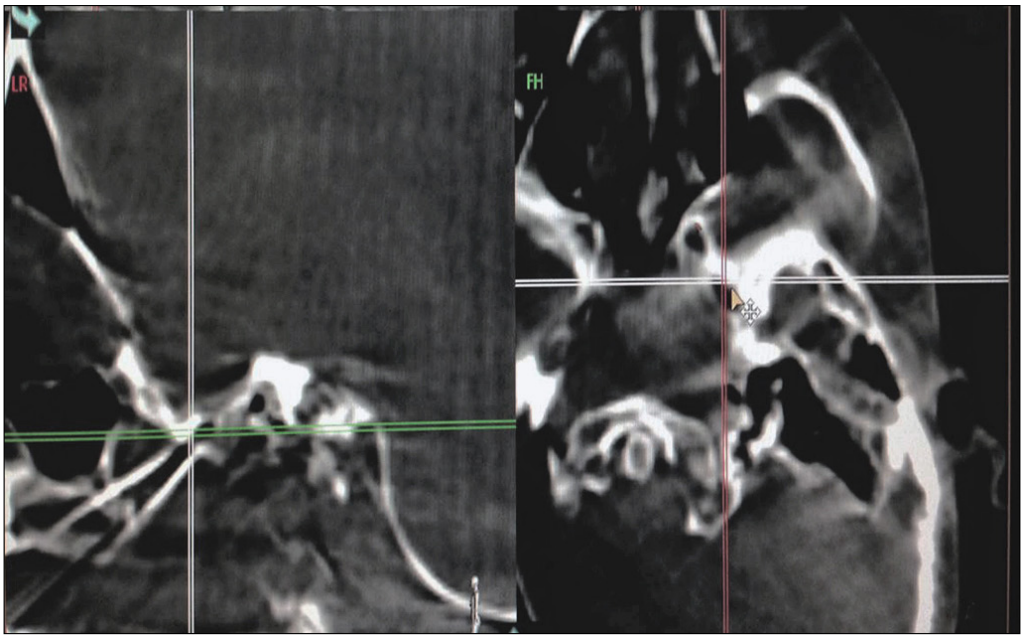

FIGURE 2 Submental view of the cannula positioned at the foramen ovale.

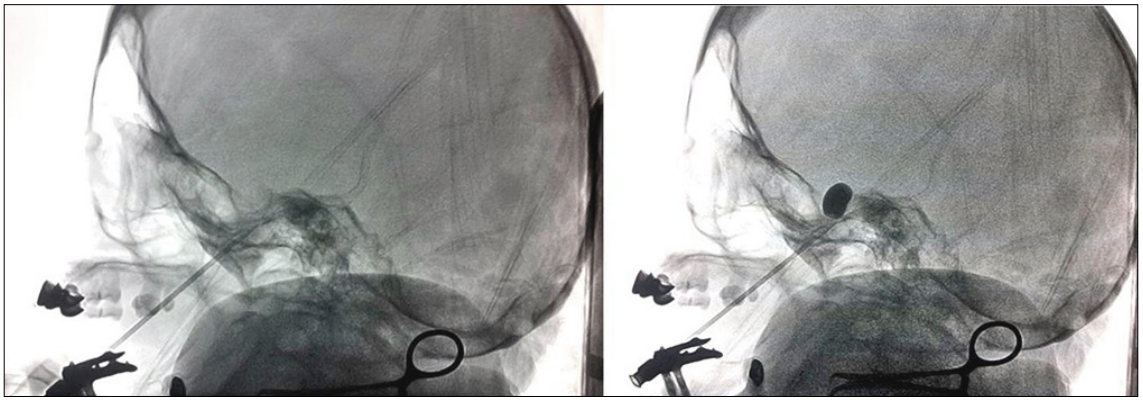

FIGURE 3 Intensifier control. Lateral view showing the pear-shaped aspect of the balloon.

mild in most cases and causes minimal deformities in adults. Skeletal manifestations may include thinned calvarium and excessive wormian bone formation.[4]

Basilar invagination is a rare complication of multiple generalized bone diseases, and may cause severe disability. The upward displacement of the basilar and condylar portions of the occipital bone may cause the foramen magnum to fold inward with subsequent translocation of the upper cervical spine into the brainstem.[7,9] This condition can result in bulbar dysfunction and myelopathy with lower cranial nerve palsies due to stretching.

Few cases in the literature described basilar invagination associated with trigeminal neuralgia.[3,8,12] This condition, due to the complexity of anatomical deformities, hinders treatment options. Microsurgical decompression may be considered as a definitive and long-term solution, but it carries a high risk, since substantial bone deformity caused by the disease may worsen after bone removal for craniotomy.

The foramen ovale approach is an alternative option, although it can prove challenging or even impossible. Normal anatomical relations are distorted by bone deformity and the lower surface of the foramen ovale may be posteromedially displaced, thus making the conventional route for percutaneous approaches virtually impossible to use.[5,7,8] The authors found that Mayfield clamps, necessary in the previous alternatives, also imply high complication risk given the potential fragmentation of wormian bones and deformity worsening.

Conclusion

In cases of OI, cannulation of the foramen ovale and classical percutaneous treatment can be a useful, safe and efficient alternative using CT-guided puncture for patients with trigeminal neuralgia and other bone deforming diseases that severely affect the skull base.

Conflict of interest

The authors declare no conflict of interest.

Resumo

Opção alternativa em tratamento da neuralgia do trigêmeo com osteogênese imperfeita

Osteogênese imperfeita (OI) é uma doença óssea que pode levar a deformidades de base de crânio, como invaginação basilar que pode provocar compressão de nervo craniano, incluindo o nervo trigêmeo. Nestes casos, a neuralgia do trigêmeo permanece como um desafio, pela anatomia distorcida e pelas deformidades. Apresentamos uma alternativa que consiste na canulação do forame oval e no tratamento percutâneo clássico. A microcompressão percutânea por balão foi realizada em uma paciente de 28 anos apresentando OI e grave neuralgia do trigêmeo, sendo realizadas tomografia computadorizada (CT) e canulação guiadas do gânglio gasseriano sem neuronavegação ou dispositivos estereotáxicos. A paciente apresentou hipoestesia à esquerda dos segmentos V1, V2 e V3, com bom controle da dor. Essa técnica alternativa com punção orientada por CT utilizando o *angiosuite* sem a necessidade de qualquer grampo de Mayfield, sistemas de neuronavegação, ou dispositivos com ou sem arcos estereotáxicos, pode ser uma opção útil, segura e eficiente para pacientes com neuralgia do trigêmeo cursando com outras doenças deformativas que afetem a base craniana de modo grave.

Palavras-chave: neuralgia do trigêmeo, dor, osteogenesis imperfeita, compressão percutânea por balão.

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Patient Concerns Inventory for head and neck cancer: Brazilian cultural adaptation

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Summary

Objective: The purpose of this study was to translate, culturally validate and evaluate the Patients Concerns Inventory – Head and Neck (PCI-H&N) in a consecutive series of Brazilian patients.
Method: This study included adult patients treated for upper aerodigestive tract (UADT) cancer. The translation and cultural adaptation of the PCI-H&N followed internationally accepted guidelines and included a pretest sample of patients that completed the first Brazilian Portuguese version of the PCI. Use, feasibility and acceptability of the PCI were tested subsequently in a consecutive series of UADT cancer patients that completed the final Brazilian Portuguese version of the PCI and a Brazilian Portuguese version of the University of Washington Quality of Life Questionnaire (UW-QOL). Associations between physical and socio-emotional composite scores from the UW-QOL and the PCI were analyzed.
Results: Twenty (20) patients participated in the pretest survey (translation and cultural adaptation process), and 84 patients were analyzed in the cultural validation study. Issues most selected were: fear of cancer returning, dry mouth, chewing/eating, speech/voice/being understood, swallowing, dental health/teeth, anxiety, fatigue/tiredness, taste, and fear of adverse events. The three specialists most selected by the patients for further consultation were speech therapist, dentist and psychologist. Statistically significant relationships between PCI and UW-QOL were found.
Conclusion: The translation and cultural adaptation of the PCI into Brazilian Portuguese language was successful, and the results demonstrate its feasibility and usefulness, making this a valuable tool for use among the Brazilian head and neck cancer population.

Keywords: surveys and questionnaires, quality of life, head and neck neoplasms, validity and reliability, outcomes research.

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Introduction

Worldwide, approximately 650,000 people per year are diagnosed with head and neck cancer.[1] The city of São Paulo has one of the highest incidence rates of laryngeal and oral cancer worldwide.[2]

Head and neck cancer and its treatment can cause significant changes in vital functions related to feeding, communication and breathing of affected patients, as well as to an individual's self-image. Such alterations can have devastating consequences on the patient's quality of life and also on their families.[3-5] The current focus of head and neck oncology is to eliminate cancer, prolong survival, obtain better functional outcomes and preserve or restore the quality of life (QOL) of affected patients. Studies of QOL using generic or specific measures usually generate data and information about the disease and the overall

impact of its treatment on the patient, including data that could be used to improve treatment support, optimize patient care, define rehabilitation necessity and goals, identify problems and preferences of patients, and also facilitate communication between patients and doctors.[6-9]

In conjunction with the Merseyside Regional Head & Neck Cancer Centre Support Group, Rogers et al.[10,11] and Ghazali et al.[12] developed the Patient Concerns Inventory – Head and Neck (PCI-H&N), which has been included in the British H&N National Annual audit (DAHNO) as an indicator of quality of care. It consists of an inventory based on the collection of items from various health-related quality of life (HRQOL) questionnaires. The PCI is a carefully designed H&N cancer-specific checklist intended to be used in consultations as part of routine outpatient care. It is holistic, self-administered and designed to achieve a more patient-focused and patient-directed medical consultation, leading to more shared decision-making and potentially better patient support and outcomes. Patients select items from the checklist that they want to discuss during the consultation and also select health professionals that they would like to see during their outpatient visit or be referred to.

The purposes of the present study were to adapt and culturally validate the PCI-H&N into Brazilian Portuguese language and to evaluate its use in a series of patients treated for head and neck cancer (HNC) in a referral cancer center in Brazil.

Method

The instrument description

The first part of the original English version of the PCI has 57 items grouped into five domains: physical and functional well-being (29 items), treatment-related (four items), social assistance and welfare (nine items), psychological, emotional and spiritual well-being (14 items), and other (free text). The second part consists of a list of 17 specialists, also grouped into five domains: physical and functional well-being (eight specialists), treatment-related (three specialists), social assistance and welfare (three specialists), psychological, emotional and spiritual well-being (three specialists), and others (free text). In both parts of the inventory, patients can select as many items and specialists as they would like.

It is important to highlight that the PCI is a checklist of issues that patients wish to talk about in their clinical consultation and is not a measure of the level of concern per se. Patients can have concerns and still not want to discuss them. It has no rating or score.

Translation and adaptation process

The translation and cultural adaptation of the PCI followed internationally accepted guidelines.[13,14] Initially, two bicultural experts and translators, who were experts in the area of healthcare, translated the original English version of the PCI into Brazilian Portuguese. A third bicultural expert compared the two versions, and a consensus version was reached. The consensus-translated version of the Brazilian Portuguese PCI was then back-translated into English by two additional bicultural experts and translators who were native English speakers in collaboration with one of the authors (SNR). To confirm the cross-cultural equivalence of the original and translated versions for semantic, idiomatic, experiential and conceptual aspects, a committee formed by three healthcare professionals with experience in HNC revised the final version, comparing it to the original one. Any discrepancies between the original and back-translated versions were resolved by repeating the process as needed.

A pretest survey was performed.

Pretest survey

The inclusion criteria for the pretest survey were: patients 18 years of age or older who were treated for upper aerodigestive tract (UADT) cancers regardless of the histological type or tumor staging. Exclusion criteria: Patients with a diagnosis of thyroid cancer or neurological changes, and/or deficits in comprehension and/or ability to communicate, as well as individuals who reported being physically and/or psychologically unable to answer the questionnaire.

A series of 20 eligible patients completed the Brazilian Portuguese PCI version for the pretest survey. Informed consent was obtained from all patients enrolled in this pretest survey.

During this pretest survey, patients were asked about possible difficulties in interpreting any words or expressions translated in the Brazilian version of the PCI, as well as limitations to complete the questionnaire. If any specific difficulty to complete the inventory was reported by more than one patient, a meeting among the research team members (Brazilian authors) was performed to adapt and modify such item as needed.

Cultural validation study

A consecutive series of UADT cancer patients meeting the same inclusion and exclusion criteria of the pretest survey completed the final version of the PCI and also the Brazilian Portuguese validated form of the University of Washington Quality of Life Questionnaire (UW-QOLv4).[15,16] Informed consent was obtained from all enrolled patients.

Based on the literature available for this type of research, there is no consensus and no general criteria to establish the required sample size for a patient-reported outcome instrument validation study. A sample size of at least 50 to 100 participants is generally recommended. These numbers of subjects ensure stability of the variance-covariance matrix.[17]

A psychometrically valid survey instrument will assess what it is meant to be measured, and the PCI evaluates whether patients want to discuss items – in practice, they either want to discuss an item or they don't. Due to its "checklist" format, PCI is not suited for psychometric testing in the usual sense applied to the majority of HRQOL survey instruments. Translation and cultural adaptation are the most important steps to assure that the properties of the original instrument are maintained in another language. Content validity is the most relevant of the PCI properties, reflecting a belief that the questions adequately cover the content of the field of study. In the original language format, content validity was thoroughly established, which ought to be maintained after proper translation into Brazilian Portuguese and subsequent verification through back-translation.

However, even considering that the most important step of cultural validation of PCI into Brazilian Portuguese is the rigorous process of translation and back-translation, we decided to test the construct validity of the Brazilian version by comparing its results with the UW-QOL scores. Construct validity is present if the scale behaves according to hypothesized relationships. We hypothesized that the composite score of the UW-QOL should be associated and correlated with the number of issues selected for discussion by the patients on the PCI.

The validated Brazilian Portuguese format of the UW-QOLv4 was used because it is a disease-specific, concise and well-validated QOL questionnaire and because its domains overlap with many of the concerns listed on the PCI. Each domain item on the UW-QOL scale is scored from 0 to 100, with the composite score being the mean of the 12 domains. Higher scores are indicative of better QOL. In our study, the UW-QOL results were analyzed considering two composite scores: "physical function" (simple average of the domain scores for swallowing, chewing, speech, saliva, taste and appearance) and "socio-emotional function" (simple average of the domain scores for activity, recreation, pain, mood, anxiety and shoulders).[15]

Statistical analysis

Nonparametric Mann-Whitney test (two categories) or Kruskal-Wallis test (three categories) were used to evaluate the association between number of items/specialists selected and patient characteristics. The Mann-Whitney test was also used to associate the composite scores of the UW-QOL (physical, socio-emotional) with the specific items/specialists selected by the patients. The associations between the scores of the UW-QOL and age, time of diagnosis and the number of items/specialists selected were assessed using Spearman's correlation coefficient. Associations between the specific items/specialists selected and patient characteristics were evaluated using Chi-square test or Fisher's exact test. The level of significance was set to 1%. The software used was R version 3.0.1. (R is a language and environment for statistical computing and graphics. R provides a wide variety of statistical and graphical techniques, and is highly extensible. It is available as free software under the terms of the Free Software Foundation's GNU General Public License in source code form).

Results

Translation process and pretest survey

As part of the translation and adaptation process, we conducted a pretest survey with 20 patients with UADT cancers (eight oral cavity, six larynx, two oropharynx, three nasopharynx and one maxillary sinus) who completed the Brazilian Portuguese version of the PCI.

There were no significant discrepancies between the translation and back-translation versions, despite the grammatical and cultural differences between the Brazilian and English populations.

However, in the pretest survey, some patients did not understand the meaning of the following terms in the first translated version: mucous, deglutition, smell, taste, percutaneous gastrostomy (PEG), fear of adverse events and coping strategies. The terms home care/Family Health Program (originally described as home care/district nurse) were erroneously interpreted as any family member or person providing help/medical assistance. The term "activity" was interpreted differently by patients, being understood as physical exercises or as activities of daily living. Patients were also confused by the terms salivation and dry mouth and were unable to distinguish and clearly define them.

Following the adequacy of equivalence and adaptations for the Brazilian population, the consensus research team then constructed a final version in Brazilian Portuguese. This final version was defined replacing or supplementing the terms that were misunderstood, misinterpreted or indistinguishable, for phlegm/secretions, swallowing (deglutition), olfaction (smell), taste (sense

of flavor), feeding tube, fear of adverse events and strategies to cope with the illness and treatment, specialist care at home, physical activity and too much salivation.

In the pretest survey, among the specialists listed in the original PCI version, dental hygienist and chaplain were not easily identified by patients, as well as emotional support therapists (a clinical specialty just below the level of a psychologist that exists in the United Kingdom, but not in Brazil). For the final consensus version, the specialty dental hygienist was removed, chaplain was replaced by the religious authority/leader of their religion, and psychologist and psychiatrist were added.

Cultural validation study

A consecutive series of 84 patients with UADT cancers were enrolled between February 2013 and June 2013 for this study, and they completed the final Brazilian Portuguese version of the PCI and the Brazilian Portuguese version of the UW-QOLv4. Most patients were male (74%), mean age of 62 years (ranging from 36 to 89 years), with low education level (57%) and histologically confirmed squamous cell carcinoma (SCC) (89%). Tumor sites were the oral cavity in 35 patients (42%), larynx in 20 patients (24%), oropharynx in 19 patients (23%), nasopharynx in three patients (4%), hypopharynx in three patients (4%), nasal and paranasal sinuses in two patients (2%), and occult primary tumors in two patients (2%). Most of them presented with tumors at an advanced stage: T3 in 19 patients (23%) and T4 in 25 (30%). Half of the patient sample was clinically negative for lymph node involvement (50%), and almost all patients were clinically negative for distant metastasis (96%). Regarding time interval between treatment and questionnaire completion, 25 patients (30%) marked less than 12 months, 22 patients (26%) between 12 and 36 months and 37 patients (44%) for 36 months or more. Thirty-four (34) patients (40%) underwent surgery and radiotherapy, 29 (35%) underwent primary radiotherapy and 21 (25%) underwent surgery only. Thirty-five (35) patients (42%) underwent chemotherapy at some point during their treatment.

The time required to fill out the PCI ranged from 6 to 20 minutes (mean: 13 minutes). The frequency of the items indicated on the PCI is described in Figures 1 and 2. Among the items listed in the inventory, issues selected most frequently by patients for discussion included the following: fear of cancer returning (57%), dry mouth (45%), chewing/eating (44%), speech/voice/being understood (43%), swallowing (40%), dental health/teeth (36%), anxiety (31%), fatigue/tiredness (30%), taste (sense of flavor) (30%) and fear of adverse events (30%) (Figure 1). The three specialists most selected by patients were speech therapist by 22 patients (26%), dentist by 20 patients (24%) and psychologist by 15 patients (18%) (Figure 2). The median (interquartile range – IQR) number of PCI items selected was eight (5-13), ranging between 0 and 42 items, with 75 patients (89%) selecting at least one item. The median (IQR) number of specialists indicated was one (0-2), ranging between zero and eight specialists, with 30 patients (36%) selecting at least one specialist.

The number of concerns and specialists selected correlated weakly with the time of diagnosis (-0.29, p=0.007 and -0.28, p=0.01), with more items selected in the case of shorter interval between the end of treatment and participation in the study, while patient age correlated with the number of specialists selected (-0.28, p=0.009) in that younger patients selected a greater number of specialists (Table 1). There were no other significant association of clinical variables studied with the number of items and specialists selected.

The mean composite score for UW-QOL was 73 (standard deviation [SD], 20). For the physical and socio-emotional domains, the scores were respectively, 78 (21) and 67 (24). The physical and socio-emotional domains of the UW-QOL were significantly correlated with the number of concerns selected (-0.46 and -0.45, both p<0.001) and with the number of specialists selected (-0.46, p<0.001 and -0.33, p=0.002). The worse the UW-QOL score, the more PCI items were selected.

Significant associations between specific issues and specialists selected with patient characteristics and with the physical and socio-emotional domains of the UW-QOL were detected. Patients under 55 years of age selected more items of dental health (50%) and fear of cancer returning (75%) than older patients. Females were more than twice as likely as males to select sleep and anxiety, with 8/22 (36%) and 12/22 (55%), respectively. Patients with post-treatment interval of 12 months or less selected considerably more items than patients with longer than 12 months treatment interval regarding to appetite (40%), cancer treatment (48%), use of a feeding tube (32%), wound healing (16%) support for the family (16%), and specialist dietician (28%) and speech therapist (52%). Appetite (42%) and appearance (46%) were about three times more likely to be selected by patients who had more extensive neck disease (N2-N3). Individuals undergoing reconstruction indicated excessive salivation (22%) more frequently than those without reconstruction (3%). The item dry mouth was indicated by 16 (35%) patients who underwent radiotherapy and 17 (81%) who did not undergo surgery. In addition, 19 patients (66%) who underwent primary radiotherapy (RT) selected dry mouth, and one-third of

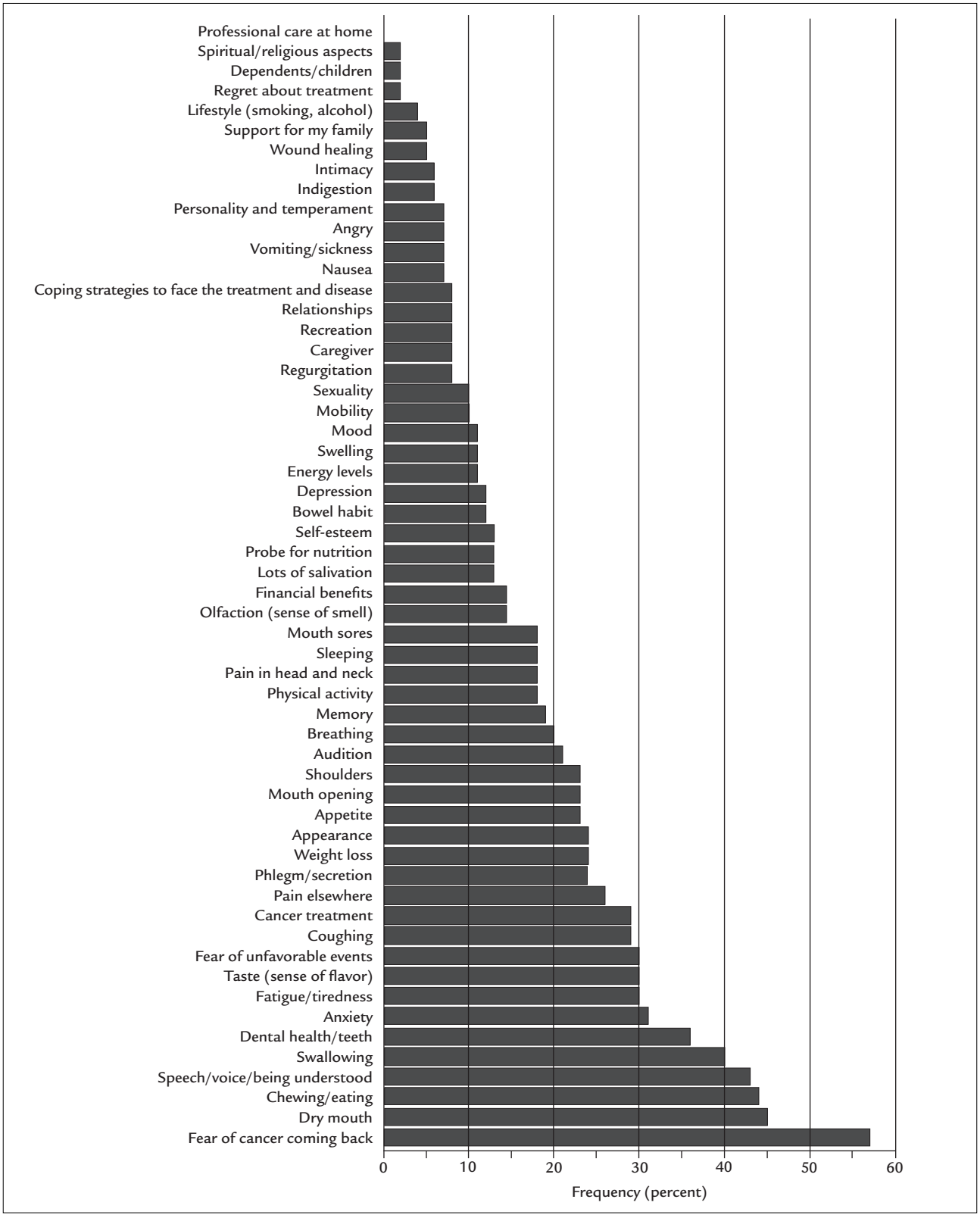

FIGURE 1 Patient Concerns Inventory issues selected most frequently by patients.

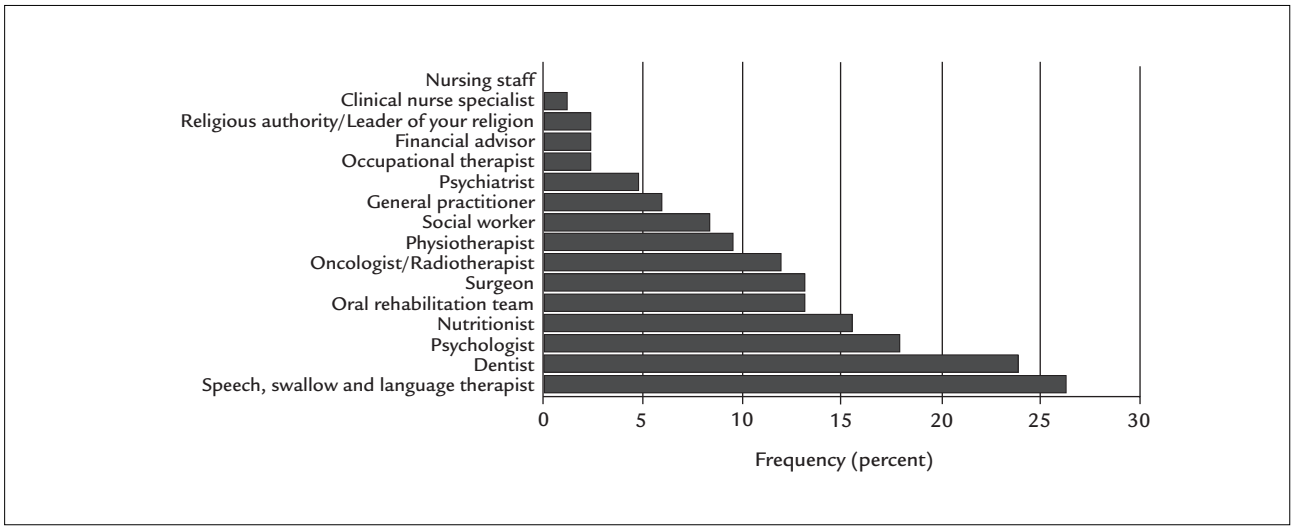

FIGURE 2 The specialists selected most by patients.

TABLE 1 Patients characteristics and the number of items and professional specialists selected in the PCI (n=84).

			Number of issues selected				Number of professionals selected			
					5+ items				2+ professionals	
		n	Median	IQR	%	n	Median	IQR	%	n
Age*	<55	20	9	5-12	80	16	2	0-4	60	12
	55-64	27	7	4-12	67	18	1	0-3	37	10
	≥65	37	9	5-14	78	29	0	0-1	22	8
Gender	Male	62	8	5-13	76	47	0	0-2	31	19
	Female	22	10	4-15	73	16	1.5	1-4	50	11
Site of tumor	Oral cavity	35	8	4-13	71	25	1	0-3	31	11
	Oropharynx	19	9	5-15	79	15	1	0-3	47	9
	Larynx	20	8	5-10	75	15	0	0-2	30	6
	Others	10	10	7-13	80	8	1	0-2	40	4
Time from diagnosis*	<12	25	13	7-15	84	21	2	0-4	60	15
(months)	12-35	22	9	4-12	73	16	0.5	0-2	36	8
	≥36	37	7	4-11	70	26	0	0-1	19	7
T stage	T1_2	38	7.5	4-12	66	25	1	0-2	37	14
	T3_4	44	8.5	6-14	82	36	0.5	0-3	34	15
N stage	N0_1	59	7	4-12	69	41	0	0-2	27	16
	N2_3	24	11	8-15	88	21	2	0-3	54	13
Reconstruction	No	38	8.5	4-12	68	26	1	0-2	34	13
	Yes	46	8	5-14	80	37	1	0-3	37	17
RT	No	16	8.5	4-16	63	10	1	0-2	38	6
	Yes	68	8	5-13	78	53	1	0-2	35	24
Treatment	Surgery, no RT	21	10	4-17	67	14	1	0-3	48	10
	Surgery & RT	34	7	5-11	76	26	0	0-1	21	7
	Primary RT	29	9	6-13	79	23	1	0-3	45	13

(*Continues*)

TABLE 1 (Cont.) Patients characteristics and the number of items and professional specialists selected in the PCI (n=84).

			Number of issues selected				Number of professionals selected			
					5+ items					2+ professionals
		n	Median	IQR	%	n	Median	IQR	%	n
Physical function (UW-QOL) ++**	<50	9	9	8-16	100	9	3	2-4	89	8
	50-74	19	12	9-16	84	16	1	0-5	47	9
	75-89	26	9	4-13	73	19	1	0-2	38	10
	90+	30	6	3-9	63	19	0	0-1	10	3
Social-emotional (UW-QOL)++*	<50	20	12	8-15	90	18	1.5	0-4	50	10
	50-74	27	9	6-14	85	23	1	0-3	48	13
	75-89	20	6	4-10	70	14	0	0-1	20	4
	90+	17	4	2-8	47	8	0	0-1	18	3

+0.001, p<0.01, Spearman correlation for the number of issues selected; *0.001, p<0.01, Spearman correlation for the number of specialists selected; ++p<0.001, Spearman correlation for the number of issues selected; **p<0.001, Spearman correlation for the number of specialists selected; none of the other patient characteristics were associated at p<0.01 with the number of issues or specialists selected according to the Mann-Whitney test (gender, clinical T, N, reconstruction, RT, surgery) or Kruskal-Wallis test (site, treatment); RT: radiotherapy; IQR: interquartile range; PCI: Patient Concerns Inventory; UW-QOL: University of Washington Quality of Life Questionnaire.

those who underwent surgery without RT selected salivation and depression (33%), while one-quarter selected strategies to confront the disease and the treatment (24%) and mood (29%). The physical domain score was significantly associated with the selection of items for appetite, mouth opening, pain in head and neck, swallowing, swelling, recreation, relationships, speech/voice/being understood and fear of adverse events as well as selection of dentist, speech pathologist and oncologist/radiotherapist. The socio-emotional domain score was significantly associated with the items for appetite, chewing/eating, mouth opening, swallowing, taste, feeding tube, speech/voice/being understood and memory as well as speech pathologist and oncologist/radiotherapist. Patients with UW-QOL composite scores of below 50 were particularly affected.

Additional concerns and specialists that were not present in the original instrument were suggested by the patients under the item "others." These items and specialists included the following: spouse/partner (twice), gastroesophageal reflux, follow-up, hair loss, choking, dementia, quality of life, transportation to hospital (displacement/cost), health insurance, dependence on others to provide support in general, diabetes, alternative treatments, burning tongue, sensation of suffocation, geriatrician, professor of physical education specialized in oncology, nose-ear-throat specialist, neurologist, acupuncturist, hematologist, vascular physician, dermatologist (four times), gastroenterologist, nephrologist, ophthalmologist and orthopedist.

