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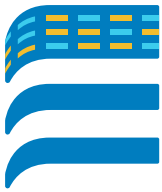

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Correspondence

Núcleo de Publicações da Comissão Científica do Pedro Ernesto (NP COCIPE)
Endereço: *Boulevard* 28 de Setembro, 77
Rio de Janeiro – RJ. CEP: 20551-030.

Telephone

(55 21) 2868 8506 | 2868 8108

Internet

bjhbs.hupe.uerj.br
E-mail: bjhbs@hupe.uerj.br

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Who takes care of the alpha male? Taboos, myths, masculinity, and male sexual health

Like other higher primates, human beings use their libido not only to perpetuate the species, but also to maintain social bonds and to develop deep relationships of affection. Human sexuality represents this set of behaviors in relation to the satisfaction of sexual desire as part of each individual's personality, as a basic need and an aspect of each person that is inseparable from other facets of life. It influences thoughts, feelings, actions and interactions and, therefore, physical and mental health.

Even if we consider that the patriarchal family pattern, structured around the alpha male, is currently changing, it still significantly influences many men who, through their identity behavior, not only cause damage to others, but especially to themselves. I am referring more specifically to the implications of the social imaginary about male sexual identity caused by the tensions arising from wanting to maintain traditional standards and the possibility of living other ways of being a man.

This situation is related to the existence of many myths and taboos about human sexuality. Sexual taboo is part of the moral sphere and is based on prejudice and the prohibition of certain sexual practices for social, political or religious reasons (e.g., masturbation). The sexual myth, on the other hand, is a fantasy, a mistaken idea about human sexuality (e.g., penis size) Figure 1. Faced with a lack of scientific information, explanations arising from common sense are disseminated as truths. This misinformation has the potential to cause serious damage to the quality of life. The good news is that myths and taboos are social constructions that vary with time and social geography, and therefore can be suppressed over time. If we want to confront these symbolic impasses, efforts must be made to create an environment that affirms and promotes sexual health, as well as a liberating sexual education that doesn't repeat oppressive patterns to imaginary alphas.

Figure 1. MOESIA INFERIOR, Nicopolis ad Istrum. Septimius Severus. AD 193-211. Æ Assarion (18mm, 3.83 g, 2h). AV KAI CE CEVHPOC, laureate head right / NIKOPOLI TWN PROC IC, ithyphallic Priapus standing left, pointing downward with right hand at his characteristic attribute, left hand on hip. Mouchmov 987; Varbanov 1789.

Eloísio Alexsandro da Silva Ruellas
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Treatment of penile curvature with aldehyde free bovine pericardium graft: an original and prospective evaluation of a cohort of patients with Peyronie's disease

Eloísio Alexsandro da S. Ruellas,[1] Igor José C. Feitosa,[1] Miguel de O. Osta,[1] Luiz Augusto Westin,[1] Tamiris M. de O. Ruellas da Silva,[1] Ronaldo Damião[1]

Abstract

Introduction: Peyronie's disease (PD) is a common condition that can cause penile curvature and/or deformities, thereby affecting the male sexual function. The most effective treatment for clinically significative and stable penile curvature due to PD is reconstructive surgery that involves techniques that do not shorten penile length. Among the techniques for the correction of curvature and restoration of penile length, the incision and/or excision of the plaque, followed by reconstruction of the defect with a graft, remains the gold standard. Although several materials have been used as a graft in the treatment of penile curvature due to PD, none have shown results good enough to be considered as the first choice. Herein, we aimed to evaluate the results of the surgical treatment of patients with penile curvature due to PD using a new biograft. Methodology: We performed the surgical management with graft technique in twelve men suffering from penile curvature due to PD. Penile curvature was measured before and one year after the reconstructive procedure. The graft of choice was bovine pericardium graft segment treated with L-Hydro aldehyde-free technology. Erectile function was evaluated by the IIEF questionnaire and penis length was measured by a rigid ruler during a pharmacologically induced erection test. Results: The overall success rate of correction of the penile curvature was 90.9% and the patients' satisfaction rate was 91.7%. No serious adverse events were found and the erectile function was impaired in 18.2% of cases. There was an increase in penile length, ranging from 0.7 to 3.0cm in 91.7% of cases. Conclusion: Correction of penile curvature due to Peyronie's disease using the technique of incision and/or excision of the plaque with an aldehyde-free bovine pericardium graft presents high success rates, both in terms of penile shaft rectification and patient satisfaction. L-hydro bovine pericardium graft is easy to handle surgically, allows a tight anastomosis and does not present serious postoperative complications. Furthermore, it can be used simultaneously with penile implants and appears to play a role in restoring penile length.

Keywords: Penis; Penile induration; Penile diseases; Surgery; Regenerative medicine.

1. Departamento de Urologia. Hospital Universitário Pedro Ernesto. Universidade do Estado do Rio de Janeiro. Rio de Janeiro, RJ, Brazil..

*Correspondence address:
E-mail: cirurgiagenital@gmail.com
ORCID: https://orcid.org/0000-0002-7216-8780

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Introduction

Peyronie's disease (PD) is a common condition affecting the male sexual function, whose prevalence can be as high as 20%.[1] PD is a fibrotic alteration of the penile tunica albuginea due to

an inflammatory process.[2] These tunica albuginea changes impair its elasticity and can, therefore, cause deformity during erection. Deformities can manifest themselves in different ways, including penile shortening, hourglass penis, and penile curvatures.

Clinically significant penile curvature impairs penetrative sex and also tends to cause psychological distress in patients.[3] Difficulties during intercourse and social stigmas caused by the deformity and/or loss of length can affect these patients emotionally, leading them to withdraw from sexual activity and greatly impairing their quality of life.

The most indicated and effective treatment for clinically significant and stable penile curvature due to PD is reconstructive surgery.[4,5] Currently, surgical techniques that do not shorten penile length are preferred, because these potentially avoid great dissatisfaction on the part of patients regarding penis size. Among the techniques that correct curvature while restoring the length and even lengthening the penis, the incision and/or excision of the plaque, followed by reconstruction of the defect with a graft, remains the gold standard.[4,5]

Although several materials have been used as a graft in the treatment of penile curvature due to PD, none have shown results that are good enough to be considered a gold standard.[6]

The aim of this study is to prospectively evaluate the results of surgical treatment using a new biograft in patients with penile curvature due to PD.

Methodology

Thirteen men suffering from penile curvature due to PD who were routinely diagnosed in a urology clinic and referred for surgical treatment were enrolled in this study. All of them signed the informed consent form. The age of the patients ranged from 42 to 72 years. Diabetes Mellitus and Arterial Hypertension were comorbidities reported by two patients. Twelve men completed the 12-month postoperative follow-up period and were reassessed with regard to satisfaction with surgery, curvature correction, presence of surgical complications, erectile function, and penile length.

Penile curvature was assessed during a drug-induced erection test with 20 micrograms of intracavernous prostaglandin E1 before surgery and during the one-year follow-up consultation. Digital images were taken, and the angle of the curvature was measured using Kelami's five line protocol[7]. A free on-line software for measuring the angle was used. A Likert scale, ranging from 1 to 5, was used to assess the subjective satisfaction of penile curvature correction. The International Index of Erectile Function (IIEF-5) was used to assess erectile function.[8] One patient was lost to follow-up. All patients underwent the technique of partial excision of the plaque + geometric relaxation incisions and implantation of a bovine pericardium graft segment treated with L-Hydro aldehyde-free technology (Vivendi UR®, Labcor, Contagem - Brazil). Three patients underwent a simultaneous semi-rigid penile implant due to associated severe erectile dysfunction.

Results

The overall success rate of the penile curvature correction was 90.9%. Eleven (91.7%) patients reported being satisfied (n=5; 41.7%) or very satisfied (n=6; 50%) with the surgical outcome of their penile curvature (Table 1). Only one patient showed dissatisfaction, the presumed reason for which was a decrease in penile size in the postoperative period. This fact was not confirmed

by objective measurements and the patient is still being followed up in a psychology clinic due to difficulties in self-perception of genital body image.

No serious adverse (grade II to V) events were found according to the Clavien Dindo scale.[9] One patient had drainage of serous fluid through the surgical wound for 19 days, without inflammatory signs, which ceased after conservative treatment. A culture of the serous fluid was performed and no growth of bacteria or fungi was found.

Table 1. Comparative results from the baseline to 12 months follow-up of patients with penile curvature due to Peyronie's disease managed with non-aldehyde bovine pericardium matrix

Patient	IIEF-6 baseline	IIEF-6 12 months	Penis Length baseline	Penis Length 12 months	Penile Curvature baseline	Penile Curvature 12 months
1	6	29*	12.3	13	90° dorsal	Non-significant
2	24	21	12	11.5	70° lateral	Non-significant
3	20	10	11.1	12.1	70° lateral 70° dorsal	Non-significant
4	21	23	13.5	14.3	45° dorsal 25° right	Non-significant
5	15	13	13	13.9	32° dorsal 40° lateral	Non-significant
6	28	24	13.8	15.3	90° dorsal 45° lateral	60° lateral
7	17	29*	13	16	80° dorsal 30° lateral	Non-significant
8	6	6*#	12.5	14	40° ventral 60° lateral	#
9	22	25	13.8	15.5	25° ventral 55° lateral	Non-significant
10	27	24	12.5	14.5	95° dorsal 40° lateral	Non-significant
11	16	12	14	14.7	70° dorsal	Non-significant
12	15	7	11.8	12.5	60° dorsal 25° lateral	Non-significant

Legend: * Penile implant; # The penile prosthesis was removed at the request of the patient
Source: The authors (2023).

Erectile function was assessed in 11 patients (Table 1). One individual could not assess the result of erectile function at the end of the study because he had been submitted to the implantation of a penile prosthesis concomitant with correction of the penile curvature with a graft and voluntarily requested the removal of the prosthesis 3 months after the operation due to personal problems, even with the perfect and recognized straightening of the penile shaft. Two (18.2%) individuals showed significant worsening of erectile function, progressing to severe erectile dysfunction (IIEF-5 score 1-10).

There was an increase in penile length in 11 of the 12 subjects (91.7%) and this ranged from 0.7 to 3.0cm (Table 1). One patient (8.3%) presented a reduction of 0.5cm in penile length, although this was not the subject of any complaint.

Discussion

The use of aldehyde-free bovine pericardium as a graft has been widely known in cardiovascular surgery for more than two decades.[10,11] Several clinical studies show that it has excellent biomechanical and long-term histocompatibility characteristics, confirming the safety of its use in humans. There is also evidence that the biointegration of the grafts occurs and promotes guided regeneration, a typical strategy of regenerative medicine based on scaffolds. The use of pericardium as a biograft for penile albuginea reconstruction has been widely described and shows a wide variation in success rates.[12,13] However, despite the fact that bovine pericardium is excellent as a raw material for the production of matrices for grafting, to our knowledge no graft processed without aldehydes throughout the entire process has been previously used in clinical studies of penile reconstruction. The use of aldehyde solutions during processing or even in the final preservation of graft matrices induces an intense inflammatory reaction that can eventually evolve into the formation of calcium deposits.[14] These alterations are very undesirable and can cause functional damage to the regenerated target organ.

Another important advantage of using aldehyde-free bovine pericardium graft is its pleasant surgical handling, allowing for easy apposition and tight suturing. Only the thickest part of the bovine pericardium was used to manufacture the final biograft and its thickness is similar to the human adult tunica albuginea. It has sufficient tear strength properties to ensure organ function until complete biointegration occurs and has a visceral and adventitial surface. The smoother and brighter visceral surface was used by us to maintain contact with the corpora cavernosa, since it induces less clot formation. Regarding this point, antithrombotic and anti-inflammatory agents were incorporated in the graft during the processing, making the microenvironment less hostile for tissue repair.[15,16] We report no complications regarding to the graft and, therefore, considered that full biointegration of the graft was achieved. Although this finding would be better evaluated with new samples obtained from biopsies, this was not the purpose of this study. Other limitations of the study were the small number of patients and the fact that it was carried out in a single center. Finally, the excellent result of this initial study provides a basis for carrying out multicenter studies with a larger sample.

Conclusion

Correction of penile curvature due to Peyronie's disease using the technique of incision and/or excision of the plaque with an aldehyde-free bovine pericardium graft has high success rates in terms of both penile shaft rectification and patient satisfaction. L-hydro bovine pericardium graft is easy to handle surgically, allows a tight anastomosis and does not present serious postoperative complications. Furthermore, it can be used simultaneously with penile implants and appears to play a role in restoring penile length.

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Prevalence of carbapenem-resistant *Enterobacteriaceae* in in- and out-of-hospital environments

Julianna G. B. Olivella,[1] Bianca de O. Fonseca,[1] Adenilson de S. da Fonseca,[3] Eduardo A. R. de Castro,[2] Alexandre R. Bello,[1] José Augusto A. Pereira[1*]

Abstract

Introduction: Healthcare is recognized as a condition of high selective pressure (SP) due to the use of antimicrobials. The emergence of community bacterial species resistant to the antimicrobials most extensively used in hospitals is of concern, as is the case with Gram-negative rods producing extended-spectrum beta-lactamases (ESBL). Hospital colonization by carbapenem-resistant Enterobacteria (CRE) can persist for many months after hospital discharge, and their encoding genes can be transferred among different species. Methodology: Because of the scarcity of options due to resistance to carbapenems, we sought to evaluate the occurrence of CRE in stool samples from outpatients and inpatients of a University Hospital, for whom clinical and epidemiological data were obtained from the analysis of medical records. After parasitological examination, stool samples from outpatients and inpatients were diluted in saline (1:20, 1:1000 and 1:2000) solution and 0.1 mL seeding was performed on MacConkey Agar (MA) containing gentamicin 8 µg/mL and MA containing cephalexin 32 µg/mL. Once isolated, strains were identified by biochemical tests and AST (with imipenem, ertapenem, and meropenem) was performed according to CLSI (2020). Results: We isolated carbapenem-resistant strains of enterobacteria in 13 (43.3%) of the 30 inpatients and in 13 (29%) of the 31 outpatients (p>0.05). In 7 (23.3%) of the 30 inpatients and in 5 (16.1%) of the 31 outpatients, we isolated CRE at 1:1000 or 1:5000 dilutions of stool samples, which correspond to "microbial loads". Ten bacterial species were isolated in the CRE related to the 13 inpatients and 13 outpatients, among whom we identified two or more species in 9 (69.2%) and 10 (76.9%), respectively. Even taking into account the limitations of the study due to the possibility of bias arising from the absence of data in the medical records, the detection of intestinal colonization with CRE in non-hospitalized individuals is of concern and may jeopardize the implementation of rational empirical therapy in patients of that community. Conclusion: It is possible that, as was the case of ESBL-producing strains decades ago, infections by carbapenem-resistant strains have become endemic in the community. In general, this development is a cause of concern, for plasmid-associated antimicrobial resistance strains, due to the fact that an often-recognized plasmid-associated fitness of bacterial cell favors persistence of the strains, even in the absence of antimicrobial (co-)selective pressures.

Keywords: Feacal enterobacteriaceae; Carbapenemases; Inpatients; Outpatients; Microbial loads.

1. Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro (UERJ). Rio de Janeiro, RJ, Brazil.

2. Serviço de Controle de Infecção Hospitalar, Hospital Universitário Pedro Ernesto, Universidade do Estado do Rio de Janeiro (UERJ). Rio de Janeiro, RJ, Brazil.

3. Departamento de Biofísica e Biometria, Universidade do Estado do Rio de Janeiro (UERJ), Instituto de Biologia Roberto Alcantara Gomes (IBRAG). Rio de Janeiro, RJ, Brazil

*Correspondence address:
E-mail: jadlerpereira@gmail.com
ORCID: https://orcid.org/0000-0003-0530-0697

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Introduction

Reports of the detection of community-acquired carbapenem-resistant *Enterobacteriaceae* (CREs) are of great concern.[1,2] Nordmann and colleagues, 2011[3] emphasize that enterobacteria are responsible for infections acquired in hospitals or in the community, since they are easily disseminated among humans because of the contamination of hands, food, and water.[3] These bacteria are recognized as especially competent in the horizontal transfer of genes carried by transposons and plasmids. Such carbapenemase-producing, multidrug-resistant strains can be considered to be endemic in various parts of the world.[4,5]

The circulation of these microorganisms and the resulting endemic condition of infections in the community is thought to be a consequence of several factors acting together: contaminated food; natural or artificial water reservoirs; poor sanitation and hygiene; and uncontrolled use of antimicrobials (including self-medication, etc.).[6,7] It is considered that, more generally, the wide global spread of antimicrobial resistance is due to the complex interactions between human health, animal breeding and veterinary science, with antimicrobial use, sanitation, hygiene, sewage and water treatment playing a significant role.

Currently, according to the World Health Organization (WHO), carbapenem-resistant CRE as well as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are the main threats to human health.[8] In 1993, in France, the first carbapenemase-producing *Enterobacteriaceae* (NmcA) were detected in *Enterobacter cloacae*.[9]

The most frequently detected carbapenemases worldwide are *Klebsiella pneumoniae* carbapenemase (KPC), Ambler class A, Verona integron-encoded metallo-beta-lactamase, type B (VIM), New Delhi metallo-beta-lactamase, class B (NDM) and oxacylinase-48, class D (OXA-48). One of the most frequently detected genes is blaKPC-2.[10] Resistance to carbapenems, as occurs in the common production of "classical" ESBLs, is encoded in bacterial mobile genetic elements.[11] It is very relevant that hospital colonization by CRE can persist for many months after hospital discharge, and that their coding genes can be transferred between different Gram-negative bacterial species.[4]

Human gut microbiota, especially, are known to be important reservoirs of CRE and other relevant pathogens. Surveillance cultures have become a practice of great relevance for the detection of multidrug-resistant bacteria that require contact prevention measures, among others, to control the spread of these potential agents of infection.[10]

Chika and colleagues, 2017[12] isolated metallo-beta-lactamase-producing GNR in rectal swabs from cattle and poultry. The authors highlight the importance of food as a transmission vehicle of CRE in the environment and emphasize the need for continuous monitoring. They highlight the importance of continuous monitoring of multidrug-resistant strains, including MBL-producing enterobacteria, to detect the occurrence of transmission, between hospital and community, in both directions. Reports of CRE infection in the community are unable to fully confirm the origin (whether hospital or community) of the strains.[13-17]

We can consider monitoring to include more than hospital colonization surveillance, despite recognizing the great importance of the latter. Thus, the aim of this study is to evaluate the occurrence of CRE in stools of patients treated at a University Hospital Outpatient Clinic.