Discussion

The assessment of HRQOL of patients with HNC is typically measured using specific questionnaires that cover a complex conceptual framework involving physical, psychological and social domains.[18] Worldwide, the most widely used questionnaires are the EORTC QLQ-H&N35,[19] the UW-QOL[3,15,16] and FACT the H&N.[20]

The conception and main goals of the PCI are different from HRQOL questionnaires available in Brazil, since it enables the health team to know in real time the priorities and concerns raised by patients, helping them to target and structure consultations and promote shared decision-making and multidisciplinary care.[10] Moreover, the PCI encompasses a wide range of different concerns in diverse areas of the patient's life, which may be affected by the disease and/or treatment. Given the importance of this topic as well as the increased survival of individuals with HNC, the availability of a specific and easy-to-use clinical tool in Brazilian Portuguese is necessary to allow physicians to identify the concerns that permeate the life of these patients during and after their treatment and to facilitate any referrals or clarifications they require. Especially in busy referral medical centers, this instrument could help to focus the clinical consultation on the patients needs, promote a more comprehensive multidisciplinary care, which could result in a more personalized approach.

Although the study consisted mainly of patients with tumors of the oral cavity, oropharynx and larynx with a few cases of nasopharynx, hypopharynx, nasal cavity, maxillary sinus, and other HNC sites, the patient sample was fully represented by different tumor stages, treatment modalities and time intervals from diagnosis. Several other concerns and specialists not in the original instrument emerged during this study, which should be considered in future refinements of the PCI, a factor already considered by the original author (SNR) since the initial conception of the PCI.[10]

Overall, patient impressions about the PCI were largely favorable, as illustrated by the following comments: "Some-

times, the doctor is unwilling to talk about some subjects; I do not know if it is lack of time or if it is because they don't want to trouble the patient... Many doctors want to protect the patient..." "Everything I feel is written there...!" "This questionnaire was very important to me; they could create an explanatory leaflet about everything that can happen to us because of the treatment..." "From the common concerns and common cases, group meetings could be established for patients and families..." "... This questionnaire is like an anticipated dialogue with the doctor...!" "This was the first time someone listened to me and asked how I felt. I have already taken this initiative with respect to the hospital, but it is the first time someone from the hospital has taken this initiative in relation to me..." "... In several years of continuous treatment at the hospital, this is the first time I've been formally consulted on topics related to the treatment. I think that, with this, I could contribute more with my experiences to enhance the hospital services..." "People being concerned about others is very assuring."

The item selected most often by patients was fear of their cancer returning (also named fear of recurrence, FoR), followed by dry mouth, chewing/eating, and the indication for a speech therapist and dentist. Other studies that used the PCI-H&N have also found FoR to be the most common concern that patients with HNC want to discuss in clinics,[10,21,22] especially in patients aged less than 65 years, who seemed to experience more significant FoR,[23,24] which is in accordance with the findings of our study. Using the PCI during clinical routine practice seems to "allow"/facilitate patients to talk about this heavy burden with the clinical team, which is usually not addressed during consultation and may cause detrimental effects on patients psychological well-being.[25]

For PCI validation, we considered that the most important step of cultural validation in Brazilian Portuguese was the rigorous process of translation and back-translation, as well as the cultural adaptation of some words and expressions not well-understood or misinterpreted by Brazilian patients. However, we decided to test the construct validity of the Brazilian version comparing its results with the UW-QOL scores. The results showed important associations between the Brazilian Portuguese version of PCI and the UW-QOL scores. Patients with low UW-QOL scores selected a higher number of issues for discussion in the PCI, confirming the hypothesized scenario of low QOL scores being related to the number of issues raised on PCI.

Overall, the results of our study demonstrated good user feasibility/acceptability of the PCI and significant correlations of PCI with clinical variables and the UW-QOL, which is in accordance with the expectations related to this instrument and demonstrates the usefulness of the PCI in our population.

The incorporation of the PCI-H&N into clinical practice has the potential of offering patients the freedom to choose whether they wish to address some of their concerns at any point during treatment with members of the clinical team, supporting the adoption of appropriate strategies and referrals, which may in turn minimize the impact of the disease and its treatment in different areas of the patient's life. In its original format created in the UK, the PCI and UW-QOL were completed by patients using a touch-screen computer (TST) and the responses were instantly available to the doctor. In the present study, touch-screen technology was not available and patients received a printed version of the translated PCI. The concomitant use of a HRQOL instrument such as the UW-QOL allows patients with any disease or treatment-related dysfunction to be identified and thus promote an opportunity for the clinician to discuss aspects where patients are performing badly even though the patient might not have selected related items on the PCI for discussion. In this context, PCI can be used alone either on paper or via touch screen or combined with a HRQOL questionnaire prior to consultation.

Conclusion

To sum up, PCI is the only clinical tool of its class currently available for patients with HNC that is fast, easy to apply, and can be used alone or in combination with HRQOL questionnaires. The translation and adaptation of the PCI into Brazilian Portuguese can be considered successful, and the results demonstrate its applicability and sensitivity, making the Brazilian Portuguese version a valuable tool that can be used in the Brazilian population. International comparison would give valuable insight into the cross-cultural patient experience of HNC survivorship.

Further studies using the Brazilian Portuguese version of the PCI must evaluate the adherence of the clinical staff to this new tool, the optimization of communication between patients and physicians, as well as verify if the previously undiagnosed concerns of the patients were actually identified and discussed, assessing if there were any changes in the number of referrals to other members of the multidisciplinary team, and exploring the changes in patient concerns over time.

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Conflict of interest

The authors declare no conflict of interest.

Resumo

Inventário de Preocupações dos Pacientes com câncer de cabeça e pescoço: adaptação cultural brasileira

Objetivo: O objetivo deste estudo foi traduzir, adaptar culturalmente e avaliar o Inventário de Preocupações dos Pacientes – Cabeça e Pescoço (IPP-CP) em uma série consecutiva de pacientes brasileiros.
Método: Este estudo incluiu pacientes adultos tratados por câncer do trato aerodigestivo superior (TADS). A tradução e a adaptação cultural do IPP-CP seguiram diretrizes internacionalmente aceitas e incluíram uma amostra piloto de pacientes que completaram a primeira versão em português do IPP-CP. O uso, a viabilidade e a aceitabilidade do IPP-CP foram testados posteriormente, em uma série consecutiva de pacientes com câncer do TADS que completaram a versão final em português do PCI e uma versão em português do questionário de qualidade de vida da Universidade de Washington (UW-QOL). As associações entre os escores físicos e socioemocionais do UW-QOL e do IPP foram analisadas.
Resultados: Vinte pacientes participaram da pesquisa piloto (processo de adaptação cultural e tradução), e 84 pacientes foram analisados no estudo de validação cultural. As questões mais selecionadas foram: medo de o câncer voltar, boca seca, mastigação/comer, fala/voz/ser compreendido, deglutição, saúde dental/dentes, ansiedade, fadiga/cansaço, paladar e medo de eventos adversos. Os três especialistas mais selecionados foram fonoaudiólogo, dentista e psicólogo. Relações estatisticamente significativas entre IPP e UW-QOL foram encontradas.
Conclusão: A tradução e a adaptação cultural do IPP para o português foram bem-sucedidas, e os resultados demonstram a viabilidade e a utilidade da ferramenta, tornando-a valiosa para uso na população brasileira com câncer de CP.

Palavras-chave: questionários, qualidade de vida, neoplasias de cabeça e pescoço, validade e reprodutibilidade, pesquisa de resultados.

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Effect of fluoride on salivary immunoglobulins and sialic acid

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Summary

Objective: The aim of our study was to evaluate the effect of fluoride on salivary immunoglobulin and sialic acid levels in children with dental fluorosis and healthy teeth who live in places with high fluoride concentration in drinking water.
Method: Fifty-one (51) healthy children between 6 and 12 years old with no caries were randomly selected from primary schools enrolled in the dental-care program operated by the Department of Pediatric Dentistry. The children were divided into two groups: group I comprised 26 children with dental fluorosis [Thylstrup–Fejerskov Dental Fluorosis Index (TFI) = 4] who lived in Isparta (2.7–2.8 ppm), and group II consisted of 25 children without dental fluorosis who were born in low-fluoride areas and had lived in Isparta for only the previous two years. Stimulated and unstimulated saliva were collected and analyzed for fluoride, salivary immunoglobulins and sialic acid levels.
Results: Sialic acid level was correlated negatively with age. Levels of secretory immunoglobulin A (sIgA) and secretory immunoglobulin G (sIgG) were higher in children with dental fluorosis compared with those in group II, although these differences were not significant.
Conclusion: Increased sIgA and sIgG levels may arrest the progression of caries in subjects with dental fluorosis. Given the risks of dental fluorosis, further studies of the effects of different fluoride levels in drinking water on salivary composition of children with mixed dentition are needed to confirm the results of our study and to provide data for comparison.

Keywords: dental fluorosis, fluoride, sialic acid, salivary immunoglobulin.

Study conducted at Süleyman Demirel University, Scientific Research Project Coordination Unit, Isparta, Turkey

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Introduction

Salivary secretions are important for the maintenance of oral health. In the human saliva, there are components that control the composition of oral microflora and the function of microorganisms. The main proteins, peptides and enzymes in human saliva have been identified and characterized.[1-4] Macromolecules in the saliva have different functions and biochemical properties.

The protective role of saliva includes a buffer effect, flow rate, immune and non-immune antimicrobial factors and minerals that support enamel tissue, such as calcium, phosphorus and fluoride.[4] Salivary flow rate, pH, buffering capacity, calcium-phosphate homeostasis and effects on bacterial metabolism are important findings of the saliva-caries interaction.[5]

Sialic acid is an important structural component of salivary glycoproteins, having an essential role in enhancing bacterial agglutination. Sialic acid-containing glycoproteins are also important structural components of the acquired pellicle and of dental plaque.[6]

Salivary secretory immunoglobulin A (sIgA) antibodies play an important role in the immune response against dental caries, which are generated by mucosa-associated lymphoid tissue (MALT) common in the mucosal immune system. These antibodies may reduce the initial adhering of bacteria to saliva-coated teeth surfaces and neutralize extracellular enzymes.[7] It has been reported that caries-free patients have significantly higher levels of naturally occurring salivary sIgA compared with caries-active subjects.[8] Results of previous studies have led researchers to believe

that there is a negative correlation between salivary sIgA and dental caries.[9,10] Several studies have reported that caries were particularly correlated with sIgA and sIgG.[11-13] In addition, many studies demonstrated that high salivary sIgA levels result in lower incidence of dental caries.[14-16]

With the increasing use of fluoride products, dental fluorosis has become more common. Dental fluorosis is characterized by enamel hypomineralisation with increased surface and subsurface porosity, which causes opacity, pitting, or staining of the enamel. Information about changes in sIgA, sIgG, and sialic acid levels in the presence of dental fluorosis is limited.

The aim of our study was to evaluate the effects of fluoride on salivary sIgA, sIgG, and sialic acid levels in children with dental fluorosis and those with healthy teeth. In addition, the effects of high levels of fluoride in drinking water on the salivary content were evaluated.

Method

The ethical committee of Süleyman Demirel University's Faculty of Medicine approved the study. All participants and their parents/guardian were informed about the study and a written consent was obtained for all children.

Isparta Province, Turkey, is a region of endemic fluorosis with high fluoride levels (2.7–2.8 ppm) in drinking water. Children in six primary schools were chosen to participate. Children and their parents filled out a total of 1,026 questionnaires with items regarding the duration of residence in Isparta, place of birth, tooth-brushing and daily dietary habits, systemic diseases, and socio-economic status. Children were examined for oral hygiene, dental plaque index and caries status. The forms were reviewed and 473 children were identified with similar features. Children were examined and oral hygiene instructions were given again at our clinic. Before starting the collection of saliva, the criteria for inclusion were that: (a) the patients should be healthy and free of systemic disease; (b) the patients should not have consumed any medications for at least 15 days before saliva collection; (c) the patients' first permanent molars should have erupted fully; (d) there should be equal portions of male and females participants; (e) and they should have the same fluorosis index.

According to these criteria, 51 healthy 6 to 12 year-old (9,15 ± 1,17) children were randomly selected from the primary schools enrolled in a dental-care program maintained by the Department of Pediatric Dentistry. The children were divided into two groups: group I comprised 26 children with dental fluorosis [Thylstrup–Fejerskov Dental Fluorosis Index (TFI) = 4][17] who lived in Isparta (2.7–2.8 ppm), and group II consisted of 25 children without dental fluorosis who were born in low-fluoride areas and had lived in Isparta for only the previous two years. The groups are summarized in Figure 1.

Saliva sample collection

A special diet was assigned to the participants for breakfast and the teachers were instructed not to give any food/beverage except water to the children after breakfast on the day of saliva collection. The saliva was collected in the morning (10-10:30 am) to minimize circadian rhythm effects. The samples were collected in a quiet, isolated, ventilated and lighted room, at room temperature.

After an initial swallow of saliva, the unstimulated saliva was collected into sterilized polyethylene cups. For saliva stimulation, participants were asked to chew on one piece (1 g) of unsweetened, unflavored chewing gum for 2 minutes. The saliva produced in the first 30 seconds was expectorated. The subjects spat 4 mL of saliva into the second sterilized cup. The unstimulated saliva was used for saliva immunoglobulin analyses and the stimulated saliva was used for salivary fluoride and sialic acid analyses.

Analysis of the saliva samples

The salivary immunoglobulin levels were evaluated using ELISA method. The salivary sialic acid levels were determined using Sigma Sialic Acid Quantitation Kit (Sigma-Aldrich Inc., Missouri, USA). The salivary levels of fluoride were analyzed by fluoride electrode.

Statistical analysis

Results were analyzed statistically by Mann-Whitney-U test and Kruskall-Wallis. All analyses were performed using the SPSS statistical package for Windows version 13.0 (SPSS, Chicago, IL, USA). All levels of significance were set at $p<0.05$.

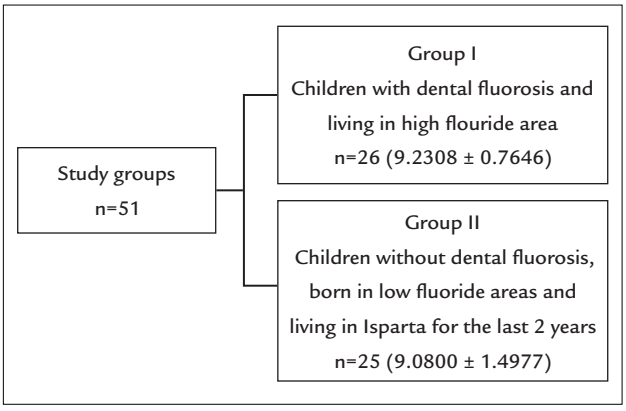

FIGURE 1 The study and control groups.

Results

Table 1 and Table 2 show the results for salivary fluoride, sIgA, sIgG and sialic acid levels in the children of both groups.

No statistically significant difference was found in salivary fluoride levels between group I and II. A significant negative correlation was found between salivary fluoride level and age ($p<0.005$, 0.025) in all subjects. No relationship was observed between dental fluorosis and salivary fluoride, sIgA, sIgG, or sialic acid level.

The sialic acid level differed significantly according to age; this level was much lower in older children.

The sIgA and sIgG concentrations were similar. A negative correlation was found between sIgA and sialic acid levels ($p<0.05$, 0.028). Salivary sIgA levels were higher and sialic acid levels were lower in group I compared to group II, but these differences were not significant.

Discussion

There are various factors affecting secretion and composition of saliva, such as age, sex, number of teeth, weight, stress, diseases or medications causing hypo salivation/hyper salivation, environmental factors and radiation.[18] According to these factors, 51 children aged 6-12 years with mixed dentition and fully erupted permanent first molars were included in our study. Despite the standardization of study factors, the groups were not equivalent in terms of the number or age of included children.

In our study, TFI, which is based on visual examination, was used to determine the severity of dental fluorosis. Children with TFI scores of 4 were included in the study. TFI scores of 5 are well-known to progress rapidly to 6, with the rate of progression increasing after the loss of surface enamel.

Despite the attention given to the collection of unstimulated saliva in our study, the movement of children's tongues and cheeks during expectoration may have caused mechanical stimulation, which would change the salivary composition.

In the study, we compared the levels of salivary fluoride of the children with dental fluorosis or healthy teeth and there was no statistically significant differences recorded. However, salivary fluoride levels decreased with increasing age in both groups. Difficulties allocating the children into groups that met all the study parameters also occurred, namely: an equal number of children in both groups with the same number of teeth could not be controlled, in addition to individual variety and effects of soft tissues and reservoir systems in the mouth, such as plaque and teeth, which affect the secretion and retention of fluoride and have different properties at different ages.

Martin-Gomez et al.[19] reported that the salivary sialic acid concentration in children with TFIs ≥ 2 was lower than that of controls. In our study, the sialic acid concentration was higher in group II compared with group I, whereas sIgA and sIgG levels were higher in the dental fluorosis group; however, these differences were not significant.

The relation between sIgA, sIgG and sialic acid levels has been investigated in geriatrics patients. It is reported that a negative correlation was found between total IgA and sialic acid.[6]

In our study, the level of sIgA was related to sialic acid and increasing of sIgA was associated with decreasing of sialic acid in mixed dentition. Our results suggest that the dental plaque is negatively affected with decreased sIgA and sialic acid levels. However, comparison of our findings from children with mixed dentition with those of other studies is difficult, given the lack of available studies conducted in different age groups.

In two different studies, breastfed infants, compared with formula-fed infants, were found to have almost two times more sialic acid in the saliva.[20,21] Salivary sialic acid was measured in children separated in three age groups: 18, 30 and 42 months. Sialic acid concentrations were higher in the first group than in the third group.[22] Although our study was performed in children but not babies, the results showed similarities with those obtained

TABLE 1 Comparison of means according to group.

Salivary parameters	Fluoride (ppm)	IgA (µg/mL)	IgG (mg/dL)	Sialic acid (µmol/mg protein)
Group I	0.0563 ± 0.0194	445.8462 ± 175.4654	1.4595 ± 1.5952	0.0214 ± 0.0089
Group II	0.0543 ± 0.0168	443.2727 ± 239.9123	1.0060 ± 0.6904	0.0223 ± 0.0076

TABLE 2 Comparison of means by sex.

Salivary parameters	Fluoride (ppm)	IgA (µg/mL)	IgG (mg/dL)	Sialic acid (µmol/mg protein)
Girl	0.0538 ± 0.1636	489.8261 ± 247.8151	1.8548 ± 1.7725	0.0250 ± 0.0053
Boy	0.0568 ± 1974	403.1200 ± 149.6340	0.8205 ± 0.3734	0.0189 ± 0.0098

from babies including level of sialic acid, which was reduced as the children aged.

Salivary sialic acid levels were compared with those of children with systemic problems. Two studies found that salivary sialic acid levels were higher in healthy individuals than in children with Down syndrome.[8,23] However, this difference was significant in only one of the two studies. Similarly, the salivary sialic acid level was found to be higher in a control group than in diabetic children.[24] Patients with cystic fibrosis have been shown to have significantly lower sialic acid concentrations compared with a control group.[25] These differences in sialic acid level have been interpreted as influenced more by the effects of systemic diseases than by local factors.[8]

Conclusion

In our study, gathering a large group of children using the same parameters to standardize was quite difficult. We conclude that sIgA and sIgG levels were higher in children with dental fluorosis than in those with healthy teeth, even though the difference was not significant. Therefore, increased levels of sIgA and IgG in dental fluorosis may arrest the progression of dental caries. Given the risks of dental fluorosis, further studies of the effects of different fluoride levels in drinking water on salivary characteristics are needed to confirm the results of our study and to provide data for comparison. Studies conducted in samples with features similar to those of our study will increase the knowledge available.

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Peripheral polyneuropathy in severely obese patients with metabolic syndrome but without diabetes: Association with low HDL-cholesterol

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Summary

Introduction: The purpose of this study was to evaluate the prevalence of peripheral polyneuropathy (PPN) in subjects with grade II and III obesity (Ob-II,III) and metabolic syndrome (MetS) but without diabetes and to investigate possible associated factors.
Method: A cross-sectional study was performed in non-diabetic Ob-II,III,MetS patients using the Michigan Neuropathy Screening Instrument (MNSI) to assess the presence of PPN.
Results: A total of 24 of 218 non-diabetic Ob-II,III,MetS patients had PPN. Based on univariate analysis, serum levels of LDL-cholesterol (p=0.046) were significantly associated with PPN, while serum triglycerides (p=0.118) and low HDL-cholesterol (p=0.057) showed a tendency toward this association. On a Poisson regression analysis, when the three possible associations were included, low HDL-cholesterol (p=0.047) remained independently associated.
Conclusion: In non-diabetic Ob-II,III,MetS patients, PPN defined by the MNSI showed a high prevalence and was associated with low levels of HDL-cholesterol. In order to diagnose that complication, neurological evaluation should be performed in these patients.

Keywords: polyneuropathies, obesity, metabolic syndrome, HDL cholesterol.

Introduction

Clinical trial results have shown that intensive metabolic control reduces the incidence and progression of neuropathy in patients with type 1 diabetes mellitus (DM1). Yet, for patients with type 2 diabetes mellitus (DM2), it is unclear that glycemic control has such a striking effect, although other microvascular complications can be clearly prevented. Since polyneuropathy occurring in patients with DM2 has been related to risk factors such as obesity, dyslipidemia, peripheral arterial disease, vitamin deficiencies and pre-diabetes, these and other factors often associated with DM2 presence could have a relevant impact as a determinant of disease onset and progression.[1-3]

Currently, few studies have investigated the association and risk factors for progression of peripheral polyneuropathy (PPN) in non-diabetic severely obese patients with metabolic syndrome (MetS). If in DM2 these associations have a great impact on PPN, patients who have them, even before presenting metabolic changes consistent with the diagnosis of DM, could already present PPN. Therefore, knowing the prevalence of PPN and its risk factors in patients with predisposition to DM could be useful to define the determining factors of PPN in obese patients with and without DM2. Considering the aspects above, we sought to establish the prevalence of PPN in obese patients grade II and III with MetS and without DM (non-diabetic Ob-II,III,MetS patients) in which possible risk factors such as hyperglycemia, dyslipidemia, increased body weight or waist circumference, use of metformin and decreased serum vitamin B12 levels could be associated with presence of PPN as defined by the Michigan Neuropathy Screening Instrument (MNSI).

Method

Subjects

A cross-sectional and prospective study including patients with grade II and III obesity (severe obesity) with MetS and without diabetes was conducted in the Obesity Treatment Center at Santa Rita Hospital, Santa Casa de Misericórdia de Porto Alegre Hospital Complex, in the period of January to December 2014. The studied subjects underwent tests and consultations according to a surgical protocol to undergo bariatric surgery. Patients were evaluated consecutively during their routine visits by two biomedical examiners who were unaware of the comorbidities related to obesity or dyslipidemia and MetS components presented by the patients. The following inclusion criteria were adopted: minimum age of 18 years; grade II and III obesity as defined by the World Health Organization (WHO)[4] (BMI ≥ 35 to 39.9 g/m^2 and BMI ≥ 40 kg/m^2, respectively); MetS as defined by the International Diabetes Federation (IDF);[5] absence of DM according to parameters of the American Diabetes Association (ADA);[6] and consent form signed for participation in the study. Exclusion criteria were: hypothyroidism (TSH levels greater than 6 mU/L); vitamin B12 deficiency (serum levels below 210 pg/mL); prior bariatric surgery; history of alcohol abuse according to the CAGE questionnaire;[7] creatinine clearance less than 60 mL/min according to the Cockcroft-Gault equation;[8] acute liver disease; leprosy; HIV positive test; nursing mothers and pregnant women; fasting glucose or A1c hemoglobin levels suggesting diagnosis of DM on the date of inclusion.

The following information was obtained from the patients' medical charts in the last three months of assessment for neuropathy: anthropometric data and blood pressure (mmHg), serum blood glucose (mg/dL) checked after fasting and again two hours after drinking a glucose-rich drink, HDL-cholesterol (mg/dL), LDL-cholesterol (mg/dL), triglycerides (mg/dL), creatinine (mg/dL), TSH (mU/L), and vitamin B12 (pg/mL). After authorizing their participation in the study, patients were asked to complete the questionnaire, which comprised questions regarding presence of neuropathy – the MNSI.[9,10] The first measures assessed were degree of neuropathy according to the MNSI and waist circumference. Impaired glucose intolerance was defined according to the ADA criteria.[6]

Metabolic syndrome was characterized according to the IDF criteria,[5] and was considered present with waist circumference ≥ 80 cm for women and ≥ 94 cm for men in addition to at least two of the following risk factors: triglycerides ≥ 150 mg/dL, HDL-cholesterol < 40 mg/dL for men and < 50 mg/dL for women, systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg, fasting glucose ≥ 100 mg/dL, previous diagnosis of DM and hypertension or patient undergoing treatment for hypertension or DM.

LDL-cholesterol was defined as increased if > 130 mg/dL, fasting blood glucose ≥ 100 mg/dL and < 126 mg/dL was categorized as impaired glucose tolerance (IGT),[6] blood glucose 2 hours after oral intake of 75 g of glucose ≥ 140 mg/dL and < 200 mg/dL was defined as IGT,[11] and creatinine > 1.4 mg/dL was defined as chronic renal disease.

Of the 315 patients, 67 were excluded for having diabetes, 23 for not meeting the criteria for MetS, three for having hypothyroidism, three for having had bariatric surgery in the past and one for having vitamin B12 deficiency. In the end, 218 individuals remained in the study.

Ethical aspects

The study was approved by the Research Ethics Committee of the Santa Casa de Misericórdia de Porto Alegre Hospital Complex and all patients were informed about this research through a Free and Informed Consent Form, according to resolution 466/2012.

Instruments

MNSI was used to assess the presence and degree of neuropathy.[9,10] This instrument was validated and yielded a specificity of 79% and a sensitivity of 61%.[9]

The MNSI questionnaire for symptoms consists of 15 questions, which were translated into Brazilian Portuguese and used to track PPN symptoms. The maximum amount of points scored by patients was 13, since questions 4 and 10 serve to assess association with peripheral vascular disease and asthenia, respectively.

The MNSI physical examination was conducted in a comfortable and calming room of the OTC, with temperature ranging between 22°C and 26°C. The patients remained seated or at supine position lying comfortably and blindfolded in order to raise the perception. A physician of our team was trained to perform this test at the University of Michigan, further instructing two of our PhD students on performing this neurological evaluation. These two biomedical scientists performed all of the tests and in case of discrepancies the physician also examined the patient and made the final decision. Examiners were blinded for the results of laboratory exams while performing the physical examination. In the physical exam, points were awarded according to results of appearance of feet, vibratory sensitivity on the hallux, sensitivity to Semmes-Weinstein monofilament applied in the same location of the tuning fork, and blow with a neurological hammer on the Achilles

tendon, just above its insertion on the calcaneus, with the patient seated and with his legs dangling (Achilles reflex). As for feet abnormalities and presence of ulceration, one point was awarded to each if the sign was present and zero point if it was absent. On the other results, one point was given when the response was absent; half a point when presented with reinforcement or decreased and zero point when present, even with reinforcement (Figure 1). In all, the points were added and a cutoff of 2.5 or more (cutoff validated for the MNSI) was defined as suggestive of neuropathy.[9]

Statistical analysis

The statistical analysis plan was defined prior to data acquisition.

Then, a univariate descriptive analysis was performed, in which all the quantitative data were described using medians and the qualitative data were described by its frequency. All categorical data were tested using Chi-square test or Fisher's exact test, if appropriate. Continuous variables were first tested using the Shapiro-Wilk test followed by Student's t-test to compare the average in case of normal distribution of the data. Mann-Whitney test was used when the distribution was not normal. Significant difference was considered when p≤0.05.

After the univariate analysis, a Poisson regression analysis was performed to assess which of the factors studied were independently associated with the occurrence of PPN. The combination of measures was evaluated using a prevalence ratio with 95% confidence interval. Variables included in the model were those that in the univariate analysis had a result of p≤0.2. The statistical program used was SPSS for Windows, version 18.

Results

In our study, all patients with physical exam positive for PPN presented at least one symptom of neuropathy. An 11% prevalence (n=24) of PPN was found in non-diabetic Ob-II,III,MetS individuals. In this study, 81.2% of individuals with obesity grade II and III with MetS and without DM were female. In Table 1, we verified features of the 218 individuals evaluated for PPN presence, according to MNSI.

According to the data obtained, there was a tendency towards higher frequency of low HDL-cholesterol in the neuropathy group compared to the non-neuropathy group (p=0.057). In both groups, most subjects had grade III obesity (75% vs. 73.2%, respectively). There were no significant differences between neuropathic and non-neuropathic patients regarding number of individuals with systemic arterial hypertension (SAH) (p=0.239), high levels of LDL-cholesterol (p=0.341), hypertriglyceridemia (p=0.219), impaired fasting glucose (p=0.610), use of metformin (p=0.606) and pre-diabetes or IGT (p=0.954).

		Yes	No	
Normal foot		(0)	(1)	
		Absent	Present	
Ulceration		(0)	(1)	
	D	.....	.....	
	E	.....	.....	
		Present	Present on reinforcement	Absent
Achilles reflex		(0)	(0.5)	(1)
	D	.....	.....	.....
	E	.....	.....	.....
		Present	Decreased	Absent
Vibration perception		(0)	(0.5)	(1)
	D	.....	.....	.....
	E	.....	.....	.....
		Present	Decreased	Absent
Monofilament 10 g		(0)	(0.5)	(1)
	D	.....	.....	.....
	E	.....	.....	.....
			Total = .../10 points	

FIGURE 1 Physical exam of the MNSI.

TABLE 1 Characteristics of 218 patients with MetS, degree II and III obesity and without DM, assessed for the PPN presence (≥ 2.5). The numbers shown are the total number of patients with the condition described in each group. The percentage of patients with the condition described in each group is shown in the parentheses.

	PPN Group (n=24)	No PPN Group (n=194)	p-value
Female sex (%)	19 (79.2)	158 (81.4)	0.784[b]
Degree II obesity (%)[c]	6 (25.0)	52 (26.8)	1.000[a]
Degree III obesity (%)[c]	18 (75.0)	142 (73.2)	
SAH (%)	15 (62.5)	92 (47.4)	0.239[a]
Low HDL-c (%)	22 (91.7)	138 (71.1)	0.057[a]
High LDL-c (%)	5 (20.8)	25 (12.9)	0.341[b]
Hypertriglyceridemia (%)	14 (58.3)	83 (42.8)	0.219[a]
High fasting blood glucose (%)	6 (25.0)	63 (32.5)	0.610[a]
High blood glucose 2h post-intake of 75 g of glucose (%)	6/21 (28.6)	57/179 (31.8)	0.954[a]
Metformin use (%)	0 (0.0)	10 (5.2)	0.606[b]

[a]Chi-Squared test.
[b]Fisher's exact test.
[c]BMI: body mass index.
SAH: systemic arterial hypertension; HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; MetS: metabolic syndrome; DM: diabetes mellitus; PPN: peripheral polyneuropathy.

Table 2 shows anthropometric and clinical data expressed as median, evaluated for the presence of PPN according to MNSI. The group with neuropathy showed higher serum levels of LDL-cholesterol compared to those without neuropathy (p=0.046). There was a tendency towards higher serum levels of triglycerides in the neuropathy group compared to the non-neuropathy group (p=0.118).

On the other hand, there was no significant difference between the results of neuropathy and non-neuropathy groups, respectively, on: age (34.5 years vs. 34 years, p=0.350), height (1.66 m vs. 1.63 m, p=0.866), weight (118 kg vs. 116 kg, p=0.443), BMI (44.9 kg/m² vs. 42.5 kg/m², p=0.207), waist circumference (123 cm vs. 122 cm, p=0.374), systolic blood pressure (SBP) (130 mmHg vs. 130 mmHg, p=0.852), diastolic blood pressure (DBP) (80 mmHg vs. 80 mmHg, p=0.341), mean blood pressure (MBP) (96.7 mmHg vs. 96.7 mmHg, p=0.442), fasting blood glucose (89.4 mg/dL vs. 93.8 mg/dL, p=0.273), blood glucose 2 hours after oral intake of 75 g of glucose (126 mg/dL vs. 126 mg/dL, p=0.889), HDL-cholesterol (43.5 mg/dL vs. 45 mg/dL, p=0.457), creatinine (0.82 mg/dL vs. 0.8 mg/dL, p=0.965), TSH (2.5 mU/L vs. 2.2 mU/L, p=0.437) and vitamin B12 (416 pg/mL vs. 449 pg/mL, p=0.432).

Based on the evaluated data, we performed a multivariate Poisson regression (Table 3) in order to assess which of the factors were independently associated with the occurrence of PPN in non-diabetic Ob-II,III,MetS individuals.

In the model, the variables used were low HDL-cholesterol, serum levels of LDL-cholesterol and triglycerides. PPN was associated independently with low HDL-cholesterol (p=0.047), but there was no association with serum levels of LDL-cholesterol (p=0.118) and triglycerides (p=0.239).

Discussion

Few studies assess the prevalence of PPN and factors associated to it in severe obesity and metabolic syndrome patients. This study aimed to evaluate the prevalence and some factors that could be associated with PPN in both men and women with severe obesity and MetS without DM. We observed an 11% prevalence of PPN in our sample with no difference in gender prevalence. Ylitalo et al., looking into data from men and women in the NHANES study, found a 10.9% prevalence of PPN in the obese group.[12] Although we found a similar number of individuals with PPN, it seems important to identify differences between Ylitalo's study and ours. In the comparative study, participants were older than 40 years, obesity was determined by a lower BMI (≥ 30 kg/m²) and participants were not selected for the presence of MetS. Moreover, diabetic patients were not excluded and the presence of PPN was evaluated using Semmes-Weinstein monofilament applying pressure on three different points at the plantar surface of each foot (method which identifies neuropathy only later than the majority of other methods).

In the literature, data is not consistent when considering the prevalence of PPN in subjects without diabetes. Singleton et al. evaluated 107 patients with idiopathic neuropathy and obtained a 34% prevalence of IGT individuals, of which 92% had neuropathic pain and 81% had sensory complaints.[13] Smith and Singleton found a 45% prevalence of pre-diabetes in patients with idiopathic

TABLE 2 Anthropometric and clinical data of 218 patients with MetS, degree II and III obesity, and without DM, assessed for the presence of PPN. These figures represent the median. The values in parentheses correspond to 25% and 75% quartiles, respectively.

	PPN Group (n=24)	No PPN Group (n=194)	p-value[a]
Age (years)	34.5 (31.5; 43.8)	34 (29; 40.3)	0.350
Height (m)	1.66 (1.60; 1.68)	1.63 (1.58; 1.70)	0.866
Weight (kg)	118 (104; 139)	116 (104; 128)	0.443
BMI (kg/m²)	44.9 (39.4; 50.8)	42.5 (39.9; 46.3)	0.207
Waist circumference (cm)	123 (118; 136)	122 (116; 131)	0.374
SBP (mm/Hg)	130 (120; 140)	130 (120; 140)	0.852
DBP (mm/Hg)	80 (72.5; 90)	80 (80; 90)	0.341
MBP (mm/Hg)	96.7 (93.3; 102)	96.7 (93.3; 103)	0.442
Fasting blood glucose (mg/dL)	89.4 (82; 99.8)	93.8 (86.8; 101)	0.273
Blood glucose 2h post-intake of 75 g of glucose (mg/dL)	126 (113; 147)	126 (106; 146)	0.889
HDL-c (mg/dL)	43.5 (37; 49)	45 (39; 50)	0.457
LDL-c (mg/dL)	128 (110; 144)	111 (89; 133)	0.046*
Triglycerides (mg/dL)	172 (97; 247)	132 (99; 192)	0.118
Creatinine (mg/dL)	0.82 (0.75; 0.90)	0.80 (0.70; 0.94)	0.965
TSH (mU/L)	2.5 (1.5; 4.5)	2.2 (1.6; 3.0)	0.437
B12 vitamin (pg/mL)	416 (343; 507)	449 (328; 655)	0.432

*Statistically significant (p<0.05).
[a]Mann-Whitney U test.
BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; MBP: mean blood pressure; HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; MetS: metabolic syndrome; DM: diabetes mellitus; PPN: peripheral polineuropathy.

TABLE 3 Multivariate Poisson regression, in order to evaluate which factors were independently associated to the occurrence of PPN in the sample of degree II and III obesity patients with MetS and without DM.

Variables	Model	
	PR (95CI)	p-value
Low HDL-c	4.12 (1.02 – 16.7)	0.047*
LDL-c	1.01 (1.00 – 1.02)	0.118
Triglycerides (mg/dL)	1.00 (0.99 – 1.01)	0.239

*Statistically significant (p<0.05).
HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; MetS: metabolic syndrome; DM: diabetes mellitus; PPN: peripheral polineuropathy; PR: prevalence ratio.

sensory neuropathy.[14] Ziegler et al. found a 13% prevalence of PPN in individuals with pre-diabetes, 11.7% in those with impaired fasting blood glucose and 7.4% in normal patients.[15] A study performed between 1999 and 2004 in individuals aged over 40 years has shown that, compared with patients without diabetes, pre-diabetic subjects had 11% higher risk of PPN.[16] Other groups have also revealed higher than expected prevalence of pre-diabetes in patients with idiopathic neuropathy in comparison with data of the healthy population.[17]

In our study, no difference was found between the percentage of patients with positive criteria for pre-diabetes (fasting hyperglycemia and altered hyperglycemia 2 hours after 75 g oral glucose intake in groups with and without neuropathy), which suggests that the loss of glycemic control is not the only mechanism determining PPN in the group with neuropathy. In addition, there was no association on univariate analysis between presence of PPN and gender, age, weight, height, BMI, waist circumference, SBP, DBP, MBP and serum levels of HDL-cholesterol, creatinine, TSH and B12 vitamin.

After a multivariate Poisson regression using low HDL-cholesterol and serum levels of LDL-cholesterol and triglycerides, only low HDL-cholesterol was demonstrated to be independently associated with the presence of PPN. Considering this study model, severely obese individuals with MetS and without DM with low HDL-cholesterol had approximately four times higher prevalence of PPN. This result is in accordance with the observation by Callaghan et al. that triglyceride levels were positively related to the risk of amputation in patients with DM2 and that HDL-cholesterol levels between 40-59 mg/dL confer protection against the probability of a lower limb amputation.[18]

Tesfaye et al. found cardiovascular risk factors associated with the development of neuropathy, such as BMI and serum levels of total cholesterol, LDL-cholesterol and triglycerides,[19] but in the KORA study,[20] serum triglycerides were not associated with neuropathic pain. In another research, there was an association of triglyc-

erides with lower limb amputation, but no association with LDL and HDL-cholesterol.[18] We observed that, although there was initially a significant association between serum levels of LDL-cholesterol and a tendency of association between serum triglyceride levels with the presence of PPN, this association was not confirmed in the multivariate regression.

Our study raised the hypothesis that non-diabetic Ob-II,III,MetS patients and with PPN would have lower levels of vitamin B12 compared to those without PPN and that there would be an association with the use of metformin, since some pre-diabetic patients receive this prescription in order to prevent progression to DM. However, the results showed no association between the presence of PPN and vitamin B12 levels or use of metformin.

Since we showed a significant prevalence of PPN in severely obese subjects with MetS without diabetes and a significant association of PPN with low HDL-cholesterol, we considered this finding as the most consistent in our study and we will make some considerations about this possibility.

In addition to being a marker for future[21] or concurrent[22] cardiovascular disease, low HDL-cholesterol is also associated with many clinical parameters such as low levels of physical activity.[22] Thus, the main recommendation made to increase HDL-cholesterol is physical exercise.[23-25] Since a study conducted by our research group showed that 1 year of Roux-en-Y gastric bypass decreased serum levels of blood glucose, total cholesterol, LDL-cholesterol and triglycerides, but did not improve HDL cholesterol,[26] it is also possible that PPN described after this kind of bariatric surgery by other authors[27] could be related to the low HDL-cholesterol levels that these sedentary patients usually present, as well as to vitamin deficiency.

Clinical and epidemiological studies have shown that HDL, a class of plasma lipoproteins, can be very heterogeneous in size and density, and have an atheroprotective role attributed to its ability to promote efflux of cholesterol from arterial macrophages loaded with cholesterol.[28,29] However, recent studies have recognized great physical heterogeneity of HDL, which is associated with its multiple functions. Both the protein and lipid components of these particles are involved in its effect. The apolipoprotein AI (ApoA-I) is quantitatively the major protein constituent having a structure suitable for transporting lipids. It readily interacts with the ATP-binding cassette transporter A1 (ABCA1) and the scavenger-receptor B-1 (SR-B1), and it activates the enzyme lecithin-cholesterol acyl transferase (LCAT), which is essential for HDL maturation. The resulting primary mature particles are HDL2 and HDL3. Moreover, ApoA-1 has antioxidant and anti-inflammatory properties, together with other enzymes. Regarding lipid fraction, an atheroprotective role has been recognized for lisosphingolipids, particularly sphingosine-1-phosphate (S1P), which is involved in the process of cholesterol reverse transfer. All these atheroprotective functions are apparently lost in the plasma of patients with systemic inflammation, coronary heart disease, diabetes and chronic kidney disease, as these patients' plasma is considered dysfunctional.[28-30] When HDL-cholesterol levels are low, as observed in our patients classified as neuropathic, the atheroprotective effect of HDL-cholesterol is probably also decreased.

On the other hand, it has been observed in vitro that HDL-cholesterol particles can be captured by injured distal axons and used for the regeneration of these fibers.[31] If the uptake of HDL from plasma through binding with SR-B1 receptors previously described as present in distal axons also occurs in vivo, lower peripheral axonal regeneration can take place in patients with low HDL-cholesterol, which would explain our findings.

Increased physical activity of animals has been associated with better autonomic function and could prevent the decrease of nerve function related to aging when they exercise on a regular basis.[32,33] In humans, in whom the presence of denervation was assessed by skin biopsy and study of intra-dermal nerves, it was observed that lifestyle changes (exercise and diet) determined not only improvement of the lipid abnormalities but also an increase of nerve fibers density in a subsequent biopsy.[34,35] These data, as well as the findings of our study, suggest that HDL-cholesterol could be either a mediator involved in this effect or a marker that indicates little physical activity, genetic predisposition to atherosclerosis, and other alterations, for example, increased oxidative stress (which would favor neuronal dysfunction). If our findings are later confirmed in studies with other population samples and other designs, there would be a common explanation for the etiology of neuropathy in sedentary subjects, especially when they are obese, with MetS and/or with DM2.

In our study, no difference was found between men and women for the presence of PPN and other studied variables. The low number of men with PPN in our sample population limited our evaluation. Increasing the number of men in the neuropathic group would allow us to analyze if all associated factors found would associate in both sexes or if any of these would prevail in male or female. Since men have a mean HDL-cholesterol level lower than that of normal women, it is possible that the higher prevalence of neuropathy and amputations found

in other studies in men is a reflection of lower HDL-cholesterol levels. Another limitation for assessing this relationship is the study design, which is cross-sectional. In a longitudinal study performed with a larger number of non-diabetic Ob-II,III,MetS patients with PPN, other associations or risk factors might be found. Therefore, cross-sectional studies with a larger sample and a higher number of men, as well as longitudinal studies, would be necessary to confirm that PPN is associated with low serum HDL-cholesterol and whether there are other associated risk factors for PPN in these patients.

Conclusion

Our study revealed an 11% prevalence of PPN in individuals with obesity grade II and III with MetS and not diagnosed with diabetes, and the presence of PPN was independently associated with low HDL-cholesterol levels. Since an increase in HDL-cholesterol is rarely achieved with the treatment of these patients, PPN often progresses in these cases, especially in patients that will become hyperglycemic or develop DM. In order to diagnose this complication, a neurological evaluation should be done when patients come for outpatient visits.

Resumo

Polineuropatia periférica em pacientes obesos graves com síndrome metabólica sem diabetes: associação com baixo HDL-colesterol

Objetivo: Avaliar a prevalência da polineuropatia periférica (PNP) em indivíduos obesos graus II e III com síndrome metabólica (Ob-II,III,SM) sem diabetes e buscar possíveis fatores associados.
Método: Em um estudo transversal, realizado em indivíduos Ob-II,III,SM e sem diagnóstico de diabetes, o Instrumento de Screening de Michigan (MNSI) foi utilizado para avaliar a presença de PNP.
Resultados: Um total de 24 de 218 pacientes Ob-II,III,SM e sem diabetes tinham PNP. Quando observamos as associações com PNP em uma análise univariada, níveis séricos de LDL-colesterol (p=0.046) estiveram significativamente associados e houve também uma tendência à associação com níveis séricos de triglicerídeos (p=0.118) e baixo HDL-colesterol (p=0.057). Em uma análise de regressão de Poisson, quando as três possíveis associações foram incluídas, baixo HDL-colesterol (p=0.047) manteve-se independentemente associado.
Conclusão: Em pacientes Ob-II,III,SM, mas sem diabetes, a PNP definida pelo MNSI tem uma prevalência elevada e está associada a baixos níveis de HDL-colesterol. Para diagnóstico dessa complicação, recomenda-se realizar o exame neurológico desses pacientes.

Palavras-chave: polineuropatias, obesidade, síndrome metabólica, HDL-colesterol.

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Should azoospermic patients with varicocele disease undergo surgery to recover fertility?

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Summary

Introduction: Varicocele disease is well-known cause of infertility in men. The presence of spermatic varices veins create a hostile environment to spermatogenesis. It results in reduced quality of the sperm production and in some cases can determine a total absence of sperm. The varicocelectomy procedure in patients with non-obstructive azoospermia (NOA) can raise the rates of sperm in the semen analysis. A positive rate for sperm, even if very low, may be sufficient to enable the capture of sperm intended for in-vitro fertilization without the use of donor sperm.
Objetive: To evaluate the raise of sperm in NOA patients with varicocele disease who were submitted to a bilateral procedure to recovery sperm production.
Method: We analized the sperm results of 25 NOA patients who undergone to a bilateral varicocelectomy procedure.
Results: From a total of 25 patients, three (12%) recovered sperm count four months after procedure. One year after the procedure, five (20%) patients recovered sperm production.
Conclusion: Patients with varicocele disease and azoospermia, without genetic changes or obstruction of the spermatic tract, should undergo surgical procedure to recover sperm.

Keywords: azoospermia, infertility, varicocele, surgery.

Introduction

Should azoospermic patients with varicocele be operated? In cases of marital male infertility evaluation, absence of sperm on a sperm test is often a frustrating and devastating result for the couple. The azoospermic "couple" is referred to an assisted reproductive program many times already condemned to resort to the use of a sperm donor. However, something else can be offered to these patients. An etiological diagnosis should be performed and, depending on the cause, treatment options can be offered. The diagnosis of varicocele disease in an azoospermic patient justifies the ressection of espermatic veins to improve spermatogenesis.

Objective

To evaluate patients with non-obstructive azoospermia (NOA) and varicocele undergoing bilateral surgical correction for recovery of sperm production.

Method

In a retrospective study conducted from 2002 to 2015 at a private urologic service, we analyzed 25 patients with combined varicocele and azoospermia (the inclusion criteria). The patients had two consecutives sperm samples showing absence of sperm cells, collected within an interval of 3 to 5 days. The semen volume should be greater than 3 mL in the studied group. Physical examination identified presence of vas deferens and uni or bilateral varicocele. The results of laboratory tests of FSH, LH, total testosterone and fructose were within the normal range. All selected patients underwent karyotyping and Y-chromosome microdeletion analysis. All 25 patients underwent bilateral surgical repair of varicocele. Sub-inguinal approach assisted by loupe magnification was performed by the surgical team. They all received the pre-surgical guidelines and signed an informed consent form. Patients were discharged from the hospital on the same day as the surgical procedure. The

first sperm count was conducted 60 days after surgery, and subsequently repeated every 60 days.

Results

Of the 25 patients, 10 (40%) presented bilateral varicocele on physical examination. Left varicocele was identified in nine (36%) patients, and six (24%) had right-sided varicocele only.

Sperm was found in three (12%) patients treated surgically, four months after the procedure. Six months after surgery, sperm was identified in five (20%) patients. After one year of follow-up, only five (20%) patients had sperm in the ejaculate (Table 1). None of the five patients presented testicular volume reduction in pre-op. The concentration of sperm in the patients operated reached 0.5 to 12 million/mL (Vr > 20 million/mL). Out of the total of 25 patients, three (12%) had changes in karyotype, and two (8%) had Y-chromosome microdeletions.

Discussion

The male factor is responsible for about 40% of cases of marital infertility. A diagnosis of azoospermia is made in up to 15% of infertile men.[1,2,4,6,7] Non-identification of sperm in the semen should always be investigated. A differential diagnosis between obstructive and non-obstructive azoospermia is fundamentally important for the treatment and prognosis of patients.[1,2,4,8] FSH, LH, total testosterone and estradiol levels combined can demonstrate the testicular function. Last but not least, measuring the level of fructose in semen offers evidence for obstructive diagnosis.[1,2,4,6,8]

Researching the medical history is crucial and can guide the diagnosis:

1. history of cryptorchidism in childhood and if and when it was corrected;
2. history of sexually transmitted diseases;
3. previous contact with gonadal-toxic agents or the use of exogenous androgen, very common in the absence of sperm;
4. story of retrograde ejaculation (in diabetics, patients with bladder voiding disorders, changes in patients undergoing prostatic surgeries) will identify patients with sperm in post-ejaculation urine analysis.

Karyotype analysis showed 15% of changes in azoospermic patients in the studied group. Regarding changes in karyotype, Klinefelter syndrome is one of the most frequent genetic alterations in azoospermic patients.[1,5-7,12,13] Y-chromosome microdeletions can be expected in approximately 15 to 20% of patients with NOA. The Y-chromosome is responsible for determining the male gender and features three regions known as AZFa, AZFb, AZFc. That is where the information for the production and maturation of sperm cells can be found.[1,5-7,12] This evaluation is truly important because patients referred for assisted reproduction can transmit genetic disorders in the absence of genetic counseling. These patients are unlikely to benefit from varicocelectomy.[4,6,8,11-13]

On physical examination, identification of the vas deferens is fundamental. The presence of ectopic testicles or testicles with reduced volumes can be indicative of testicular failure or poor response to surgical procedure. These patients have a poorer prognosis.[1,3,4,9]

Until recently, the presence of varicocele was not acknowledged in these patients. Varicocele repair should not be considered in obstructive seminiferous tubules. It has a prevalence of 15% in the general population and occurs in 30 to 40% of infertile patients.[1-4]

A diagnosis is made during the physical exam with the patient being brought to a standing position, at room temperature. Doppler ultrasound can be used as a supplementary method.[1-3]

Varicocele is a vascular disease that affects testicular veins. Its presence causes an inappropriate environment for the production and development of sperm.[1-4] The disease can change the concentration, motility, morphology and structure of sperm DNA.[1-4]

Pathological sperm vein reflux leads to the accumulation of CO_2 and free radicals, lowering local concentrations of O_2, which affects the functioning of Leydig cells and Sertoli cells. These cells are responsible for the production of testosterone and sperm, respectively. The varicocele can cause atrophy and calcification, hindering cell development and espermatogenesis.[1-3,5,6,11-13]

TABLE 1 Results of the sperm analysis after varicocelectomy.

Varicocele	Cases treated surgically	%	Sperm positive 4th month	Sperm positive 6th month	Sperm positive 12th month
Bilateral	10	40			
Left	9	36	3 patients	5 patients	5 patients
Right	6	24			
Total	25	100	12%	20%	20%

Note that even one year after surgery, the rate of sperm recovery was not greater than 20%.

In the studied group, 25 patients underwent bilateral procedure, although only ten presented bilateral varicocele veins. Out of 25 patients operated, five (20%) benefited from varicocelectomy. Despite not reaching a minimum concentration of sperm, i.e. 20 million/mL, the recovery of spermatogenesis allowed the use of the patient's own semen for assisted fertilization techniques.[5,6,8-10,13]

Semen cryopreservation was suggested to five patients. None of the patients who recovered some production presented testicular volume reduction, noting that testicular volume reduction can be a prognostic of poorer results.[1,8,9] Patients with no sperm found in the sperm control exam were referred to a human assisted reproduction service.

The five patients with genetic changes were advised of the possibility of choosing a sperm donor.

The other 15 patients with a diagnosis of idiopathic azoospermia were advised to undergo TESE, micro-TESE, TESA and PESA. These techniques are used to collect sperm from the testicle or epididymis. The sperm sample could be used for cryopreservation or assisted reproductive procedures (Table 2).[1,7-9,12-16]

TABLE 2 Findings and actions after one year of varicocelectomy.

Varicoceles operated	n	%	Patients with sperm retrieved	With alterations in the karyotype	With Y-chromosome microdeletions	Idiopathic patients
Bilateral	10	40				
Left	9	36	5 patients	3 patients	2 patients	15 patients
Right	6	24				
Total	25	100	20%	12%	8%	60%
Actions			Cryopreservation of sperm	Use of sperm donor		TESA PESA TESE
				Human reproduction service		

Note that 1/5 of the operated patients can use their own sperm.

Conclusion

Patients with varicocele and azoospermia and without genetic changes or obstruction should be treated surgically with varicocelectomy.

Although small, the postoperative recovery of sperm would allow the couple to undergo assisted reproductive procedures without the need of a sperm donor. Thus, patients with NOA associated with varicocele veins should be made aware of the small chance of success in recovering sperm production after surgery, and of the purpose of surgery.[7-13,16]

Conflict of interest

The authors declare no conflict of interest.

Resumo

Pacientes com azoospermia e varicocele devem ser submetidos à cirurgia para recuperar a fertilidade?

Introdução: A varicocele é sabidamente uma das causas de infertilidade nos homens. A presença de veias espermáticas dilatadas pode criar um ambiente hostil para a espermatogênese. Isso é causa de baixa qualidade e quantidade da produção do esperma; em alguns casos, pode determinar uma ausência total de espermatozoides. O procedimento de varicocelectomia em pacientes com azoospermia não obstrutiva pode aumentar as taxas de espermatozoides na análise do sêmen. Uma taxa positiva para o esperma, mesmo se muito baixa, pode ser suficiente para permitir a coleta e destinar-se ao processo de fertilização *in vitro*. Isso sem a necessidade de usar esperma de doador.

Objetivo: Avaliar o aumento de espermatozoides em pacientes com varicocele associada à azoospermia não obstrutiva.

Método: Foram analisados os espermas de 25 pacientes azoospêrmicos não obstrutivos submetidos a procedimento de varicocelectomia bilateral com magnificação microcirúrgica.

Resultados: De um total de 25 pacientes, em três (12%) ocorreu recuperação da contagem de espermatozoides quatro meses após o procedimento. Após um ano de procedimento, em cinco (20%) ocorreu a recuperação.

Conclusão: Pacientes com ausência de espermatozoides e varicocele, sem alterações genéticas, devem ser submetidos a tratamento cirúrgico a fim de recuperar a produção de espermatozoides.

Palavras-chave: azoospermia, infertilidade, varicocele, cirurgia.

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Implications of alcoholic cirrhosis in atherosclerosis of autopsied patients

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Summary

Introduction: Alcoholism is a major public health problem, which has a high social cost and affects many aspects of human activity. Liver disease is one of the first consequences of alcohol abuse, and steatosis, liver cirrhosis and hepatitis may occur. Other organs are also affected with pathological changes, such as pancreatitis, cardiomyopathies, dyslipidemias and atherosclerosis.
Objective: To identify the occurrence and degree of atherosclerosis in alcohol-dependent individuals with liver cirrhosis, observing macroscopic and microscopic changes in lipid and collagen deposits and in the liver. We also aimed to verify the association of lipid and collagen fiber deposits with gender, age and body mass index, and to relate alcoholism, liver cirrhosis and atherosclerosis.
Method: We performed a study based on autopsy reports of patients with alcoholic liver cirrhosis, with analysis of aorta and liver fragments to verify the occurrence and degree of atherosclerosis, as well as collagen contents.
Results: Microscopic atherosclerosis was higher in young subjects (early injury) and in patients with alcoholic liver cirrhosis. The macroscopic analysis of atherosclerosis in aortas showed that patients in more advanced age groups presented more severe classifications. Atherosclerosis, both micro and macroscopically, and the percentage of fibrosis in the liver and aorta were more expressive in females.
Conclusion: Cirrhotic patients presented a higher percentage of fibrosis and lipidosis, and may represent a group susceptible to the accelerated progression of cardiovascular diseases. Investigative studies contribute to targeting health-promoting interventions, reducing the mortality and costs of treating cardiovascular disease.

Keywords: atherosclerosis, alcoholic liver cirrhosis, autopsy.

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Introduction

Alcoholism is a major public health problem and has a high social cost, affecting various aspects of human activity.[1] In the liver, the alcohol produces toxic products such as acetaldehyde and acetic free radicals, highly reactive and potentially damaging to liver cells.[2] Acetaldehyde is one of the causes of liver fibrogenesis, which triggers an interrelationship between two cell types resident in the hepatic sinusoids: Kupffer cells and stellate Ito cells.[3]

Liver disease is one of the first consequences of alcohol abuse.[4,5] Some complications include steatosis, liver cirrhosis and hepatitis, but other organs are also affected, with pathological changes such as pancreatitis, cardiomyopathy, cardiac arrhythmias, hypertension, hemorrhagic outbreak, anemia, cancer, immunosuppression, sudden death, dyslipidemia and atherosclerosis.[1,6,7]

These products can interfere with the normal metabolism of other nutrients, particularly lipids, and contribute to liver cell damage. Atherosclerosis is the term used to describe "damage to the large and medium-sized arteries with deposit of yellow plaques containing cholesterol and lipoid material." Such plaques – formed by the proliferation of smooth muscle cells, cholesterol deposition and infiltration of mononuclear cells – reduce

endothelial function, limiting the effective diameter of the vessels, and can also cause thrombosis, which can trigger various kinds of strokes, such as sudden death from myocardial infarction.[8]

Several risk factors have been described for atherogenic development, such as dyslipidemia, hypertension, diabetes, smoking, alcohol abuse and physical inactivity. Lipid profile has been widely studied for its strong association with atherogenesis.[9] The most characteristic component of atherosclerosis is chronic inflammation of the artery walls, pointing out the importance of inflammation for the severity of the disease.[10]

Regarding the advances in diagnostic and therapeutic areas, cardiovascular disease remains a major cause of mortality worldwide[11] and, in view of the epidemiological profile of the Brazilian population, further research is essential to identify risk groups and enable interventions to prevent the incidence and progression of these diseases. In addition, current treatment methods must be improved, seeking new therapeutic targets and specific biomarkers that prove to be useful in the medical practice. Classic research related to the circulatory system mainly involves histopathologic descriptions of pathophysiological processes.[11]

Our study aimed to quantify collagen fibers in the aorta and liver of autopsied patients with and without alcoholic cirrhosis, and to associate the percentage of these fibers with the degree of atherosclerosis, age and BMI of patients, in order to validate the hypothesis that patients with cirrhosis have a higher degree of atherosclerosis and higher percentage of collagen fibers both in the liver and in the aorta.

Method

This study was approved by the Ethics Committee of the Federal University of Triângulo Mineiro (UFTM, in the Portuguese acronym), under number 888.

The sampling comprised 30 samples of aorta and 30 of liver from autopsies routinely performed at the Clinical Hospital of the Federal University of Triângulo Mineiro, in the city of Uberaba, State of Minas Gerais, between 1983 and 2008. The information and the fragments were collected by two pathologists responsible for the autopsies performed by the Division of General Pathology of the Clinical Hospital of the UFTM.

Based on autopsy reports, 15 patients were selected, aged over 18 years and with alcoholic liver cirrhosis. For the control cases, 15 patients without alcoholic cirrhosis were selected, matched for gender, color and age and with no cardiovascular disease, autopsied at the same period. Thirty aortic fragments were removed transversely, measuring about 20 x 1 mm, and 30 liver fragments of the right lobe were collected, with approximately 2 x 2 cm.

As a contributor to the nutritional assessment of patients, the body mass index (BMI) was calculated using the following formula: weight (kg)/height^2 (m). Patients were classified according to BMI as underweight (BMI less than 18.5 kg/m^2), normal (between 18.5 and 24.99 kg/m^2), overweight (between 25.0 and 29.99 kg/m^2) or obese (over 30.0 kg/m^2). For this classification, the standards of the World Health Organization were applied (WHO, 2004).

Macroscopic analysis of the aorta

Macroscopic degree of atherosclerosis was classified into mild, moderate or severe. From this criterion, the observers of this study described quantitatively the atherosclerotic arteriosclerosis. For this evaluation, the extent of atheromatous plaques and the intensity of fibrosis and calcification were considered.[12]

The aortas were evaluated with the aid of a standard scale of 12.0 cm. Each examiner subjectively measured the degree of atherosclerosis using a non-millimetric scale, marking a point on the scale. Next, with a ruler, the distance from 0.0 to the point marked on the scale was measured. The observers standardized atherosclerosis, according to its intensity, categorizing as mild from 0.1 cm to 4.0 cm, moderate from 4.1 cm to 7.0 cm, and severe from 7.1 cm to 12.0 cm.[12]

Microscopic analysis of aorta and liver fragments

The slides were stained with hematoxylin and eosin (HE) for semi-quantitative evaluation of lipid deposits, analyzed under common light microscopy. After this analysis, the degree of lipid deposit was classified as mild, when the involvement of the section analyzed was less than 25%; moderate from 26 to 50%, and severe if greater than 50%. The morphological evaluation method of the aortas was adapted from other studies.[13,14]

Slides stained with picro-sirius were used for quantification of collagen fibers in the inner layer of the aorta (Figures 1 and 2) and throughout the extension of the liver cut, examined under polarized light with a 20x objective, final magnification of 800x. The analyses of collagen fibers were performed by morphometric software using the automatic image analysis system KS-300® (Kontron-Zeiss).

Statistical analyses

Initially, a Microsoft Excel® spreadsheet was produced and data were analyzed using Graphpad Prism®5.0 software. The normality of the data was checked with Kolmogorov-Smirnov test. Since the distribution was non-

-normal, we used Mann-Whitney test to compare the two groups and Kruskall-Wallis test for comparison between three or more groups. The Spearman correlation coefficient was used to correlate two variables. Differences were considered statistically significant at $p<0.05$.

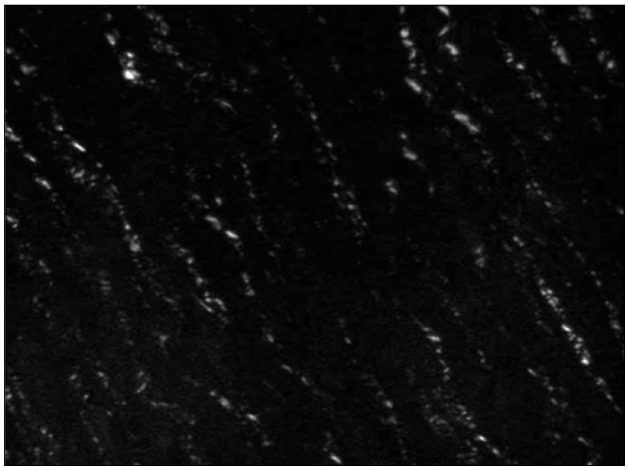

FIGURE 1 Histologic section of the intima layer in human aorta under polarized light, stained with picro-sirius, with end magnification of 800x. Discrete fibrosis is observed.