Methodology

We sought to evaluate the occurrence of CRE in stool samples from outpatients and inpatients of a University Hospital, whose clinical and epidemiological data were obtained from the analysis of clinical records. The use of antimicrobials prior to hospitalization, prior hospitalization or treatment in out-of-hospital healthcare environments were considered to be exclusion criteria. Stool samples obtained for parasitological examination from outpatients and inpatients were diluted in saline (1:20, 1:1000 and 1:2000) solution and 0.1 mL seeding was performed on MacConkey Agar (MA) containing gentamicin 8 µg/mL and MA containing cephalexin 32 µg/mL. Isolated strains were stained using the Gram method and observed by microscopy. The strains were identified by biochemical tests and AST for carbapenems (with imipenem, ertapenem, and meropenem) and for several other antimicrobials, performed according to the stipulations of the Clinical and Laboratory Standards Institute (CLSI).[18] The data obtained were analyzed using the Statistical Package Epi InfoTM - CDC/USA. The study was approved by the Ethics Committee of the Pedro Ernesto University Hospital (CEP/HUPE), under the number CAAE 44685015.9.0000.5259/2015.

Results

We isolated strains of carbapenem-resistant *Enterobacteriaceae* in 13 (43.3%) of the 30 inpatients and in 9 (29.0%) of the 31 outpatients ($p>0.05$), which is not statistically significant. In 7 (23.3%) of the 30 inpatients and in 5 (16.1%) of the 31 outpatients, we isolated CRE at 1:1000 or 1:5000 dilutions of stool samples, which correspond to "microbial loads" (Table 1). Ten bacterial species were isolated both among the CRE originating from the 13 inpatients and those isolated from the 13 outpatients, in which we identified two or more species in 9 (69.2%) and 10 (76.9%), respectively (Table 2).

It seems important, here, to report the diversity of species isolated in a large proportion of the isolated cases (individual stool samples) from the stool of both inpatients and outpatients (Table 2). We found multiple CRE resistance profiles in individual stool samples (from the same patient) for both types of patients. The association of several determinants associated with carbapenem resistance for the different isolated strains for both inpatients and outpatients is, therefore, evident.

Discussion

Even taking into account the limitations of the study due to the possibility of bias arising from the absence of data in the medical records, the detection of and intestinal colonization with CRE in non-hospitalized individuals is cause for concern. Furthermore, our results may possibly be subject to some bias, since the fecal samples were obtained for parasitological examination and may include patients presenting dysbiosis, which favors bowel colonization by antimicrobial resistant bacteria. Analyses involving stool dilutions allowed the detection of CREs in some cases in dilutions of up to 1/5,000.

Nordmann and colleagues, 2011[3] found few reports of community-acquired CRE infections; however, considering the numbers of cases in endemic areas worldwide, the true number may, in fact, be higher. In fact, the authors assert that selective pressure processes may occur due to structurally unrelated antimicrobials for which carbapenem-producing strains express resistance genes (co-selection), promoting the spread of these strains. Case reports exist of

Table 1. Frequencies of patients with CRE isolation at different dilutions of stool samples. For each patient, we considered the highest dilution at which a strain of CRE was isolated

Highest dilution where CRE were found	Number of outpatients	Number of inpatients
1:20	8 (62%)	6 (46%)
1:1000	3 (23%)	2 (15%)
1:5000	2 (15%)	5 (39%)
Total	13 (100%)	13 (100%)

Source: The authors (2022).

Table 2. Distribution of *Enterobacteriaceae* species isolated from patient stools by origin and by dilution of clinical specimen

Numbers of carbapenem-resistant *Enterobacteriaceae* strains									
Bacterial species	Inpatients				Outpatients				Total
	Dilutions of stool			Subtotal	Dilutions of stool			Subtotal	
	1:20	1:1000	1:5000		1:20	1:1000	1:5000		
Escherichia coli	6	6	0	12 (27%)	5	1	0	6 (21%)	18 (25%)
Klebsiella pneumoniae	5	2	2	9 (21%)	0	0	0	0	9 (13%)
Morganella morganii	5	1	1	7 (16%)	2	0	0	2 (7%)	9 (13%)
Enterobacter cloacae	1	1	1	3 (7%)	6	0	0	6 (21%)	9 (13%)
Citrobacter freundii	3	1	0	4 (9%)	2	1	1	4 (14%)	8 (11%)
Klebsiella oxytoca	0	1	1	2 (5%)	1	1	1	3 (11%)	5 (7%)
Citrobacter diversus	0	0	0	0	4	0	0	4 (14%)	4 (5%)
Pantoea agglomerans	2	0	2	4 (9%)	0	0	0	0	4 (5%)
Enterobacter aerogenes	1	0	0	1 (2%)	1	0	0	1 (4%)	2 (3%)
Providencia stuartii	1	0	0	1 (2%)	0	0	0	1 (4%)	2 (3%)
Enterobacter asburiae	0	0	0	0	0	1	0	1 (4%)	1 (1%)
Enterobacter sakasakii	1	0	0	1 (2%)	0	0	0	0	1 (1%)

Source: The authors (2022).

CRE infections with a presumed community origin, but it is difficult, from these sources, to exclude the occurrence of exposure to hospital or other healthcare environments – a possible source of colonization with CRE and/or other antimicrobial multidrug-resistant bacteria.

Okazaki and colleagues, 2015[13] report on community pyelonephritis, with no history of hospitalization. However, the patient resided in a "nursing home". Such environments are consid-

ered a risk factor for colonization by multidrug-resistant strains.[11] In fact, the authors draw attention to the importance of emergency services professionals considering the occurrence of multidrug-resistant microorganism infections, even in non-hospital institutions. Song and colleagues, 2019[16] report a hospital outbreak of KPC2-Kp infections in Seoul, South Korea. This outbreak was well-characterized, by phenotypic and molecular analysis, as originating from hospitalization of a patient with community-acquired infection (*index* case), where conjugative plasmid carrying the gene *kpc* was detected. From the information provided in the study, we cannot totally exclude the occurrence of healthcare-associated infection (HAI) in this patient index. Khatri and colleagues, 2015[14] report the successful treatment of a pregnant patient with pyelonephritis caused by KPC-producing *Klebsiella pneumoniae*. The patient did not report previous hospitalization, but reportedly lived with her mother, who was undergoing cancer treatment in out-of-hospital healthcare units. It seems that the importance of this contact in the eventual acquisition of CRE cannot be dismissed. In the Brazilian context, Azevedo and colleagues, 2019[15] detected, as CRE, an expressive proportion of *K. pneumoniae* isolated from urinary tract infections considered to be community-based. From 48 strains of *K. pneumoniae* isolated from "community" patients with urinary tract infection, 29 (60.4%) were found to be multidrug-resistant strains. The authors detected 46 genes specific for beta-lactamases in 27 (56.3%) samples. They found 73 genes encoding virulence factors in 30 strains (62.5%). The authors, however, do not report on the possible occurrence of prior hospitalization of patients. They draw attention to the need for epidemiological surveillance to prevent patients being discharged from hospital, thus preventing colonization that leads to the spread of resistant strains and the occurrence of infections in the community.

Reports of CRE infection in a community invariably raise some degree of doubt about the origin of the strains. We can consider a genuine community infection to be true if it occurs thanks to "circulating" CRE, relatively independent of institutional environments under high selective antimicrobial pressure. According to Nordmann and colleagues, 2011,[3] it is possible that, and of great concern for carbapenem resistance, a process similar to that for ESBL-producing strains decades ago will occur, causing endemic infections in the community. Furthermore, it is important to consider that, unlike what may be considered the selective pressure capable of favoring ESBL-producing strains in the community in general, the restrictive use of carbapenems "out-of-hospital" can delay the establishment of CRE. However, plasmids containing both the gene for carbapenem resistance and "classic" ESBLs have been described in CRE strains.[1]

The combination of several determinants associated with carbapenem resistance, particularly the resistance markers for aminoglycosides and quinolones for the different stool strains isolated, in patients from both origins, is very likely a sign of plasmid-encoding.[10] Plasmids that carry gene coding for resistance to carbapenems often also contain gene coding for ESBLs, in addition to other determinants of resistance. Also, plasmids containing two different genes codifying for carbapenemases have been reported.[19] Furthermore, many of the different plasmids already characterized in several plasmids of different incompability (Inc) groups are transferable by bacterial conjugation.[20]

It is likely that poor hygiene and sanitary conditions, environmental contamination, and/or contamination of the food and water used, as well as uncontrolled use of antimicrobials in India (as occurs in the Asian continent in general) may act as risk factors for the acquisition of CRE and ESBL-producing *Enterobacteriaceae* (ESBLPE). Such strains can be acquired by travelers. The authors recommend that patients receiving healthcare while traveling abroad should undergo surveillance cultures for colonization by these microorganisms upon hospitalization.[6]

Woerther and colleagues, 2017[21] concluded from an extensive review of international travel, particularly to sub-tropical regions, that these are important risk factors for ESBLPE colonization. We can consider that – even while acknowledging some differences – Brazilian sanitary and hygienic conditions are very often precarious, and may identify settings recognized as favorable for contamination, persistence, and circulation of CRE in the community.

CRE have been detected in the community environment in Guanabara Bay, Rio de Janeiro.[22] De Araújo and colleagues, 2016[23] detected different gene coding for the production of carbapenemases (bla_{KPC}, bla_{NDM}, bla_{GES}, and $bla_{OXA48\text{-}like}$ genes) in *Enterobacteriaceae* in two water bodies (matrices) in Rio de Janeiro, Brazil. The authors warn of the possibility of transmission of these strains and genes to the community.

We found multiple CRE resistance profiles in individual stool samples of both inpatients and outpatients. These are strains of different *Enterobacteriaceae* species isolated from the stool of the same patient. It seems valid to consider that the diversity of species may caused by the very diversity of contaminating strains in the environment, whether hospital or otherwise, or by successive contamination events. Hospital environments, especially some departments (e.g. ICUs), are recognized as being intensely and widely potentially contaminated (as to the diversity of multidrug-resistant strains, in general). But it should be considered that, very often, gene coding for carbapenem resistance is located in plasmids transferable by bacterial conjugation. We cannot rule out the transfer of plasmids among strains (possible even from different species) in the context of the microbiota of individual patients.

Considering what has been reported and discussed above, the stool colonization of patients with community-acquired infections and their domestic contacts may be considered CRE reservoirs, and can determine the risk of dissemination of resistant strains to healthy individuals in the community.

Cano and colleagues, 2018,[24] in a prospective study during an outbreak of KPC-Kp infections in a Spanish hospital, validated both models determining scores indicative of the occurrence of infection (not just sepsis) in patients with intestinal colonization, and a mortality prediction model for established infections. This result allowed the establishment of the most appropriate empirical therapy according to the clinical situation.

Nordmann and colleagues, 2011[3] alert that high rates of carbapenemase-producing *E. coli* infections could be reached worldwide. According to the authors, unlike viral epidemics, such as the H1N1 pandemic, the CRE epidemic would not spontaneously cease.

Conclusion

Our study has limitations because it is based on analyses of clinical material not obtained specifically for the proposed objective, but this material allowed the detection of intestinal colonization of inpatients and outpatients by different species of enterobacteria showing phenotypes of resistance to carbapenems. Analyses involving stool dilutions allowed the detection of CREs, in some cases in dilutions of up to 1:5,000. It is possible that, as occurred with ESBL-producing strains decades ago, infections by carbapenem-resistant strains will become endemic in the community.

Potential conflict of interest

The authors declare no conflicts of interest.

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Smoking in post-bariatric patients without regular medical follow-up and associated weight loss and regain: a cross-sectional study

Marcela P. Paula,[1] Eline C. Romagna,[2,3] Diogo M. F. Mattos,[4] Karynne G. Lopes,[2,3]
Luiz Guilherme Kraemer-Aguiar[2,3,4*]

Abstract

Introduction: Obesity and smoking are associated with an increased risk of cardiovascular disease, reduced quality of life and premature mortality. The relationship between smoking and body weight changes in patients who underwent bariatric surgery is unclear. Objective: We studied post-bariatric patients without any current medical follow-up and compared smokers and non-smokers with regard to weight loss and regain. Methodology: Ninety-four patients post-Roux-en-Y gastric bypass (n=80) or Sleeve gastrectomy (n=14), aged 42±9 years, body mass index (BMI)=32.9±6.5kg/m2, were recruited in public outpatient care and allocated into two groups according to time since surgery < or ⩾5 years (G<5y or G⩾5y, respectively). They were further divided into smokers or non-smokers. Clinical history, physical examination, anthropometrics and hemodynamics measurements were obtained. Results: The prevalence of smoking was 12.8%. BMI, neck circumference, pre-surgical BMI, and rate of weight regain (RWR) were higher in the G⩾5y group than in the G<5y (p⩽0.03) one. No differences in excess weight loss (EWL) were observed between smokers and non-smokers in both groups (p⩾0.15). Higher RWR was detected in non-smokers in G<5y (p=0.03), while no differences between smokers and non-smokers were found for RWR in G⩾5y (p=0.37). Conclusion: Smoking habits do not appear to influence weight loss after surgery. However, a higher weight regain was detected in non-smokers who had less than 5 years since surgery.

1. Departamento de Endocrinologia, Hospital Federal da Lagoa, Rio de Janeiro, RJ, Brazil.

2. Programa de Fisiopatologia Clínica e Experimental, Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

3. Endocrinologia, Departamento de Medicina Interna, Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

4. Programa de Pós-Graduação em Engenharia Elétrica e de Telecomunicações, Universidade Federal Fluminense, Niterói, RJ, Brazil.

5. Unidade de Obesidade, Centro de Pesquisa Clínica Multiusuário, Hospital Universitário Pedro Ernesto, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

*Correspondence address:
E-mail: lgkraemeraguiar@gmail.com
ORCID: https://orcid.org/0000-0002-3528-5881

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Introduction

Excess body fat contributes to several health problems, including the increased risk of metabolic, cardiovascular, pulmonary, and hepatic diseases, cancer, osteoarthritis and premature

mortality.[1-4] Individuals with obesity, even without metabolic abnormalities, present a higher risk of coronary heart disease, cerebrovascular disease, and heart failure compared to normal-weight metabolically healthy individuals.[5] It is known that adipose tissue is an endocrine organ that regulates energy metabolism, insulin sensitivity and vascular homeostasis, and when dysfunctional, links obesity with many metabolic and cardiovascular disorders, and that a pro-inflammatory state probably contributes to these disorders.[6]

Bariatric surgery is considered to be an effective method of long-term treatment for severe obesity.[7] Successful bariatric surgery improves the quality of life and obesity-related metabolic comorbidities, increases self-awareness about unhealthy food habits and active lifestyle, promotes self-confidence, and improves mood and self-esteem.[8] However, the habit of smoking continues to be a concern in clinical practice.[9] For the last twenty years, smoking has been considered a neurobehavioral disease caused by nicotine addiction.[10] In smokers with obesity, medical and surgical interventions may have substantial health impacts.[11]

Abstinence from smoking is recommended for at least 6 weeks before surgery.[9,12] Smokers with obesity have an increased risk of early postoperative morbidity and mortality compared to non-smokers.[13] Infections, prolonged intubation/reintubation, sepsis, shock, and length of hospitalization may occur in these patients.[13,14] In addition, they are at higher risk of long-term cardiometabolic events.[13] Nevertheless, the resumption of smoking can occur in the post-operative period due to worries about decreased weight loss or weight regain.[8,15]

The lack of post-operative follow-up compliance is a concern, since it ensures the benefits of the surgical procedure and the screening of health status and lifestyle habits, such as smoking[9,16] Lack of counseling on smoking habits may have a negative impact on several surgical outcomes.[17] Therefore, we invited post-bariatric patients with no regular medical follow-up and compared smokers and non-smokers with regard to weight loss and regain.

Methodology

Patients

We invited 132 patients who had previously submitted to bariatric surgery to our unit and asked whether they would like to volunteer for this study and to start their follow-up in the outpatient care unit of the University Hospital. A criterion for inclusion was being at least one year without any regular medical follow-up. At the time of their first appointment, none of these patients had any clinical follow-up. Of the 132 patients contacted, 21 declined to participate, one was pregnant, and six dropped out during the study and 10 had <24 months after surgical procedure. A total of 94 patients with post-Roux-en-Y gastric bypass or Sleeve gastrectomy (RYGB, n=80, and SG, n=14), physically inactive, 87.2% females, aged=42±9 years, body mass index (BMI)=32.9±6.5kg/m², excess of weight loss (EWL)=88.7±18.6%, rate of weight regain (RWR)=22.9±20.3%, and mean time since surgery=6.1±4.0 years were included in the study. Patients were allocated to groups according to time since surgery, whether < or ⩾5 years (G<5y or G⩾5y, respectively), and evaluated for smoking status, EWL and RWR, obtained by self-report. Current smoking status was defined as having smoked at least once during the previous week.[8] None of the patients were using post-surgical weight loss medication or were submitted to revisional bariatric surgery. Information about the type of surgery was reported by all participants and later confirmed by digestive endoscopy.

Study design and ethics

This cross-sectional study occurred between November 2018 and December 2019 in the outpatient care unit, a public health unit of the State University of Rio de Janeiro (RJ, Brazil). Clinical history, physical examination, anthropometric and hemodynamic measurements were obtained. This study was approved by the local Ethics Committee (CAAE: 07662918.1.0000.5259) and registered at ClinicalTrials.gov (NCT04193384). All procedures were performed in accordance with the ethical standards of the Helsinki Declaration. A signed consent form was obtained from each participant.