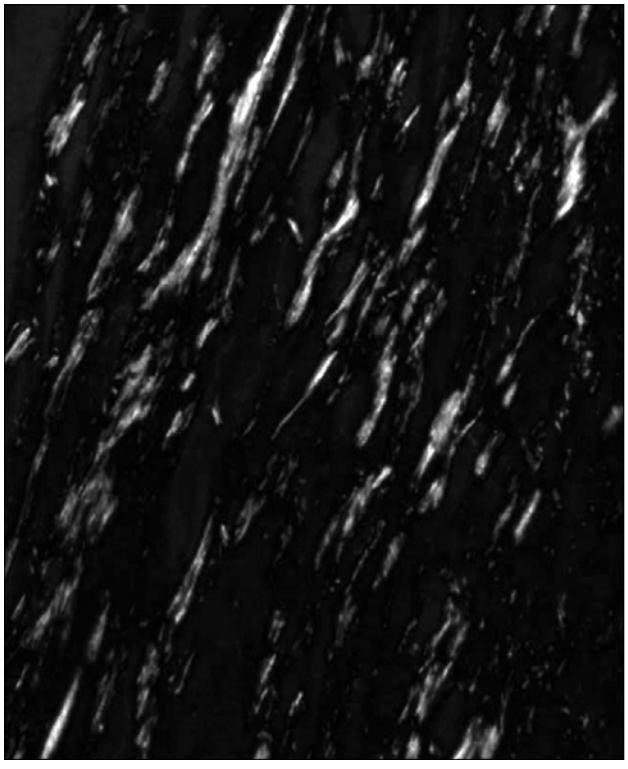

FIGURE 2 Histologic section of the intima layer in human aorta under polarized light, stained with picro-sirius, with end magnification of 800x. Pronounced fibrosis is observed.

Results

Thirty patients were selected, with 15 cases of alcoholic liver cirrhosis found in the analyzed period, corresponding to 4.2% of autopsy reports, and 15 without cirrhosis. The patients were divided into four groups: group 1 (mild atherosclerosis, without cirrhosis), group 2 (moderate and severe atherosclerosis, without cirrhosis), group 3 (mild atherosclerosis, cirrhotic), and group 4 (moderate and severe atherosclerosis, cirrhotic). The general characteristics of the samples were analyzed with descriptive methods presented in Table 1.

TABLE 1 General characteristics of the samples.

Data	Cirrhotic (n=15)	Non-cirrhotic (n=15)	Total (n=30)
Atherosclerosis			
Mild	10	13	23
Moderate and severe	5	2	7
Gender			
Female	4	5	9
Male	11	10	21
Color			
White	12	10	22
Non-white	3	5	8

By associating liver fibrosis with cirrhosis and atherosclerosis, we found that cirrhotic patients with mild atherosclerosis showed a higher percentage of liver fibrosis than cirrhotic patients with moderate and severe atherosclerosis ($p<0.0001$).

As for aortic fibrosis, cirrhotic patients had a significantly higher percentage of fibrosis than the non-cirrhotic ones ($p<0.0001$). Among those with moderate and severe atherosclerosis, the degree of atherosclerosis was higher in cirrhotic patients ($p<0.0001$).

Considering the association of BMI with cirrhosis and atherosclerosis, cirrhotic patients with mild atherosclerosis had higher BMI than those with moderate and severe atherosclerosis ($p=0.0017$).

Analyzing all patients, in general and in groups, both BMI and macroscopic atherosclerotic severity significantly increased with age ($p<0.0001$ for all correlations). However, with advancing age, aortic fibrosis decreased significantly ($p<0.0001$) in groups 3 and 4 (cirrhotic).

In all groups, the BMI increased significantly with liver fibrosis ($p<0.0001$ for all correlations). The macroscopic degree of atherosclerosis increased significantly with the increase of liver fibrosis in all groups ($p<0.0001$). Aortic fibrosis decreased significantly with hepatic fibrosis only in group 3 ($p<0.0001$).

Discussion

Our analysis led to the confirmation of the hypothesis that cirrhotic patients have a higher degree of atherosclerosis and aortic fibrosis than non-cirrhotic ones. Furthermore, we observed that liver fibrosis increases with BMI and the intensity of atherosclerosis. This shows a possible link between the two diseases, and the worsening of liver disease could influence the formation of atherosclerotic plaques.

There is increasing evidence that atherosclerosis starts to develop early in life, progressing to more advanced stages since young adulthood.[15] We also noted a higher degree of atherosclerosis and aortic fibrosis in patients with alcoholic liver cirrhosis. A possible explanation for this would be that the vitamins involved in homocysteine metabolism might be reduced in chronic alcoholism, thus leading to the accumulation of homocysteine, primary indicator of atherogenesis.[7] Furthermore, it has been discussed that increased levels of homocysteine in patients with alcoholic liver disease also have consequences to the liver, triggering endoplasmic stress[16] and inhibiting the production of adiponectin, a hormone that induces lipolysis,[17] stimulates fatty acid oxidation[18] and inhibits the production of hepatic glucose.[19]

Adipose tissue plays a crucial role in the regulation of fatty acid homeostasis in the entire body. The idea of a dynamic adipose tissue as an endocrine organ is being increasingly recognized, which is very important for the regulation of metabolism in health and disease conditions. Adipocytes secrete proteins called adipokines, such as adiponectin, resistin, Il-6, TNF-alpha, and leptin;[20,21] and overweight individuals have altered secretion of adipokines, increasing the production of TNF-alpha and IL-6 by macrophages, which fuels inflammatory processes.[22] Lifestyle is a significant factor in the development of hepatic steatosis, since it can promote weight gain, ultimately leading to obesity, generating an increase in inflammatory cytokines and insulin resistance that can, in turn, lead to inflammation of visceral fat and increased accumulation of fat in the liver. This cycle of responses of the organism to obesity and the development of hepatic steatosis trigger chronic inflammation (increased C-reactive protein, interleukin 6 and tumor necrosis factor alpha), hypercoagulation, hyperlipidemia (increased levels of triglycerides, decreased HDL-cholesterol levels and possible increased LDL), which increases the risk of heart disease.[23] This information corroborates our findings, where patients with higher BMI showed a higher degree of cirrhosis and atherosclerosis.

Analysis in the aortas showed that older patients presented more intense macroscopic atherosclerosis. This may be due to progression of the disease; in more advanced stages, there is the formation of fibrous connective tissue, associated with a lipid core, called fibroatheroma.[24]

Regarding fibrosis in the liver of cirrhotic patients, the high incidence may have been a result of repeated use of alcohol.[3] Triglyceride storage in the form of VLDL is a physiological mechanism that prevents the accumulation of neutral fats in the liver, since VLDL is carried away from the organ. However, excess ethanol causes changes in the triglyceride storage mechanism, and this causes severe cellular stress, resulting in the accumulation of neutral fats in hepatocytes, which can later lead to fibrosis and cirrhosis.[25]

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Resumo

Implicações da doença hepática alcoólica na aterosclerose de pacientes autopsiados

Introdução: O alcoolismo é um grande problema de saúde pública, de elevado custo social e que afeta vários aspectos da atividade humana. Hepatopatia é uma das primeiras consequências do abuso de álcool, podendo ocorrer esteatose, cirrose hepática e hepatite. Outros órgãos, porém, também são afetados, ocorrendo alterações patológicas, como pancreatite, cardiomiopatias, dislipidemias e aterosclerose.
Objetivo: Identificar a ocorrência e a intensidade de aterosclerose em alcoolistas com cirrose hepática, observando alterações macro e microscópicas do depósito lipídico e de fibras colágenas e fígado. Verificar a associação de depósito lipídico e de fibras colágenas com gênero, idade e índice de massa corporal (IMC). Relacionar alcoolismo, cirrose hepática e aterosclerose.
Método: Foi realizado estudo com base em laudos de autópsias de pacientes com cirrose hepática alcoólica, sendo estudados aortas e fígados para verificar a ocorrência e a intensidade de aterosclerose, bem como a quantidade de colágeno encontrada.
Resultados: A aterosclerose microscópica foi maior em jovens (lesão inicial) e em pacientes com cirrose hepática alcoólica. A análise macroscópica da aterosclerose nas aor-

tas mostrou que pacientes com faixas etárias mais avançadas apresentaram classificações mais intensas. A aterosclerose, tanto micro quanto macroscopicamente, e a porcentagem de fibrose no fígado e na aorta foram mais expressivas no gênero feminino.

Conclusão: Os pacientes cirróticos apresentaram maior porcentagem de fibrose e lipidose, e podem representar um grupo susceptível à acelerada progressão de doenças cardiovasculares. Estudos investigativos contribuem para o direcionamento das intervenções promotoras da saúde, reduzindo a mortalidade e os custos no tratamento das doenças cardiovasculares.

Palavras-chave: aterosclerose, cirrose hepática alcoólica, autópsia.

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HbA1c levels in individuals heterozygous for hemoglobin variants

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Summary

Objective: To evaluate the levels of glycated hemoglobin (HbA1c) in patients heterozygous for hemoglobin variants and compare the results of this test with those of a control group.

Method: This was an experimental study based on the comparison of HbA1c tests in two different populations, with a test group represented by individuals heterozygous for hemoglobin variants (AS and AC) and a control group consisting of people with electrophoretic profile AA. The two populations were required to meet the following inclusion criteria: Normal levels of fasting glucose, hemoglobin, urea and triglycerides, bilirubin > 20 mg/dL and non-use of acetylsalicylic acid. 50 heterozygous subjects and 50 controls were evaluated between August 2013 and May 2014. The comparison of HbA1c levels between heterozygous individuals and control subjects was performed based on standard deviation, mean and G-Test.

Results: The study assessed a test group and a control group, both with 39 adults and 11 children. The mean among heterozygous adults for HbA1c was 5.0%, while the control group showed a rate of 5.74%. Heterozygous children presented mean HbA1c at 5.11%, while the controls were at 5.78%. G-Test yielded p=0.93 for children and p=0.89 for adults.

Conclusion: Our study evaluated HbA1c using ion exchange chromatography resins, and the patients heterozygous for hemoglobin variants showed no significant difference from the control group.

Keywords: glycated hemoglobin A, ion exchange chromatography, hemoglobins.

Introduction

Glycated hemoglobin (HbA1c) is used to assess the efficacy of diabetes treatment and is a paramount control for better quality of life among patients. The test reveals the mean blood glucose in the last four months before the test, based on the turnover of hemoglobin, which lasts 120 days.[1] Clinical data show that the mean level of blood glucose in the 20 to 30 days preceding blood sampling contributes around 50% of the end result of HbA1c, while the mean level in the prior 90 to 120 days contributes only 10%. Thus, this method can detect large variations in blood glucose.[2,3]

HbA1c was defined as a diagnostic method for diabetes mellitus, with a threshold of 6.5% for adults. The test should be performed using a method that is certified by the National Glycohemoglobin Standardization Program (NGSP) and standardized or traceable to the Diabetes Control and Complications Trial (DCCT) reference.[4]

For the measurement of HbA1c, it is desirable that laboratories use NGSP certified methods, and it is important to note that high-performance liquid chromatography (HPLC) is not the only method available for the measurement of HbA1c. Several other diagnostic sets that use non-HPLC methods are also certified. The following methods were also evaluated by the NGSP: turbidimetric immunoassay, electrophoresis, ion exchange chromatography and enzymatic assay.[5-8]

HbA1c has several advantages over fasting glycemia, including greater convenience, since fasting is not necessary, evidence suggesting greater pre-analytical stability, and fewer day-to-day disturbances during periods of stress and disease.[4]

For a reliable HbA1c result, some possible interferers must be considered, including the labile fraction and sample preservation.[9,10] In addition to the variables mentioned above, some factors such as hyperbilirubinemia, hypertriglyceridemia, hyperuremia, chronic alcoholism, chronic ingestion of salicylates and opiate dependence can significantly alter HbA1c results, producing falsely increased results.[2] False-low results are observed in the presence of large amounts of vitamins C and E, which are described as inhibitory factors for hemoglobin glycation.[2,6,7,9,11,12]

Hemoglobinopathies and hemoglobin variants are also interfering factors, the latter being characterized by changes in hemoglobin structure caused by differences in the amino acid sequence of globin chains.[2,7,9]

Hemoglobin (Hb) is a spheroidal molecule, which is located inside the red blood cells and whose main function is the transport of oxygen to the tissues.[13] Its structure is composed of four subunits formed by two pairs of identical polypeptide chains, called globins, each bound to a heme group. The hemoglobin profile of a normal adult contains about 97% of HbA (two α-globin chains and two β-globin chains, represented as $\alpha 2\beta 2$), 2% of HbA2 ($\alpha 22$) and 1% of fetal Hb (HbF, represented as $\alpha 2\gamma 2$), which is the predominant Hb during intrauterine life.[10]

HbA1c is a subtype derived from the binding of HbA1 by means of an irreversible non-enzymatic reaction between blood glucose and the N-terminal amino acid valine of the beta chain.[14]

The change in hemoglobin levels observed in heterozygous individuals may interfere with test results, which is the case of HbA1c.[15] Thus, our objective in the present study was to verify A1c levels in patients heterozygous for hemoglobin variants.

Method

This is an experimental study, based on the comparison of HbA1c test results from two different populations: a test group represented by individuals heterozygous for hemoglobin variants (AS and AC) and a control group consisting of people with electrophoretic profile AA confirmed by hemoglobin electrophoresis at alkaline pH. Both populations were required to meet the inclusion criteria: Normal levels of fasting glucose, hemoglobin, urea and triglycerides, bilirubin > 20 mg/dL, and non-use of acetylsalicylic acid (ASA). All of the above factors, if changed, can trigger falsely high or low results.[2,6,7,9,11,12]

In addition to the HbA1c test, other tests were performed in the two populations to contribute to the interpretation of HbA1c, such as: fasting blood glucose and blood counts, as well as tests to identify possible interferers: urea and triglycerides. Still assessing interferences, we checked if the samples were icteric, as bilirubin levels above 20 mg/dL can cause falsely high HbA1c results. We paid special attention to ascorbic acid, which influences the assessment of HbA1c. It is a volatile compound and, after gathering sampling, requires a period of 90 minutes to be separated from the rest of the blood sample and proceed to dosing.[2,7,9]

The heterozygous participants were selected from the Clinical Laboratory (LAC, in the Portuguese acronym) database at Pontifícia Universidade Católica de Goiás (PUC Goiás), and invited to participate in the research. A Free and Informed Consent Form with the Code of Approval by the Ethics Committee (no. 4.1.1.00-0 PUC Goiás) was promptly presented to them.

Whole blood samples were collected in three tubes stored and distributed as follows: a tube containing EDTA (ethylenediaminetetraacetic acid) for blood counts and HbA1c; a fluoride tube for blood glucose dosing; and a tube for serum separation and dosing of triglycerides and urea.

HbA1c was measured using a column method of ion-exchange resins to separate HbA1c, which was subsequently read on a CELM (Cia. Equipadora de Laboratórios Modernos) spectrophotometer, with the aid of the HbA1c dosing kit Gold Analisa Diagnóstica Ltda© (Lot: 30031).

HbA1c levels read by column chromatography are equivalent to those obtained with HPLC, which is certified by the NGSP, and can be converted to standard HPLC method from the International Federation of Clinical Chemistry (IFCC) using the formula: $IFCC=(10.93 \times NGSP)-23.50$.[16,17]

Blood glucose levels were measured using a glucose oxidase method, ELITechGroup© (Lot: 13-0096), while the blood counts were assessed with Sysmex© XE-2100 automated hematology analyzer.

To eliminate the interferers from the HbA1c test, urea, triglycerides and bilirubin were dosed in the patients' serum. An enzymatic colorimetric test was used to estimate serum urea and bilirubin, and peroxidase-oxidase for the analysis of triglycerides, whose dosages were measured using Selectra® XL – Vitalab. In order to validate the fasting blood glucose, urea, triglycerides and bilirubin tests, three control sera were used, namely positive and normal controls branded ELITechGroup© (Lot: 5564 A and Lot: 3575 B, respectively) and the control serum of the National Program of Quality Control (PNCQ) (Lot: 3575).

The study divided groups by age group according to Ministry of Health criteria: children and adolescents aged < 20 years, adults aged 20 to 60 years.[18]

We evaluated 50 individuals heterozygous for hemoglobin variants and 50 controls from August 2013 to May 2014.

Data were tabulated using statistical software, Microsoft Excel® and GraphPad Software, Inc., version Prisma 6. Comparison of HbA1c levels between individuals heterozygous for hemoglobin variants and controls with no change in hemoglobin structure was performed based on standard deviation, mean and G-Test.

Results

We analyzed 50 patients heterozygous for hemoglobin variants with normal blood glucose levels, of which 23 were male, representing 46% of the study population, and 27 were female, totaling 54%. Of the total 50, 16% are AC and the remaining 84% are AS.

Dividing the group by age, we found 39 adults with a mean age of 34.6 years (21 ± 38 years) and 11 children and adolescents with a mean age of 5.9 years, ranging from 1 to 13 years. In this group, no interferer was observed, as described in Table 1.

TABLE 1 Assessment of interferers in a heterozygous population.

Tests	Mean	Minimum value	Maximum value	Reference value
Urea	27 mg/dL	17 mg/dL	39 mg/dL	15-40 mg/dL
Triglycerides	113 mg/dL	80 mg/dL	143 mg/dL	< 150 mg/dL
Total bilirubin	0.9 mg/dL	0.6 mg/dL	1.2 mg/dL	≤ 1.2 mg/dL

Source: Clinical Laboratory at PUC Goiás.

The mean values of HbA1c in heterozygous children were at 5.11%, with a minimum of 3.9% and maximum of 5.97%. The mean fasting glucose in this group was 89.56 mg/dL, ranging from a minimum of 75 mg/dL to a maximum of 97 mg/dL, while mean hemoglobin was 12.9 g/dL, ranging from 12.1 g/dL and 14 g/dL.

The mean A1c of heterozygous adults was close to the values found for children, at 5.0%, ranging from a minimum of 3.1% to a maximum of 6.9%. As for mean fasting blood glucose, the adult population presented 84 mg/dL, ranging from a minimum of 69 mg/dL to a maximum of 97 mg/dL. Mean hemoglobin was at 13.8 g/dL, ranging from 12.3 g/dL to 15.8 g/dL.

In order to compare the data, we analyzed the same tests in 50 patients without heterozygosity for hemoglobinopathies, with normal glycemic levels, of which 71.4% were female. This group included 11 children and adolescents, with a mean age of 10 years (6 ± 14 years). The following results were found in this class: HbA1c 5.78%, fasting blood glucose 78.8 mg/dL and hemoglobin 14 g/dL.

With regard to the adult control population, 39 people with mean age of 45 years (22 ± 37 years) were analyzed. We found in this group the following mean test results: HbA1c 5.84%, ranging from 4.1% to a maximum of 6.9%, fasting blood glucose at 89.3 mg/dL, ranging from 60 mg/dL to 99 mg/dL, and mean hemoglobin at 13.6 g/dL, ranging from a minimum of 12 g/dL to a maximum of 15.7 g/dL.

HbA1c standard deviation of the children was then calculated, reaching the result of 0.40 in the control group and 0.75 in the test group. The standard deviation from the adult test population was also calculated, which was 1.09 and the control was 0.57.

The value of the G-test was also determined, representing the result of HbA1c in the normal group compared to the altered group, reaching p=0.93 for children and p=0.89 for adults (Figures 1 and 2).

Of the 50 heterozygous individuals, ten had results below the lower limit of 4%. Of these, eight were adults and two were children, of whom nine were female. The electrophoretic pattern of this group was verified and eight fit the AS profile, while two were AC.

Discussion

This study showed significant similarity with the findings of Menezes et al.,[19] whose comparative inferential analysis of HbA1c was: AA, AS, AC and SS obtained from blood samples from 150 participants, including diabetics, was not significant, the same being observed in our study with 100 subjects who had normal glycemic levels.

Yedla, Kuchay and Mithal[20] evaluated the levels of fasting blood glucose, postprandial blood glucose, fructosamine and HbA1c (immunoassay technique) of a diabetic patient, resulting in higher levels of fasting blood glucose, postprandial blood glucose and fructosamine, but not HbA1c, which remained normal. They concluded that the result was underestimated, and a different technique should be performed for this type of examination.

According to the study, ion exchange chromatography (HPLC) as a method used to measure HbA1c is not influenced by heterozygous hemoglobinopathies, as Copeland et al.[21] report that some methodologies may have interference from heterozygous hemoglobins.

In a study by Camargo and Gross,[22] the authors analyzed more than 25,000 samples and found patients heterozygous for HbS, HbC or HbD, noting that the presence of a hemoglobin variant may yield lower (false) results. However, anemia is also a source of negative interference. The hematological status should be considered for the correct interpretation of GHb results.

Despite cation exchange, HPLC is the reference method, and the presence of hemoglobin variants may interfere with levels of HbA1c, yielding false high or low levels

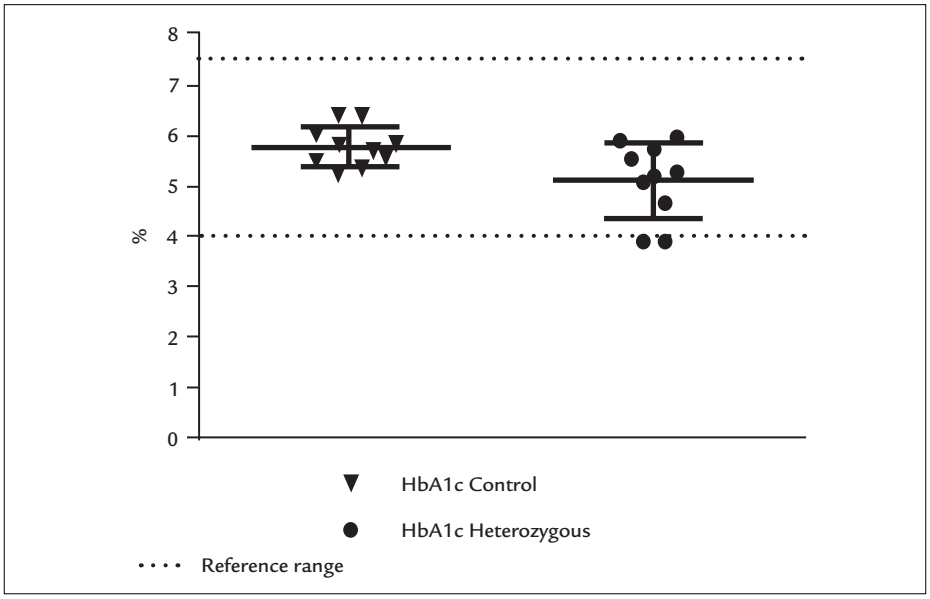

FIGURE 1 HbA1c comparison between control and heterozygous children.

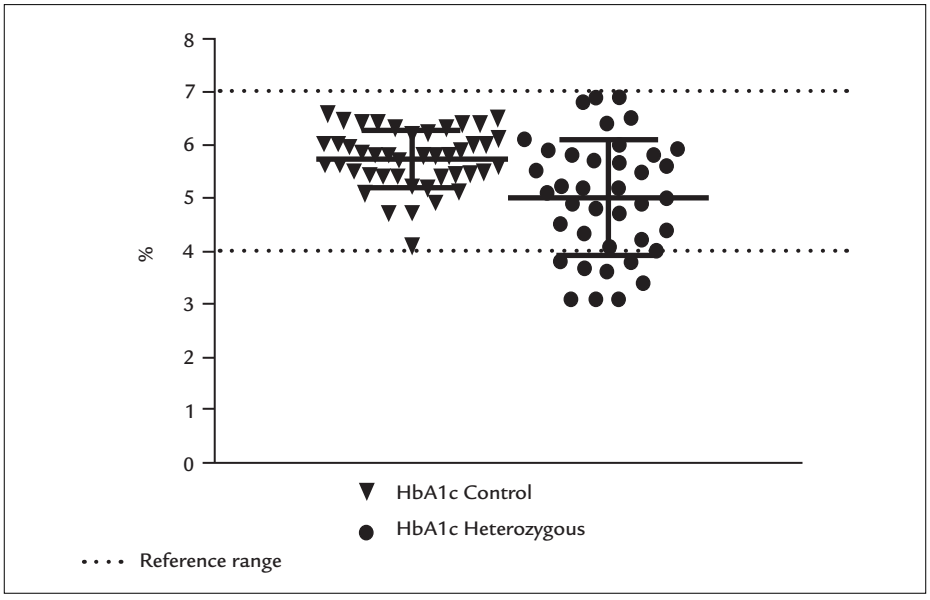

FIGURE 2 HbA1c comparison between control and heterozygous adults.

when the hemoglobin variant or its glycosylated form cannot be separated from hemoglobin A or HbA1c.[23,24] In contrast, heterozygous hemoglobin S and C samples did not affect the assay.[25]

Not only hemoglobinopathies, including thalassemia syndromes, but also factors that affect red blood cell survival, age, uremia, hyperbilirubinemia and iron deficiency can affect the results of HbA1c tests. Racial and ethnic differences, genetic variation in hemoglobin glycation and assay methodology may also influence the results.[26]

New methods are being developed, such as mass spectrometry, which is based on the suppression of fluorescence of a boron eosin in acid solution, and shows minimal interference of the hemoglobin variants.[27-30] Despite these valuable attributes, cost of equipment and complexity of the operation limit its use.[31]

According to studies, the presence of genetic variants of hemoglobin under heterozygous conditions may interfere with the measurement of HbA1c, resulting in falsely high or decreased values, depending on the type of test used.

Dosage of HbA1c does not apply in homozygous conditions to anomalous hemoglobins under any method, because, in these cases, hemoglobin A is practically absent. However, when an interfering hemoglobin variant is visualized or suspected, samples should be analyzed using an alternative method. In such cases, tests such as fructosamine and/or glycated albumin may be useful.[2,21,31]

Bouzid et al.[32] suggest that laboratory professionals add a footnote that reads: "Quantification of HbA1c was not possible using this method due to the presence of homozygous HbS or HbC variants or heterozygous SC compounds. We suggest fructosamine or another alternative." This would provide the physician with the information needed to properly manage these diabetic patients.

Lower levels of hemoglobin affect A1c measurement and, since women have lower hemoglobin levels than men, the A1c result will suffer interference and the reference value should be different.[33]

Conclusion

Our study evaluated HbA1c using ion exchange chromatography, and the patients heterozygous for hemoglobin variants did not present a significant difference compared to the control group.

Based on the results of this study, members of the medical community should consider an assessment of laboratory results due to the differences in method employed for diagnostic support. When assessing laboratory results, the interpretation must include patient history, which may contain data related to the ethnic and socioeconomic characteristics of that specific population.

Resumo

Dosagem de hemoglobina glicada em heterozigotos para hemoglobinas variantes

Objetivo: Avaliar os níveis de hemoglobina glicada em pacientes heterozigotos para hemoglobinas variantes e comparar os resultados deste exame com grupo controle.
Método: Trata-se de um estudo experimental, baseado na comparação do exame de hemoglobina glicada de duas populações diferentes, sendo um grupo teste, representado por indivíduos heterozigóticos para hemoglobinas variantes (AS e AC) e um grupo controle, constituído por pessoas com perfil eletroforético AA. As duas populações verificadas devem obedecer ao critério de inclusão: glicemia de jejum, hemoglobina, ureia e triglicérides normais, bilirrubina > 20 mg/dL e não fazer uso de ácido acetilsalicílico. Foram avaliados 50 indivíduos heterozigotos e 50 controles no período de agosto de 2013 a maio de 2014. A comparação dos valores de hemoglobina glicada entre indivíduos heterozigóticos e controle foi realizada por meio do desvio padrão, média e teste G.
Resultados: O estudo analisou um grupo teste e um grupo controle, ambos com 39 adultos e 11 crianças. A média dos adultos heterozigotos para HbA1c foi de 5,0%, o grupo controle apresentou índice de 5,7%. Já as crianças heterozigóticas obtiveram média de HbA1c de 5,11%, enquanto as normais apresentaram valores médios de 5,78%. O valor do teste G foi de p=0,9 para crianças e p=0,89 para adultos.
Conclusão: Este estudo avaliou HbA1c pela metodologia de cromatografia de coluna com resinas de troca iônica, em que pacientes com heterozigoses para hemoglobinas variantes não apresentaram uma diferença significativa em relação ao grupo controle.

Palavras-chave: hemoglobina A glicosilada, cromatografia por troca iônica, hemoglobinas.

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Patient-reported measures of quality of life and functional capacity in adhesive capsulitis

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Summary

Objective: To evaluate patient-reported measures of quality of life and functional capacity in adhesive capsulitis before and after suprascapular nerve block treatment; to analyze the influence of clinical and sociodemographic variables on both the outcome and correlation between the respective instruments.

Method: A prospective clinical study was performed with adhesive capsulitis patients. Inclusion criteria were clinical diagnosis of adhesive capsulitis and a shoulder imaging test. The WHOQOL-BREF and DASH instruments were administered before and after treatment. A Constant test score of 55 points was used as a cutoff point for discontinuation of treatment. Mean values were compared using paired t-test and Wilcoxon. The Pearson or Spearman coefficients were used for correlation analysis. Multiple linear regression analysis was carried out using variables with $p<0.20$ as predictors in univariate analysis and WHOQOL domains and DASH as outcomes. The significance level was 5%.

Results: Forty-three patients were evaluated. The comparison between WHOQOL-BREF and DASH mean values before and after the blocks, $p<0.05$. DASH correlated negatively with the physical, psychological and environmental WHOQOL-BREF domains. Older patients and those with higher levels of education influenced the improvement in patients' quality of life and functional capacity.

Conclusion: The improvement of adhesive capsulitis with treatment involves an improvement in quality of life and functional capacity. The greater functional capacity of the shoulder matches a better quality of life for patients. Age and education level are the variables that most influence improvement in quality of life and functional capacity.

Keywords: quality of life, adhesive capsulitis, shoulder pain, sickness impact profile, nerve block.

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Introduction

Adopting a quality of life (QoL) assessment in clinical practice can assist therapy, allowing a more holistic approach to the individual that goes beyond the treatment of his disease. The difficulties in including this evaluation in clinical practice reside mainly in the health professionals' lack of information about its uses and applicability.[1-3]

Since this assessment reflects the perspective of the patient, and not that of scientists and health professionals, it is widely accepted as an indicator of health in medical interventions.[3] Several tools may be used for this purpose, depending on the situation.[1-4] The World Health Organization Quality of Life (WHOQOL) questionnaire was initially developed as an expanded questionnaire, the WHOQOL-100,[2] and later as the WHOQOL-BREF.[5] The WHOQOL-BREF is an abbreviated version consisting of the 26 questions on the WHOQOL-100 with the best psychometric performance.[5]

The Disabilities of the Arm, Shoulder, and Hand Questionnaire (DASH) is a self-administered regional questionnaire which assesses symptoms and the functional capacity (FC) of the affected upper limb. It was developed by the American Academy of Orthopedic Surgeons in collaboration with several other organizations.[6]

Adhesive capsulitis is a crippling, chronic and extremely painful disease that affects movement of the shoulder joint. The impairment of everyday activities such as bathing, getting dressed and driving interferes significantly with an individual's QoL.[7] There are numerous treatments, including suprascapular nerve blocks (SSNB),[7-9] which is an effective and safe procedure in the treatment of chronic diseases affecting the shoulder. The suprascapular nerve is the most important sensory nerve in the shoulder and is susceptible to blocking with local anesthetics. The large number of sympathetic fibers that this nerve supplies to the shoulder joint capsule was one of the reasons for choosing to treat adhesive capsulitis with SSNB.[8]

Studies of adhesive capsulitis patients most often involve symptoms such as pain and range of motion scales to analyze treatment results.[9,10] QoL and FC simultaneous assessment is not included in these studies, so this paper may contribute to patients' perception of their health status and treatment efficacy.