Anthropometric and hemodynamic measurements

Body mass and height were measured using an electronic scale and stadiometer (Welmy™ W300A, São Paulo, SP, Brazil). Neck, waist, and hip circumferences were measured twice by the same trained evaluator. BMI and waist-to-hip ratio were calculated. The EWL and RWR were obtained as follows: a) EWL = (pre-surgical weight - nadir weight)/(pre-surgical weight - ideal weight for BMI of 25 kg/m²)*100, expressed in %; and (b) RWR = (current weight-nadir weight) (pre-surgical weight - nadir weight)*100, expressed in %. The pre-surgical and nadir weight were self-reported by patients, and obtained at two points in time, during the first appointment and 6 months later. Blood pressure and heart rate were measured twice in a sitting position by a semiautomated oscillometric (G-Tech™ BSP11, Hangzhou, Zhejiang, China) device, according to standard recommendations.[18]

Statistical analysis

Data normality was tested using the Shapiro-Wilk test. Between-group differences were compared by unpaired Student-t or chi-square tests and the results were expressed as mean ± standard deviation or frequency (n,%), as appropriate. Pearson correlation coefficients were calculated to test associations between data obtained by self-report (pre-surgical weight and nadir weight). All calculations were performed by NCSS™ statistical software (Kaysville, UT, USA) and KNIME 4.1.0 (KNIME AG, Zurich, Switzerland). A $p \leqslant 0.05$ was considered significant.

Results

A total of 12.8% of patients were smokers, 16% had hypertension, 5.3% had type 2 diabetes *mellitus*, and 4.3% had dyslipidemia. The majority of patients had bariatric surgery performed in the private healthcare system (80%). Anthropometric/hemodynamic measurements and surgery data are displayed in Table 1. The groups were similar in regard to all variables, except for BMI, neck circumference, pre-surgical BMI, and RWR which were higher in G≥5y than G<5y. The correlations between the two pre-surgical weights and nadir weights obtained by self-report were highly associated (r=0.97 and r=0.94; p=0.001, respectively).

Figure 1 presents the EWL and RWR of the groups studied. There were no differences in LWR between smokers and non-smokers in both groups ($p \geqslant 0.15$). Higher RWR was detected in non-smokers than smokers in those who had less time since surgery (p=0.03). On the other hand, no differences were detected between smokers and non-smokers in G≥5y (p=0.37).

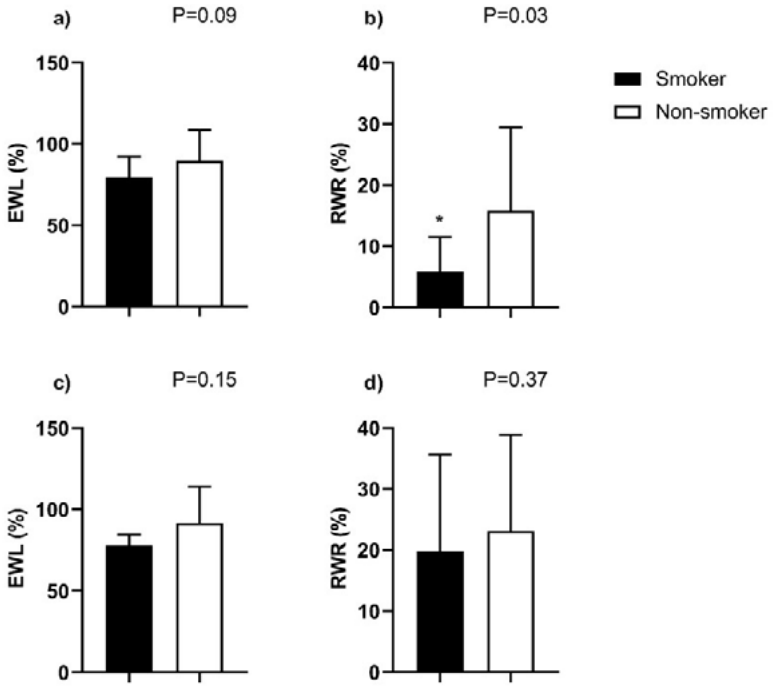
Table 1. Anthropometric/hemodynamic measurements and bariatric surgery data of post-bariatric patients with no regular medical follow-up.

Variable	Pooled sample (n=94)	G<5y (n=55)	G≥5y (n=39)	*p* value
BMI (kg/m²)	32.9±6.5	30.7±4.9*	35.7±7.4	<0.001
Neck circumference (cm)	36.0±3.8	35.3±3.0*	36.7±4.2	0.03
Waist-to-hip ratio	0.83±0.13	0.82±0.20	0.81±0.17	0.80
SBP (mmHg)	124.7±15.7	123.3±15.5	126.8±16.1	0.14
DBP (mmHg)	79.2±11.7	78.4±11.8	80.1±11.7	0.24
Heart rate (bpm)	77±12	75±11	78±10	0.10
Pre-surgical BMI (kg/m²)	48.6±7.7	47.4±7.0*	50.4±8.4	0.03
Nadir weight (kg)	75.8±15.4	74.7±13.6	76.6±17.4	0.27
EWL (%)	88.7±18.6	88.2±18.5	89.9±18.8	0.33
RWR (%)	22.9±20.3	14.5±13.2*	33.7±22.9	<0.001

Legend: G<5y, time since surgery <5 years; G≥5y, time since surgery ≥5 years; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; EWL: Excess weight loss; RWR: Ratio of weight regain *p value, unpaired Student t-test; results expressed as mean±SD

Source: The authors (2022).

Figure 1. Excess weight loss and ratio of weight regain of post-bariatric patients.



Legend: Excess weight loss (EWL, a,c) and ratio of weight regain (RWR, b,d) of studied groups (time since surgery <5 years and ≥5 years, respectively).*P value, unpaired Student t-test; results expressed as mean±SD.

Source: The authors (2022).

Discussion

The present study anaklysed post-bariatric patients with no regular medical follow-up, comparing those with time since surgery < *versus* ⩾5 years according to smoking habits and their relationship to weight loss and regain. All these data were obtained during their first appointment, immediately after consent was given to participate in the study. As expected, the BMI, neck circumference, pre-surgical BMI, and RWR were higher in those with more time since surgery. Loss of weight did not differ between smokers and non-smokers in both groups, while a higher rate of weight regain was detected in the non-smokers than in smokers in those with less than 5 years since surgery.

Bariatric surgery is an effective treatment for obesity because it yields substantial and sustained weight loss and reduces cardiometabolic risk factors.[19,20] Our data confirmed these findings, since the mean EWL was 88.7% and only 16% of the patients had hypertension, 5.3% had type 2 diabetes mellitus, and 4.3% had dyslipidemia. However, high RWR was detected in our sample, especially in those with more time elapsed since surgery (mean of 33.7%). Although multidisciplinary follow-up is recommended,[9] non-compliance with treatment is considered a significant problem by health professionals, since this is associated with an increased risk of weight regain, and consequently, the risk of relapse of comorbidities, reduced quality of life, and several psychological problems.[21,22]

The total number of bariatric surgery operations performed worldwide continues to rise, with nearly 256,000 procedures performed in the U.S. in 2019.[23] The situation in Brazil is similar. According to a survey released by the Brazilian Society of Bariatric and Metabolic Surgery between 2011 and 2018, the number of bariatric surgeries performed increased by 84.73%. However, the lack of medical follow-up in the long-term post-surgery gives cause for concern. In our study, 80% of patients had surgeries performed in a private healthcare system, but failed to maintain a regular medical follow-up due to their declining socioeconomic conditions.

Smoking was self-reported by 12.8% of our cohort. Smoking behavior may vary at different times post-surgery.[15] King and colleagues, 2022[15] detected a rate of increase from 9.6% one-year post-RYGB to 14% in 7 years. Therefore, it is possible that more patients will relapse or start smoking as time passes since surgery. This possibility is very worrying given the relationship between obesity and smoking in relation to increased risks of cardiovascular and chronic obstructive pulmonary diseases, type 2 diabetes *mellitus*, cancers, and also mortality.[11]

A recent systematic review suggested that smoking presents little to no impact on weight loss after surgery compared to abstinence from smoking.[24] Our findings also showed no difference in loss of weight between smokers and non-smokers. However, a higher weight regain was detected in non-smokers with less than 5 years since surgery. Previous studies have documented a relationship between smoking and lower BMI compared to non-smokers in the general population.[25] It is well-known that BMI increases after cessation of smoking, which can be observed even up to 20 years after cessation, according to the Framingham Heart Study.[26] The physiological mechanisms associated with weight gain after smoking cessation are not well understood.[27,28] But, among the factors recognized as being involved are the increase in energy expenditure and the suppression of appetite induced by nicotine, which lead to reduced food intake and changes in eating behavior that alter the energy balance.[27,28]

In recent years, concerns have been raised about the associations of weight gain with poorer clinical outcomes that could attenuate the benefits of quitting smoking.[29-31] Kim and col-

leagues, 2018[29] showed a reduction in the risk of myocardial infarction and stroke regardless of post-cessation BMI change. More recently, a cohort study conducted by Sahle and colleagues, 2021[11] demonstrated that adults who stopped smoking had less risk of mortality than those who continued to smoke, regardless of weight gain. All these data provide important evidence for health care professionals to advise patients before and after bariatric surgery on the importance of clinical and behavioral treatment for both smoking cessation and weight management, and the resultant effects on general health.

This study has some limitations. The cross-sectional design limits our discussion, precluding causal relationships. In addition to that, we considered current self-reported smoking status, and some patients may have stopped or decreased their use of tobacco some time earlier. Although all patients achieved an adequate EWL, different medical teams and experiences with bariatric procedures may imply different surgical technical factors among patients. Finally, considering that weight loss and regain are multifactorial, other etiological factors could be associated with changes in these post-surgery outcomes.

Conclusion

A high prevalence of smokers was detected in our cohort of patients who underwent bariatric surgery and had been without regular medical follow-up for at least one year. The habit of smoking does not appear to influence weight loss after surgery. However, smoking was associated with a lower weight regain in the first 5 years since surgery. Our findings suggest that post-surgery medical follow-up compliance is important to maintain or promote healthier habits and the benefits of surgery in long-term. Further research is warranted to investigate the relationships between smoking and changes in body weight in post-bariatric patients, especially regarding the assessment of cardiovascular risk.

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Conflict of interest

No conflicts of interest.

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Education on blood donation and transfusion from the perspective of a state university extension project

Karoline P. Roriz,[1*] Thayani Cristina N. C. Ferreira,[2] Pablo Antony C. Machado,[2] Kallic B. Fonseca,[4] Lucas S. Ferreira,[1] Andreza B. R. de Oliveira,[1] Regina Márcia R. de Oliveira,[4] Samantha V. Baião,[4] Cirley S. da Silva,[4] Flavia M. G. de C. Bandeira[3]

Abstract

Introduction: The extension project "Permanent education on transfusion therapy: promoting better care from donor to recipient" is aims to impact the learning process on safe blood donation and transfusion through education, social and cultural influences on students and health professionals, as well as on the community. Objective: To promote educational activities and tools related to safe blood donation and transfusion. Methodology: Dissemination of information about blood donation and transfusion topics through social media, educational activities, including other extension projects, and conversation circles were planned. Indicators, such as the number of posts on social media, number of people reached, and events held from March 2021 to August 2022, were considered for quantitative analysis. Results: Thirty-six posts on the project's Instagram® feed reached 270 people. Two events were registered at the University Extension Department and were executed. A conversation circle about blood donation in the context of the LGBTQIA+ community attracted 19 participants and a multidisciplinary team. One action for the "World Blood Donor Day", contributed to a 54% increase in the number of candidates for donation to the blood bank at Pedro Ernesto University Hospital, compared to the previous year. There was a discussion on refusal to receive transfusion based on the book "The Children Act", by Ian McEwan, involving another extension project called "Canga Literária". Conclusion: The extension projects play an important role in education and allowed the inclusion of content that is not part of the medical syllabus, contributing to the dissemination of knowledge.

1. Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro. Rio de Janeiro, RJ, Brazil.

2. Centro Biomédico. Faculdade de Enfermagem, Universidade do Estado do Rio de Janeiro. Rio de Janeiro, RJ, Brazil.

3. Disciplina de Hematologia e Hemoterapia, Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro. Rio de Janeiro, RJ, Brazil.

4. Serviço de Hemoterapia, Hospital Universitário Pedro Ernesto, Universidade do Estado do Rio de Janeiro. Rio de Janeiro, RJ, Brazil.

*Correspondence address:
E-mail: karolineroriz01@gmail.com
ORCID: https://orcid.org/0009-0004-1724-9862

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Introduction

Blood donation and transfusion involve technical, ethical, emotional and philosophical issues that are not always discussed during the training of students and healthcare professionals and are rarely brought up for discussion in the non-academic community. Considering the relevance of university extension projects,[1] and their potential for cultural and social change, an extension project from the Medical Science Department of the State University of Rio de Janeiro (UERJ) was initiated in 2021. The project focused on the educational aspects of blood donation and transfusion as an opportunity to develop teaching strategies within and outside the walls of the university, reinforcing the role of university extension in promoting the democratization of educational and social actions.

The extension project "Permanent education on transfusion therapy: promoting better care from donor to recipient", registered under number 6457 in the Extension Department (DEPEXT) of UERJ, considers the relevance of extension in the process of education and behavioral change from a social and cultural perspective in students, faculty, healthcare professionals and the community, with regard to blood donation and transfusion. Therefore, the project includes actions that involve different sectors of UERJ and the external community, and has the Hemotherapy Service of the Pedro Ernesto University Hospital (HUPE) as its main source of educational material. This multi- and transdisciplinary project includes the active participation of students from medicine and nursing schools at UERJ and various other professionals.

In addition to epidemiological and social changes, the COVID-19 pandemic has fostered technological advances that are aligned with the aspirations of this project, transforming the environment of social media networks, telemedicine and teleconferences into viable tools for the dissemination of knowledge. This work aims to highlight some educational actions on safe blood donation and transfusion from the perspective of an extension project.

Methodology

Goals were established to disseminate information about safe blood donation and transfusion through distance learning education platforms, social media (such as Instagram®), an open dialogue with the public and collaboration with other university projects. Actions undertaken between March 2021 and August 2022 were compiled and analyzed, in order to quantify their outreach potential. The activities were planned and executed according to a calendar and schedule established by the project members, with full involvement of the project's students at all levels of planning and execution. The activities were conducted both remotely and in person. The indicators analyzed were the number of social media posts, people reached, and events organized and executed.

Results

During the period under analysis, 434 followers were counted on the project's Instagram® feed, @hemoterapia.uerj, of which 308 (71%) resided in the city of Rio de Janeiro. With regard to the age breakdown of these followers, 325 (75%) were between 18 and 34 years old, and 342 (79%) identified as a woman. Thirty-six posts were published on this page, which reached 270 people (Table 1). From June to August 2022, project posts received 453 "likes" and 47 people shared the contents of the page. Three events were registered in the DEPEXT of UERJ and executed by the project, two of which were open discussion sessions and one was related to World Blood Donor Day (Table 2).

Table 1. Project Instagram's insights between September 2021-August 2022

Insights	Total N (%)
Followers	434 (100%)
Reach	270
Posts published	36
Stories published	78
Average engagement value	26
Between 18-34 years old	325 (74,88%)
Older than 34 years old	109 (25.12%)
Male	92 (21.24%)
Woman	342 (78.76%)
From Rio de Janeiro	308 (70.97%)
From other cities	126 (29.03%)

Source: The authors (2022).

Table 2. Events created and completed by the project from March 2021 to August 2022

Conversation circle about Blood donation in the LGBTQIA+ context
Conversation circle about Myths and Facts about Blood Donation"
World Blood Donor Day campaign

Source: The authors (2022).

One event that involved another extension project from UERJ was held in June 2022 and happened via Teams software as a synchronic activity on the book "The Children Act".[2] The first conversation circle (Figure 1) was an in-person event and discussed the topic of blood donation in the LGBTQIA+ context. It was attended by 19 people and facilitated by nurses, hematologist physicians, a social worker, and a representative from “Rio Sem LGBTfobia” program, which is part of the public LGBTQIA+ program of the Rio de Janeiro state government. The second conversation circle, held in September 2022, addressed the topic “Myths and Facts about Blood Donation”, with 84 registered participants, of whom 37 (44%) attended, creating an opportunity to clarify many issues related to blood donation and transfusion raised by the participants (Figure 2). The event related to “World Blood Donor Day”, which took place on 14 June 2022 at the Hemotherapy Service of the HUPE, included the active participation of the project, resulting in a 54% increase in the presence of blood donation candidates at the HUPE blood bank and a 59% increase in the number of blood bags collected in comparison with 2021 (data obtained from the Hemote Plus system, used in the Hemotherapy Service of the HUPE). The interaction with another extension project "Canga Literária" from UERJ focused on the topic of blood transfusion among Jehovah's Witnesses, covered in the book "The Children Act" (Ian McEwan, 2014),[2] which served as inspiration for the discussion. The event had the active participation of 18 people via Teams® who read the book and introduced their impressions and questions.

Figure 1. Conversation circle on blood donation by LGBTQIA+

Figure 1. Conversation circle on "Myths and Facts about Blood Donation"

Discussion

The tripod of teaching, research, and extension, contained in Article 207 of the Brazilian Federal Constitution,[3] seeks to strengthen student and teacher training with the purpose of contributing to better living conditions and increased well-being for the people and the country. Resolution No. 7 of December 18, 2018, from the Ministry of Education (MEC),[4] presents guidelines for university extension and mentions the urgency of incorporating extension activities into undergraduate curricula in Brazil.

Blood transfusion is one of the most frequent treatments in the world,[5] and for this to occur, healthcare professionals must have full knowledge of its functions and adverse effects. In addition, the population needs to understand the importance of blood donation and, therefore, must also be provided with information on safe blood transfusion. Extension projects are relevant in providing guidance to the population on blood donation and serving as institutional support for clarification on citizenship issues.[1]

In Brazil and around the world, education on hemotherapy and transfusion medicine in medical schools is deficient, insufficient, or even non-existent.[5-8] Extension plays a key role in the propagation of education and allows for the insertion of content that is not part of the undergraduate curriculum, with the active participation of students. This is the premise of the "Permanent education on transfusion therapy: promoting better care from donor to recipient" extension project. With just over one year of existence, the project seeks to identify unmet needs on the topic through posting of content on social networks, where users can leave questions that are explored in conversation circles, Instagram® posts, and virtual or face-to-face meetings. The focus of the information is directed at both healthcare professionals and the lay community. Social media networks have become a valuable resource for information, including teaching, and have gained strength during the COVID-19 pandemic and their use for the dissemination of knowledge should be maximized.[9,10] They constitute an important supporting tool for the project.

In summary, understanding that extension is an educational tool, discussion groups that explored questions related to blood donation by men who have sex with other men (MSM) and myths and facts about blood donation were also created and encouraged to bring up concerns. Those who signed up for events were invited to ask questions at the time of registration, which were explored during the meetings. From this standpoint, project members were able to realize how much misinformation and doubt exist about several topics. Blood transfusion in the context of people with religious beliefs that do not accept this procedure is an unmet need that needs to be explored inside and outside the university gates.

The limitations of this extension project are related to the difficulties faced by university students in including extension activities in their curriculum, which is often already highly demanding; the lack of scholarships for those involved in the projects; difficulties in raising funds for events; and problems in recruiting people with available time to dedicate to the project. Extension plays a key role in the dissemination of education and allows the inclusion of content that is outside the curriculum of undergraduate studies, in addition to helping bring the university closer to the community,[11] with the active participation of the students. It plays a relevant role in the dissemination of knowledge and engagement with the community and, in the context of the project under analysis, highlights the importance of extension in the impact of transfusion education.

Some actions for the near future are already in the planning phase, one of which is the implementation of an evaluation form on knowledge of hemotherapy and transfusion medicine for medical and nursing students and residents, which will serve as a basis for the development of educational strategies within the university departments involved.

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Gene regulation of MMPs and TIMPs by somatostatin in human fibroblasts

Guilherme N. Carvalho,[1*] Jean N. Santos,[2] Nara S. Araujo,[3] Patrícia R. Cury[3]

Abstract

Introduction: Somatostatin (SST) is a commonly found neuropeptide with general inhibitory functions. The aim of this study is to evaluate the effect of somatostatin in different concentrations on the mRNA expression of MMPs and TIMPs in cultured, human, gingival fibroblasts. Methodology and resources: Human gingival fibroblasts were stimulated with 10^{-4}, 10^{-9} or 10^{-12}M somatostatin DMEM without fetal calf serum; while untreated fibroblasts served as controls. After the incubation period, the RNA was extracted and the first-strand cDNA was synthesized. Alterations in the expression of MMP-1, MMP-2, MMP-3, MMP-7, MMP-11, TIMP-1 and TIMP-2 mRNA were evaluated using real-time polymerase chain reaction (PCR). β-actin mRNA expression was used to normalize the data. Results: After 24 hours of treatment and at the highest concentration, SSTinduced a down-regulation of MMP-1, -2, -3 and -7 expression, and an up-regulation of MMP-11 expression; while at the lowest concentration the substance induced an up-regulation of MMP-1, -2, -3, TIMP-1 and -2 expression. Similar effects were observed after 72 hours of treatment, except for the up-regulation of TIMP-2 at the higher SST concentration, as well as an up-regulation on MMP-7 and -11 expression and a down-regulation of MMP-2 and TIMP-2 expression at the lower SST concentration. Discussion: The modulation of inflammation by SST is still unclear. The findings of this study suggest that SST can modulate the gene expression of MMPs and TIMPs by cultured fibroblasts and that its effects depend on the concentration. This may represent one of the mechanisms of inflammation suppression by SST.

1. Programa de Pós-graduação em Odontologia e Saúde, Universidade Federal da Bahia, Faculdade de Odontologia, Salvador, Bahia, Brazil.

2. Departamento de Patologia Oral, Universidade Federal da Bahia, Faculdade de Odontologia, Salvador, Bahia, Brazil.

3. Departamento de Periodontia, Universidade Federal da Bahia, Faculdade de Odontologia, Salvador, Bahia, Brazil.

*Correspondence address:
E-mail: guilhermencarvalho@hotmail.com
ORCID: https://orcid.org/0000-0002-3595-2496

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Introduction

Homeostasis in the organism is regulated by three interlinked structures: the endocrine, nervous and immune systems.[1] It is increasingly clear that exchanges of information among these

systems are facilitated by the endocrine and/or paracrine release of hormones, neuromodulators and cytokines by any of these systems and by the shared expression of reciprocal receptors for these mediators. Neuropeptides are released from the unmyelinated nerve endings within the central lymphoid organs and peripheral tissues. In addition to their role in endocrine tissues, neuropeptides also play a regulatory role in the human immune system.[2] At the same time, neural cells function as receptors for cytokines, which are released from the immune system in a paracrine fashion and affect neural growth and differentiation.[3] In addition, immune cells can themselves produce neuropeptides, which influence nervous or immune cells in a paracrine or autocrine fashion.[3]

Somatostatin (SST) is a commonly found neuropeptide with a general inhibitory function on hormone release in the anterior pituitary and the gastrointestinal system4. SST also works as a neurotransmitter, immunomodulator, and suppressor of angiogenesis and cell proliferation.[5,6]

SST is released from the capsaicin-sensitive sensory nerve terminals. In the peripheral nervous system, it is found in sympathetic and sensory neurons innervating the lymphoid organs[7]. SST is also found in lymphocytes, macrophages and thymic dendritic cells, as well as in dendritic-type cells near or within the epithelium and single nerve fibers close to the epithelium.[8]

In inflammations, SST shows an anti-inflammatory behavior, generally inhibiting immunoglobulin synthesis, T cell proliferation and splenocyte proliferation[6,9]. In addition, it suppresses the inflammation system by inhibiting the activity of activated dendritic cells on prime T cells[10] and by inhibiting the release of IFN-γ from human T lymphocytes.[11]

Many chronic immune-mediated diseases in which SST is up-regulated, including system lupus erythematosus, rheumatoid arthritis and periodontitis,[12-14] have an important participation of matrix metalloproteinases (MMPs) in their pathogenesis.[15,16]

Usually, MMPs participate in numerous tissue-remodeling processes, in which they are responsible for extracellular matrix degradation. They are involved in physiological processes, such as embryonic development, postpartum involution of the uterus, bone and growth plate remodeling, ovulation, and healing of wounds[17]. Their activity is controlled by the action of tissue inhibitors of matrix metalloproteinases (TIMPs), and an imbalance between levels of MMPs and TIMPs can result in high tissue loss.[17,18]

In periodontitis, MMP regulation seems to occur through interactions between cell-surface receptors and the extracellular matrix, cytokines and growth factors[19]. The expression of MMPs and TIMPs by gingival fibroblasts is regulated by hydrocortisone, epidermal growth factor and substance P *in vitro*.[20-22] However, the impact of SST on the gene expressions of MMPs and TIMPs has not been previously evaluated.

Therefore, we hypothesized that SST can modulate the inflammation response by acting directly on fibroblasts, regulating the expression of MMPs and TIMPs. Thus, the aim of the present study was to investigate whether somatostatin at different concentrations can regulate the expression of the mRNA for MMP-1, -2, -3, -7 and -11, and TIMP-1 and -2 in cultured human gingival fibroblasts.

Methodology and resources

Cell culture in monolayer

The current protocol was approved by the local research Ethics Committee under the number 203/01, and the research was ethically conducted in accordance with the Helsinki Declaration (World Medical Association).

Human gingival fibroblasts obtained from non-smoking, periodontally and systemically healthy gingival explants, were used at the fifth passage[21,22]. Explants were rinsed for 30 seconds in 70% alcohol and stored in Dulbecco's modified Eagle's medium (Sigma, St Louis, MO, USA) containing 10% antibiotic/antimycotic solution (Sigma). The explants were rinsed in phosphate-buffered saline (Sigma) containing 1% antibiotic/antimycotic solution, finely minced into portions of 1–2mm^3, rinsed three times in phosphate-buffered saline and placed in tissue culture dishes. After 24 hours, a thin layer of Dulbecco's modified Eagle's medium containing 10% antibiotic solution was added to the dishes and the medium was supplemented. Within 14–20 days, outgrowth cells were observed. After reaching approximately 70% confluence, the fibroblasts were harvested with trypsin (0.1% Trypsin + 0.1% EDTA, pH 7.2; Sigma) and then subcultured. For the experiments, fibroblasts were plated in 60mm diameter dishes at 3 x 10^5 cells/ml. These cells were maintained in Dulbecco's modified Eagle's medium containing 10% fetal calf serum (Donor Bovine Serum; Gibco, Grand Island, NY, USA) and 1% antibiotic/antimycotic solution. When the culture reached approximately 80% confluence, the cells were washed in phosphate-buffered saline and incubated for 24h in Dulbecco's modified Eagle's medium without fetal calf serum. Cell cultures were then exposed to somatostatin (Sigma) at 10^{-4}, 10^{-9} or 10^{-12} M for 24 and 72 hours in DMEM without fetal bovine serum. Untreated cells served as controls. All experiments were performed in triplicate.

Sample preparation

After incubation, 100-µL aliquots of fibroblast culture medium were collected. Ten microlitres of protease inhibitor cocktail (Sigma) were added and the aliquots were stored at -70°C. The fibroblasts were then trypsinized, resuspended in 100µL of phosphate buffered saline, lysed by adding 0.9mL of TRIzol® Reagent (Invitrogen, Carlsbad, CA, USA) and their RNA was extracted according to the manufacturer's protocol. The A_{260}:A_{280} ratios were measured using a spectrophotometer and were always >1.8. Digestion of single- and doublestranded DNA was performed using DNase I Amp Grade (Deoxyribonuclease I, amplification grade; Invitrogen) according to the manufacturer's instructions. The first-strand cDNA was synthesized. Briefly, 1µg of RNA sample, 1µL of random primer (Random Primers; Invitrogen), 1µL of 10mM dNTP Mix (Invitrogen) and distilled water to 13µL were added to a microcentrifuge tube, heated to 65°C for 5 minutes and chilled on ice. Then, 4µL of 5x First-Strand Buffer and 2µL of 0.1M dithiothreitol were added and incubated at 37°C for 20 seconds. One microlitre of Moloney Murine Leukemia Virus Reverse Transcriptase (Invitrogen) was added and the tubes were incubated at 37°C for 50 minutes.

Real-time PCR

Alterations in the expression of mRNA for the MMP-1, MMP-2, MMP-3, MMP-7, MMP-11, TIMP-1 and TIMP-2 genes were evaluated using real-time PCR (7300 Real Time PCR System, Applied Biosystems, Foster City, CA, USA) and SYBR Green PCR Master Mix (Applied Biosyste-

ms). β-actin mRNA expression was used as a control to normalize the gene expression data. All real-time PCR assays were performed in 96-well optical plates (Applied Biosystems) using the following cycling parameters: 50°C x 2 minutes and 95°C x 10 minutes; PCR cycling for 40 cycles at 95°C x 15 seconds (denaturation), 60°C x 1 minute (annealing) and a dissociation cycle (95°C x 15 seconds, 60°C x 30 seconds, and 95°C x 15 seconds).

Oligonucleotide primers for MMP-1, -2, -3, -7, -11 and β-actin were designed from sequences in the GenBank database using Custom Primers-OligoPerfect™ Designer (Invitrogen). One sample of PCR product obtained with each set of primers was fully sequenced (MegaBace, Amersham Bioscience Corp, CA, USA). The sequencing reactions were prepared using DYEnamic™ ET Dye Terminator Cycle Sequencing Kit for MegaBACE™ DNA Analysis Systems (Amersham Bioscience Corp) in accordance with the manufacturer's protocol. The samples were precipitated using Isopropanol, resuspended in MegaBACE loading solution and sequenced. The primer sequences were deposited in GenBank under appropriate accession numbers, as previously described.[21,22] Primer sequences for TIMP-1 and -2 were obtained from the literature.[23,24] Negative controls with SYBR Green PCR Master Mix (Applied Biosystems) and water were performed for all reactions. To analyze gene expression, the mean (± standard deviation) C_T values (the point at which the amplification curves cross the threshold line, which was adjusted to 0.9) were calculated for each set of reverse transcribed mRNA triplicates. The difference between the expression of the target and the endogenous control gene (β-actin) was then calculated (ΔC_T), and the difference between target gene expression in somatostatin-treated cells and the control cells was computed ($\Delta\Delta C_T = \Delta C_T$ for somatostatin-treated cells minus ΔC_T for control cells). The range of gene expression for each somatostatin concentration was then estimated from the relation $2_T^{\Delta\Delta C}$.

Data analysis

The collected data from all groups were imported to Statistical Package for Social Sciences (SPSS) for Windows software, version 26.0 (SPSS Inc., Chicago, IL, USA). A Kruskal–Wallis ANOVA, followed by the Mann–Whitney test, was used to evaluate the significance of the differential effect of SST on the different target genes and to test the significance of the effect of different SST concentrations on each target gene expression. Significance level was set at 5%.

Results

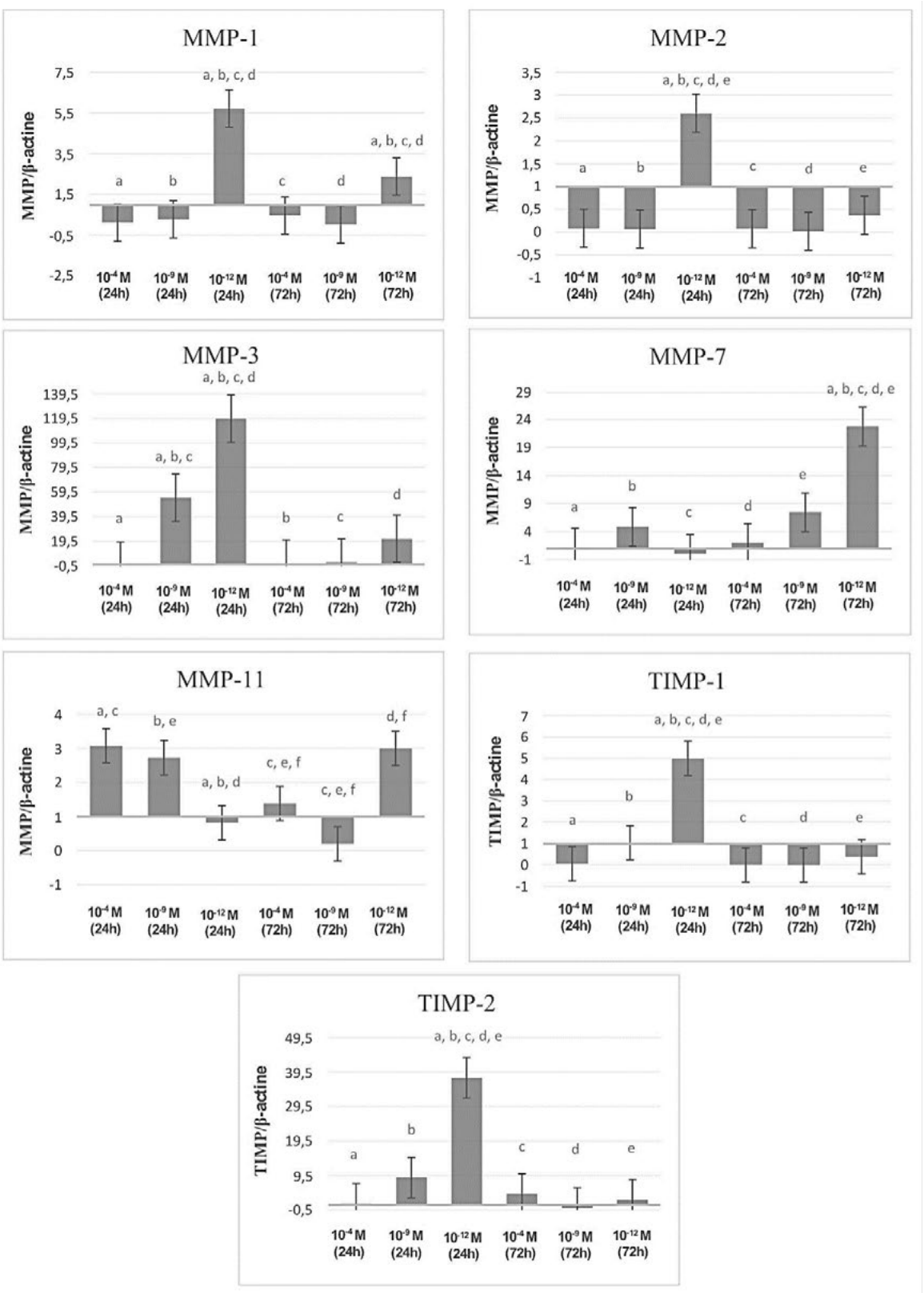
The effect of somatostatin on mRNA expression of MMP-1, MMP-2, MM-3, MMP-7, MMP-11, TIMP-1 and TIMP-2 genes in gingival fibroblasts, is illustrated in Figure 1.

Somatostatin (SST) had no significant effect on β-actin mRNA expression ($p=0.44$), validating the usefulness of the latter as an internal control.

The comparison of different genes showed significant ($p<0.01$) differences in the ratio of up/down-regulation; except for the comparisons between MMP-7 x TIMP-2, MMP-1 x MMP-11 and TIMP-1, MMP-3 x TIMP-2, and MMP-11 x TIMP-1 ($p>0.05$).

At the highest SST concentration and 24-hour exposure, MMP-1 and -2 were down-regulated; however, they were up-regulated at the lowest SST concentration. For 72 hours, SST down-regulated their expression; except for the lowest dose/72-hour exposure, which up-regulated the MMP-1 expression ($p<0.05$) by a factor of 2.

Figure 1. Effect of somatostatin on mRNA expression of MMP-1, MMP-2, MM-3, MMP-7, MMP-11, TIMP-1 and TIMP-2 genes in gingival fibroblasts as detected by semi-quantitative, real-time RT-PCR



Legend: The values (mean±SD) represent the gene expression range for each somatostatin concentration (10^{-12}, 10^{-9}, 10^{-4}M) in 24 and 72 hours, estimated using the expression: $2^{-\Delta\Delta C}_{T}$.Values greater than 1 indicate up-regulation, and values smaller than 1 indicate down-regulation. For values followed by the same letter, the difference was statistically significant.

MMP-3 was down-regulated at the highest SST concentration and up-regulated at the lowest concentration ($p<0.05$). MMP-7 was highly up-regulated at the lower SST concentration in 72-hour treatment; otherwise, MMP-7 was down-regulated or less up-regulated at higher SST concentrations ($p<0.05$). At 24-hour treatment, MMP-11 was up-regulated at higher SST concentrations and down-regulated at the lowest concentration. On the other hand, at the lowest SST concentration, MMP-11 was up-regulated when the exposure time was increased to 72 hours ($p<0.05$).

TIMP-1 was up-regulated at the lowest SST concentration at 24 hours of treatment, and longer exposure resulted in a down-regulation ($p<0.05$). TIMP-2 was highly up-regulated at lower SST concentrations at 24h; however, at 72 hours it was highly up-regulated at the highest SST concentration ($p<0.05$).