The general aim of this study was to evaluate patient-reported measures of QoL and FC in adhesive capsulitis before and after SSNB treatment. In addition, the influence of clinical and sociodemographic variables on both QoL and FC outcome and on the instruments' various domains, and the correlation between the respective instruments were analyzed.

Method

A prospective clinical study was performed with a cohort of adhesive capsulitis patients. The participants were patients selected during routine visits at a specialized clinic located in an orthopedic hospital.

Adhesive capsulitis was clinically diagnosed when there was constant pain lasting more than four weeks, with limitation on active and passive movements of the shoulder: anterior elevation to 130° (Figure 1), external rotation to 50° (Figure 2) and internal rotation to L5 (Figure 3).[10]

Inclusion criteria were a clinical diagnosis of adhesive capsulitis; existence of shoulder X-ray exams with three views (true AP, axillary profile and scapular profile) and MRI scan in the previous 30 days; not under any concomitant adhesive capsulitis treatment, no subacromial space injection in the previous 15 days; and glycosylated hemoglobin less than or equal to 7% in case of associated diabetes. Exclusion criteria were concomitant pathologies such as complete lesion of the rotator cuff, instability, glenohumeral arthrosis and locked dislocation of the shoulder; stroke sequelae (hemiplegia or paresis); recent breast surgery; current chemotherapy or radiotherapy treatment; adhesive capsulitis with bilateral involvement and previous surgery on the affected shoulder.

Before each SSNB, the Constant score was calculated. The Constant test is a clinical method of functionally evaluating the shoulder. It combines an analysis of individual parameters with a 100-point scoring system: the higher the score, the better the functional index.[11] The cutoff point for interrupting blocks and making an overall assessment of patients' quality of life and FC using the WHOQOL-BREF and DASH was a constant score of 55 points or higher. The test was administered at the beginning of every clinical visit before any other procedure.

The SSNBs were performed on an outpatient basis following Dangoisse's technique[12] without the aid of a peripheral nerve stimulator or imaging techniques: 8 mL of bupivacaine hydrochloride 0.5%, with epinephrine

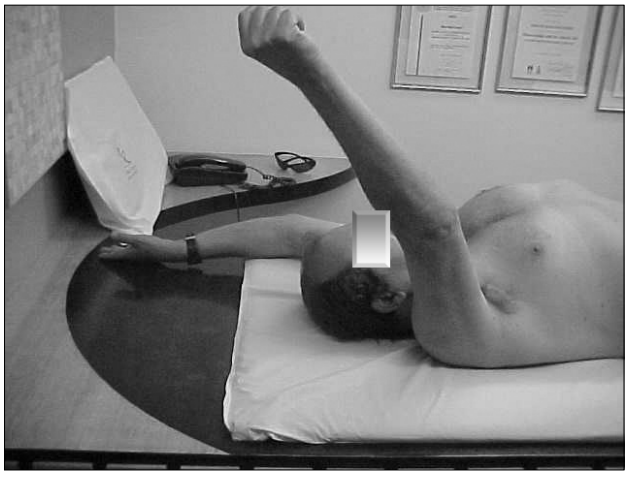

FIGURE 1 Anterior elevation limitation of the right shoulder.

FIGURE 2 External rotation limitation of the right shoulder.

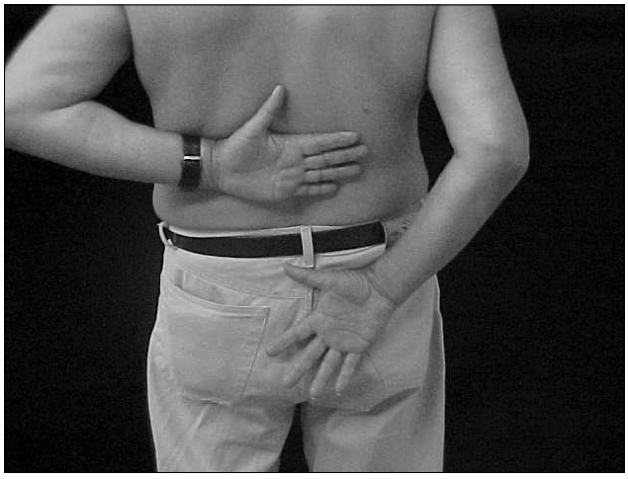

FIGURE 3 Internal rotation limitation of the right shoulder.

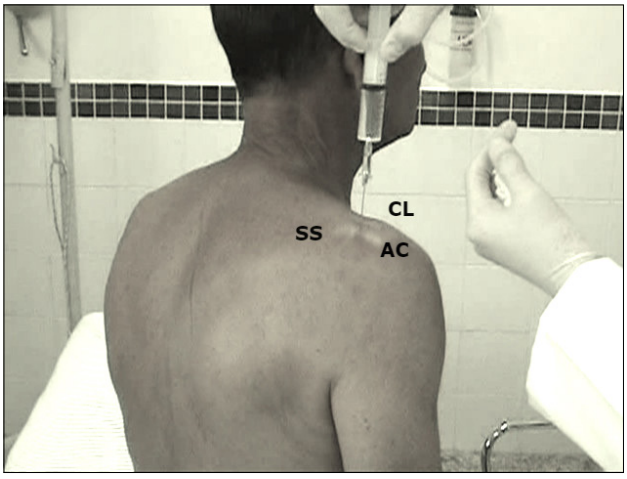

FIGURE 4 Suprascapular nerve block and anatomical landmarks.
SS: spine of the scapula; AC: acromion; CL: clavicle.

bitartrate 1:200,000 and without the association of corticosteroids, were used (Figure 4). The interval between blocks was seven days.

The sociodemographic variables were considered according to predetermined categories: age (complete years); gender (male/female); ethnic group (white/nonwhite); occupation (employed / unemployed); educational status (years of formal education); monthly income more or less than five minimum salaries; and marital status (married/divorced/single/widowed).

Clinical variables were pain and active range of motion on Constant's scale[11] where zero equals severe pain and 15 equals no pain. Anterior elevation and abduction were measured using a goniometer. The variables of dominance (right-handed/left-handed), sleep (unaffected/affected) and side of the affected shoulder (right/left) were also studied. Zuckerman's classification[13] was used to classify the disease and its clinical severity.

The outcome variable was QoL and FC. The evaluation took place before and after treatment with SSNB using the instruments WHOQOL-BREF (QoL), which the final score can range from zero to 100 (zero corresponding to the worst QoL and 100 to the best QoL),[5] and DASH (FC), which score range from zero to 100 (the higher the score, the greater the level of functional disability).[6] The questionnaires were self-administered.

This study was approved by Dr. Henrique Santillo Suleide – SES/GO research ethics committee on 6/23/2010 under number 0014.0.177.000-10.

Statistical analysis

Data were recorded on a spreadsheet (Microsoft Office Excel) and analyzed using the Statistical Package of Social Sciences (IBM – SPSS 20.0). Quantitative variables were reported as mean, median and standard deviation.

Chi-square test was used to study associations involving qualitative variables. After Shapiro-Wilk was conducted to test quantitative variables for normality, Mann-Whitney and Kruskal-Wallis or the parametric student t-test and ANOVA were applied.

The internal consistency of the instruments was analyzed by calculating Cronbach's alpha. The mean values found in each domain of the WHOQOL-BREF and DASH before and after treatment with SSNB were compared by paired t-test or the Wilcoxon test if the distribution was not parametric.

Analysis of correlation between the DASH variables and the WHOQOL domains used the Pearson or Spearman correlation depending on the type of distribution identified. The correlation analysis parameters were: $0.80 < r < 1$ = very high; $0.60 < r < 0.80$ = high; $0.40 < r < 0.60$ = moderate; $0.20 < r < 0.40$ = low; $0 < r < 0.20$ = very low.

To determine the influence of the independent variables on the various WHOQOL domain and DASH scores both before and after treatment, a multiple linear regression analysis was performed, considering the WHOQOL domains and the DASH as the endpoints of each model. The independent variables that were used were those presenting significance values at $p<0.20$ in the univariate analysis. For statistical tests, the level of significance was set at 0.05.

Results

The total number of study participants was 43. The mean age was 54.7 years (minimum 40 and maximum 75) and 23 (53.5%) were female. The majority (60.5%) had more

than eight years of formal education. The secondary form of the disease occurred in 65.1% of cases, in 11.6% due to hypothyroidism and in 4.7% to diabetes mellitus. The left shoulder was affected in 25 (58.1%) cases. The sociodemographic and clinical data of these adhesive capsulitis patients are shown in Table 1.

Cronbach's alpha values for WHOQOL-BREF and DASH, 0.91 and 0.95, respectively, were exactly the same before and after treatment with SSNB. There was improvement in QoL in all domains of the WHOQOL-BREF ($p<0.001$), least consistently in the social domain ($p=0.049$). There was also a significant increase in the functional capacity of the affected shoulder when assessed using DASH (Table 2).

Before treatment with SSNB, DASH correlated only with the physical domain of the WHOQOL-BREF ($r=-0.583$), but after this therapy there was significant correlation with the physical ($r=-0.580$), psychological ($r=-0.521$) and environmental ($r=-0.370$) domains. This means that the DASH value decreased as shoulder function improved and that QoL improved in the various domains.

TABLE 1 Sociodemographic and clinical data of the study population.

Variables		n	%	p*
Age group	40 |---- 50	14	32.6	0.977
	50 |---- 60	15	34.9	
	60 +	14	32.6	
Gender	Female	23	53.5	0.647
	Male	20	46.5	
Ethnic group	White	34	79.1	0.000
	Non-white	09	20.9	
Employment	Yes	20	46.5	0.647
	No	23	53.5	
Educational status	Illiterate	01	2.3	0.000
	Until 4 years of formal education	08	18.6	
	5 to 8 years of formal education	08	18.6	
	More than 8 years of formal education	26	60.5	
Monthly income	1 to 5 minimum salaries	27	62.8	0.000
	More than 5 minimum salaries	12	27.9	
	No salary	04	9.3	
Marital status	Single	01	2.3	0.000
	Married	32	74.4	
	Divorced	07	16.3	
	Widower	03	7.0	
Side	Right	18	41.9	0.286
	Left	25	58.1	
Dominance	Right-handed	41	95.3	0.000
	Left-handed	02	4.7	
Classification	Primary	15	34.9	0.047
	Secondary	28	65.1	
Pain	Mild	02	4.7	0.000
	Moderate	20	46.5	
	Severe	21	48.8	
Sleep	Affected	39	90.7	0.000
	Unaffected	04	9.3	
Severity	Mild	10	23.3	0.000
	Moderate	27	62.8	
	Severe	06	14	

*Chi-square test.

TABLE 2 Mean scores of WHOQOL-BREF domains, DASH and comparison between pre- and posttreatment of patients with adhesive capsulitis using suprascapular nerve block (n=43).

Domains	Mean	Median	SD	CI	p
Physical 1	45.34	46.42	19.75	39.27-51.43	0.000**
Physical 2	67.85	67.85	12.87	63.89-71.82	
Psychological 1	63.95	66.66	16.33	58.93-68.92	0.000*
Psychological 2	73.54	79.16	15.77	68.69-78.40	
Social 1	68.21	66.66	19.26	62.29-74.15	0.049*
Social 2	73.83	75	19.29	67.90-79.77	
Environment 1	60.24	62.50	15.62	55.44-65.06	0.001**
Environment 2	66.42	68.75	15.44	61.67-71.18	
DASH 1	61.68	64.16	18.71	55.92-67.44	0.000**
DASH 2	42.11	38.33	18.30	36.48-47.74	

*Wilcoxon test; **t-paired test; 1=before; 2=after.

Before treatment, severe pain was the only independent variable that influenced the low values in the physical domain significantly, while younger subjects (p=0.03) and those with less education (p=0.02) influenced the low scores in the environmental domain. The influence of the female gender as an independent variable (p=0.004) was reflected in higher DASH scores.

Older patients with higher educational levels influenced posttreatment improvement of QoL in the physical, psychological and environmental domains of the WHOQOL-BREF and also influenced increased functional capacity of the shoulder assessed using DASH (Table 3).

Discussion

This study confirms that adhesive capsulitis is more predominant in females in the 5th and 6th generations,[14] since it is a disease commonly associated with other systemic and non-systemic disorders[15] found in 65.1% of cases, of which 11.6 % were secondary to hypothyroidism. Bilateral disease was one of the study's exclusion criteria and, thus, it was not possible to compare our data with those in the literature, where the prevalence of bilateral disease is reported to be between 20 and 40%.[10,14,15]

The analysis of the results of disease treatment through scales assessing range of motion and strength only provides researchers with a single-faceted view of the patient's health conditions.[9,16,17] However, the progress of research on QoL outcomes contributes to the perception of patients regarding their health and life by providing a validated subjective score of their symptoms, free from medical bias.[1,3,4,6,14]

The main objective of this study was to assess QoL and FC in adhesive capsulitis patients using two assessment instruments, WHOQOL-BREF and DASH. Gupta et al. studied the impact of adhesive capsulitis in diabetic and elderly patients but used the Oxford Shoulder Score and Short Form-36 (SF-36) to do so. They concluded that adhesive capsulitis worsened the diabetics' quality of life.[18]

The course of this disease is prolonged,[14,19] greatly impairing sleep and the patients' everyday activities and, therefore, their physical, psychological and social QoL as reflected in the various WHOQOL-BREF domains before SSNB treatment. Baums et al. used the SF-36 to examine the QoL of adhesive capsulitis patients before surgical release and found that the physical component was considerably affected.[20] The DASH score confirms major functional disability caused by adhesive capsulitis in the affected shoulder as cited in the literature.[7,9,14]

SSNB is one of the treatment options for adhesive capsulitis.[8,16] It was used alone in the present research, on a weekly basis, with no simultaneous physiotherapy or any other additional method. Mitra et al. used a protocol of SSNB associated with three additional procedures for a synergistic therapeutic effect. Despite improvement in shoulder function, the authors were unable to say which fraction of the protocol was most effective.[21] Shanahan et al. carried out SSNB without the aid of a nerve stimulator or imaging techniques, as in our study, but used 1 mL of 40 mg methylprednisolone, associated with bupivacaine 0.5%.[22] Neither Mitra nor Shanahan assessed QoL as the outcome.

Importantly, after SSNB, mean QoL scores in all of the WHOQOL-BREF domains increased. This means that QoL that was low in all domains but lowest in the physical domain improved significantly after procedure. The domain that improved the least was the social domain, probably because adhesive capsulitis does not cause a

TABLE 3 Results of multiple linear regression analysis of the WHOQOL-BREF domains and DASH after treatment among adhesive capsulitis patients.

Explanatory variables	PD β-Standardized	Ps D β-Standardized	SD β-Standardized	ED β-Standardized	DASH β-Standardized
Constant	43.10	33.85	10.69	24.09	108.01
Pain	0.16	----------	----------	----------	----------
Ethnic group	----------	----------	0.43**	----------	----------
Age group	0.41*	0.38*	0.31	0.33*	-0.32*
Educational status	0.31*	0.47**	0.55**	0.48**	-0.34**
Gender	0.25	----------	----------	----------	----------
Marital status	----------	----------	----------	----------	----------
Monthly income	-0.15	-0.12	----------	0.00	-0.01
Employment	----------	----------	-0.28	----------	-0.25
Side	----------	----------	----------	----------	----------
Dominance	----------	----------	----------	----------	----------
Classification	----------	----------	----------	----------	----------
Sleep	----------	0.10	-0.02	0.16	-0.11
Severity	-0.16	----------	----------	----------	----------
R2	0.40	0.29	0.51	0.31	0.42
Adjusted R2	0.25	0.21	0.41	0.23	0.33
F	2.83	3.89	5.32	4.29	4.47
Significance F	0.01	0.01	0.00	0.00	0.00

PD: physical domain; Ps D: psychological domain; SD: social domain; ED environment domain. *p<0.05; **p<0.01.
Variable coding: pain (severe=0, moderate=5, gentle=10); gender (female=0, male=1); ethnic group (white=1, non-white=2); educational status (illiterate=1, up to four years=2, five to eight years=3, more than eight years of formal school=4); marital status (single=1, married=2, divorced=3, widowed=4); monthly income (1 to 5 minimum salaries=1, more than 5 minimum salaries=2, without salary=3); employment (yes=1, no=2); side (right=1, left=2); dominance (right-handed=1, left-handed=2); classification (primary=1, secondary=2); sleep (affected=0, unaffected=2); severity (mild=1, moderate=2, severe=3); age group (40 |-- 50 =1, 50 |---60 = 2, 60 + = 3).
----------: variable was not included in the model of that respective domain.

major impact on personal relationships with friends and relatives. On the other hand, the mean DASH score fell, indicating a decline in shoulder disability and consequent increase in the functioning of the affected shoulder.

A moderate correlation between DASH and the physical domain of the WHOQOL-BREF before therapy indicates that functional disability of the shoulder in adhesive capsulitis has similar effects on the physical aspect of QoL. However, an analysis of the correlation between the instruments after the blocks revealed a moderate negative correlation between the DASH and the physical and psychological domains, and a weak negative correlation between DASH and the environmental domain. This shows that the less severe the shoulder disability, the better the QoL.

The purpose of DASH is to assess the function of the affected region and the incapacity component of this function. The emphasis is on the physical aspect, with a particular focus on musculoskeletal disorders. Thus, the most expected correlation of DASH was with the physical domain of WHOQOL-BREF, which was in fact the highest (r=-0.580). It may be that the small number of questions assessing patients' social function was insufficient to generate a correlation between DASH and the WHOQOL--BREF social domain.

Pain when adhesive capsulitis patients completed the initial questionnaire was the only independent variable that significantly influenced the physical domain of the WHOQOL-BREF. As 95.3% of cases had moderate to severe pain, this symptom could explain the low score in this domain before treatment (45.34). Our study also revealed that shoulder pain affected the patients' sleep, daily activities and ability to work. These items were part of the list of questions in this domain and the responses given suggested low QoL.

Multiple linear regression analysis after SSNB revealed that better educated patients positively influenced QoL in all domains of the WHOQOL-BREF. They also positively affected functional capacity as indicated by lower DASH scores. Except in the social domain, older patients positively influenced QoL on the WHOQOL-BREF and also influenced better functioning of the affected shoulder as measured by DASH.

The absence in the literature of studies comparing domain means before and after treatment and of analyses of

the influence of the independent variables on the QoL of adhesive capsulitis patients made it impossible to compare the results of our study. Orthopedic diseases greatly impact patients' lives, affecting daily activities and consequently QoL.[23-25] This study is therefore justified and this health indicator, QoL, needs to be evaluated more often, as already seen in other medical specialties.[1,4,26-28]

The simultaneous use of the WHOQOL-BREF and DASH represents an important contribution to the literature because there is a lack of knowledge regarding how these two instruments correlate in relation to this specific disease. Now it is possible to understand the QoL and FC in adhesive capsulitis as measures reported by the patients, before and after treatment. Pre-treatment QoL was low, particularly in the physical domain with impaired function of the shoulder. This explains why patients need medical care for both physical and social rehabilitation. After treatment, improved functional capacity of the affected shoulder led to an increase in QoL scores.[6,23,29] Older and better educated patients weighed on the change in scores.

The strengths of our study lie in its prospective design, the use of assessment instruments for evaluating QoL and FC, well-defined exclusion criteria and the absence of similar research in the literature. Importantly, the clinical diagnosis of adhesive capsulitis was confirmed by imaging studies. Limitations include not having a control group treated with physical therapy or placebo for comparison, sampling that was not probabilistic, and a non-blinded outcome assessment.

In conclusion, SSNB improves the QoL and FC of patients with adhesive capsulitis. The greater functional capacity of the shoulder matches better QoL of patients. Age and education level are the variables that most influence improvement in QoL.

Conflict of interest

The authors declare no conflict of interest.

Resumo

Medidas relatadas pelos pacientes sobre qualidade de vida e capacidade funcional em capsulite adesiva

Objetivo: Avaliar as medidas relatadas pelos pacientes sobre qualidade de vida e capacidade funcional em capsulite adesiva antes e após tratamento com bloqueios do nervo supraescapular; analisar a influência das variáveis clínicas e sociodemográficas em ambos os desfechos e a correlação entre os respectivos instrumentos.

Método: Estudo clínico prospectivo foi realizado em pacientes com capsulite adesiva. Os critérios de inclusão foram diagnóstico clínico de capsulite adesiva e realização de exames de imagem do ombro. Os instrumentos WHOQOL-BREF e DASH foram aplicados antes e após o tratamento. Utilizou-se o escore de Constant com ponto de corte em 55 para a interrupção do tratamento. As médias dos valores foram comparadas pelos testes t-pareado e Wilcoxon. Na análise de correlação foi utilizado o coeficiente de Pearson ou de Spearman. Foi realizada análise de regressão linear múltipla utilizando como preditoras as variáveis com p<0,20 na análise univariada e como desfechos os domínios do WHOQOL e o DASH. O nível de significância foi de 5%.

Resultados: Foram avaliados 43 pacientes. A comparação entre as médias dos valores do WHOQOL-BREF e DASH antes e após os bloqueios apresentou um p<0,05. Houve correlação negativa entre o DASH e os domínios físico, psicológico e ambiental do WHOQOL-BREF. Os pacientes mais velhos e com maior grau de escolaridade influenciaram a melhora da qualidade de vida e capacidade funcional dos pacientes.

Conclusão: A melhora da capsulite adesiva com o tratamento implica melhora da qualidade de vida e capacidade funcional. Quanto maior a capacidade funcional do ombro, melhor a qualidade de vida dos pacientes. A faixa etária e o grau de escolaridade são as variáveis que mais influenciam a melhora da qualidade de vida e capacidade funcional.

Palavras-chave: qualidade de vida, capsulite adesiva, dor de ombro, perfil de impacto da doença, bloqueio nervoso.

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Effects of ozone on the pain and disability in patients with failed back surgery syndrome

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Summary

Introduction: Low back pain is one of the painful disorders of higher prevalence. It has several etiologies and surgery may be indicated in the presence of neurological deficits or compression syndromes. However, in up to 40% of cases, patients develop worsening of pain and failed back surgery syndrome (FBSS), which is an important cause of chronic pain with high morbidity and disability. In the last two decades, ozone has been shown to be a new therapeutic option for FBSS due to its analgesic and anti-inflammatory properties.
Objective: To evaluate the effect of ozone therapy on pain and disability in patients with failed back surgery syndrome.
Method: We selected 19 patients undergoing epiduroscopy and injection of ozone. Patients were evaluated preoperatively and 21 days after the procedure, using the following instruments: Visual Analogue Scale (VAS), Brief Pain Inventory, Roland-Morris Questionnaire Disability, Oswestry Disability Index (ODI), Neuropathic Pain Symptom Inventory and Douleur Neuropathique 4.
Results: The patients showed significant pain relief, but no improvement was observed in the functional scales.
Conclusion: Our results suggest that epidural ozone therapy can be a treatment option in FBSS to reduce the intensity of the pain.

Keywords: low back pain, chronic pain, failed back surgery syndrome, ozone, epiduroscopy, Visual Analogue Scale.

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Introduction

Low back pain (LBP) is one of the most prevalent pain disorders,[1] and a frequent cause of morbidity and disability. It is estimated that about two-thirds of the population experience LBP at some point in their life, which is an important cause of sick leave in working-age adults.[1,2] In the US, the prevalence of chronic LBP can reach 30%, costing the country approximately US$ 90 billion. In Brazil, there are no national statistics. A cross-sectional study conducted in Rio Grande do Sul found a prevalence of 4.2% for chronic LBP.[3]

LBP may be primary or secondary, and either present or not neurological impairment. The origin of the pain can be mechanical-degenerative, traumatic, congenital, neoplastic, inflammatory, infectious or metabolic,[1,4,5] the first being more frequent. Many patients with LBP have musculoskeletal or degenerative changes that do not need specific treatment and are usually self-limiting. In more than 85% of LBP cases, there is no specific cause.[6] Most cases of LBP are acute (also known as lumbago), have a good prognosis and usually resolve within four weeks. Some patients may have persistent symptoms and the improvement period may extend up to 12 weeks (subacute LBP).[1,7] However, a minority of patients develop persistent pain for more than 12 weeks (chronic LBP), and pain and disability may persist relatively constantly.[1,8]

Several factors may contribute to the aggravation and chronification of LBP syndromes, such as smoking, obesity, chronic diseases, educational level, posture habits and other conditions that compromise quality of life.[4,9] Imaging studies of the spine may reveal abnormalities such as disc degeneration, vertebral disc herniation and

joint facet arthropathy, which may not correlate with clinical findings, particularly in the elderly.[4,10]

As an initial therapeutic measure for LBP, rest is indicated in all cases.[11] Symptomatic treatment can be performed with analgesics, non-hormonal anti-inflammatory drugs, corticosteroids, muscle relaxants, benzodiazepines or tricyclic antidepressants, according to the underlying disease, as well as physiotherapy and weight control.[1,4,5] In cases where conservative treatment was not satisfactory, when there are neurological deficits and/or compressive syndromes, surgery should be considered.[12,13] Of the surgical indications associated with LBP, disc herniation is one of the most frequent. Surgery may be performed through an open approach (hemilaminectomy plus flavectomy at the level of the involved spinal root, as well as hernia repair)[2] or using a minimally invasive procedure.[12] According to Martin et al.,[14] the failure rates of surgery for degenerative diseases of the lumbar spine may exceed 40% in the first year. Therefore, there may be poorly indicated surgeries, which contributes to the worsening of pain and favors a progression to failed back surgery syndrome (FBSS).[2]

FBSS is defined as "spinal lower back pain of unknown origin that persists at the same site of the original pain despite surgical interventions, or appears after the procedure."[2] Despite the term "unknown origin," it is believed that the causes of FBSS include disc infection,[15] epidural fibrosis and local arachnoiditis,[16] and even joint facet instability due to reduced intervertebral space and consequently altered vertebral angle. FBSS is more frequent as myofascial pain syndrome (MPS), being diagnosed in 85.7% of these patients.[2] MPS is a regional musculoskeletal pain derived from trigger points with motor dysfunction and autonomic phenomena, which usually affect one or more muscle groups. A neuropathic pattern, alone or associated with MPS, is less frequent.[2]

Among the treatment modalities for cases that progress to FBSS, conservative behaviors, such as medication and rehabilitation, often yield unsatisfactory results.[17] Retrospective studies suggest that surgical revisions tend to have lower rates of improvement than the initial procedure.[18-20] Some authors mention that lysis of epidural adhesions may be a therapeutic option,[2,21] but there is no consensus.[22] Treatment with spinal cord stimulation[23,24] and intrathecal drug delivery[25,26] produce good results, but these are procedures that involve high cost and limited availability. Nevertheless, compared with the costs related to hospital stay and reoperation, these procedures may actually cost less and provide better long-term results.[27]

In the last two decades, the application of ozone has emerged as a potential therapeutic option for patients with FBSS. It is suggested that ozone is useful for treating LBP due to its analgesic and anti-inflammatory properties.[28,29] Although ozone therapy is not validated to treat FBSS, its cost is low and it is a minimally invasive procedure, which opens a new therapeutic horizon for the treatment of FBSS, a condition known to be debilitating and whose treatment is often frustrating for both physicians and patients.

Method

A retrospective analysis was carried out including 19 patients treated with epiduroscopy for adhesiolysis[30] and 20 mL of a ozone-oxygen mixture at the concentration of 30 ug/mL from January 2013 to June 2014, following the protocol published by Magalhães.[28] The patients came from the Pain Center of the Department of Neurology of the Faculty of Medicine of the University of São Paulo and were selected according to the following criteria:

- Inclusion criteria: Age between 18 and 70 years including both sexes; being able to inform the requested data, either in the presence of chronic radiating LBP or not, resulting from herniated disc for more than one year; having undergone surgery and progressed without improvement, presenting symptoms and diagnosis of FBSS.
- Exclusion criteria: Diagnosis of blood dyscrasia, hemophilia, hemolytic anemia, oncological diseases, acute or chronic infections, pregnancy, psychiatric conditions, diabetic neuropathy; diagnosis of favism and hyperthyroidism, which are contraindications to ozone therapy; presence of lumbosacral abnormality that could make the procedure unfeasible; other abnormalities in the spine such as segmental instability, canal stenosis, spondylolisthesis or scoliosis > 20°.

Pain and disability assessments were performed based on specific scales and validated in Portuguese. Visual Analogue Scale (VAS) and Neuropathic Pain Symptom Inventory (NPSI) were used to assess pain. The Oswestry Disability Index (ODI) and the Roland Morris Disability Questionnaire were used for disability. The Brief Pain Inventory (BPI) assesses both pain intensity and disability caused by it, and therefore was also included. The Neuropathic Pain Diagnostic Questionnaire (Douleur Neuropathique 4 – DN4) was used to diagnose neuropathic pain. The selected patients were interviewed and examined until a week before and 21 days after epiduroscopy with ozone therapy, and the scales were applied on both occasions.

Results

The patients' ages ranged from 24 to 66 years (mean 46.1), 63.16% were male and 36.84% were female. Just under a third had attended higher education. Half of the patients were on leave from work because of pain, unemployment or retirement. Smoking was reported by 21.1% and alcohol abuse by 10.5% of the patients. Associated diseases included type 2 diabetes mellitus, dyslipidemia, fibromyalgia and bipolar disorder. Other diseases such as tuberculosis, Hansen's disease, Chagas disease and neoplasms were not observed in the sample. The main diagnoses associated with pain were radiculopathy (57.89%), followed by low back and leg pain (36.84) and low back pain (5.26%). In only three cases the pain was related to a history of trauma to the lumbar spine. The mean time to onset of pain was 12.5 years. More than one surgery was indicated for the treatment of pain in 57.9%, and in 63.16% of the cases lumbar spine arthrodesis was performed, without significant pain improvement (Table 1).

TABLE 1 Clinical characteristics of patients.

Characteristic		n (%)
Mean age (SD)*	46.1 ± 10.7	
Sex	Male	12 (63.16)
	Female	7 (36.84)
Education	Primary and middle school	10 (52.63)
	High school	4 (21.05)
	Higher studies	5 (26.32)
Occupation	Homemaker	1 (5.26)
	Active	5 (26.32)
	On sick leave**	5 (26.32)
	Retired	3 (15.79)
	Unemployed	5 (26.32)
Comorbidities	Diabetes	3 (15.79)
	Dyslipidemia	1 (5.26)
	Fibromyalgia	2 (10.53)
	Bipolar affective disorder	1 (5.26)
Habits	Alcohol abuse	2 (10.53)
	Smoking	4 (21.05)
Trauma-related pain	Yes	2 (10.53)
	No	16 (84.21)
Site of pain	L5	14 (73.68)
	L5 + S1	4 (21.05)
	S1	1 (5.26)
Duration of pain	12.5 ± 6.5	
Number of surgeries	1	8 (42.11)
	2	6 (31.58)
	≥ 3	5 (26.32)
Arthrodesis	Yes	12 (63.16)
	No	7 (36.84)
Additional treatments	Physiotherapy	19 (100)
	Acupuncture	17 (89.47)
	Trigger point deactivation	1 (5.26)
	Psychotherapy	8 (42.11)
	Previous epiduroscopy	4 (21.05)

*Mean age plus standard deviation (SD); **Patient on sick leave through the social security system.

Of the 19 patients, 18 scored higher than or equal to 4 on the DN4 questionnaire, being diagnosed with neuropathic pain. However, during clinical evaluation, we observed that 17 patients had a mixed pain pattern, 12 with predominantly neuropathic pain (PNP) and seven with predominantly non-neuropathic pain (PNNP).