Discussion

Our results showed that 24-hour exposure of gingival fibroblasts to SST at a high concentration (10^{-4} M) induced a down-regulation of MMP-1, -2, -3 and -7 expression, and an up-regulation of MMP-11 expression; while at a low concentration (10^{-12}M) the exposure induced an up-regulation of MMP-1, -2, -3, TIMP-1 and -2 expression. Seventy-two hours of treatment still resulted in an up-regulation on TIMP-2 expression at the highest SST concentration, as well as an up-regulation of MMP-7 and -11 expression and a down-regulation of MMP-2 and TIMP-2 expression at the lowest SST concentration. Therefore, the functions of neuronal SST in inflammation are related to a variety of mechanisms, including the effect on immune cells,[9-11] the inhibitory effect on pro-inflammatory neuropeptide release[25] and, as our results suggest, the effect on MMP and TIMP expression in fibroblasts.

The low concentrations of SST in this study are similar to those described during inflammation. The serum concentration of SST in patients with rheumatoid arthritis aged 55 years or older is 1.5×10^{-11}M[26] and the intravitreal levels of SST in diabetics is 4.5×10^{-11}M.[27] Physiological SST concentrations are heterogenous in the organism and the SST concentration of the periodontium was not measured, although our results indicate that it is above 10^{-12}M and below 10^{-9}M.

The anti-inflammatory effect of SST affects many tissues. SST suppresses the inflammatory effect in cutaneous lymphocytic inflammatory and tumoral infiltrates.[28] In human retinal pericytes, SST can neutralize the effects of pro-inflammatory factors, up-regulate pro-apoptotic mediators and down-regulate pro-survival factors, mediated by microglia.[29] In acute pancreatitis, an increase in SST concentrations occurs, which is correlated with interleukin-6 levels.[30] In pancreatic fistulas in rats, the efficacy of the SST analogue in reducing inflammation is higher than that of other drugs, with greater reduction in interleukin-6 and tumor necrosis factor-α concentrations.[31] Systemic or intra-articular injection of SST prompted a reduction of joint inflammation in experimental animals with CFA-induced arthritis.[32] Chronic arthritis in rats is accompanied by the release of SST into the circulatory system, which, in turn, leads to diminished inflammatory response by: (a) inhibiting the release of proinflammatory neuropeptides from afferent nerve endings; (b) acting directly on blood vessels by decreasing vasodilation and plasma protein extravasation; and (c) inhibiting immune cell functions.[33]

This anti-inflammatory effect of SST is related, at least in part, to its action on fibroblasts. The way in which process occurs, however, remains unknown. In synovial membranes of patients

with rheumatoid arthritis, the exposure of fibroblasts to octreotide, an SST analogue, results in the inhibition of pro-inflammatory cytokines (interleukin-15 and tumor necrosis factor-α) and increased concentrations of anti-inflammatory cytokine (interleukin-10).[34]

Proinflammatory neuropeptides, such as substance P (SP) and calcitonin gene-related peptide, are up-regulated in primary afferent neurons in acute and chronic inflammation,[35] and SST, which is an antinociceptive and anti-inflammatory neuropeptide, is also regulated in inflammation.[12,13,21]

The presence of neuropeptides was verified in the gingival crevicular fluid and the periimplant sulcus in healthy and diseased gums. Diseased sites presented an increase in pro-inflammatory neuropeptides and a decrease in neuropeptides related to immunosuppressive effects.[36] In periodontitis, SST mRNA is up-regulated in the mandibular division of the ipsilateral trigeminal ganglion[14] and in SST-immunoreactive cells in the gingival of phenytoin-treated patients.[37] The degree of tissue destruction in those inflammatory diseases may be related to the balance of pro- and anti-inflammatory and nociceptive peptide expression, which regulates the expression of MMPs, the main enzyme associated with extracellular matrix degradation.

SP at a high non-physiological concentration induces a high up-regulation of MMP-1, 2, 3 and 11 and of TIMP-1 and 2 expressions by gingival fibroblasts,[22] which can result in tissue breakdown. Furthermore, according to the present study, SST at high concentration, in general, induces down-relation of these genes, which can inhibit tissue breakdown. The controversial findings for the different MMPs expressions in this study may be related to their functions. MMP-11 has low destructive potential and is incapable of degrading proteins with major relevance in the extracellular matrix,[38] while MMP-7 shows similar levels in crevicular fluid to patients with periodontal diseases in comparison with healthy patients, indicating a role in innate host defense of periodontal tissues.[39]

Our results may clarify how SST can modulate the inflammatory process. However, this is an *in vitro* study, and its findings may not represent how this process actually occurs *in vivo*. New research on cell viability using these concentrations of SST should be performed, in an attempt to ensure that they are not toxic. Furthermore, the lack of western blotting to confirm real-time PCR findings is another limitation.

Conclusion

Somatostatin can modulate the expression of MMPs and TIMPs in cultured gingival fibroblasts. In general, at high concentrations SST down-regulates MMP expression and up-regulates TIMP-1 expression,. This may represent one of the mechanisms of inflammation suppression by somatostatin.

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Rupture of pancreatic pseudocyst and therapeutic conduct: a case report

Victor P. Graciano,[1*] Andressa C. Rodante,[2] Idari F. O. Netto,[3] Alessandro S. Pin,[3] Ludymilla O. P. Lacerda,[4] Gabriela M. Pontes,[4] Ricardo D. Marciano[1]

Abstract

Introduction: To report on a clinical case of a pancreatic pseudocyst that evolved to rupture and to understand the main therapeutic approaches adopted for pancreatic pseudocysts, thus fostering a better approach to such cases. Case report: Female patient, 30 years old, with pseudocystoadenoma and acute abdomen due to cystic rupture. Discussion: The therapeutic forms for pancreatic pseudocysts are expectant management, drainage (endoscopic and percutaneous) and surgical treatment. Results: The best approach addresses the treatment of the patient's clinical condition and pancreatic pseudocyst. For this to happen, a clinical evaluation, by means of complementary examinations, is required.

1. Centro Universitário de Mineiros. Trindade, GO, Brazil.

2. Hospital de Urgência de Goiânia. Goiânia, GO, Brazil.

3. Centro Universitário de Goiatuba, Unicerrado. Goiatuba, GO, Brazil.

4. Universidade de Rio Verde Campus Aparecida de Goiânia, Faculdade de Medicina. Aparecida de Goiânia, GO, Brazil.

*Correspondence address:
E-mail: victorgraciano41@gmail.com
ORCID: https://orcid.org/0000-0003-3643-2481

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Introduction

Pancreatic pseudocysts are, in most cases, a complication of acute pancreatitis; however, they can be caused by cystic pancreatic neoplasms, which correspond to about 10% of cystic lesions. There are several ways to classify cystic formations of the pancreas. These include division by epithelial lining, which can be of type 1, 2 or 3. The first is defined as absence of epithelial lining (pseudocyst), and is the factor that differs from type 2, in which an epithelial lining (mucinous cystadenomas and serous cystadenomas) exists. Finally, type 3 is characterized by the degeneration of solid lesions (papillary cystic-solid tumors, ductal adenocarcinomas, and neuroendocrine tumors).[3]

Pseudocysts are composed of a collection of fluids, rich in amylase, usually close to the head and body region of the pancreas, which encapsulates and forms cystic lesions of peripancreatic and/or intrapancreatic origin. This cystic formation is characterized by a well-defined capsule with solid material inside and an absence of epithelial lining. The collection accumulates in a more localized way and becomes delimited after 4 weeks. After this period, it may present a necrotic character with solid characteristics.[5]

This study aims to report a clinical case of pancreatic pseudocyst and to discuss the main therapeutic forms adopted for this pathology, thus showing the most appropriate conduct for these rare cases.

Case report

A 30-year-old female patient, a chronic alcoholic, reported that she had started to experience epigastric pain, nausea and weight loss about 2 months previously. It evolved with asthenia, hyporexia, vomiting (containing only food remains) and a continuous band-like pain in the abdomen with irradiation to the back when the pain intensified. She did not report fever, dyspnea, dysuria or other complaints.

The exam requested was an abdominal tomography, which showed a unilocular expansive lesion in close contact with the body of the pancreas and without communication with the main pancreatic duct.

The case evolved into an acute clinical picture of intense, colicky pain, with an intensity of 10/10, located in the hypochondrium and left flank. In addition, she was sweating, tachycardic, hypotensive (90/50mmHg), with a rigid abdomen, presenting pain on superficial and deep palpation, with pain on sudden decompression in the hypochondrium and left flank. An exploratory laparotomy was performed. Intraoperatively, a greenish fluid with associated debris was identified, the result of the rupture of a pseudocyst. Pseudocyst-gastric bypass, pseudocyst drainage, exhaustive lavage and closure in layers were performed.

Discussion

Numerous therapeutic approaches exist to treat pancreatic pseudocysts that result from acute pancreatitis or from an exacerbation of chronic pancreatitis. The interventions that may be adopted are expectant management, endoscopic drainage, percutaneous drainage, and surgical treatment. In the case of the last three interventions and in the absence of complicating factors, the procedure can be delayed for up to 6 months after the initial episode of pancreatitis, since this allows the wall of the pseudocyst to thicken and mature.[6]

Expectant management

The choice of expectant management is especially appropriate in the case of small, asymptomatic pseudocysts. Spontaneous resolution is common, especially in clinical cases that occur after an episode of acute pancreatitis. It is worth mentioning that stable pseudocysts, i.e., those that do not increase in size, rarely cause symptoms and the gold standard for the treatment of uncomplicated pseudocysts is conservative. Conservative actions include the use of analgesics, antiemetics and a hypocaloric diet.[6]

Endoscopic drainage

This type of procedure is often used when the pseudocyst is close to the stomach and duodenum. It is a viable alternative for pseudocysts in which the site comprises dozens of collateral vessels. When symptoms, infection or pseudocyst growth occur for at least 4 weeks after the onset of pancreatitis and no complications arise, endoscopic ultrasound-guided drainage is the gold standard treatment.[1,7]

Percutaneous drainage

This type of procedure is performed only in critically ill patients who cannot tolerate surgical or endoscopic procedures, or in those with an infected or complicated immature pseudocyst, since percutaneous drainage does not require wall maturation before intervention.[6]

Surgical procedure

Surgical treatment is the most invasive and definitive form of resolution of pancreatic pseudocyst presentations. Drainage can be either external or internal. The first is used in the case of pseudocysts, whether complicated or not, when the wall covering the collection still does not present an adequate consistency for internal drainage to be carried out, but this treatment is currently in disuse. Internal drainage is the surgical treatment most often used by surgeons today. For this to occur, the exact location of the pseudocyst must be known.[6]

Internal surgical drainage

The type of procedure used will depend on the location and characteristics of the pseudocyst. The location can be in the head, body or tail, and treatment will depend on whether the pseudocyst is fistulized or not, as will be classified in the following text.

If the pseudocyst is in the head of the pancreas and does not have a fistula with a pancreatic duct, the best procedure is to perform an omentoplasty. On the other hand, if a fistula is present between the pancreatic duct and the cystic formation, an anastomosis between the pseudocyst and the digestive tract is the preferred procedure. Care must be taken with drainage in the second duodenal portion to avoid injury to the duodenal papilla or the terminal bile duct. If the pseudocyst is in the body of the pancreas and no fistula exists, the recommended procedure is unfolding with omentoplasty. In the case of fistulization between the pancreatic duct and the cyst, a central pancreatectomy or anastomosis between the pseudocyst and the digestive tract is performed. In cases where the pseudocyst is in the tail of the pancreas, with a fistula between the pancreatic duct and the cyst, the recommended procedure is a distal pancreatectomy or budding procedure with omentoplasty.[3]

This classification has a level of evidence-5 and recommendation-4 (expert opinion), and all types of procedures must be associated with the use of albendazole, 1 week before surgery and up to 2 months postoperatively.[2]

In the clinical case under discussion, an abdominal tomography was performed, which showed a unilocular expansive lesion in close contact with the body of the pancreas and with no communication to the main pancreatic duct, in addition to lateral rejection of the stomach. With these findings, it was possible to suggest the course of treatment to be performed. Among the available treatments, the most viable ones could be surgery with anastomosis between the pseudocyst and the gastric tract, endoscopic drainage, expectant management, or even percutaneous drainage, due to the location of the liquid collection.

However, since the patient presented unstable hemodynamic activity, extreme weight loss and subacute malnutrition, the initial management was preoperative nutrition. However, after 20 days, the clinical picture worsened with the appearance of a possible acute abdomen, presence of abdominal distension, flat abdomen, tachycardia and intense acute pain. Associated with this condition, imaging tests found a large amount of free fluid in the abdominal cavity. At

that moment, the main hypothesis was a ruptured pancreatic pseudocyst and, in these circumstances, the best course of action was an exploratory laparotomy with pseudocystogastroanastomosis, including collection of material for culture without biopsy of the ruptured material.

In pseudocysts with a retrogastric location, the main invasive surgical technique for drainage is pseudocystogastroanastomosis (anastomosis of the pseudocyst with the posterior gastric wall), which is known as the *Jurasz* technique. This aims to establish communication of the pseudocyst with the anterior wall or back of the stomach, so that the collection drains into the stomach.[4]

Conclusion

The best management for cases of pancreatic pseudocyst will depend on the patient's clinical state, the complications present and their location.

A review of the bibliography led to the conclusion that, due to the specific case and the numerous forms of treatment, initially, the patient was hemodynamically unstable, malnourished and incapable of being subjected to definitive invasive procedures. However, after evolving into a clinical picture of acute abdomen with a rare rupture of the pseudocyst, the best therapeutic approach was an exploratory laparotomy with pseudocystogastroanastomosis, due to its location in the body of the pancreas.

Therefore, one can conclude that the ideal scenario in cases of cystic lesions of the pancreas is clinical evaluation, imaging and programming of the most appropriate therapy, especially because the majority of these cases are pancreatic pseudocysts after episodes of acute pancreatitis. However, as an acute situation of urgency existed in the case under analysis, the surgical procedure was mandatory, with no time to investigate the etiology of the cystic lesion initially found. The patient had a good postoperative evolution, with no signs of recurrence or growth of the lesion, a fact that suggests that the condition was a pancreatic pseudocyst and not a neoplastic cystic lesion of the pancreas.

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Compensatory treatment for hyperdivergent skeletal Class II using temporary anchorage device

Luíza T. Vilela,[1*] Leonardo Koerich,[2] Katherine Judith C. M. P. Silver,[1] Margareth Maria G. de Souza,[1] Antônio Carlos de O. Ruellas[1]

Abstract

Introduction: The treatment of hyperdivergent skeletal Class II is one of the most challenging tasks facing orthodontists. With the advent of temporary anchorage devices, patients who were previously submitted to orthognathic surgery may be favored by a counter-clockwise rotation of the mandible, resulting from vertical control of the posterior teeth. Also, cases with increased incisor display at rest may benefit from these biomechanics in the anterior segment. Methodology and resources: This case report illustrates the successful treatment of a patient with hyperdivergent skeletal Class II malocclusion resulting in an unaesthetic smile with excessive gingival display when smiling and absence of passive lip seal. Results: The temporary anchorage device produced a suitably functional and aesthetic result, with the correction of the increased gingival exposure, passive lip sealing and improved angulation of the mandibular plane. In addition to the conventional cephalometric superimposition, three-dimensional superimposition was performed and evaluated to validate the treatment outcome. Discussion: Although it does not replace orthognathic surgery, this modality of treatment may benefit patients who are unwilling to undergo a more invasive procedure.

1. Departamento de Odontopediatria e Ortodontia. Universidade Federal do Rio de Janeiro. Rio de janeiro, RJ, Brazil.

2. Private Practice - Charlotte, North Carolina, United States.

*Correspondence address:
E-mail: luizavilela@ortodontia.ufrj.br
ORCID: https://orcid.org/0000-0002-6306-8868

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Keywords: Gummy smile; Three-dimensional superimposition; Orthodontic anchorage procedures; Skeletal anchorage.

Introduction

Treatment of hyperdivergent skeletal Class II is one of the most challenging tasks facing orthodontists. The use of appropriate orthodontic devices is very important to control the vertical dimensions during the orthodontic treatment of hyperdivergent patients.[1] This condition is often caused by clockwise rotation of the mandible or excessive vertical growth of the posterior segments,[2] particularly maxillary vertical alveolar growth.

Surgical-orthodontic treatment is often the best approach when the condition has a skeletal origin, such as vertical maxillary excess. However, patients are not always willing to undergo

surgery, which makes compensatory treatment an option.[2,3] To achieve success, the nonsurgical treatment needs to induce a counterclockwise rotation of the mandible.[2,4]

The advent of temporary anchorage devices (TADs, mini-implants, MI, or mini-screws), by which the direction and the amount of force are carefully controlled, enables the successful achievement of maxillary molar intrusion.[5,6] Skeletal anchorage has enabled the treatment of some problems that were previously treated only by orthognathic surgery.[7] Patients who are referred for orthognathic surgery may be favored with the intrusion of the posterior teeth through the use of TADs and resulting counterclockwise rotation of the mandible.[8]

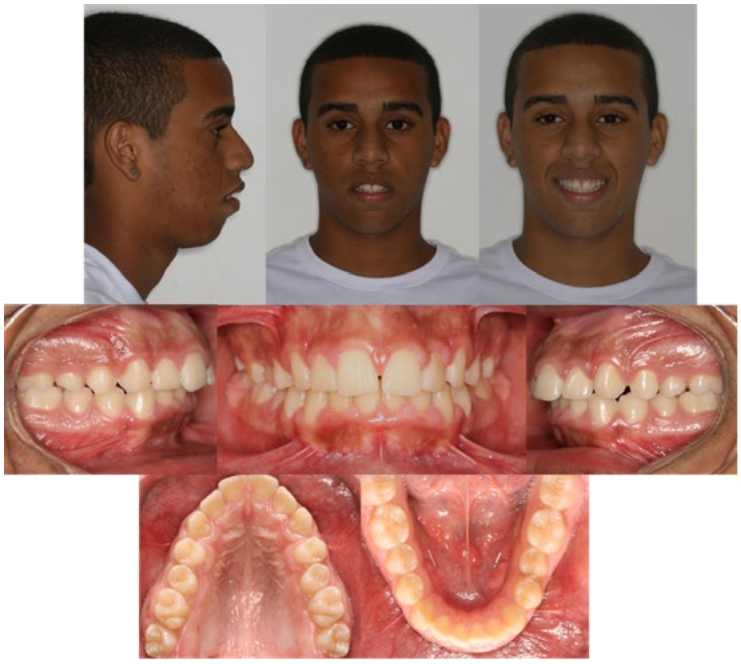
The widespread use of TADs is attributable to their relatively simple installation and to the fact that force can be applied immediately after installation. Therefore, the purpose of this report is to present a treatment option for a hyperdivergent skeletal Class II patient with increased gingival display and increased lower facial height.

Diagnosis and etiology

A 14-year-old male patient in good general health sought orthodontic treatment at the orthodontic clinic of the Federal University of Rio de Janeiro. He was unhappy with the position of his anterior teeth.

Extra-oral evaluation revealed a convex Class II profile. No evident facial asymmetry was found. The patient had a hyperdivergent facial pattern, increased lower facial height with (clockwise) mandibular rotation, retrognathic chin, incompetent lips at rest, short nasolabial angle and obtuse mentolabial angle. The pre-treatment intra-oral photographs (Figure 1) revealed a Class I molar relationship with mild dental crowding in the mandibular dentition, moderate overbite and a 5.0mm overjet. The lower midline was deviated by 1mm to the left. There was Bolton discrepancy of 3.7mm of excess in the lower arch, including 1.7mm in the anterior region.

Figure 1. Pretreatment facial and intraoral photographs.



Source: The authors (2023).

Evaluation and analysis of cephalometric radiography showed absence of facial asymmetries and revealed skeletal Class II (ANB = 9.7o) with protrusive maxilla (SNA = 89.2o) and high mandibular plane (SNGoGn = 41.4o). The upper incisors were upright in relation to the NA line. The lower incisors were protruding and projected in relation to the NB line, but relatively well-positioned in relation to the mandibular plane (IMPA). The results of the cephalometric analysis are presented in Table 1 and Figure 2.

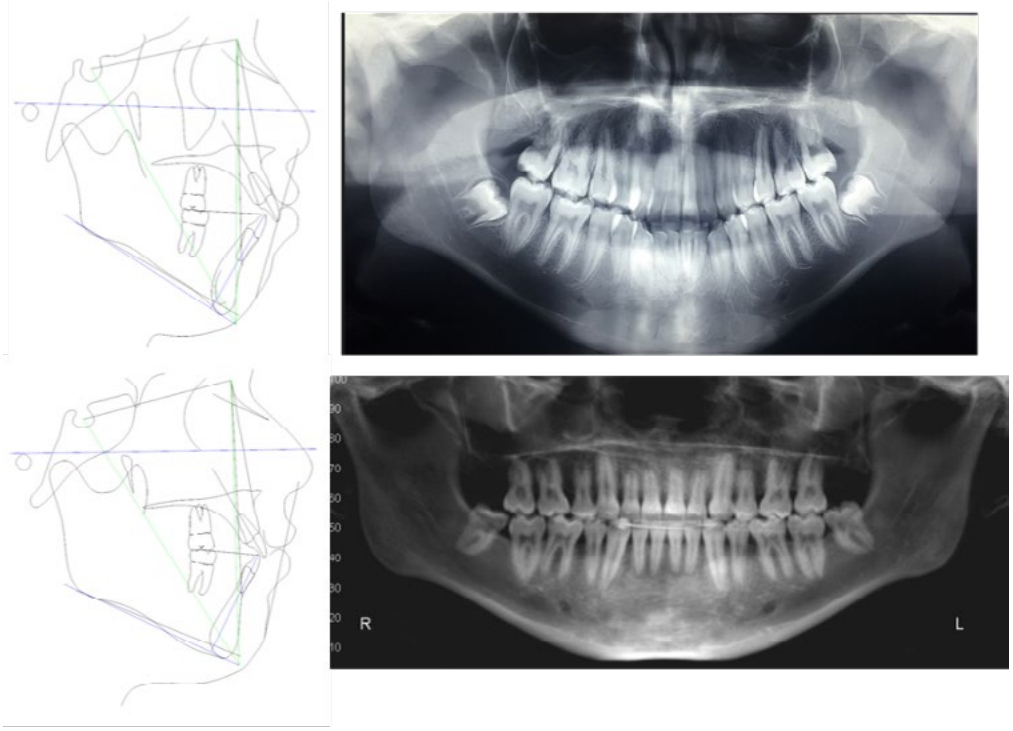
Table 1. Cephalometric measurements

Measurement	Pretreatment	Posttreatment
SNA	89.2	87.5
SNB	79.5	80.1
ANB	9.7	7.4
SND	75.6	75.7
NA.A.Pog	21.4	16.9
SN.GoGN	41.4	35,7
FMA	31.1	31,0
SGn-FH	58.9	58.4
U1-NA (mm)	2.8	0.9
U1-NA (°)	18	20.2
L1-NB (mm)	12.1	9.3
L1-NB (°)	30.9	29.2
IMPA	88.1	91.4
Pl.Ocl.SN	17.5	21.5
S.Ls	5	3.3
S.Li	10	4.4

Legend: SNA: SNA angle, indicates the position of the maxilla; SNB: SNB angle, indicates the position of the mandible; ANB: ANB angle indicates the maxilla-mandible relationship in the anteroposterior direction; SND: angle formed by line SN to point D; NA.A.Pog: convexity angle; SN.GoGN: angle formed by lines S-N and Go-Gn; FMA: angle formed by the mandibular plane and the Frankfurt plane; SGn-FH: U1-NA (mm): the distance between the tip of the upper incisor and a line from nasion to point A; U1-NA (°): angle that measures the inclination of the upper incisors; L1-NB (mm): distance from the most anterior part of the lower incisors to the NB line; L1-NB (°): angle that measures the inclination of the lower incisors; IMPA: angle between the mandibular plane and the long axis of the lower central incisor; Pl.Ocl.SN: angle formed between the SN line and the Occlusal Plane S.Ls: distance from the most prominent point of the upper lip to the S line; S.Li: distance from the most prominent point of the lower lip to the S line.

Source: The authors (2023).

Figure 2. Panoramic radiograph and lateral cephalometric tracing: A) Pretreatment; B) Posttreatment.



Source: The authors (2023).

Treatment objectives

Orthodontic treatment was introduced with the aim of: (1) alignment and leveling of the upper and lower teeth; (2) retraction of the maxillary and mandibular incisors, providing normal overjet and overbite; (3) vertical control using MI to allow for mandibular autorotation; and (4) functional correction to achieve competent lips and to reduce mentalis muscle strain.

Treatment alternatives

The first treatment option suggested was orthognathic surgery with the aim of obtaining skeletal correction and a pleasant facial profile. The procedure would combine jaw surgery with maxillary impaction, using counterclockwise mandibular rotation to reduce the long lower facial height and genioplasty to balance the facial profile. However, the patient and his family refused to accept the surgical proposal.

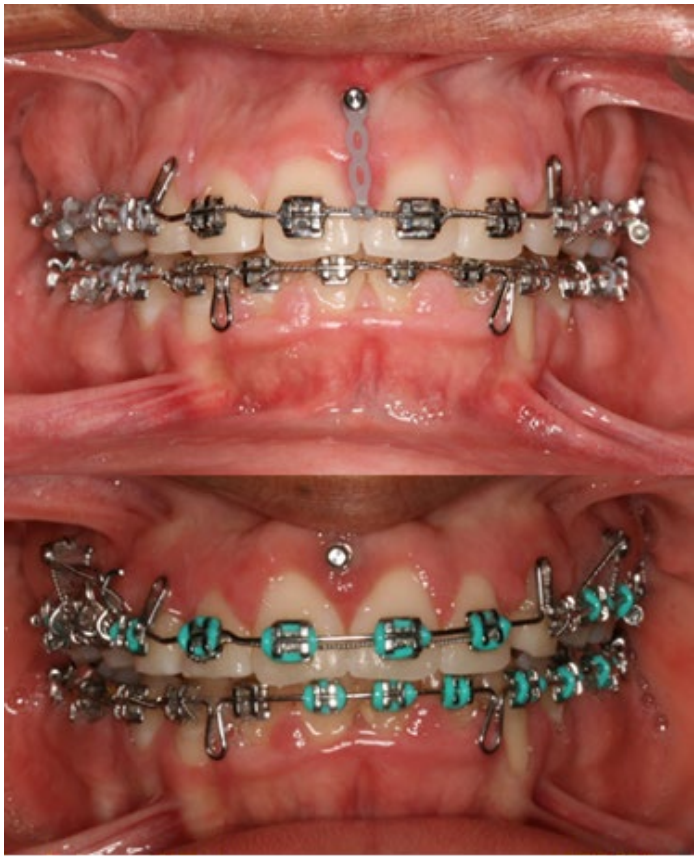
The second alternative was an orthodontic camouflage treatment, with the extraction of the 4 second premolars and introduction of directional force using TADs (mini-implants) to promote maxillary teeth intrusion or to restrain the maxillary vertical alveolar growth. TADs can provide absolute anchorage not only for anteroposterior movements, but also for intrusions of the maxillary anterior and posterior teeth. This treatment would facilitate a counterclockwise mandibular response, leading to a more prominent chin and a balanced facial profile.

Treatment progress

Standard edgewise orthodontic accessories were used. After extraction of the upper and lower second premolars, a pair of 0.018" stainless steel (SS) archwires were made for alignment and

leveling, followed by a 0.018"x0.025" SS archwire and the distalization of the first premolars. Canine retraction was subsequently performed, using a power chain with simultaneous loss of antero-posterior anchorage, particularly in the lower arch. Upper and lower 0.019"x0.025 SS archwires were made with tear drop loops on the distal side of the lateral incisors for incisor retraction with 1.0mm activation and incorporation of a gable effect in the upper archwire. The retraction started in the lower arch and was followed by upper arch retraction. A TAD (8mm x 1.5mm, Morelli, Brazil) was inserted in the upper midline (between the roots of upper central incisors) for intrusive biomechanics with a power chain supported directly from the TAD to the archwire with approximately 45g (0.45 Newtons) of initial force (Figure 3A). This force was gradually increased to 80g (0.8 Newtons). One TAD was also placed on each posterior side, between the maxillary first molars and maxillary first premolars for vertical control of the maxillary posterior teeth, linking the archwire to the TAD with a power chain (Figure 3B). After reaching adequate vertical control, a metallic tie was placed to link the TAD to the archwire and stabilize the anterior and posterior segments until the orthodontic appliance was removed.

Figure 3. MI in the midline, in order to intrude the anterior segment and MI posterior to upper space closure phase through sliding-jig distalization mechanics.



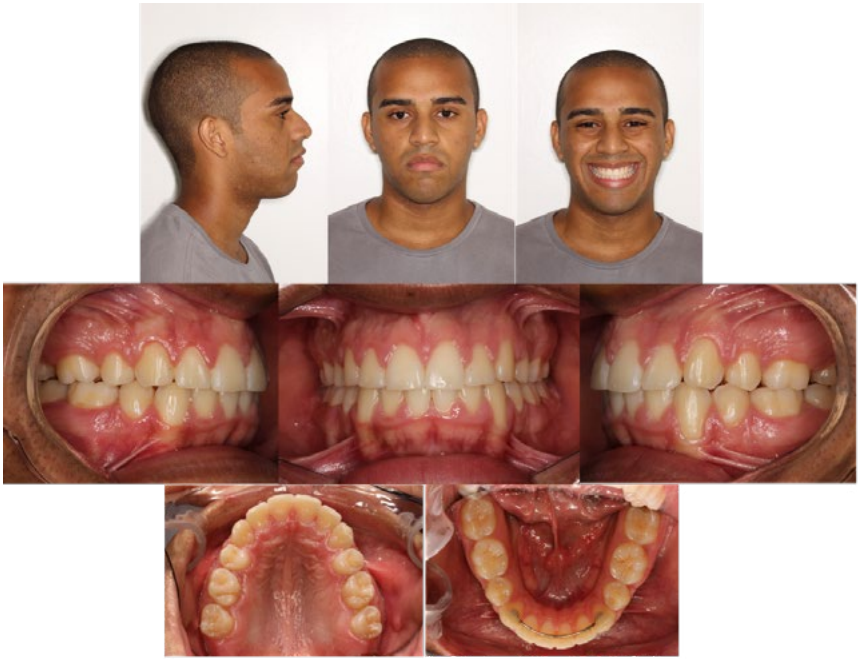
Source: The authors (2023).

Treatment results

Photographs were taken after debonding (Figure 4). Retention consisted of 3x3 lower bars bonded only at canines in the mandibular arch (lifetime) and a circumferential removable retainer in the maxillary arch (20 hours/day, first year; 12 hours/day, second year).

Evaluation of the treatment results showed a well-balanced and harmonious face resulting from less protrusive lips, improved lip seal, reduction of mentalis muscle strain, adequate display of incisors at rest, shorter lower anterior facial height and a more prominent chin.

Figure 4. Posttreatment facial and intraoral photographs.



Source: The authors (2023).

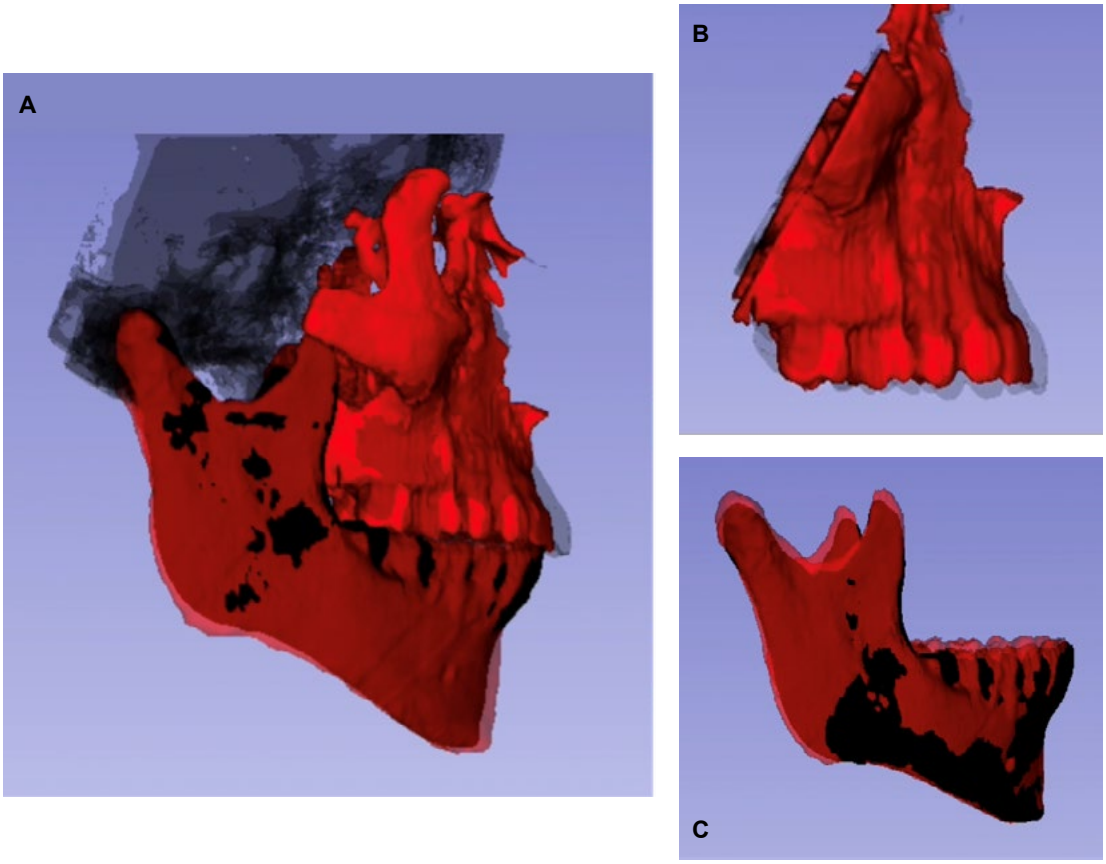
The excessively gummy smile was corrected by use of intrusive forces on the maxillary arch. An acceptable intercuspation of the teeth, good arch form, normal overjet and overbite relationship are shown in the intraoral photographs. A slight lower midline shift to the left occurred. Radiographic examination showed that good root parallelism was obtained and that the upper incisors presented mild apical root resorption (Figure 2B). The patient was referred for extraction of lower third molars.

Pretreatment and post-treatment 3D superimposition were performed (Figure 5), which demonstrated the achievement of a proper mandibular response (counterclockwise rotation) through correct directional forces (Figure 5A). The maxillary anterior teeth were retracted and intruded, while the maxillary posterior teeth were intruded (Figure 5B). The mandibular superimposition (Figure 5C) displays small condylar growth and slight incisor retraction. The results of the post-treatment cephalometric analysis are displayed in Table 1.

Discussion

The aim of this paper was to describe the orthodontic treatment with TADs of a patient with hyperdivergent skeletal Class II, retrognathic chin, excessive upper incisor display at rest, an unaesthetic gummy smile and absence of passive lip seal. Controlling the vertical dimension can be challenging, especially because high mandibular angles tend to increase during facial development.[1] Since the intrusion of posterior upper teeth is a difficult movement for patients with long faces, associated orthognathic surgery may be required. According to Wang et al,[2] or-

Figure 4. Three dimensional superimposition: A) cranial base superimposition; B) maxillary superimposition; C) mandibular superimposition.



Source: The authors (2023).

thodontic surgical treatment is often implemented in order to achieve a successful treatment for patients with skeletal involvement, clockwise mandibular rotation and gummy smile.

The effectiveness of using temporary anchorage devices during orthodontic treatment has been highlighted in previous studies,[6,9,10] since different types of orthodontic tooth movement can be achieved through their use. Furthermore, the use of TADs offers better anchorage control, in addition to eliminating the dependence on patients' compliance with the wearing of rubber bands and extra-oral appliances.[8]

In this case, the prediction of growth potential revealed that the patient had already passed the peak of the growth spurt, but some facial growth could still be expected. The biomechanics with TADs were intended to restrain the remaining vertical alveolar growth and effectively achieve intrusion of the upper teeth. These biomechanics corroborate those used by other authors,[2,6,11] who performed compensatory orthodontic treatment of skeletal Class II patients with severe high mandibular angle. TADs were used for vertical control, intrusion of both the anterior and posterior segments, and consequent favorable counterclockwise rotation of the mandible, thereby achieving successful results. According to a recent systematic review and meta-analysis,[10] mini-implants seem to be more effective than conventional anchorage for vertical control of class II treatments in adolescents after pubertal growth peak when extraction is prescribed.