There was a mean reduction in the VAS scale from 8.47 to 7.05, which was statistically significant (Figure 1A). There was a significant reduction in NPSI from 62.74 to 54.21 points (p=0.034). In the ODI assessment, no significant improvement in disability was observed after the procedure (p=0.217). A similar result was observed with the Roland-Morris questionnaire, which obtained a mean decrease from 14.47 to 13.84, although not statistically significant (p=0.438). Functional impairment was also evident in the BPI of the patients, since in the field that assessed interference in daily activities there was a nonsignificant reduction from 7.63 to 6.71. However, similarly to VAS and NPSI, BPI showed a statistically significant reduction in pain intensity from 7.68 to 6.58 (Table 2).

Comparing the results obtained in the PNP and PNNP groups, no significant difference (p>0.05) was observed in the postoperative reevaluation scores, both for VAS (Figure 1B) and for the other scales.

Discussion

The first records of the use of ozone for medicinal purposes date back to the early twentieth century during the First World War. Its germicidal and analgesic effect was observed in the treatment of post-traumatic gas gangrene in German soldiers.[31]

Currently, ozone used for therapeutic purposes is a mixture of oxygen and ozone at 5%. The gas has a cell oxidation and diffusion capacity ten times higher than that of oxygen. These characteristics suggest that, when ozone comes into contact with biologically active tissue, it reacts with numerous organic molecules forming sev-

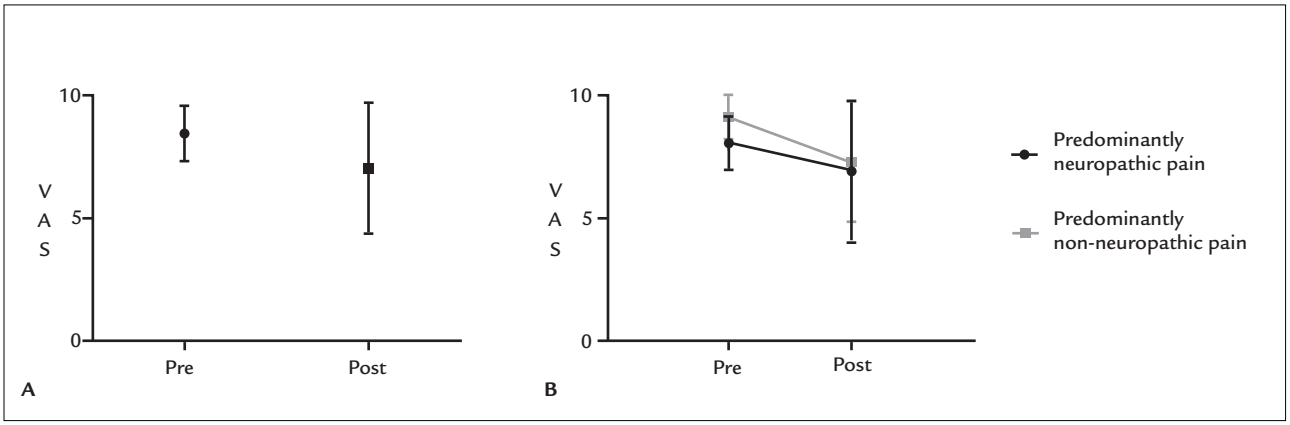

FIGURE 1 A. Statistical analysis of VAS after epiduroscopy with ozone injection, evidencing a significant reduction in scores (Student's paired t-test with p<0.05). B. Comparison of VAS in patients with PNP and PNNP pain (Repeated Measures ANOVA with p>0.05).

TABLE 2 Mean of results obtained from the scales applied before and 21 days after the procedure (n=19).

Scales		Pre-surgery*	21 days*	p**
Visual Analogue Scale (VAS)		8.47 (± 1.12)	7.05 (± 2.68)	0.029
Neuropathic Pain Symptom Inventory (NPSI)		62.74 (± 20.99)	54.21 (± 28.50)	0.034
Oswestry Disability Index (ODI)		37.58 (± 5.58)	35.84 (± 7.62)	0.217
Roland-Morris Disability Questionnaire		14.47 (± 5.08)	13.84 (± 6.32)	0.438
Brief Pain Inventory (BPI)	Intensity	7.86 (± 1.11)	6.58 (± 2.15)	0.005
	Interference	7.63 (± 1.54)	6.71 (± 2.45)	0.054

*Scale values expressed as mean and standard deviation; **Student's paired t-test.

eral enzymatic and non-enzymatic buffering systems, with anti-inflammatory and analgesic action.[31-33]

Ozone's mechanism of action in the treatment of FBSS is little known. Current hypotheses suggest that its effect could occur through chemical adhesiolysis of scar fibrosis associated with pain and dehydration of the herniated disc contents,[31] as well as the activation of cytokines that would inhibit proinflammatory factors associated with chronic pain.[32] It is believed that ozone may cause local vasodilation, favoring the neutralization of acidosis and the induction of antioxidant enzymes associated with analgesia, as well as stimulating the activation of the descending antinociceptive system, blocking the transmission of pain to the thalamus and cortex.[33]

Interest in the effects of ozone therapy to treat FBSS has been growing in the last two decades. Intradiscal and paravertebral ozone injection associated with a steroid and local anesthetics have presented significant results. Gallucci et al.[34] compared the effect of adding ozone to a mixture of steroid and anesthetic in 159 patients. They reported that the ozone-treated group showed more improvement than the patients who did not receive it within six months of follow-up. In addition to the role of ozone in FBSS, Bonetti et al.[35] suggest that its analgesic action is also reproducible in chronic LBP and lumbago with sciatica.

When evaluating the intraforaminal effect of ozone versus steroidal infiltration in 306 patients, the authors observed that those treated with the oxygen-ozone mixture showed more improvement than the group treated with corticosteroids alone. In a meta-analysis by Magalhães et al.,[29] the level of evidence attributed to long-term pain relief was II-3 in the case of intradiscal ozone and II-1 for paravertebral ozone. Although the evidence corroborates the efficacy of intradiscal and paravertebral ozone injection, there is still little data on epiduroscopy-assisted use. In a pilot study with 13 patients published by Magalhães et al.,[28] the effect of the ozone-oxygen mixture versus oxygen in the epidural space was compared after adhesiolysis. Patients with PNNP presented a significant reduction in pain and disability according to ODI, whereas in the PNP group this reduction was not significant, which

shows that ozone therapy may present better results to decrease pain and disability in patients with PNNP.

In addition to pain, FBSS causes great incapacity, insecurity and feelings of disability, conditions that can compromise the professional and family life of patients, causing great damage to their overall function and quality of life. In a cross-sectional study with 177 patients with chronic LBP, Salvetti et al.[36] found the prevalence of disability at 68%.

All patients evaluated in our study had a high degree of disability due to FBSS. During the evaluation 21 days after epiduroscopy, a significant decrease in pain intensity on the VAS and BPI scales (intensity field) was observed in 19.34% and 16.28% of patients, respectively. The reduction in intensity was consistent with the reduction in paroxysm and duration of pain found on the NPSI scale. However, this result was not seen in the functional evaluation using the ODI and Roland-Morris scales, which presented a non-significant decrease in the scores (Table 2) that evaluated daily activities such as personal care, sleep, social and sexual function, and mobility. This result may be related to the homogeneity of the sample, which includes only patients diagnosed with FBSS who presented moderate to high degree of disability, while other studies evaluated mixed populations, with other causes of chronic LBP. As the occurrence of litigation was not investigated in these patients, it is not possible to ascertain the effect of such variable in this population. Epiduroscopy-assisted ozone therapy is considered a low risk procedure with few complications, including accidental dural injury and epidural bleeding. Doses of ozone above the therapeutic range of 10 to 40 ug/mL may cause vagal vessel reflex, syncope and gas embolism.[28] No complications were reported in this series.

Conclusion

FBSS-related pain is a debilitating condition that is difficult to treat. Ozone therapy has emerged with a therapeutic option in the management of pain and disability in these patients. The results of our study confirm its usefulness and show a decrease in pain intensity in patients with both PNP and PNNP. However, double-blind and controlled studies are needed to understand the long-term effects of ozone and to determine the effectiveness of the procedure.

Resumo

Efeitos do ozônio na dor e na incapacidade em pacientes com síndrome dolorosa pós-laminectomia

Introdução: A dor lombar é um dos distúrbios dolorosos de maior prevalência. Tem diversas etiologias e, na presença de déficits neurológicos ou síndromes compressivas, pode ser indicada cirurgia. Entretanto, em até 40% dos casos os pacientes podem evoluir com piora da dor e síndrome dolorosa pós-laminectomia (SDPL), que se constitui em uma importante causa de dor crônica com grande morbidade e incapacidade. Nas últimas duas décadas, o ozônio tem se mostrado uma nova opção terapêutica para a SDPL em virtude das suas propriedades analgésicas e anti-inflamatórias.

Objetivo: Avaliar o efeito da ozonioterapia na dor e na incapacidade de pacientes com SDPL.

Método: Foram selecionados 19 pacientes, submetidos a epiduroscopia e aplicação de ozônio. Os pacientes foram avaliados no pré-operatório e 21 dias após o procedimento, por meio de Escala Visual Analógica, Inventário Breve de Dor, Questionário Roland-Morris de Incapacidade, Oswestry Disability Scale, Inventário de Sintomas de Dor Neuropática e Questionário de Dor Neuropática.

Resultados: Os pacientes apresentaram redução significante nos escores das escalas de avaliação de dor; porém, essa redução não foi observada na avaliação da incapacidade funcional.

Conclusão: Os dados obtidos sugerem que a ozonioterapia epidural pode ser uma opção de manejo da SDPL na diminuição da intensidade da dor.

Palavras-chave: lombalgia, dor crônica, síndrome pós-laminectomia, ozônio, epiduroscopia, Escala Visual Analógica.

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Burnout syndrome prevalence in physiotherapists

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Summary

Objective: To evaluate burnout syndrome in its three aspects, jointly as well as independently, in physiotherapists from the Extremadura region (Spain).
Method: Analytic descriptive epidemiological transversal trial in primary care and institutional practice, with physiotherapists practicing in Extremadura who met the inclusion criteria, after having signed an informed consent form. Emotional exhaustion, depersonalization and low professional accomplishment were the outcomes measured.
Results: Physiotherapists from Extremadura show a 65.23 point level of burnout syndrome, according to the Maslach Burnout Inventory questionnaire. Therefore, they are positioned in the middle of the rating scale for the syndrome, and very near to the high level at starting score of 66 points.
Conclusion: Physiotherapists in Extremadura present moderate scores for the three dimensions of burnout syndrome, namely, emotional exhaustion, depersonalization and low professional accomplishment. For this reason, they are in the moderate level of the syndrome and very near to the high level, which starts at a score of 66 points. No relation between burnout syndrome and age has been found in our study.

Keywords: burnout, physiotherapy, physical therapy.

Introduction

Healthcare personnel represents one of the working groups among which the majority of burnout syndrome (BOS) studies have been carried out. Along with educators, this group is probably the most affected by BOS.[1] The majority of health professionals get involved for many hours with problems and worries of the patients they treat. Changes in the health sector generate increased competitiveness, leading to difficult situations that health professionals have to adapt to continuously. All of the situations above can disrupt the physical and psychological integrity of these professionals.[2]

Different studies demonstrate the occurrence of BOS in several professions. Many of these studies are focused in the health sector. However, few of them show its prevalence among physiotherapists, despite this being one of the most vulnerable groups. Wolfe[3] was the first to study BOS in physiotherapists, and he concluded that these professionals weren't exempt from suffering from this pathology.

The interest of this project is assessing the prevalence of BOS among physiotherapists who work in the Extremadura region (Spain). Physiotherapists who work in The Regional Health Service (public health), as well as those who work in the private sector were included.

The aim of our study was to assess BOS in its three dimensions: emotional exhaustion (EE), depersonalization (Dp) and low professional accomplishment (LPA) in physiotherapists working in Extremadura, as a group and independently. This will make it possible for us to know the syndrome's prevalence as well as its relations to sociodemographic and working variables, and the groups of affected physiotherapists.

Method

Participants

We have designed a descriptive and analytic transversal epidemiologic study. It has been carried out in the Auto-

nomic Community of Extremadura. Participants were physiotherapists working in the region.

Inclusion criteria were: to be in active employment since the January 1, 2010 and to have been working at least one year, in a public or private practice. Exclusion criteria were: to be in sick leave at the moment of data collection or to not meet the inclusion criteria.

Procedures

To take part in the study, each participant was sent an envelope by regular mail, including: two questionnaires, one being the Maslach Burnout Inventory[4] (MBI) and the other a sociodemographic and working variables questionnaire (SDLVQ); an informed consent with a cover letter; and the instructions. A pre-paid postage envelope was also sent for document return.

Instruments

SDLVQ: It consists of a general-purpose questionnaire specifically made for the study performance. It collects sociodemographic and working characteristics of the subjects. MBI:[4] It is a questionnaire where the subjected is asked a series of questions on feelings and thoughts related to his or her work interactions. It consists of 22 items that are assessed by a Likert-type scale. The subjects are assessed through a range of six adverbs of frequency, from "never" to "daily" for each of the described by the items.

The three subscales of the MBI[4] are constituted by three factors: EE, Dp and LPA at work.

Since our study assesses BOS in physiotherapists, the scale used will be the MBI-HSS, addressed to healthcare professionals, which is considered the classical version of the MBI,[4] because it was the first to be elaborated. This scale consists of three subscales that measure the frequency in which professionals perceive EE. The professional feels he cannot give more of himself during his working time, in the emotional dimension or in the affective one. Moreover, they experience feelings of loss of hope, defencelessness, physical and psychological weakness, Dp, as well as a negative attitude towards patients, a dehumanized vision of the patient, a feeling of LPA, negative self-evaluation in respect to his work performance, a feeling that the work is not worth the trouble and since nothing can be changed at work it is not worth trying anything new. This dimension, LPA, constituted as a reversed way to the other two dimensions (EE and Dp), has been criticized by some authors, because it can be a cause of disagreement among studies. Recently, working with items that assess inefficiency at work has been proposed as an alternative to reverse the non-efficacy items of the MBI. Moreover, some studies[5] have defended that while EE and Dp are clearly interrelated, LPA seems to be an independent and parallel variable, more related with the context "self-efficacy," which is a modulator of BOS. However, in our study we have used the dimension LPA.

BOS is classified in its three dimensions as: low, moderate and high level, according to the score achieved in each subscale.

Statistical analysis

Data was analyzed using SPSS 19.0 software. A value of $p<0.05$ was adopted for statistical significance. One hundred and fifteen (115) professionals were studied in all. Significant correlation between joint numeric response (EE, Dp, LPA and total MBI) and the 18 categorical variables were searched by carrying out MANOVA test. The categorical variables that did not provide significant result in MANOVA weren't considered in the rest of the study. For the remaining categorical variables and for every component of MBI, ANOVA with Bonferroni method and Student's t-test were applied in order to understand the meaning of those correlations. Correlations between categorical variables as well as correlations between the components of MBI were also considered.

Results

The sample included 584 physiotherapists at first, and 22.43% of them replied a total of 131 questionnaires. After having applied inclusion and exclusion criteria, the sample remained in 116 subjects.

After carrying out the MANOVA test for every categorical variable, the only ones that provided significant results (we understand this as proof of correlation between these categorical variables and MBI) were: number of working days per week, type of working day, number of hours of direct attention to patients and family, number of patients and type of practice. The results are summarized in Table 1. We emphasized averages that turned out to be significantly higher according to ANOVA or Student's t-test.

LPA is clearly higher in the case of split shift working day as well as in private practice. Nevertheless, it can be understood as a redundancy since both categories are strongly associated, as we can see in Figure 1.

We can also note that more than 40 hours of direct attention is linked to higher scores in EE, and that more than 20 patients treated per day is associated with higher scores both in EE and Dp. Curiously, none of these five categorical variables, but number of working hours per week, which is also correlated with type of practice (Figure 2), provided any significant result for joint MBI. This fact can

TABLE 1 Components of burnout syndrome for the main categorical variables; mean (± standard deviation).

		N	EE	Dp	LPA	MBI
Total		115	20.02 (11.33)	7.45 (5.42)	37.77 (7.39)	65.23 (14.37)
Number of working hours per week	< 38.5	52	18.08 (10.62)	7.42 (5.5)	36.46 (7.86)	61.96 (14.26)
	> 38.5	63	21.71 (11.78)	7.35 (5.33)	38.89 (6.91)	67.95 (14.11)
Type of working day	Consecutive	43	19.23 (10.69)	8.09 (8.81)	35.19 (7.97)	62.51 (14.21)
	Split shifts	73	20.48 (11.74)	7.07 (5.19)	39.29 (6.63)	66.84 (14.31)
Number of hours of direct attention to patients and family	< 10	8	12.63 (8.65)	4.50 (3.66)	43.63 (5.53)	60.75 (11.76)
	11-20	12	14.50 (10.81)	4.75 (3.86)	40.08 (8.24)	59.33 (13.42)
	21-40	65	20.05 (10.39)	7.68 (5.38)	36.72 (7.19)	64.45 (14.04)
	> 40	22	25.05 (13.08)	8.09 (6.17)	39.73 (7.18)	72.86 (14.36)
Number of patients treated per day	< 20	55	16.93 (10.23)	6.36 (4.86)	38.84 (7.15)	62.13 (13.49)
	> 20	54	22.46 (11.39)	8.87 (5.79)	37.00 (7.79)	68.33 (14.72)
Type of practice	Public	24	19.17 (9.57)	8.12 (5.31)	34.42 (6.98)	61.71 (12.44)
	Private	78	19.68 (11.54)	6.85 (5.21)	39.27 (6.70)	65.79 (14.87)
	Both	13	24.31 (13.09)	10.15 (6.44)	35.46 (9.78)	69.92 (13.35)

EE: emotional exhaustion; Dp: depersonalization; LPA: low professional accomplishment; MBI: Maslach Burnout Inventory.

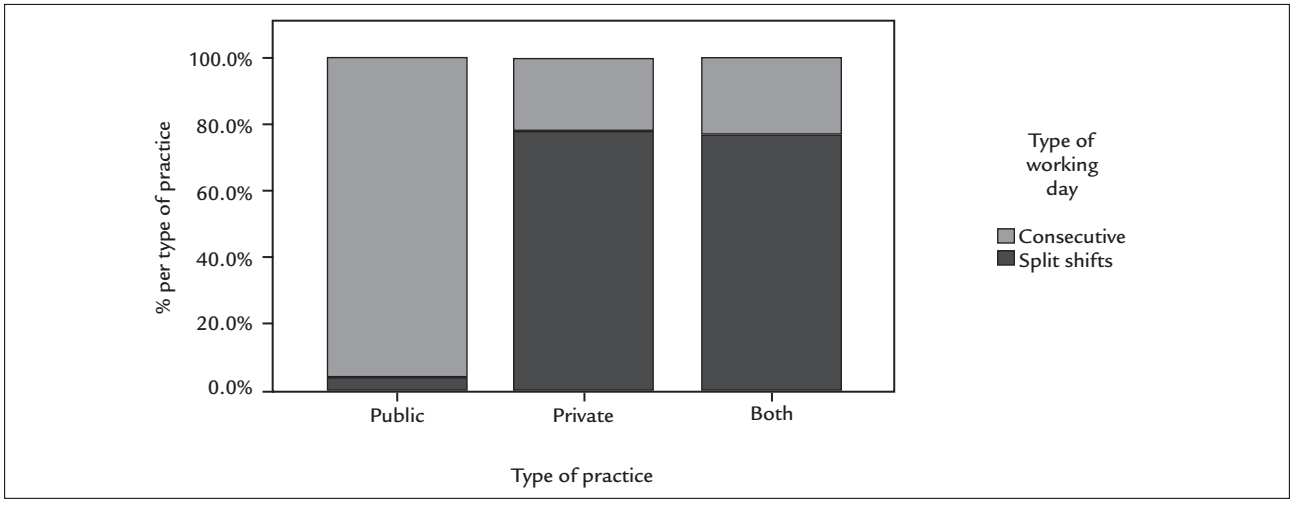

FIGURE 1 Type of working day per type of practice.

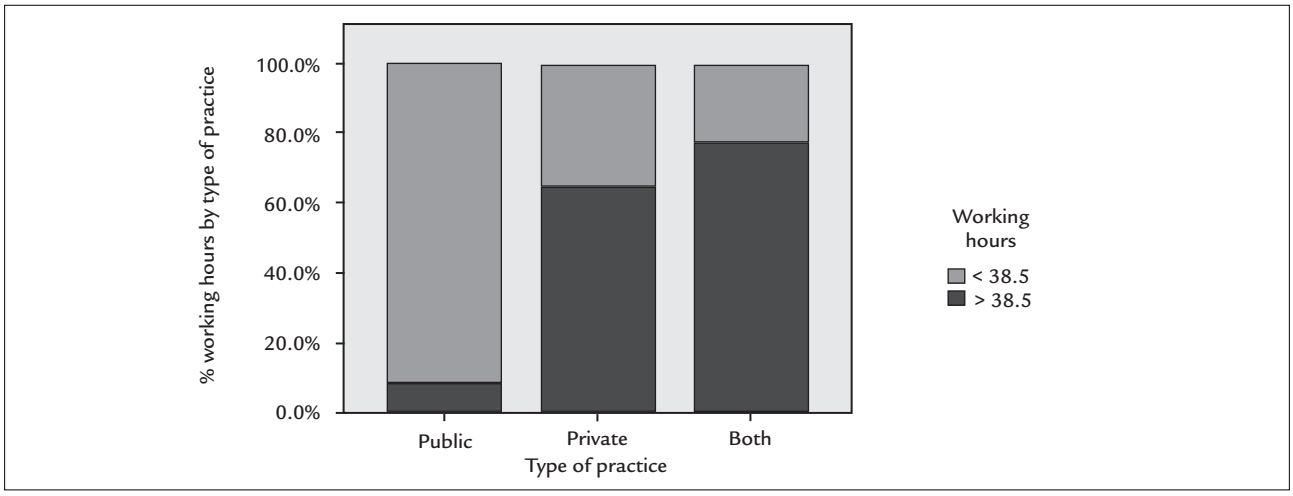

FIGURE 2 Working hours per week by type of practice.

be statistically explained by LPA, providing significant inverse correlation with EE and Dp (r=-0.27 and r=-0.33, respectively), so that the different scores in Burnout I compensated most of the other variables.

Discussion

Some of the issues that have had an important influence in the results of our research are:

- Physiotherapist collaboration in our study was very low. Perhaps the low level of collaboration could be due to the questionnaires being sent by mail instead of an interview. The reviewed literature reflects lower rates of reply when the questionnaires are sent by mail. However, we thought that sending questionnaires through mail would be the best procedure in order to respect anonymous participation, as well as to reach to the higher possible number of professionals. This is because the Extremadura region comprises a very large territory.
- We avoided all bias selection risk. Questionnaires were sent to all physiotherapists from Extremadura and therefore they were all included in the study. No previous selection of professionals was done.
- In addition, a third party carried out the statistical analysis to avoid information bias.
- We must mention that in the reviewed literature, trials about BOS are very frequent. There are several trials focusing on healthcare personal, but very few focus on physiotherapists, therefore making data comparison with existing studies difficult.

Physiotherapists included in our study had a moderate level of BOS in its three dimensions: EE, Dp and LPA. This tendency is in accordance with our literature references. Comparing our results with those of similar studies, we can see that in a trial done with physiotherapists in the Murcia region[6] only 4% of participants showed high level of BOS, which is in correlation with other similar foreign studies.[7] In a study made in Mexico[8] about BOS in physicians and nurses, 36% of the sample showed moderate-high levels of BOS. If we compare our results with those obtained from other health professionals, we can see that physiotherapists show BOS levels similar to physicians and nurses. In a study[9] performed in the Madrid region, BOS levels and their three dimensions showed a moderate level in 50% of the participating nurses.

Regarding controlled variables and BOS components, we have observed no significant relation between BOS and the participants' age, which is in accordance with the reviewed literature. So, the age of physiotherapists does not seem to have any influence in the syndrome. However, there is an adjustment period, at the beginning of the physiotherapist's professional development, where they are especially vulnerable to the development of BOS.

Marital status influences the final stages of BOS, as well as the levels of its three dimensions. With the data shown above, we reaffirm the theory that BOS is associated with people who do not have a stable partner and, although there is no consensus, it seems that the familiar environment cushions the effects of work stress and compensates emotional over implication in work, as affirmed by Dale and Weinberg.[10]

After having analyzed the bibliography, we can see that neither the number of working hours per week nor the type of shifts in a working day can negatively affect the levels of BOS and its three dimensions. In a study carried out by Bernaldo de Quirós and Francisco and Labrador,[11] the authors show a strong correlation between work time (spent in the exercise of profession) and BOS. The study of Serrano Gisbert et al.[6] shows that almost 75% of physiotherapists who suffer from BOS work more than seven hours per day. The study by Gran, Suñer and García[12] shows highest levels of Dp in professionals who work night shifts. Data presented in our study affirm these theories, because physiotherapists who work split shifts and more than 38.5 hours per week are those who present the highest levels of BOS. In relation to the three dimensions, for working day, moderate levels are present in both groups, except for Dp, a disturbance that is highest in those professionals who work less than 38.5 hours per week; however, they are in the moderate level range. LPA is low in physiotherapists who work split shifts. The number of hours of direct attention to patients and family represents a factor that influences negatively the levels of BOS and its three dimensions, because professionals who dedicate more than 40 hours per week to this issue are those who present the highest level of EE and Dp. Both parameters show moderate levels. LPA is lower in professionals who dedicate less than 10 hours per week to direct attention to patients and family, representing moderate levels. Burnout syndrome reaches its highest levels in those who dedicate more than 40 hours per week of direct attention to patients or family. We can establish that this group is highly affected by BOS. A study carried out in a sample of physiotherapists from a health service in Andalucía[13] did not find any significant statistical relation between BOS and the number of hours dedicated to direct patient attention.

With respect to the number of patients attended to daily and BOS, our results are very similar to those mentioned in the previous paragraph. Professionals who attend

to more than 20 patients per day have the highest levels of EE, Dp and BOS. Both EE and Dp dimensions present moderate levels and BOS presents high levels. LPA is lower in those who attend to less than 20 patients per day, showing moderate levels. This is in accordance with reviewed authors such as Atance Martínez[14] and another study carried out in The Madrid Regional Rehabilitation Service.[15] In the latter, BOS is associated to excess of work. These results differ from those obtained by Castro Sánchez et al.[13] in a sample of 46 physiotherapists from a health service in the Andalucía region where a significant relation between BOS and the number of daily treated patients was noted.

Finally, in our study, EE is high in physiotherapists who work in private practice. Dp is moderate in those who have both, private and public practice. LPA is low in those who work in private practice. Burnout syndrome levels are high in those who work both (private and public). There are very few studies in this field. If we compare our results with those obtained by other authors, such as Schuster et al.[7] or Serrano Gisbert et al.,[6] our results would not be in accordance with them. The relation between the number of working hours per week and the kind of work day shows that more than half of the physiotherapists who work split shifts work more than 38.5 hours per week. Conversely, most of those who work on consecutive days carry out less than 38.5 hours per week.

We have not found any studies regarding work day in respect to public or private practice performance. However, with our results, we can affirm that more than half of the physiotherapists who have participated in the study work in private practice and, since physiotherapy is a healthcare profession, it is in the private sector that assistance time is higher and better adapted to the type of patients.

Both who work more than 38.5 hours a week and who work less than 38.5 hours a week do not present differences in the number of hours of direct care to patients or their relatives. In both groups, more than half of the participants dedicate between 21 to 40 hours per week to it. Regarding these data, we can establish that more than 50% of a physiotherapist's working day is dedicated to direct attention, thus taking time that could be dedicated to other important activities such as study, research and training. These results are in accordance with the research done by Schuster et al.,[7] who affirm that factors such as excess of activity represent negative prediction variables towards work, thus making health professionals very frequently a risk group for this kind of disturbance development.

We must point out that more than half of the physiotherapists who work in private practice do it for over 38.5 hours per week; and more than half of those who work in public practice do it for less than 38.5 hours per week. This is so because, in Spain, the public sector's working week never exceeds 35 hours.

Conclusion

Physiotherapists from the Extremadura region have a BOS level of 65.23 points, according to the MBI questionnaire.

A high burden of care, whether considering the number of patients or the number of hours of direct care), raises the scores of EE and Dp. The total number of hours is reflected in the total. Typically, in the private sector, starting day is associated with a high score in LPA.

No relation between BOS and age has been found in our study.

Conflict of interest

The authors declare no conflict of interest.

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Knowledge and attitudes towards dementia among final-year medical students in Brazil

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Summary

Objective: Among all countries, Brazil is expected to have the sixth largest elderly population in 2025. Dementia syndromes are prominent among aging-related diseases. Despite the necessity of and curriculum for training in geriatric medicine to make recommendations on an approach to this theme, adequate training appears to be infrequent. The present study aimed to evaluate the knowledge about dementia and students' attitude towards it during the last semester of the medical course in two of the most important Brazilian medical schools.
Method: In our study, a sample of 189 students was invited to complete questionnaires comprising demographic and professional topics, knowledge with respect to cognitive alterations in the elderly and attitudes in dealing with an elderly patient with dementia.
Results: A total of 155 students accepted to participate in the study; 92(59.7%) considered that they had good training in cognitive alterations during their undergraduate medical course, while 67 (58.8%) of them declared having had only theoretical training. Regarding knowledge, the students obtained a mean of 6.9, out of a scale from 0 to 14 points. As for attitudes, the students agreed that they can contribute to the life quality of the patient and of the caregiver, and that it is useful to provide the diagnosis to the family.
Conclusion: The findings of this study are relevant for overturn the educational barriers of physicians in relation to the care of patients with dementia.

Keywords: aged, dementia, health knowledge, attitudes and practice in health, medical students.

Study conducted at Faculdade de Medicina de Botucatu da Universidade Estadual Paulista (FMB-Unesp), Botucatu, and Escola Paulista de Medicina da Universidade Federal de São Paulo (EPM-Unifesp), São Paulo, SP, Brazil

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Introduction

Aging of the population is a worldwide phenomenon.[1] Among countries in 2025, Brazil is expected to have the sixth highest number of the elderly.[2] Dementia syndromes are prominent among aging-related diseases, and dementia from Alzheimer's disease (AD) is the most prevalent. In the USA, in 2015, about 5.3 million persons were diagnosed with AD.[3] In Brazil, projections indicate that the prevalence of dementia will rise, reaching 7.9% of the elderly aged 65 years or older by 2020.[2]

Early diagnosis of dementia allows the patient to have access to several treatment options, as well as appropriate multidisciplinary care, facilitating the planning of future care.[3] However, studies show that patients with dementia are not diagnosed, especially by a general practitioner.[4-6] In Brazil, a single published study showed that cognitive decline of the elderly is infrequently detected by general practitioners.[4] Many physicians who have recently graduated from medical school opt for working in the Brazilian Public Health System, which is responsible for attending to about 75% of the population.[7] Despite the necessity of and curriculum for training in geriatric medicine to make recommendations on an approach to this theme, adequate training appears to occur infrequently.[8]

In Brazil, the National Policy for the Elderly has among its main guidelines the promotion of healthy aging including assistance to obtain specific necessities for the health of the elderly together with training of specialized human resources. Despite this law, there is a notorious lack of professionals with specialized training for attending the demands of this population.[9]

The Brazilian government has invested in the reorganization of basic medical attention, trying to make academic products (professionals, knowledge and services) adequate for social needs.[10]

In this context, the present study aimed to evaluate the knowledge about dementia and students' attitude towards it during the last semester of the medical course in two of the most important Brazilian medical schools.

Method

The project was approved by the Committee for Research Ethics of the two institutions involved.