Intrusion of the maxillary molars is difficult to accomplish through traditional methods of anchorage.[1,6,8] One of the limitations is related to the proximity of the upper molar roots to the sinus floor. According to Abdulghani et al,[12] intrusion of the upper molars of hyperdivergent patients is subject to a higher risk of root resorption due to the possible risk of cortical bone encroachment. We recommend a careful evaluation of the CBCT image before planning the intrusive treatment.

In 1967, Creekmore[1] mentioned that many orthodontic problems could be solved if the vertical growth of the face could be controlled. Orthodontists have tried to successfully control the vertical dimension of their patients and obtain mandibular counterclockwise autorotation using different approaches and this subject has been discussed in the literature. Wang et al[13] reported a satisfactory correlation between the amount of upper maxillary repositioning (after surgical maxillary impaction) and mandibular autorotation. Another successful method was reported by Kassem[7], who achieved mandibular autorotation with the intrusion of maxillary posterior teeth anchored in TADs (mini-plates), and confirmed by Kim et al,[14] who used mini-implants. Our treatment results for the described case report are similar to those described by Kassem[7] and Kim et al,[14] who obtained intrusion of the posterior segment with consequent mandibular autorotation and a more prominent chin. The findings of previous authors[15] show that adolescent patients tend to display more favorable effects from mandibular counterclockwise autorotation than adult patients.

Hart et al,[15] when performing open bite treatment in patients with and without growth, reported that the intrusion of upper posterior teeth using TADs is an effective non-surgical treatment modality, confirming the results found in the present case report. The camouflage treatment required extractions and differential displacement of teeth to compensate for jaw discrepancy (upper incisors displayed larger retraction than lower incisors, as shown in Figure 4), which can be an option to avoid the expenses and risks associated with orthognathic surgery.

The stability of this type of treatment may be questioned. The literature reports that the stability of molar intrusion using TADs can be considered relatively similar to that associated with surgical approaches, since 10 to 30% of relapse occurs.[16] Dental intrusion and associated orthopedic corrections, resulting from the use of TADs, remained stable post-treatment for growing patients.[17] The application of an appropriate retention method after debonding effectively enhances the long-term stability of total arch intrusion treatment.[18] The recovery of proper muscular function (passive lip seal, nasal breathing, normal swallowing and speech) is very important and usually requires an interdisciplinary approach for the treatment of hyperdivergent cases with incompetent lips and mouth breathing.

In the reported case, part of the mentalis muscle strain still persists, due to the reduction in upper lip length, but the smile is esthetically pleasant at the end of the orthodontic treatment. The lower midline shift could have been better managed during the space closure phase. TADs could also have been used in the posterior lower dentition to restrain mandibular alveolar growth and to obtain an even shorter lower third of the face. A genioplasty could improve the facial esthetics but, as mentioned above, the patient has refused to undergo surgery. The case has been followed-up for 5 years and has remained stable overall.

Conclusions

A patient with skeletal class II malocclusion with high mandibular plane was successfully treated using TADs for maxillary vertical control. The results of the orthodontic treatment were achieved: a Class I molar and canine relationship; successful esthetics and function, as evidenced by adequate gingival display when smiling; adequate incisor display at rest; more prominent chin; and harmonious facial profile. This modality of treatment may benefit patients who are unwilling to undergo a more invasive procedure.

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BJHBS

Impact of zinc supplementation on male infertility: a systematic review

Jean F. de Lima,[1] Natália V. S. Daniel,[1] Luciana Pietro[2*]

Abstract

Introduction: According to the World Health Organization (WHO), infertility is defined as a failure in fertilization without any use of a contraceptive method for at least 12 consecutive months, with the individual being of reproductive age and having an active sexual life, where male infertility has been assuming a prominent position. This condition is considered to be of high importance in the contemporary world. Objectives: To systematically review the literature on the impact of oral zinc supplementation on male infertility and to assess whether its intake exerts a therapeutic effect, based on the evaluation of seminal parameters, according to manuals published by the WHO. Methodology: A systematic review was carried out by means of a search, in the English and Portuguese languages, for original articles in the Pubmed, Science Direct, Lilacs and Scielo scientific databases. Results: The intervention groups ranged from 18 to 77 individuals and the control groups ranged from 8 to 113 individuals. Regarding age, the average age of the participants in the studies was 31 years. The daily dose of zinc administered in the trials varied from 66mg to 500mg and its use time ranged from three to six months. In the end, 87.5% of the studies showed positive results on the sperm parameters evaluated after the intervention. Conclusion: The results were mainly attributed to the protective effect that the trace element provided, reducing the lipid peroxidation index of plasma membranes and improving DNA integrity, thus increasing the fertilization capacity.

1. Departamento de Nutrição, Universidade Paulista Unip. Campinas, SP, Brazil.

2. Departamento de Biomedicina, Universidade Paulista Unip. Campinas, SP, Brazil.

*Correspondence address:
E-mail: lucianapietro1@gmail.com
ORCID: https://orcid.org/0000-0002-8511-2196

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Introduction

Most couples demonstrate a desire to have children, especially with the passing of the years and the achievement of maturity, when it becomes one of the most prevalent desires in adult individuals. However, not every couple can spontaneously achieve pregnancy and some will

require treatment to reach this goal. Different approaches will be necessary, depending if the case is one of infertility or sterility.[1]

According to the World Health Organization (WHO),[2] infertility is defined as a failure to achieve fertilization without use of any contraceptive method for at least 12 consecutive months, when the individual is of reproductive age and has an active sex life.

The terms infertility and sterility are often confused and interpreted as being equal. Infertility is characterized as a situation that can be reversible, where individuals who are in this state can become fertile again. In contrast, sterility is a permanent condition.[3]

According to Bhongade,[4] male infertility has been assuming an increasingly prominent position, since it is already considered of high importance in the contemporary world and involves psychological aspects that need to be taken into account.[4]

A male factor check is obligatorily carried out during the first visit due to its usually simple, non-invasive and relatively low-cost nature. Findings in the literature note that, in general, one in every six couples has difficulties in having children and that male infertility is a primary or associated cause in half of these cases. This explains why the evaluation of the male factor is mandatory and should consist of a clinical history, physical examination, sperm analysis and endocrine profile.[5]

After performing the spermogram test, different results regarding the seminal parameters are obtained. A specific nomenclature exists for each situation, which only classifies the quality of the semen and does not suggest any biological etiology.[6] These terms are used to describe samples with values outside the reference range and therefore possibly coming from a different population. Much of semen classification refers to a single parameter; however, normozoospermia refers to three sperm parameters considered normal: count, motility, and morphology. Thus, deviations from the reference interval for each parameter can be described individually (Table 1).[6]

One of the factors that can jeopardize the quality of spermatozoa, and consequently cause changes in the spermogram, is the accumulation of free radicals (reactive oxygen species - ROS). These are molecules derived from highly reactive oxygen that are characterized by the presence of unpaired electrons in the valence shell. They play an important role in homeostasis and cell signaling, and are produced in small amounts by sperm cells. Free radicals provide beneficial functional effects, ranging from sperm capacitation and regulation of the maturation state to the increase of cell signaling pathways.[8]

However, high levels of ROS can exert negative effects on sperm function, resulting in male infertility. Due to increased DNA damage and lipid peroxidation, these effects are visible in the presence of exaggerated levels of free radicals in seminal plasma.[9] However, free radicals are counterbalanced by antioxidants that help maintain homeostasis at the ideal reduction potential for adequate sperm function.[10]

In addition, there is the seminal fluid, which is a compound rich in antioxidant substrates that nourish and protect the sperm, arranged in enzymatic and non-enzymatic antioxidant systems. The enzymatic system is composed of natural sperm enzymes and originates from the prostate (superoxide dismutase, glutathione peroxidase and catalase), unlike the non-enzymatic system which is composed of several components that originate in food.[11,12]

Table 1. Nomenclature of seminal parameters and their definitions

Term	Description
Aspermia	Without semen (no or retrograde ejaculation)
Asthenozoospermia	Percentage of spermatozoa with progressive motility below the lower reference limit
Asthenoteratozoospermia	Percentages of progressively motile and morphologically normal sperm below lowest thresholds
Azoospermia	No spermatozoa in the ejaculate (given as a limit of quantification for the valuation method employed)
Cryptozoospermia	Sperm absent in fresh preparations but observed in centrifuged pellet
Hemospermia	Presence of erythrocytes in the ejaculate
Leucospermia	Presence of leukocytes above the threshold value in the ejaculate
Necrozoospermia	Low percentage of live sperm and high percentage of immobile sperm in the ejaculate
Normozoospermia	Total number of sperm and % of sperm progressively mobile and morphologically normal, the same or above lower reference limits
Oligoasthenozoospermia	Total number of sperm and % of sperm progressively moving below the lower reference limits
Oligoasthenoteratozoospermia	Reported total number of sperm and % of both progressively motile and morphologically normal sperm below reference limits lower
Oligoteratozoospermia	Total number of spermatozoa and % of morphologically normal spermatozoa below the lower reference limits
Oligozoospermia	Total number of sperm below lower reference limits
Teratozoospermia	% of morphologically normal sperm below lower reference limits

Source: Nomenclature related to semen quality, WHO Manual.[7]

In a situation where the amounts of ROS produced increase excessively, or where the antioxidant function fails, the balance between oxidation and reduction is disrupted, resulting in oxidative stress. Therefore, sperm cells contain very low levels of enzymatic antioxidants, which are insufficient to achieve protection against high concentrations of ROS.[13]

In recent decades, several advances have occurred in the area of male infertility, such as sperm function tests, analyses of oxidative stress and sperm DNA fragmentation, providing a better understanding of the true male reproductive potential.[14]

Oxidative stress is still considered an essential factor that can exert considerable influence over the reproductive outcome. Finding its cause and treating it by reducing ROS and/or therapy with antioxidants is an interesting strategy for a possible reversal of this condition. However, no clear consensus has been reached on the actual effectiveness of this possible therapy.[15]

Although ROS are necessary for the normal physiological function of sperm, out-of-balance oxidative stress can cause increased susceptibility to DNA damage, potentially leading not only to infertility, but also to miscarriage or genetically inherited mutations that cause deficiencies.[16]

Therefore, zinc is essential for testicular steroidogenesis, testicular development, synthesis and secretion of luteinizing and follicle-stimulating hormones, testosterone synthesis, gonadal differentiation, sperm formation and maturation, acrosomal reaction, acrosin activity and fertilization.[17,18]

Zinc's function is vital to reproductive potential. It is believed that seminal zinc derives almost exclusively from prostatic secretions. Motility and structural integrity of spermatozoa are significantly influenced by the mineral since zinc deficiency has been associated with male sterility and subfertility. It is one of the most important antioxidant elements in seminal plasma fluid and appears to protect sperm from bacteria and chromosomal damage.[19]

This study aims to systematically review the literature on the impact of oral zinc supplementation on male infertility, in order to assess whether zinc intake exerts a therapeutic effect based on the evaluation of seminal parameters.

Methodology

Searches for articles were carried out in two bibliographic databases, namely: PubMed (National Library of Medicine) and Science Direct, and duplicate references were excluded. The descriptors used comprised combinations in English of: "supplementation", "effects", "male infertility", "zinc sulfate", and "seminal parameters". During the search, some filters were applied, such as research article, male, in addition to the or/and operators for combining the terms used.

This review included research articles published in English on interventions with oral supplementation of zinc sulfate in infertile men proven through collection from spermogram and analysis of seminal parameters. No time limit was established regarding the year of publication of the articles, onlyoriginal studies, both cross-sectional and longitudinal, were included. As exclusion criteria, review articles as well as animal and in vitro studies were not considered. Studies that did not apply zinc or use the analysis of seminal parameters as a method to evaluate results according to the manual for collection, processing, and analysis of human semen by the World Health Organization were also excluded from this review.

The selection of articles initially involved an analysis of the titles and abstracts of the works. Studies not excluded during this phase were read in full, so that they could be fully evaluated as to their fulfillment of the eligibility criteria. After this procedure, eight studies, published from 1998 to 2014, were selected for the present review.

Results

Table 2 presents data collected from the eight articles included in the review, which were published from 1998 to 2014. The studies were carried out in different countries, 37.5% of them in the Netherlands,[20,23,24], 25% in Iraq,[25,26] 25% in Kuwait[11,22] and 12.5% in Iran,[21] and examined groups of patients attending infertility clinics. Of the eight, seven are transverse and one is longitudinal.

All of studies followed the precepts of the Human Semen Collection, Processing and Analysis Manual published by the World Health Organization, although they differ with regard to the version of the manual used. Of the total, 50% of the studies used the 1992 version,[20,22-24] 37.5% used the 1999 version[11,21,26] and 12.5% used the 2010 version.[25] All the authors used atomic absorption spectrophotometry to determine the amount of seminal zinc.

A food frequency questionnaire for assessing the approximate intake of dietary zinc was not applied to research participants, except in the case of Wong and colleagues,[20] who used the determination of foods rich in zinc as a criterion for the exclusion of participants. The other works did not report the use of this criterion as a methodology.

The target audience consisted of fertile and infertile men. The intervention sample groups ranged from 18 to 77 individuals, and the control groups ranged from 8 to 113 individuals. The average age of the participants in the studies was 31 years. All the studies began by carrying out a survey of the pathological conditions of the participants in the infertility clinics, seeking to select men with a proven infertility factor, through the evaluation of sperm parameters, who were in a position to gain significant benefits from the intervention. The studies excluded those already on medication or supplementation with antioxidants, patients with endocrinopathies and varicocele, alcoholics, men with infertile partners and smokers (with the exception of Raigani and colleagues,[21] who included fertile and infertile smokers in their sample). In those studies that also used fertile men as a sample, the selection was based on proven fathers or those with a pregnant partner and diagnosis of normozoospermia by a spermogram test.

The daily dosage of zinc sulfate administered between the studies ranged from 66mg to 500mg and use time ranged from three to six months.

Table 2. Summary of the main results obtained after the intervention with oral zinc sulfate supplementation

Author, year and country	Sample group	Daily dose of Zn and period	P value	Results of sperm parameters
Omu et al., 1998[22] (Kuwait)	n= 97 (I: 49 e C: 48)	500 mg 3 months	P<0,05	Positive effects (↑count and ↑ motility)
Wong et al., 2002[20] (Netherlands)	n= 71 (I: 23 e C: 48)	66 mg 6 months	P<0,05	Positive effects (↑count)
Ebisch et al., 2003[23] (Netherlands)	n= 190 (I: 77 e C: 113)	66 mg 6 months	P>0,05	No significant changes
Ebisch et al., 2006[24] (Netherlands)	n= 40 (I: 18 e C: 22)	132 mg 6 months	P<0,05	Positive effects (↑count)
Omu et al., 2008[11] (Kuwait)	n= 45 (I: 11 e C: 8)	400 mg 3 months	P<0,05	Positive effects (↑ motility, ↑ PM integrity, ↓ DNA fragmentation)
Hadwan et al., 2012[25] (Iraq)	n= 74 (I: 37 e C: 37)	440 mg 3 months	P<0,05	Positive effects (↑ motility and ↑ morphology)
Raigani et al., 2014[21] (Iran)	n= 42 (I: 24 e C:18)	440 mg 4 months	P= 0,05	Positive effects (↑ chromatin integrity)
Hadwan et al., 2014[26] (Iraq)	n= 120 I: 60 e C: 60	440 mg 3 months	P<0,05	Positive effects (↑count, ↑ motility and ↑ morphology)

Legend: *I: intervention, *C: control, *NR: not reported, *Zn: zinc, *PM: plasma membrane.

The study by Omu and colleagues[22] used medical records and tests to select 100 infertile patients with a diagnosis of proven asthenozoospermia based on the criterion of impaired total motility of at least 40% . The patients belonged to the Kuwait Maternity Andrology Clinic and were randomly divided into two groups, half of which took zinc supplementation of two capsules a day of 250mg each for three months, while the other half served as a control. At the end of the period, semen samples were collected again and compared with the control group. The result of zinc therapy significantly improved sperm parameters in relation to count and progressive motility. In addition, 11 pregnancies were recorded in the intervention group.

The participants in the study by Wong and colleagues[20] were 108 fertile individuals and 203 subfertile individuals recruited from two infertility clinics in the Netherlands. They were carefully selected and randomly distributed into eight groups, of which six were intervention and two control. Two of the intervention groups (one composed of infertile individuals and the other of fertile ones) received a zinc supplement, containing a daily dose of 66mg, for six months. At the end of the period, they were evaluated again and their results were compared with the base values recorded at the beginning. The result was that the sperm count increased significantly in the infertile group, while the count increased slightly in the fertile group but not in a statistically significant way.

The study by Ebisch and colleagues[23] examined 113 fertile individuals and 77 subfertile individuals with polymorphism in the gene of the enzyme methylenetetrahydrofolate reductase (MTHFR). They were patients from two infertility clinics in the Netherlands and were randomly assigned to four groups, in which the zinc sulfate group received 66mg daily for six months. Other groups received placebos or folic acid (5mg) combined with zinc. In the final evaluation, after the intervention, the samples were collected and analyzed. The results showed that the intervention group that received only zinc did not present statistically significant differences.

In the next study by Ebisch and colleagues,[24] 49 fertile men and 40 with idiopathic infertility were recruited from the same sites as in the previous study and were randomized into four groups, where two (fertile and infertile) groups received a dose of 132 mg of zinc sulfate for six months, while placebos were applied to the other two groups. After the study period, the men were reassessed based on their seminal parameters, which showed a significant increase in the count. Other sperm parameters were not evaluated in this study.

In the study by Omu and colleagues,[11] 45 men with asthenozoospermia and normal sperm concentration were recruited from the same infertility clinic as the previous study in Kuwait. After randomization, the participants were split into four groups, namely: group A with zinc only (n=11) 200mg twice a day; group B (n=12) zinc 200mg + vitamin E 10mg twice a day; group C (n=14) zinc 200mg + vitamin E 10mg + vitamin C 5mg twice a day, all for three months; and group D (n=8) served as a control. For inclusion in the review, only the result sof the group that solely used zinc were taken into consideration, although there was no difference in the outcome measures between zinc by itself and zinc with other antioxidants. Concentration and morphology improved, but not statistically enough compared to the control group. However, motility increased twice as much and membrane integrity improved, decreasing sperm DNA fragmentation.