A sample of 189 students included 74 students from Faculdade de Medicina de Botucatu, São Paulo State University (FMB-Unesp), and 115 from Escola Paulista de Medicina, Federal University of São Paulo (EPM-Unifesp). Five students from FMB-Unesp were lost (who did not sign the Free and Informed Consent Term), as well as 29 students from EPM-Unifesp (ten with whom contact could not be made and 19 who alleged lack of time for data collection). The final sample included 155 (89%) students, 69 from FMB-Unesp (93% of FMB population) and 86 from EPM-Unifesp (75% of EPM population).

The inclusion criterion was to be properly enrolled in the undergraduate medical course of one of the two medical schools; there were no criteria for exclusion.

The participants were invited to answer three questionnaires: 1) Demographic and professional questionnaire developed by the researchers for this specific study, which describes the profile of medical training and their prior training to detect cognitive alterations; 2) Questionnaire of the knowledge of the physician with respect to cognitive alterations in the elderly; and 3) Questionnaire on attitudes in dealing with an elderly patient with dementia.[8,11] Questionnaires 2 and 3 were culturally adapted to Brazil and published elsewhere.[11]

The responses to questionnaire 1 were displayed as absolute and relative numbers. The frequencies of correct responses regarding general knowledge (questions 1 to 14), epidemiological knowledge (questions 1 to 5), diagnostic knowledge (questions 6 to 11) and management knowledge (questions 12 to 14) were obtained from questionnaire 2. Questionnaire 3 provided frequencies of responses for each question.

Results

The total of students evaluated was 155. Their mean age was 25.20 at FMB-Unesp (SD = 1.8) and 25 years at EPM-Unifesp (SD = 1.8).

According to Table 1, 92 (59.74%) considered that they had good training in cognitive alterations during their undergraduate medical course, while 67 (58.8%) of them declared having had only theoretical training. As to extracurricular courses, 142 (93.42%) reported taking them during their undergraduate course.

According to Table 2, questions 3, 4, 5, 7, 8, 11 and 12 yielded a higher percentage of correct answers. The students obtained a mean of 6.9 points in the general knowledge section of the questionnaire, based on a total scoring scale from 0 to 14 points.

According to Table 3, the students agreed that they can contribute to the quality of life of both patient and caregiver, and that it is useful to provide the diagnosis to the family.

Discussion

The present study is pioneering in the verification of the knowledge about dementia and Brazilian medical students' attitude towards it. We observed that most students recalled having had good fundamental knowledge in cognitive alterations during their undergraduate medical course, which would have been essentially theoretical, while almost all reported taking extracurricular courses on the subject during their undergraduate course.

We did not find any research studies in the literature that dealt with the study of knowledge and attitudes of students in the final year of medical school regarding patients with dementia. A single Brazilian study has investigated knowledge and attitudes towards dementia by medical residents.[12]

The results of our study regarding the profile of training received by the students during the medical course contrast with other studies in the area, given that 92 (59.74%) of the students analyzed reported having received good training in cognitive alterations, whereas in the other studies, there was variation from 29 to 47.6%.[8,13,14]

The medical students analyzed achieved a mean of 6.9 in the general score of the knowledge questionnaire (on a scale ranging from 0 to 14 points), with the highest percentage of correct answers being in questions on epidemiology. A study[15] from 2010 that compared general practitioners who graduated in 1990 and those recently trained revealed that, in general, the score of correct answers was low (between zero and two out of 10 questions), observing that the gap in graduation time did not determine significant differences in relation to knowledge in dementia. Similarly, an-

TABLE 1 Aspects of the medical students' training in dementia during their undergraduate program.

Item	Students from FMB-Unesp	Students from EPM-Unifesp	Total
Sex			
Male	27 (17.42%)	51 (32.90%)	78 (50.32%)
Female	42 (27.10%)	35 (22.58%)	77 (49.68%)
Did you have good training in cognitive alterations?			
Yes	41 (26.62%)	51 (33.12%)	92 (59.74%)
No + do not remember	27 (17.53%)	35 (22.73%)	62 (40.26%)
Did not respond		1	
If yes, the training in cognitive alterations was:			
Only theoretical	27 (23.68%)	40 (35.09%)	67 (58.77%)
Theoretical and practical	25 (21.93%)	22 (19.30%)	47 (41.23%)
Did not respond	17	24	
Have you taken any extracurricular courses on the subject?			
Yes	62 (40.79%)	80 (52.63%)	142 (93.42%)
No	5 (3.29%)	5 (3.9%)	10 (6.58%)
Did not respond	2		

TABLE 2 Correct answers in the knowledge questionnaire.

	Question	Correct answer n (%)
Epidemiology	1. General clinic with a list of 1,000 persons aged 60 years or older must wait to have the following approximate number of persons with dementia on this list:	44 (28.39%)
	2. Starting from 65 years of age, the prevalence of dementia is:	43 (27.74%)
	3. One of the risk factors for developing Alzheimer's disease is:	125 (80.65%)
Diagnosis	4. All of the following are potentially treatable etiologies of dementia, except:	119 (76.77%)
	5. Patients with suspected dementia must be evaluated as soon as possible, since:	96 (61.94%)
	6. Which of the following procedures is necessary to confirm definitively that the symptoms are caused by dementia?	20 (12.90%)
	7. Which of the alternatives is not necessary in the initial evaluation of a patient with suspected dementia?	126 (81.29%)
	8. Which of these alternatives can resemble dementia?	125 (81.29%)
	9. When a patient presents a sudden onset of confusion, disorientation and incapacity to maintain attention, this clinical picture is more compatible with a diagnosis of:	33 (21.29%)
	10. Which of the following options is almost always present in dementia?	9 (5.81%)
	11. Which of the following clinical findings best differentiate vascular dementia from the dementia of Alzheimer's disease?	106 (68.39%)
Management	12. The effect of anti-dementia medications act on:	109 (70.32%)
	13. Which statement on the treatment of demented patients with depression is true?	60 (38.71%)
	14. For what purpose does the ABRAZ Brazilian association supply information to patients and caretakers?	62 (40%)

TABLE 3 Distribution of attitudes towards dementia.

Attitudes	Agree completely (1)	Agree (2)	Neither agree nor disagree (3)	Disagree (4)	Disagree completely (5)
1. Much can be done to improve the quality of life of caregivers of persons with dementia	106 (69.74%)	42 (27.63%)	4 (2.63%)	–	–
2. The families prefer to be informed about the dementia of their relative as rapidly as possible	55 (36.18%)	61 (40.13%)	29 (19.08%)	6 (3.95%)	1 (0.66%)
3. Much can be done to improve the quality of life of persons with dementia	97 (63.82%)	53 (34.87%)	2 (1.32%)	–	–
4. Providing the diagnosis is generally more useful than harmful	71 (46.71%)	69 (45.39%)	10 (6.58%)	2 (1.32%)	–
5. Dementia is better diagnosed in specialized service units	45 (29.61%)	59 (38.82%)	29 (19.08%)	18 (11.84%)	1 (0.66%)
6. The patients with dementia can drain resources with little positive result	5 (3.33%)	39 (26%)	42 (28%)	55 (36.67%)	9 (6%)
7. It is better to speak with the patient utilizing euphemisms	-	3 (1.97%)	27 (17.76%)	87 (57.24%)	35 (23.03%)
8. Treating dementia tends to be more frustrating than gratifying	3 (2.01%)	18 (12.08%)	49 (32.89%)	66 (44.30%)	13 (8.72%)
9. Directing families to specialized services is not worth the effort when they do not want to use them	1 (0.66%)	25 (16.56%)	37 (24.50%)	73 (48.39%)	15 (9.93%)
10. The primary care team has a very limited role in the care of persons with dementia	3 (1.97%)	12 (7.89%)	15 (9.87%)	77 (50.66%)	45 (29.61%)

other study[8] found that general practitioners presented a low level of knowledge in dementia and in that same study they obtained worse scores in questions of epidemiology and found that poorer general knowledge in dementia was associated with less communication of the dementia diagnosis to the patients. Based on this, the authors concluded that educational support should be concentrated on epidemiological knowledge. Thus, the possibility may be considered that, in prior studies, if the students herein evaluated, upon commencing as professionals, would maintain open communication with their patients as was shown in "attitude four" in which 71 (46.71%) of the students agreed that "Providing a diagnosis generally is more useful than harmful," demonstrating a positive attitude in relation to communication with demented patients.

Most of the students demonstrated a positive attitude toward AD patients, a fact corroborated by previous studies that found an association between diagnostic competence (related to knowledge) and positive general attitudes.[8,16,17]

Despite the expressive increase in this social and professional demand worldwide, little has been accomplished in relation to the training of professionals in dementia.[18] A study involving 14 European countries found that little has been done in relation to the training of professionals in dementia and that, in many countries, there are no educational programs directed toward this matter nor financial support.[19] Based on this premise, aging-related diseases must be part of the curriculum of medical students recommended in the curricular correspondence matrix.[20,21] Training of these professionals is of vital importance, and must focus on the attitudes of medical students, which are molded during their years of training, given that such attitudes have great impact on the care provided to elderly patients when they become health professionals. Thus, so that these professionals may diagnose and treat adequately, it becomes necessary that they have greater proximity to the care of demented seniors.[22]

Some limitations must be considered: only two medical schools were analyzed among 268 throughout Brazil;[23] the curricula were not analyzed in detail; the specific instrument utilized in our research cannot cover the questions and attitudes considered in the most exhaustive and adequate manner possible. In this manner, the comparisons made must be analyzed with caution as they deal with different populations (students in their final year of medical school versus graduated physicians), keeping in

mind that the literature on these topics is scarce for final-year medical students' considering the knowledge and attitudes related to patients with dementia.

In conclusion, the findings of our study are relevant for introducing and implementing the knowledge and attitudes that would help overcome the educational obstacles of physicians in relation to the care of patients with AD.

Acknowledgments

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Conflict of interest

The authors declare no conflict of interest.

Resumo

Conhecimentos e atitudes em relação à demência de estudantes de medicina brasileiros no final da graduação

Objetivo: Em 2025, o Brasil terá a sexta maior população de idosos do mundo. Destacam-se, dentre as doenças relacionadas com o envelhecimento, as síndromes demenciais. Apesar da necessidade de o currículo para a formação médica conter tópicos em geriatria, isso parece ocorrer com pouca frequência. O presente estudo tem como objetivo avaliar conhecimento e atitudes em relação à demência de alunos do último semestre do curso médico em duas das mais importantes escolas médicas brasileiras.
Método: Neste estudo, 189 alunos foram convidados a responder questionários que compreendem temas demográficos e profissionais, conhecimento sobre alterações cognitivas em idosos e atitudes frente a um paciente idoso com demência.
Resultados: Um total de 155 estudantes aceitou participar do estudo; 92 (59,7%) relataram ter obtido uma boa formação em alterações cognitivas durante o curso de graduação em medicina, e, entre estes, 67 (58,8%) relataram ter tido apenas uma base teórica. Quanto ao conhecimento, os alunos obtiveram uma média de 6,9, considerando uma escala de pontuação de 0 a 14 pontos. Considerando as atitudes, os estudantes concordaram que eles podem contribuir para a qualidade de vida do paciente e de cuidadores, e que é útil dar o diagnóstico para a família.
Conclusão: Os resultados deste estudo são relevantes para discutir as barreiras educacionais dos médicos em relação ao tratamento de pacientes com demência.

Palavras-chave: idoso, demência, conhecimentos em saúde, atitudes e práticas em saúde, estudantes de medicina.

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Use of anastrozole in the chemoprevention and treatment of breast cancer: A literature review

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Summary

Aromatase inhibitors have emerged as an alternative endocrine therapy for the treatment of hormone sensitive breast cancer in postmenopausal women. The use of third-generation inhibitors represented by exemestane, letrozol and anastrozole is currently indicated. Anastrozole is a nonsteroidal compound and a potent selective inhibitor of the aromatase enzyme. Although a few studies have shown that its pharmacodynamic and pharmacokinetic properties may be affected by interindividual variability, this drug has been recently used in all configurations of breast cancer treatment. In metastatic disease, it is currently considered the first-line treatment for postmenopausal women with estrogen receptor-positive breast tumors. Anastrozole has shown promising results in the adjuvant treatment of early-stage breast cancer in postmenopausal women. It has also achieved interesting results in the chemoprevention of the disease. Therefore, due to the importance of anastrozole both for endocrine treatment and chemoprevention of hormone-sensitive breast cancer in postmenopausal women, we proposed the current literature review in the SciELO and PubMed database of articles published in the last 10 years.

Keywords: aromatase inhibitors, chemoprevention, breast neoplasms, pharmacokinetics.

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Introduction

Breast cancer is one of the most commonly diagnosed types of cancer in women, presenting high incidence rates in developed regions of the world compared with developing ones. Incidence rates of the disease range from 27 cases per 100,000 women in Eastern Africa to 96 cases per 100,000 women in Western Europe.[1,2] Breast cancer is characterized as a multifactorial disease and its development has been reported as the result of complex interactions between an individual's genoma and the environment.[3] Prolonged exposure to estrogene plus progesterone plays a significant role in the etiology of breast carcinoma and biosynthesis pathway of estrogens is thus an important therapeutic target.[4]

The main enzyme involved in estrogen biosynthesis is CYP19A1 or aromatase that belongs to the cytochrome P450 family and is predominantly located in the liver, adrenal glands and fatty tissue.[5] However, the source of estrogen varies widely between premenopausal and postmenopausal women (Figure 1).[6] In premenopausal women, the main source of estrogen is the ovary, while in postmenopausal women, estrogen is derived from the conversion of androgens into estrogens (through the aromatase enzyme). In particular, testosterone is converted into estradiol, androstenedione into estrone and 16-alpha-hydroxytestosterone into estriol (Figure 2), originating from the peripheral tissues, including the skin, fatty tissue and breast. Therefore, the aromatase enzyme directly affects estrogen biosynthesis in the breast and it is believed that this enzyme plays an important role in the progression of breast cancer.[7,8]

Aromatase inhibitors (AIs) have recently been approved as a first-line endocrine therapy for postmenopausal women with hormone-sensitive and metastatic breast cancer.[9,10] There are three generations of AIs and the last, represented by exemestane, letrozol and anastro-

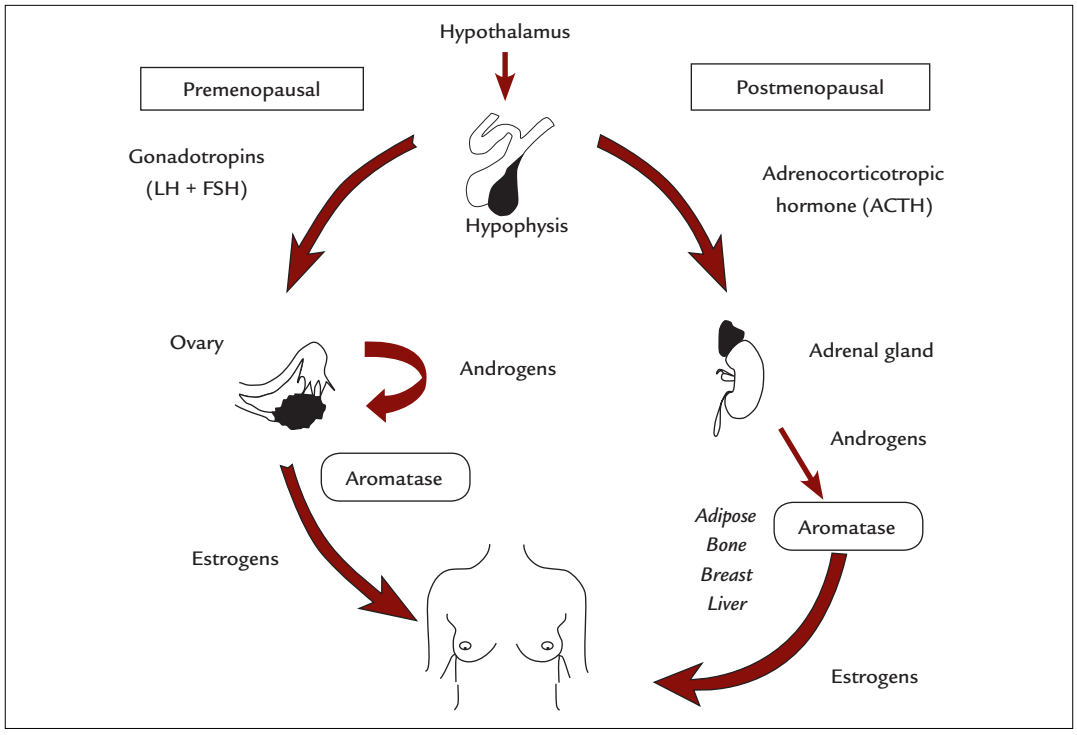

FIGURE 1 Estrogen production in premenopausal and postmenopausal women.

LH: luteinizing hormone; FSH: follicle-stimulating hormone.

Source: adapted from Freedman et al.[6]

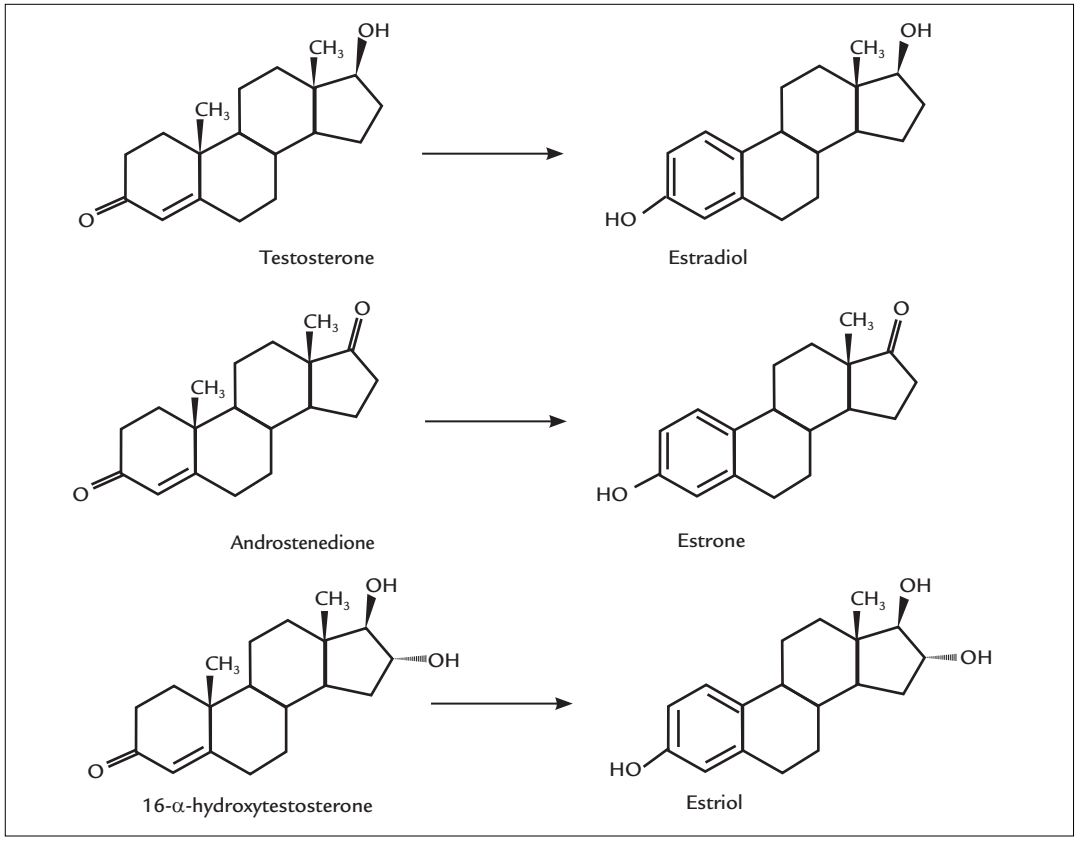

FIGURE 2 Molecular structure of the androgen substrates of aromatase and the corresponding estrogen products.

Source: adapted from Di Nardo and Gilardi.[8]

zole, is the one with the most widely recommended drugs, due to their high specificity for the aromatase enzyme and less adverse effects compared with the previous generations of AI drugs (Figure 3). Exemestane is a steroidal compound that forms covalent bonds with aromatase, and this type of inhibition is irreversible and can only be overcome by the synthesis of a new enzyme. Letrozol and anastrozole are nonsteroidal compounds with reversible action and are competitive AIs.[11,12]

Anastrozole and all third-generation compounds have become endocrine drugs of choice for postmenopausal breast cancer patients, as they are associated with a stronger activity and better general tolerability compared with tamoxifen (TAM), a first-generation selective estrogen receptor modulator (SERM)[13] that has been associated with potentially fatal adverse effects such as an increased incidence of endometrial cancer, thromboembolism and cerebrovascular event.[14] However, there are few studies particularly on the pharmacodynamic and pharmacokinetic properties of anastrozole and its use in the chemoprevention and treatment of breast cancer.

In this review, we will discuss the pharmacodynamic and pharmacokinetic properties of anastrozole, as well as its use in the chemoprevention and treatment of breast cancer.

Pharmacodynamic properties of anastrozole

Anastrozole is a derivative of benzotriazole marketed as ARIMIDEX® by AstraZeneca Pharmaceuticals LP. Similarly to other AIs, it has an inhibitory action on aromatase, thus blocking the conversion of testosterone into estradiol and androstenedione into estrone (Figure 4).[15-18] Inhibition of the aromatase enzyme occurs particularly through competitive binding of aromatase to the heme group of cytochrome P450, decreasing estrogen biosynthesis in the peripheral tissues of the body and in the breast.[19]

Anastrozole has significant effects on breast cancer treatment and, therefore, it is currently used as first-line treatment in estrogen receptor (ER)-positive postmenopausal women, particularly to treat locally advanced or metastatic breast cancer. Furthermore, it is also indicated for early cancer treatment, tumor chemoprevention and postmenopausal women using TAM, especially if the drug is used during a prolonged period of time and has been indicated in the disease's recurrence, i.e., as another therapeutic endocrine option.[9,10]

Prolonged use of anastrozole has no effects on the concentrations of steroid hormones cortisol, aldosterone, androstenedione and 16-hydroxyprogesterone, confirming that it is highly selective for the inhibition of aromatase without interfering in other pathways of adrenal stereoidogenesis. The lack of alterations in luteinizing hormone and folllicle-stimulating hormone demonstrates that anastrozole has no estrogenic, progestational or androgenic activity, and it does not affect the synthesis of gonadotropins.[8,20]

Pharmacokinetic properties of anastrozole

Some studies were conducted in an attempt to identify the daily dose required for anastrozole to promote aromatase inhibition and decrease estrogen synthesis. The results showed that a daily dose of 1 mg of anastrozole was the minimum capable of consistently supressing estrone and estradiol at the limit detectable by radioimmunoassay.[18,21] However, recent studies have reported that a daily dose of 1 mg may not benefit all breast cancer patients because interindividual variability may alter the efficacy and tolerability of anastrozole,[22,23] interfering in its pharmacodynamic and or pharmacokinetic properties.[24]

Administered orally during fasting, anastrozole is rapidly absorbed. After meals, however, it has a slower absorption rate. In the recommended dose of 1 mg, anastrozole achieves maximum plasma concentrations within 2 hours after its administration and, after seven days, approximately 90 to 95% of its plasma concentrations are obtained. Less than 10% of anastrozole is excreted in the form of unaltered drug, while 60% are excreted as metabolites.[17,18]

Anastrozole is metabolized in the liver, involving N-dealkylation, hydroxylation and glucuronidation reactions, leading to a mean plasmatic half-life of 50 hours, which indicates that the administration of a single daily dose of the drug is adequate. The three main metabolites of anastrozole observed in the plasma and urine of human patients are: triazol, hydroxy-anastrozole glucuronide and anastrozole glucuronide. Triazole is the main metabolite; however, it is inactive and does not suppress, along with two other metabolites, the activity of aromatase. The excretion of these metabolites is mainly through urine.[22,25]

The main side effects of anastrozole use include hot flashes (35%), asthenia (17%), headache (13%) and edema (10%). Nausea is the most common gastrointestinal side effect (19%), while diarrhea, constipation, abdominal pain and anorexia are less frequently reported (8%).[26] Nevertheless, in addition to these effects, studies have recently identified the presence of muscle and joint pain, as well as a significant increase in the loss of bone mass, leading to increased incidence of osteopenia and osteoporosis.[23,27]

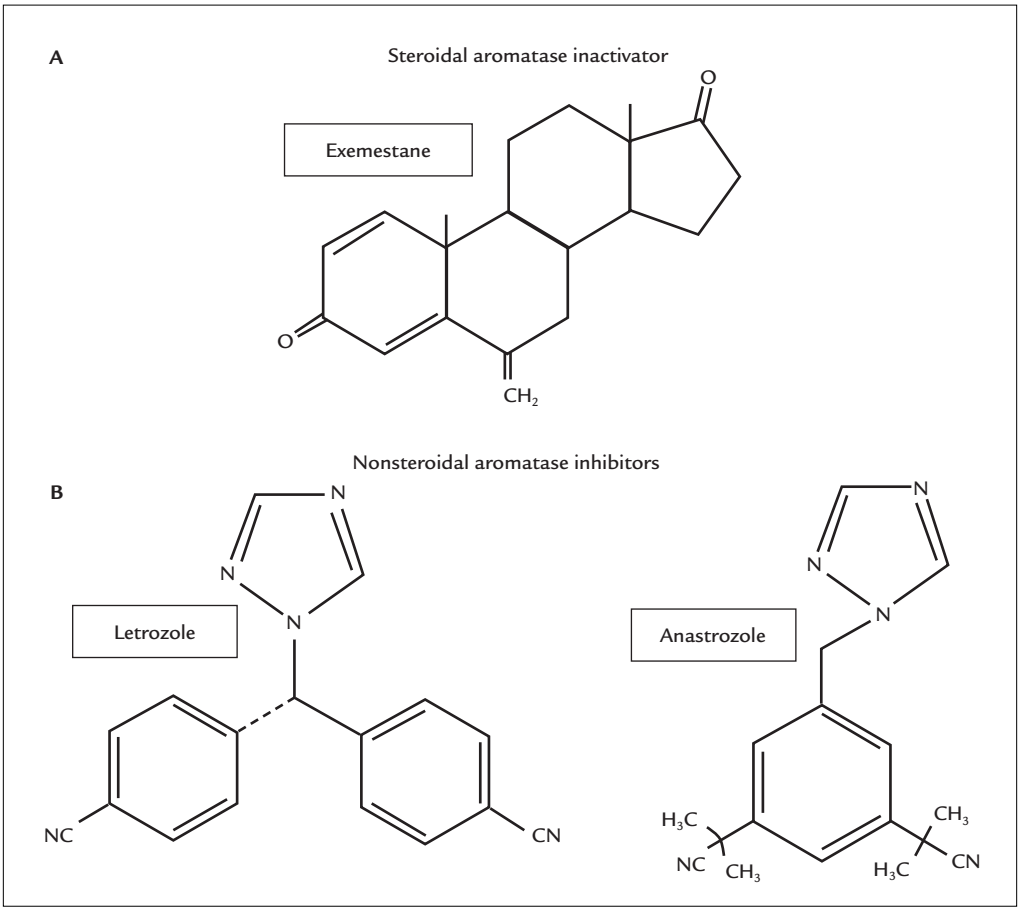

FIGURE 3 Chemical structures of currently used antiaromatase compounds. A. Steroidal aromatase inactivator. B. Nonsteroidal aromatase inhibitors.
Source: adapted from Geisler and Lønning.[7]

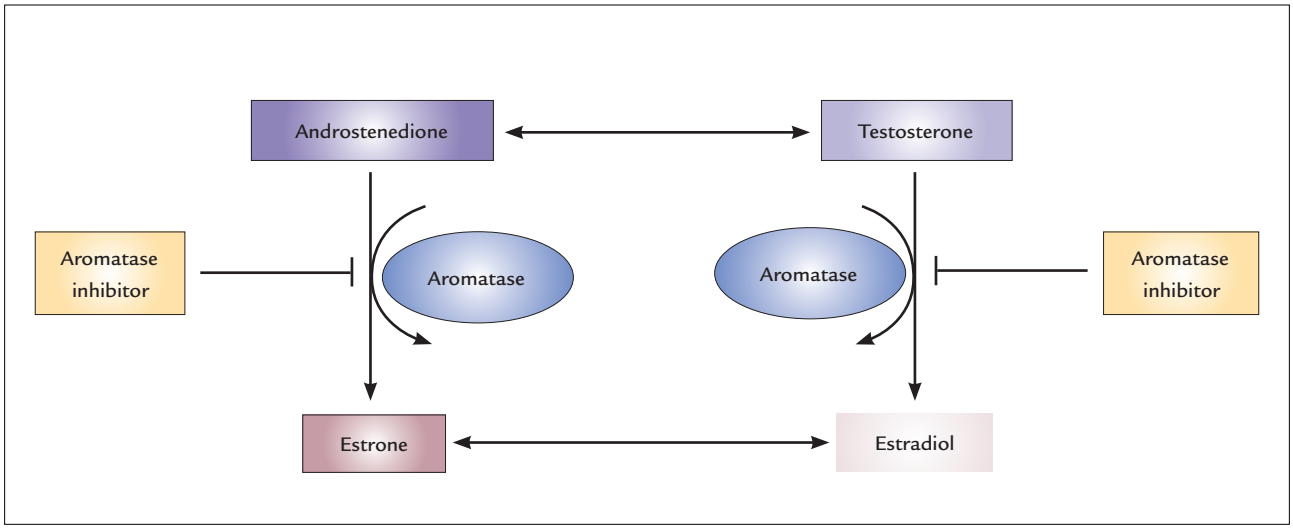

FIGURE 4 Mechanism of action of aromatase inhibitors.
Source: adapted from Johnston and Dowsett.[18]

Metastatic setting

AIs, including anastrozole, are commonly used for first-line treatment of hormone receptor-positive postmenopausal women with metastatic breast cancer.[28] The combination strategy of AIs and the selective ER degrader fulvestrant has been investigated. The Fulvestrant and Anastrozole Combination Therapy (FACT) trial was a randomized study involving 514 postmenopausal women allocated into a treatment group with anastrozole and another group that received a combination of fulvestrant and anastrozole. This trial did not reveal significant differences in median time of disease progression and overall survival when both groups were compared.[29] In contrast, the Southwestern Oncology Group (SWOG) S226 trial had a design similar to the FACT trial and included 707 patients, who demonstrated not only a significant improvement in disease-free survival (DFS) but also advantages in overall survival (OS). This study involved a large number of patients with recurrent metastasis who had not received previous endocrine therapy and who seemed to have more benefits from the combination of fulvestrant and anastrozole.[30]

The Faslodex Versus Exemestane With/Without Arimidex (SoFEA) trial aimed to evaluate endocrine resistance in postmenopausal patients through the combination of fulvestrant and anastrozole versus fulvestrant versus exemestane, and did not demostrate any benefits to the groups of patients treated with fulvestrant and exemestane alone. These data suggest that treatment with fulvestrant combined with anastrozole may offer advantages to postmenopausal women with metastatic recurrence and there seems to be no significant benefit from the combination of fulvestrant and anastrozole in patients with endocrine resistance who had previously received endocrine therapy.[31]

Adjuvant setting

The use of AIs has been widely investigated in postmenopausal women with early breast cancer.[32] Several strategies have been investigated comparing the benefits of AIs and TAM in the adjuvant setting.[33] For initial treatment strategy for breast cancer, both anastrozole and letrozol have demonstrated a significant improvement in DFS after 5 years of study compared to the use of TAM alone or combined with anastrozole during the same time period. Nevertheless, a significant improvement in OS was not observed in these studies, probably due to crossover of a considerable number of patients after publication of the studies. Despite this fact, anastrozole and letrozol seemed to be better tolerated, with fewer serious adverse events related to treatment, in comparison to TAM. Contrary to the benefit derived from the combination of AIs and the selective ER modulator, the administration of anastrozole combined with TAM in the ATAC study demonstrated damaging effects on DFS compared to the sole use of anastrozole.[34]

In an attempt to change strategies, some clinical trials compared 5 years of TAM with the sequential TAM treatment for 2-3 years followed by AIs.[35-38] All these trials demonstrated a significant improvement in DFS among patients who received sequential treatment compared to TAM alone. Furthermore, a meta-analysis conducted on adjuvant breast cancer treatments indicated that the AIs anastrozole, letrozol and exemestane consistently presented lower recurrence rates compared to the use of TAM, either as initial therapy or after 2-3 years of TAM therapy. These studies provide clear evidence that AIs (anastrozole, letrozol and exemestane) achieve a significant reduction in the recurrence rates of early breast cancer.[31]

The Breast International Group (BIG) 1-98 trial was the only study that directly compared a sequential AI treatment followed by TAM, the inverse sequence, and the initial treatment with AIs and TAM only. There was no statistically significant difference between both arms of sequential treatment. Nevertheless, a higher number of relapses occurred during the first years of TAM treatment followed by AI, particularly in patients with lymph node involvement. Therefore, this study suggests that patients at high-risk or those with axillary nodal involvement should initially use anastrozole or letrozol; in case of intolerance due to the use of these AIs, a change to TAM after 2-3 years on AIs may be considered, since there was no statistically significant difference in DFS among patients who received 5 years of AI treatment compared with 2-3 years of AI treatment followed by TAM.[35]

Neoadjuvant setting

The use of anastrozole in the neoadjuvant setting for the treatment of early-stage breast cancer was investigated by a study that compared anastrozole and TAM as neoadjuvant therapy for breast cancer in premenopausal women receiving goserelin. It was observed that responsiveness improved after 24 weeks of treatment in women using anastrozole. This study suggests that the combination of anastrozole and goserelin represents an alternative to neoadjuvant treatment and an option for premenopausal women with early stage breast cancer.[39]

Chemoprevention

The side effects of AIs are limited compared to those of TAM (risk of endometrial cancer and venous thrombo-

embolism), and effectiveness of AIs in breast cancer treatment has been demonstrated. Therefore, there has been great interest in the use of AIs for breast cancer chemoprevention. However, in the configuration of the chemoprevention programs, AIs have not yet been approved.[40,41] In recent years, two clinical trials have reported their main results on cancer prevention using AIs: the Mammary Prevention 3 Study (MAP3) and the International Breast Cancer Intervention Study-II (IBIS-II).

The MAP3 trial involved the randomization of 4,500 postmenopausal women at risk of developing breast cancer, who were allocated into two groups, exemestane and placebo, for 5 years. After a mean 35 month follow-up period, a 65% reduction in invasive breast cancers was observed in women using exemestane and no effect was observed in ER-negative women. No significant differences in side effects were found between groups, suggesting a good risk--benefit profile. However, the limitation of this study was the short follow-up period, which was only 35 months. Due to this, the MAP3 trial does not allow for any conclusions on the long term safety and efficacy of this drug.[42]

The aim of the IBIS-II trial was to evaluate the efficacy and safety of anastrozole for breast cancer prevention in high-risk postmenopausal women. The IBIS-II assessed only anastrozole versus placebo due to more effective action of anastrozole in breast cancer chemoprevention of postmenopausal women compared to tamoxifen.[43] For that trial, postmenopausal women (n=3,864) were randomized into a group using 1 mg of anastrozole daily or a group treated with a placebo. After a median follow-up period of 5 years, it could be observed that anastrozole significantly reduced the diagnosis of invasive breast cancer, and ductal carcinoma in situ, similarly to the results reported in the MAP3 trial. Also similarly to the MAP3 trial, it was found that anastrozole had no effect on ER-negative women. The strengths of this study were the large number of cancers reported and the mean follow--up period of 5 years. All women in the study continue the long-term follow-up in a blinded fashion. This is important because the long-term global efficacy of anastrozole and other AIs has not yet been established for healthy postmenopausal women who are at increased risk of developing breast cancer.[43]

The decline in invasive breast carcinoma observed with the use of exemestane and anastrozole was greater than that observed with TAM or any other SERM, and these results indicate that both drugs are attractive options for the prevention of breast cancer in postmenopausal women at increased risk of developing the disease.[40]

Animal models

The chemopreventive effects of anastrozole have also been investigated in animal models. Significant improvement in tumor supression was observed after induction of premenopausal breast carcinogenesis in rats. The incidence of tumor supression was 40% with the use of a dose of 0.5 mg/kg of anastrozole. In addition, there were no adverse effects on the genital system, lipid and bone metabolism of the rats. Nevertheless, an increase in body weight was observed.[44]

A similar study evaluated the side effects of anastrozole as a chemopreventive agent in the induction of premenopausal breast carcinogenesis in rats. In rats given a dose of 0.5 mg/kg of anastrozole, there were no macroscopic alterations in the uterus and vagina, histological exam showed no atrophic changes in the endometrium and vaginal epithelium, although the myometrium was significantly thicker. Changes in lipid metabolism and serum levels of sex hormones were not observed. However, a significant increase in cortical bone thickness and body weight was found.[45]

Conclusion

Anastrozole is one of the third-generation AIs, a highly competitive and selective inhibitor of aromatase, which is the enzyme responsible for the conversion of androgens into estrogen in postmenopausal women. Large studies have demonstrated the effective use of a daily dose of 1 mg of anastrozole in these women. Anastrozole has a significant action in metastatic disease, as well as in the adjuvant treatment and chemoprevention of breast cancer. However, recent studies have shown that interindividual variability may affect pharmacodynamic and pharmacokinetic properties while using this dose in some women. Anastrozole may be used as an option for efficient and tolerable endocrine treatment in all stages of cancer in the majority of postmenopausal women.

Resumo

Uso do anastrozol na quimioprevenção e no tratamento do câncer de mama: uma revisão da literatura

Os inibidores de aromatase têm emergido como uma endocrinoterapia alternativa para o tratamento de câncer de mama sensível a hormônios em mulheres pós-menopáusicas. A utilização de inibidores de terceira geração, representados por exemestano, letrozol e anastrozol, é atualmente indicada. Anastrozol é um composto não esteroide e um inibidor potente e seletivo da enzima aro-

matase. Embora alguns estudos tenham demonstrado que as suas propriedades farmacodinâmicas e farmacocinéticas podem ser afetadas pela variabilidade interindividual, esse fármaco tem sido recentemente utilizado em todas as configurações de tratamento do câncer de mama. Na doença metastática, é atualmente considerado o tratamento de primeira linha em mulheres pós-menopáusicas com tumores de mama e receptor de estrogênio positivo. O anastrozol tem mostrado resultados promissores no tratamento adjuvante do câncer de mama em estágio inicial em mulheres na pós-menopausa. Ele também conseguiu resultados interessantes na quimioprevenção da doença. Portanto, em virtude da importância do anastrozol tanto no tratamento endócrino quanto na quimioprevenção do câncer de mama hormoniossensível em mulheres na pós-menopausa, propusemos a atual revisão da literatura na base de dados SciELO e PubMed de artigos publicados nos últimos 10 anos.

Palavras-chave: inibidores da aromatase, quimioprevenção, neoplasias da mama, farmacocinética.

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Cytopathologic evaluation of patients submitted to radiotherapy for uterine cervix cancer

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Summary

Cervical cancer is an important public health problem. Pap smear is the leading strategy of screening programs for cervical cancer worldwide. However, delayed diagnosis leads to more aggressive and less effective treatments. Patients with uterine cervix malignancies who are referred for radiotherapy have advanced-stage disease, which results in high rates of locoregional recurrence. The use of radiotherapy as a treatment for cervical cancer causes morphological changes in neoplastic and non-neoplastic epithelial cells, as well as in stromal cells, which make it difficult to diagnose the residual lesion, resulting in a dilemma in cytopathological routine. Based on the difficulties of cytopathologic evaluation for the follow-up of patients treated with radiotherapy for cervical cancer, our objective was to describe the actinic cytopathic effects. Our paper was based on a structured review including the period from June 2015 to April 2016, aiming at an exploratory-descriptive study. Bibliographic investigations were carried out through selection and analysis of articles, list of authors and keywords, selection of new articles focused on the analysis of bibliographic references to previously selected documents, as well as textbooks of recognized merit. The most incident actinic cytopathological alterations as described in the literature are: cellular gigantism, nuclear and cytoplasmic vacuolization, dyskeratosis, bi- and multinucleated (B/M) cells, macro and multiple nucleoli, anisokaryosis, anisonucleolosis and nuclear pyknosis. To date, a protocol has not been established that can precisely differentiate the morphological characteristics between benign cells with actinic effects from recurrent malignant cells on post-radiotherapy smears.

Keywords: radiotherapy, uterine cervix neoplasms, actinic effects, cytopathology.

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Introduction

Cervical cancer is an important public health problem worldwide. Its incidence is higher in less developed countries, compared to the more developed ones.[1] The disease usually begins after the age of 30 years, and its risk increases quickly until it reaches a peak between the ages of 50 and 60 years. According to Instituto Nacional de Câncer (Inca, in the Portuguese acronym), 16,340 new cases of cervical cancer were expected in Brazil in 2016, with an estimated risk of 15.85 cases per 100,000 women. In the Northern Region, for example, this malignant tumor is the most incident among the female population.[1]

Pap smear (Papanicolaou) is the leading strategy of screening programs for cervical cancer worldwide. In Brazil, the strategy recommended by the Ministry of Health is cytopathological examination in women aged 25 to 64 years. For an effective cervical cancer control program, the organization, integrity and quality of services and actions in the care chain must be guaranteed, as much as patient treatment and follow-up.[2,3] Pap smears are considered highly effective, low-cost, and are well accepted by the population.[4]

Delays in diagnosis, on the contrary, lead to more aggressive and less effective treatments, in addition to raising hospitalization costs and mortality rates. As an example, a large proportion of Brazilian women do not regularly undergo cervical cancer screening due to shyness, fear, or lack of awareness, and are thus excluded from prevention and detection measures.[4]

Patients with uterine cervix malignancies who are referred for radiotherapy have advanced-stage disease, which results in high rates of locoregional recurrence.[5] In cases of cervical cancer, cytopathological examination should be performed to control possible residual neoplasm or recurrence of neoplasm after radiotherapy.[2,6,7]

Follow-up of cervical cancer patients treated with curative intent is based on the premise that early detection of a recurrence would result in decreased morbidity and mortality from this disease. Currently, follow-up protocols vary widely, especially in relation to the number of tests and intervals. There are no formal recommendations for an ideal program to monitor these patients. However, the importance of performing periodic exams (physical, cytopathological, colposcopic and imaging) is a consensus.[2,3,8]

According to the handbook of gynecologic oncology practice by Hospital A.C. Camargo (*Manual de condutas em ginecologia oncológica*, 2010), clinical and colpocytological reevaluations every 3-4 months in the first 2 years, with intervals of 6 months from the third to the fifth year of follow-up and annual return after 5 years, are recommended for follow-up of patients irradiated due to cervical cancer, in addition to individualized imaging tests.[8]

The use of radiotherapy as a treatment for cervical cancer causes morphological changes in neoplastic and nonneoplastic epithelial cells, as well as in stromal cells. These alterations make it difficult to diagnose the residual lesion, resulting in a dilemma in cytopathological routine.[9] Actinic cellular atypia may produce false-positive results, but also false-negatives, given the difficulty in collecting adequate samples due to changes in the anatomy of the cervix and vaginal canal, mainly caused by brachytherapy.[10] Subjectivity in the interpretation of changes is also a limitation of the method.[2]

Based on the difficulties of cytopathologic evaluation for the follow-up of patients treated with radiotherapy for cervical cancer, our objective was to describe actinic cytopathic effects in the follow-up of patients with cervical cancer after radiotherapy.

Method

This paper was based on a structured review that included the interval from June 2015 to April 2016, and followed the methods proposed by Villas et al.[11] and Mendes et al.,[12] aiming at an exploratory-descriptive study. Bibliographic investigations were carried out through selection and analysis of articles, list of authors and keywords, selection of new articles focused on the analysis of bibliographic references to previously selected documents, as well as textbooks of recognized merit. The main purpose of exploratory-descriptive studies is to characterize aspects of a given research object compared to previously accumulated knowledge. They are particularly suitable because the object of study is not recurrent in the literature.[13] Data collection included journals indexed in the following databases: MedLine, LILACS, PsycINFO, SciELO Brasil, and the CAPES Portal: http://periodicos.capes.gov.br. There was no time limitation, but articles published between 2005 and 2016 were prioritized.

Theoretical basis

Conceptually, ionizing radiation consists of electromagnetic waves with enough energy to cause electrons to detach from atoms and molecules, changing their structure in a process known as ionization. As a result, they become electrically charged. There are several types of ionizing radiation and each has different penetration power, causing different degrees of ionization in matter.[14,15] Ionizing radiation penetrates according to its type and energy. While alpha particles can be blocked by a sheet of paper, beta particles require a few millimeters of a material such as aluminum, to block them, while high-energy gamma radiation requires dense materials to block it, such as lead or concrete.[14,15]

Ionizing radiation can occur naturally, for example, by the decomposition of natural radioactive substances such as radon gas. The rate at which a radionuclide decomposes (becomes less radioactive) is called half-life, which is the time it takes for a radioactive material to reduce its activity by half. Depending on the radionuclide, this can range from fractions of a second to millions of years. It is possible to measure radiation in various materials, even at very low levels, and the amount of measured radioactivity is expressed as a concentration.[14,15]

Biological effects of radiation

Ionizing radiation interacts with living matter in a process that takes place at the atomic level. Biological molecules are mainly constituted by atoms of carbon, hydrogen, oxygen and nitrogen that can be ejected when irradiated. The transformation of a macromolecule by the action of radiation promotes harmful consequences that can be observed in the cells. Likewise, the generation of new chemical entities in the system also has an impact on the irradiated cell. On the other hand, water molecules are the most abundant in the human body, with about 2×10^{25} molecules of water per kilogram, allowing us to state that, in case of exposure to radiation, the molecules affected in greater numbers will be those of water that suffer ra-

diolysis. After ionization, the water molecules undergo an electronic rearrangement with the possibility of producing free radicals due to the presence of atoms whose last layer does not have the number of electrons that would give stability to the structure.[15-18]

DNA is a macromolecule responsible for encoding the molecular structure of all cellular enzymes, and it is key to the process of establishing biological damage. By undergoing direct (ionizing) or indirect (through free radical attack) radiation action, DNA is exposed to basically two types of damage: gene mutation and lysis.[16-19]

DNA lesions are the most biologically important because they can compromise vital processes such as cell replication and transcription.[20] The different lesions produced by radiation, if left unrepaired, can compromise important biological functions such as DNA transcription and replication, leading to cell death. Failure to repair damage leads to mutagenesis when they are present in the DNA during replication.[21]

The distribution and repair of lesions caused in DNA depend on the nucleotide sequence, whether or not they are in transcribed regions, and the accessibility to DNA by its association with chromosomal proteins.[22] Despite the ability of human cells to remove nucleotides damaged by radiation by means of excision mechanisms, some lesions remain in the genome. Radiation-induced carcinogenesis involves the inactivation of one or more tumor suppressor genes or the activation of pro-oncogenes. The disease can also result from a gene product altered by a mutation.[22]

Radiotherapy

Radiotherapy is a method capable of destroying tumor cells by employing a beam of ionizing radiation. A precalculated dose of radiation is applied at a given time to a volume of tissue encompassing the tumor, seeking to eradicate all tumor cells with the least possible damage to the surrounding normal cells, which play a vital role in the regeneration of the irradiated area. Ionizing radiation is electromagnetic or corpuscular in nature and carries energy. By interacting with the tissues, they produce fast electrons that ionize the medium and create chemical effects such as water hydrolysis and the breakdown of DNA strands. Cell death can then occur through a variety of mechanisms, from the inactivation of systems that are vital for the cell to its inability to reproduce. Tissue response to radiation depends on many factors, such as tumor sensitivity to radiation, location and oxygenation, as well as the quality and amount of radiation, and the total time it is administered. In order for the biological effect to reach a greater number of neoplastic cells and tolerance of normal tissues to be respected, the total dose of radiation administered is usually fractionated in equal daily doses when external therapy is used.[3,23]

The rate of tumor regression represents the degree of sensitivity of the tumor to radiation. It depends fundamentally on its cellular origin, its degree of differentiation, oxygenation and the clinical presentation. Most radiosensitive tumors are radiocurable. However, some tumors spread despite local control and others have their sensitivity so close to that of normal tissues that it is not possible to apply the eradication dose. Local curability is only achieved when the dose of radiation applied is lethal to all tumor cells, but does not exceed the tolerance of normal tissues.[3,23]

Radiotherapy is used in approximately 60% of all diagnosed cases of malignant tumors, including those most prevalent in Brazil, namely prostate, lung, breast and cervical cancers. This means that, out of every 100 patients, 60 will undergo radiotherapy in one of their evolutionary stages.[19,23] In recent times, the most significant development in the treatment of locally advanced cervical carcinoma has been the introduction of radiochemotherapy. However, there are some impediments to its administration, including elderly patients, patients with pre-existing diseases and patients who refuse chemotherapy. There are also financial issues, such as the cost of chemotherapy and the cost of managing toxicities.[19]

Cervical cancer

Squamous or epidermoid carcinoma accounts for more than 80% of malignant cervical neoplasms. Adenocarcinoma (endocervical adenocarcinoma, endometrioid, clear cells, adenocystic, adenosquamous) is a less frequent type that affects the glandular epithelium and corresponds to about 10% of the cases.[1,8] Other histopathological types that appear less frequently are sarcomas (embryonal rhabdomyosarcoma [children] and leiomyosarcoma), melanoma, small cell carcinoma (neuroendocrine), and metastatic carcinoma.[8,24]

The main risk factor for the development of cervical cancer is persistent infection with human papillomavirus (HPV) associated with cofactors, especially immunosuppression and smoking. The mean age of women with this diagnosis is 51.4 years. Squamous carcinoma progresses from precursor stages, the so-called intraepithelial lesions. Low-grade lesions may progress over time to high-grade lesions. Then, neoplastic cells can rupture the basement membrane and invade the underlying stroma. However, some tumors do not appear to start as low-grade lesions, evolving from high-grade lesions from the beginning.[7,25]

Microinvasive carcinoma represents the initial stage of stromal infiltration by neoplastic cells that ruptured the basement membrane, measuring up to 5 mm deep and 7 mm wide in the underlying cervical stroma. However, it is only diagnosed microscopically.[8,25-27] From the practical point of view, it is impossible in smears to accurately ensure that a carcinoma lesion is microinvasive. The cytological pattern may resemble a high-grade squamous intraepithelial lesion or an invasive carcinoma. The category of high-grade squamous intraepithelial lesion with suspected invasion characteristics (Bethesda System) or high-grade intraepithelial lesion, which cannot rule out microinvasion (Brazilian Nomenclature for Cervical Reports), can be applied when neoplastic cells in syncytial clusters exhibit occasional nucleoli and parachromatin clearing. Frankly invasive squamous carcinoma shows in histopathological examination nests of neoplastic cells infiltrating the stroma beyond 5 mm depth from the basement membrane.[25-27]

Differential diagnoses of cervical cancer

In cervical neoplasms, differential diagnoses are essentially made by a process of elimination.[26,27] The most common include:

- Squamous intraepithelial lesions, especially keratinizing ones (absence of tumor diathesis, absence of "clear spaces" in nuclei characterizing the irregular distribution of chromatin, absence of nucleolus in other non-keratinized abnormal cells).
- Repair process (rare isolated cells, lower nuclear-cytoplasmic ratio, less significant abnormalities in chromatin distribution, absence of tumor diathesis).
- Atrophy (absence of irregularity in nuclear margins, absence of irregularities in chromatin distribution).
- Cytopathic changes by herpes virus (multinucleation, nuclear molding and chromatin rarefaction in other cells).
- Effect of radiotherapy/chemotherapy (macrocytosis, polychromasia, cytoplasmic vacuolization, chromatin with "blurred" appearance).
- Poorly differentiated endocervical or endometrial adenocarcinoma (glandular arrangements and spherical arrangements, columnar cells in endocervical adenocarcinoma, frequent vacuolation and common neutrophil infiltration in endometrial adenocarcinoma, sometimes eccentric nuclei, with less hyperchromasia and more frequent and prominent nucleoli, absence of keratinized cells).
- Metastatic adenocarcinoma (characteristics similar to those described above).[26-29]

Post-radiotherapy effects on cells

Cellular and molecular changes induced by radiotherapy include: nuclear DNA destruction or damage, inhibition of protein synthesis, and denaturation/coagulation of proteins. Normal cells are also compromised, resulting in their death or in nuclear and cytoplasmic changes that may persist for many years.[19,29,32,33] Cervical smear is considered an excellent method of investigation in the follow-up of patients undergoing radiation therapy for cervical cancer. The finding of malignant cells that persist after treatment or that reappear soon after allows immediate clinical and/or surgical intervention before the onset of any symptoms. It is important to note that after the beginning of radiotherapy, during a period between four and eight weeks, cervical smears will show abundant necrotic material, with many inflammatory cells and occasional malignant cells. Cytological examination, therefore, is not indicated at this stage to assess whether the neoplasm persists. After this period, all malignant cells disappear, and an atrophic cytological pattern is established.[19,27,29,32,33] Cellular changes are related to both the acute and to the chronic phases following radiation therapy. It is not always easy to differentiate these changes from those of malignant cells. On the other hand, a cytopathologist with little experience in this area may underestimate the changes, missing the opportunity to detect early or recurrent cancer.[19,29-31]

Persistent or recurrent carcinoma is diagnosed in the cervicovaginal smear when malignant cells are identified in the course of radiotherapy or immediately after its completion. It indicates radioresistance of the neoplastic cells, being thus related to a poorer prognosis. Persistent malignant cells exhibit, in addition to irradiation-related alterations, others that are associated with malignancy. Thus, in addition to pleomorphism and hyperchromasia that are common in cells affected by irradiation, there is thickening of the nuclear margins and a well-preserved, coarse and irregularly distributed chromatin. Increased nuclear-cytoplasmic ratio is also observed. The presence of keratinized, pleomorphic cells may indicate malignancy. The most reliable criterion to establish the viability of neoplastic cells is the finding of mitoses.[26,27,34]

Since radiotherapy is associated with characteristic cytological changes, including nuclear activation, increased cytoplasm (with preservation of the nuclear-cytoplasmic ratio), cytoplasmic vacuolization, eosinophilia, polychromasia, multinucleated giant cells, nuclear membrane blebbing and nuclear vacuolization, as well as repair cells, atypical stromal cells, endothelial cells and macrophages,[29,31,35] cytological samples should be collected a few months after radiotherapy. However, it is

true that radiotherapy is associated with unsatisfactory samples. According to Wright et al.,[33] liquid-based cytology considerably reduced the occurrence of unsatisfactory results in the studies performed by them, with only 2.7% of the cytopathological exams (8 of 294) being described as unsatisfactory. They concluded that the use of ThinPrep to perform post-irradiation Pap smears is associated with a high satisfactory cytology rate. Other studies using the SurePath method also corroborated these results.[29,31,36-41] However, the method is still too expensive to be used applied on a large scale.[29]

Cells with actinic effects may be confused with dyskaryotic atypia and produce false-positive results. False-negatives may also occur as a result of actinic changes, as well as difficulty in collecting adequate samples because of changes in the anatomy of the cervix and vaginal canal, especially with brachytherapy, leading to inadequate treatment. It is a consensus among professionals who perform microscopic analyzes (cytopathologists, cytologists and cytotechnicians)[42] that it is often difficult to differentiate actinic alterations in normal cells from cellular atypia in recurrent tumors.[2]

Results

Post-radiotherapy cytopathologic diagnosis

Several methods have already been used to test the radiosensitivity of cervical cancer. Some measure the tumor response and others measure host response, such as the cytopathologic method.[43] The quantitative and qualitative analysis of SR-sensitive and RR-resistant cells, as described by Graham,[44] served as a parameter for post-radiotherapy diagnosis for a long time, but today it is no longer used in most services.[2,9,34] Several authors have didactically classified cytological changes caused by radiation as immediate or chronic, delimited in annual periods,[34,45] or acute, intermediate or chronic.[9]

Although the morphology generates difficulties to define all the cytological changes induced by radiation, it is still considered by several authors an effective means of post-therapeutic control.[45,46] In order to improve cytological diagnosis, methods such as computerized cytometry, specific immunoreactions, immunocytochemistry, and other techniques have been used. However, to date there is no effective protocol to predict the biological behavior of some cell types found in post-radiotherapy smears.[45-48]

Post-radiotherapy cytopathologic criteria

In general, almost all cells undergo radiation-induced changes.[45] Cellular alterations, despite the previously mentioned pattern, can display, depending on gravity, a wide and complex series of morphological modifications, with the appearance of bizarre cytological formations that are difficult to interpret.[49] Table 1 shows the main cytological findings in post-radiotherapy smears.[2,9,34,43-49]

TABLE 1 Main cytological findings induced by radiation in cervicovaginal smears.

Increased cytoplasm
Cytoplasmic vacuolization
Cytoplasmic degeneration
Cytoplasmic pallor
Cellular atrophy
Cellular gigantism
Amphophilia
Dyskeratosis
Pleomorphism
Nuclear increase (without compromising the nuclear-cytoplasmic ratio)
Nuclear vacuolization
Nuclear degeneration
Nuclear pallor
Hyperchromasia
Dyskaryosis (present in malignant cells)
Mitosis (typical or atypical)
Binucleation
Multinucleation
Karyorrhexis
Nuclear pyknosis
Anisokaryosis
Necrosis
Leukocyte infiltrate
Multinucleated giant cells
Repair cells
Macro and multiple nucleoli
Anisonucleolosis

Conclusion

To date, a protocol has not been established to precisely differentiate the morphological characteristics of benign cells with actinic effects from recurrent malignant cells on post-radiotherapy smears. However, there are several studies aimed at minimizing occasional diagnostic difficulties. The information presented here allows for a critical and reflexive analysis of the knowledge about the impact of radiotherapy on epithelial cells, allowing us to point out difficulties, limitations and potentialities that affect the medical practice and the care provided during cytopathological follow-up of patients submitted to cervical cancer radiotherapy.

The most incident actinic cytopathological alterations as described in the literature are: cellular gigantism, nuclear

and cytoplasmic vacuolization, dyskeratosis, bi- and multinucleated (B/M) cells, macro and multiple nucleoli, anisokaryosis, anisonucleolosis and nuclear pyknosis (Figure 1).

The difficulties pointed out show the importance of considering in future studies the experience of the professionals involved in the analysis of irradiated cells and a reflection on the subjectivity of the method. In general, our review provided insights to help coordinate further training for professionals dedicated to the analysis and diagnosis of cells under actinic effects, in addition to recommending complementary studies using techniques that contribute to the understanding of actinic alterations in order to increase prognostic acuity.

Resumo

Avaliação citopatológica no seguimento de pacientes submetidas à radioterapia por câncer de colo uterino

O câncer de colo uterino configura-se como um importante problema de saúde pública. O teste citopatológico é a principal estratégia de programas de rastreamento dessa neoplasia maligna em todo o mundo. Entretanto, a demora no diagnóstico ocasiona tratamentos mais agressivos e menos efetivos. Pacientes com neoplasia maligna de colo uterino que são encaminhadas para radioterapia apresentam doença em estádios avançados, e esse fato determina altos índices de recidiva locorregional. A utilização da radioterapia como tratamento do câncer do colo uterino provoca alterações morfológicas não só nas células epiteliais neoplásicas e não neoplásicas como também nas células estromais, o que dificulta o diagnóstico da lesão residual e resulta em um dilema na rotina citopatológica. Com base nas dificuldades da avaliação citopatológica do seguimento das pacientes pós-radioterapia, o objetivo deste trabalho foi descrever os efeitos citopáticos actínicos. O trabalho teve como base uma revisão estruturada no período de junho de 2015 a abril de 2016, visando a um estudo exploratório-descritivo. As investigações bibliográficas foram realizadas por meio de seleção e análise dos artigos, lista de autores e palavras-chave; seleção de novos artigos focada na análise de referências bibliográficas dos documentos previamente selecionados e livros-texto de relevância conceitual. As alterações citopatológicas actínicas mais incidentes descritas na literatura são: gigantismo celular, vacuolização nuclear e citoplasmática, disceratose, bi e multinucleações, macro e múltiplos nucléolos, anisocariose, anisonucleolose e picnose nuclear. Até o momento, não se conseguiu estabelecer um protocolo que possa diferenciar precisamente as características morfológicas entre células benignas com efeitos actínicos das células malignas recidivantes em esfregaços pós-radioterapia.

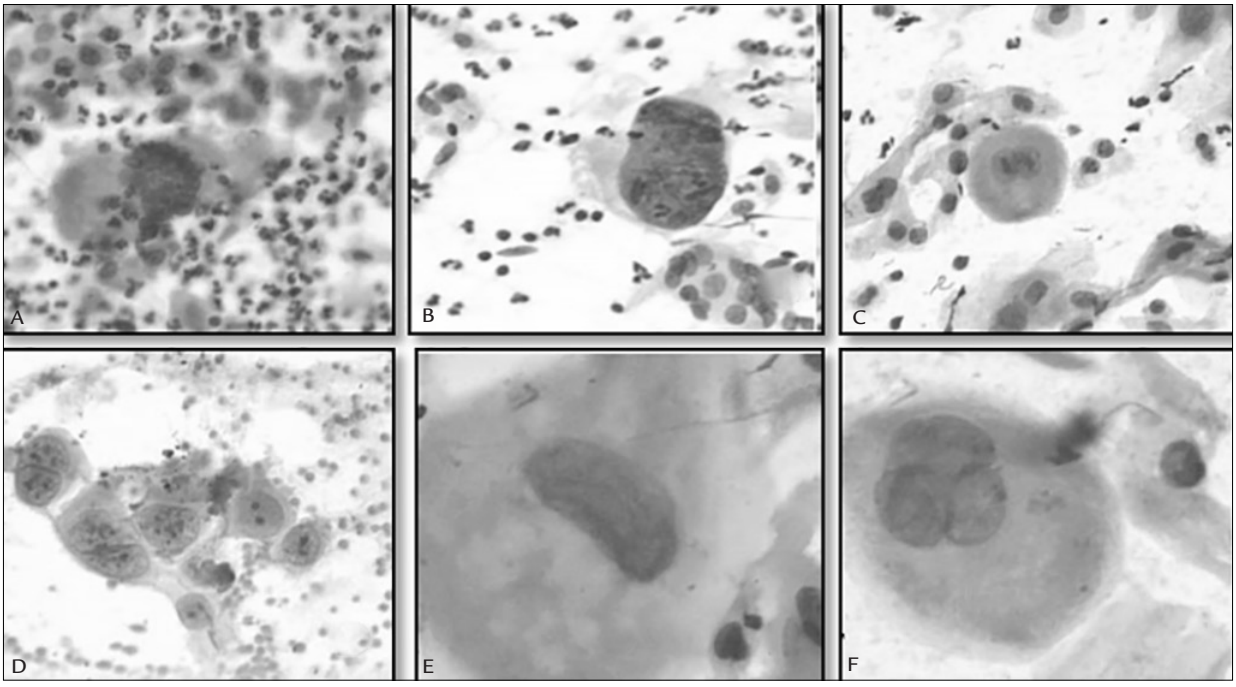

FIGURE 1 Actinic cytopathological aspects.
Source: Padilha et al.[2]

Palavras-chave: radioterapia, neoplasias do colo do útero, efeitos actínicos, citopatologia.

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