The study by Hadwan and colleagues[25] included 37 fertile patients and 37 with asthenozoospermia from the Hilla Maternity Infertility Clinic, in Iraq. Only the subfertile group was treated with 220mg zinc sulfate capsules twice a day for three months, while the fertile group was allocated as control. After this period, the intervention group was reassessed and there was an improvement in all sperm parameters, but only progressive motility and morphology, as well as seminal volume, presented statistically significant differences.

The study by Raigani and colleagues[21] used 83 patients diagnosed with oligoasthenozoospermia collected from an infertility clinic in the city of Tehran, in Iran, who were randomly distributed into four groups with different supplements (G1: folic acid; G2: folic acid and zinc; G3: zinc; G4: placebo). Doses of folic acid and zinc sulfate were 10mg and 440mg, respectively, and all groups received the supplement for four months. Only information related to the isolated zinc intervention was included in this review. After analysis, the results showed an in-

crease in concentration. However, the results were not statistically significant for this and the other sperm parameters between the intervention groups, when compared with the samples collected at the beginning of the study and the control group. Even so, a significant increase in integrity chromatin took place.

In the subsequent work by Hadwan and colleagues,[26] the authors evaluated 120 men, half of whom were fertile and the other half subfertile (asthenozoospermics), who were patients of the same infertility clinic as their previous study in Iraq. They used practically the same methodology, dosage, time and evaluation of results. This time the results reported were an increase in all sperm parameters (concentration, motility, and morphology).

Discussion

Male subfertility is a condition where the individual has a low sperm quality, usually of multifactorial etiology arising from genetic and environmental factors to which individuals are exposed, linked to imbalances between oxidative stress and antioxidant substances. In recent years, several studies have investigated interventions that include the supplementation of antioxidant substances with the aim of reversing the intense lipid peroxidation, and consequently, the DNA fragmentation of spermatozoa of some groups of infertile men.[20]

The main instrument used to support the studies were the WHO manuals, which underwent three changes in their reference values over the course of twenty years. Ongoing updates of their reference values confound the evidence surrounding the potential impact of antioxidants. This disadvantage is most evident in the inclusion criteria used by the studies in patients who were considered to have abnormal sperm quality before the 2021[7] update to the WHO reference values and were labeled as “normal” after its implementation. However, several studies have reported improvements in basic semen parameters after oral ingestion of zinc, alone or in combination with other associated micronutrients.

With the exception of Wong and colleagues,[20] who used the factor as a selection criterion for their sample, the vast majority (87.5%) of the studies did not investigate the participants' dietary factors. The use of this methodology leads to clearer and more reliable results. After all, the causes of possible nutrient deficiencies, especially those with antioxidant characteristics, could provide an idea of the possible etiology of infertility.

Regarding the results, 87.5% of the studies showed positive results in relation to the sperm parameters evaluated after intervention, even with different aspects. The only exception was the work of Raigani and colleagues,[21] who did not find significant results for the parameters of concentration, motility and morphology between the sample from the control group and the intervention group but found a significant increase in sperm chromatin integrity. Despite same dosage and administration time as Hadwan and colleagues,[25,26] Raigani did not verify improvement in sperm parameters of motility and morphology, as Hadwan observed in his works. This may be explained by the methodology used and the population assessed.

However, according to Raigani and colleagues,[21] the association of zinc with folic acid allows significant improvements in the parameters of morphology and sperm count, since zinc can potentially contribute to improvements in the parameters of evaluation of semen quality, while folic acid prevents the occurrence of aneuploidy (change in the number of chromosomes) and thus future errors in cell division after fertilization of a normal ovum[30].

On the other hand, Ebisch and colleagues[23] came to different conclusions in their two studies. The first study, in which Wong[20] participated, included individuals with polymorphism in the methylenetetrahydrofolate reductase (MTHFR) enzyme gene, a condition that disrupts the folic acid metabolism, which affects protein synthesis and, consequently, cell division. In this same study, the results do not show significant differences between the groups, unlike Wong and colleagues[20] who used the same dosage and duration and obtained positive parameters in the intervention group in the study he led. This outcome reinforces the idea that, unlike genetic factors, nutritional factors are changeable.[20]

In their next work, in 2006, Ebish and colleagues,[24] doubled the dosage (from 66mg to 132mg) over the same six months and used another sample model, this time defining criteria that excluded a possible genetic cause of infertility. They found improved parameters in sperm concentration per milliliter of semen.

Although this improvement in sperm count does not always result in sperm concentrations greater than the reference value of 20 million cells/ml, the increase observed in research suggests a beneficial effect on the quantitative aspect of spermatogenesis. This conclusion is supported by non-randomized controlled studies showing that oral zinc supplementation improves sperm concentration in males with idiopathic asthenozoospermia or oligozoospermia.[27,28]

The two works by Omu and colleagues[11,22] selected asthenozoospermic individuals and used dosages of 500mg and 400mg administered over three months. In both cases, a beneficial impact was recorded. In the first study, conducted in 1998,[22] the intervention improved concentration and motility. In the second study,[11] conducted ten years later, the researchers found increased motility, improvement in membrane integrity and a lower rate of fragmentation of sperm DNA.

All authors cited in this review used zinc dosages well above the tolerable intake levels (Tolerable Upper Intake Level - UL) for the sex and life cycle of the evaluated individuals, which is only 40mg daily for men over 19 years old, according to the Dietary Reference Intakes (DRI)[29]. Despite not being administered an extended period, this fact may be linked to the dropout rate of participants in some studies, due to reports of strong gastrointestinal discomfort described only by Omu and colleagues,[22] and Wong and colleagues.[20]

However, according to the Lifestyle Medicine approach, it appears that six pillars underlie the therapeutic use of lifestyle, which, consequently, will interfere with fertility. These are: adequate nutrition; regular practice of physical activity; quality of sleep; avoidance of exposure to toxic substances; control of stress; and positive personal relationships. These factors are correlated with increased oxidative stress, which in turn directly influences spermatogenesis.[31,32]

The presence of fatty acids and substances that promote the unbalanced generation of reactive oxygen species in the body directly influences spermatogenesis. This fact negatively contributes to the oxidative balance in the testicular cell environment,[33] such that adoption of the "prudent pattern", i.e., a high consumption of fruits, vegetables, fish and whole grains, results in a lower rate of fragmentation of sperm DNA; while adoption of the "traditional Dutch diet" pattern, i.e., high consumption of potatoes, meat and whole grains associated with low consumption of alcoholic beverages and sweets, has positive effects on sperm concentration, noting that in both evaluated patterns have foods with antioxidant characteristics.

Another study[34] with 336 individuals diagnosed with infertility compared the influence of a dietary pattern rich in antioxidants ("prudent") with a dietary pattern rich in saturated fatty ac-

ids and hypercaloric ("western") on sperm concentration, testosterone levels and sperm index of DNA fragmentation. The work reported positive results with the adoption of the "prudent" dietary pattern, while the opposite was registered for individuals with the "western" pattern.

Therefore, it appears that semen analyzes should always be interpreted with caution. After all, data can be easily influenced by intraindividual biological fluctuations and reflected in sperm parameters, in addition to possible limitations and inaccuracies of the methods used.[35,36]

Conclusion

In view of the evidence gathered from this systematic literature review, following the methodologies described above, one can conclude that interventions with zinc supplementation have a significant impact on the improvement of sperm parameters for concentration, motility, and morphology. This result is due to the protective effect provided by the trace element, which reduces the lipid peroxidation index of the plasmatic membranes and, consequently, improves the integrity of the DNA, thus increasing the fertilization capacity of these spermatozoa.

Although this review only investigated the variable of a single supplement in the condition of male subfertility, other studies have found superior beneficial effects obtained through zinc supplementation associated with other micronutrients, a fact that opens the door to conducting more clinical studies aimed at supporting the evidence already elucidated and improving therapy techniques.

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Introduction letter: a letter that must come with the submitted paper and contains at least the following information:

A statement that the paper has not been submitted for publication in another journal;

Recommendation of two reviewers (specialists) for consulting in the scientific field of the submitted paper + e-mail, preferably who are not from the same institution as the authors. The Editorial Board may or may not choose any of these consultants;

Conflicts of interest statement: state if the authors have any conflicts of interest. Conflicts of interest are those with potential influence over the published content, compromising the objectivity, integrity, or perceived value of the paper;

Author information: to provide full name and institutional affiliations of every author, and a mailing address of the main author (only e-mail) and ORCID, that is a persistent digital identifier (an ORCID iD) that you own and control, and that distinguishes you from every other researcher (https://orcid.org/). Authors will be required to objectively state that the submitted paper consists of original content, informing it has not been previously published nor is it being analyzed with this intent elsewhere.

If the authors had assistance from technical writers or language reviewers, it must be explicitly stated in the introduction letter, along with the assurance that the authors are fully responsible for the scientific content of the paper.

Authorship information: scientific authorship must be limited to those who contributed with intellectual work, with actual collaboration in the research. Therefore, to be considered an author, each contributor must meet the following conditions: (a) significant contribution to the creation and design of the study or to the analysis and interpretation of its results; (b) substantial contribution to the production of the paper, or critical review of its intellectual content, and (c) approval of the final version for publication. Leading or supervising a research lab/group does not in itself qualify as authorship. Sole contributions to fund raising or to data gathering also do not qualify as authorship. To ensure transparency in this aspect authors are expected to include a statement of authorship detailing the role of each author in the study and in the production of the paper. In the absence of this authorship statement within the introduction letter, the paper will be disqualified for analysis.

The letter must be signed by the main author, who will represent all other authors in this document.

Title page: this page must contain title and author information as follows:

title (English) 100 characters maximum, counting spaces;

short title (English) 50 characters maximum, counting spaces;

the name of each author with their affiliation in this particular order: first name, abbreviated middle names, last name. Department (or service). Course. University (or institution). City, state/province/ territory, country.

contact information for an author: first name, abbreviated middle names, last name, e-mail.

Types of papers

1. Original papers: Papers resulting of original research. Maximum of 5,000 words (excluding abstract and references) and five images or tables. Maximum of 40 listed references. They must be submitted in the following format:

abstract: must be written in English with a maximum of 250 words. Must follow the structured abstract model, with mandatory introduction, objective(s), methodology and resources, results and discussion, conclusion(s). It is well known that the abstract gets more visibility and distribution than the full text of the paper. Therefore, it must contain the essential information in the paper, but cannot be just a patchwork of sentences from it. It must be succinct and direct, highlighting what is most important in the full text in order to encourage a full reading. In the conclusion, all results must be related to the objectives of the study. The discussion must assert the contribution of the results to the body of knowledge about the subject of research.

keywords: three to six terms related to the subject must be given, separated by semicolons, according to MeSh (Medical Subjects Headings) for English.

Full text

Introduction: it must be short and present the purpose (context and justification) of the study, including a short review of relevant studies about the subject, mentioning any recent progress, and referencing just what is appropriate.

Methodology and resources: this section must briefly present all the information needed for other researchers to replicate the study. Adopted procedures must be clearly described, as must the analyzed variables and tested hypotheses. Definitions must be given whenever necessary. Population, sample, and measurement instruments must be described and information about data gathering and processing must be given. If possible, validity scores must be included. Methods and techniques used must be duly detailed, including statistic methods. New or substantially modified methods must be described, with a justification for its use and mention of its limitations. Research ethics must be observed. Authors must explicitly state that the research was done within ethical standards and with the approval of an ethics committee.

Results: this section must be a concise report of all new information found, with minimum personal bias and judgment. The data must be presented in a logical sequence, starting with the most important information. Data from tables and images must not be repeated, but briefly referred to. It must state the significance of the new data and the relevance of the new findings in relation to established theories and to scientific literature. In this section must also be mentioned the limitations of the present work, as well as its implications for future research. Finally, conclusions must be included in this section, always related to the initially stated objectives.

Acknowledgments: must be concise and limited to people and institutions that contributed to the research in some degree, but could not be included as authors.

In-text citations: BJHBS follows the Vancouver style, according to the general rules of The NLM Style Guide for Authors, Editors, and Publishers, second edition (www.ncbi.nlm.nih.gov/books/NBK7256/). For in-text citations, use Arabic numerals superscript, 1 without spaces, right after a word or punctuation: "Parkinson's Disease1 description began in the 1950s,2 when..." In some cases, the names of the authors may figure in the text: "Phillips12 analyzed several conditions of..."; and up to two authors can be named: "Handel and Matias15 conducted a study about..." However, when the number of authors is three or more, the first author must be named along with the expression "and colleagues.": "Silveira and cols.13 have proposed a new methodology..."

References: all referenced cited in-text must be in the reference list. References shall follow the Vancouver style, according to the general rules of The NLM Style Guide for Authors, Editors, and Publishers, second edition (www.ncbi.nlm.nih.gov/books/NBK7256/). They are limited to published material, papers, and abstracts. Authors are responsible for providing precise and complete references. In references with more than one author, authors up to three must be named. From there on, an "et al" must follow the first three authors. There must be no more than 40 references.

Tables and/or images: up to a maximum of five, including the authorship and/or source.

Tables: must be created in dedicated software, such as Excel. The width must be proportional to one page in the current layout. The font must be Arial, size 9, single space. Tables must be imported to and submitted in a text file: .doc/.docx (Microsoft Word), .rtf (Rich Text Format), or .odt (Open Document Text). They must be assigned a number in ascending order and receive a title and/or subtitle explanation. They must also be referenced within the text. The content of a table must not replicate that of an image nor vice versa. Their numbers must be assigned according to

the order in which they are referenced in-text. All abbreviations must be explained with a legend below the table. There must be the source from which the table was extracted and/or the authorship of it, this information must be written below the table, after the legend for the abbreviations, if any.

Images: can be photos, illustrations, graphics, drawings, etc. Images must be submitted as separate files (.tiff or .jpeg). They must be assigned a number in ascending order and receive a title and/or subtitle explanation. They must also be referenced within the text. All abbreviations must be explained with a legend below the image. There must be the source from which the image was extracted and/or the authorship of it, this information must be written below the image, after the legend for the abbreviations, if any.

2. Clinical cases:

Case report: usually it describes one to three patients or a family case. The text must be up to 2,000 words long, with up to three tables or images and up to 25 references. The abstract must be no more than 100 words long.

Clinical case solution: it must contain a step- by- step description of the decision process of clinical cases. Patient information must be presented to one or more clinical experts in stages (text in bold) to simulate the way information is made available in clinical practice. The expert must answer (text in regular font) as new information is added, sharing their reasoning/arguments with the reader. The text must be up to 2.,500 words long, and must have up to 15 references.

3. Literature review:

It must be about subjects relevant to medical practice. These will form a section about the common theme of each issue. These are limited to 5,000 words (excluding abstract and references) and a maximum of five images and/or tables. Maximum of 40 listed references. Literature reviews will be submitted for the editorial board analysis under invitation by the guest editor of this section, and must conform to the following standards:

Title page: this page must contain title and author information as follows:

Title (in English) 100 characters maximum, counting spaces;

Short title (in English) 50 characters maximum, counting spaces;

the name of each author with their affiliation in this particular order: first name, abbreviated middle names, last name. Department (or service). Course. University (or institution). City, state/province/ territory, country.

contact information for an author: first name, abbreviated middle names, last name, e-mail.

Abstract: must be written in English with a maximum of 250 words. Must follow the structured abstract model, with mandatory introduction, objective(s), methodology and resources, results and discussion, conclusion(s). It is well known that the abstract gets more visibility and distribution than the full text of the paper. Therefore, it must contain the essential information in the paper, but cannot be just a patchwork of sentences from it. It must be succinct and direct, highlighting what is most important in the full text in order to encourage a full reading. In the conclusion, all results must be related to the objectives of the study. The discussion must assert the contribution of the results to the body of knowledge about the subject of research.

keywords: three to six terms related to the subject must be given according to MeSh (Medical Subjects Headings). Keywords must be separated by semicolons.

Literature reviews may fall into two types:

a. Systematic review and meta-analysis - Through a synthesis of original studies' results, the paper must answer specific relevant health sciences questions about the theme of its issue (see BJBHS's focus). It must detail the search process to find the original studies, selection criteria, and synthesis procedures for the results of the reviewed studies (which may or may not be meta-analysis procedures).

b. Narrative/critic review - Narrative or critic review has a descriptive discursive character, and aims to offer a broad presentation and to discuss themes of scientific interest within the health field. It must have a clear formulation of the scientific subject of interest, a theoretical-methodological critic of the reviewed works, and a conclusive synthesis. It must be elaborated by experienced researchers in the field in question or by renowned experts of notorious knowledge.

Acknowledgments: must be concise and limited to people and institutions that contributed to the research in some degree, but could not be included as authors.

In-text citations: BJHBS follows the Vancouver style, according to the general rules of The NLM Style Guide for Authors, Editors, and Publishers, second edition (www.ncbi.nlm.nih.gov/books/NBK7256/). For in-text citations, use Arabic numerals superscript,[1] without spaces, right after a word or punctuation: "Parkinson's Disease[1] description began in the 1950s,[2] when..." In some cases, the names of the authors may figure in the text: "Phillips[12] analyzed

several conditions of..."; and up to two authors can be named: "Handel and Matias[15] conducted a study about..." However, when the number of authors is three or more, the first author must be named along with the expression "and cols.": "Silveira and cols.[13] have proposed a new methodology..."

References: all referenced cited in-text must be in the reference list. References shall follow the Vancouver style, according to the general rules of The NLM Style Guide for Authors, Editors, and Publishers, second edition (www.ncbi.nlm.nih.gov/books/NBK7256/). They are limited to published material, papers, and abstracts. Authors are responsible for providing precise and complete references. In references with more than one author, authors up to three must be named. From there on, an "et al" must follow the first three authors. There must be no more than 40 references.

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4. Other submissions:

Editorial: it is a commentary on or analysis of papers in a given issue. It may include an image or table and be no more than 750 words long, containing up to five references. It will be written by the editor in chief or by an invited contributor at their request.

Editorial comment: it's a complementary text done by an invited editor, generally specialist in a controversial topic, in order to bring a critical overview to the discussion. It may include an image or table and be no more than 750 words long, containing up to five references. It will be written by the editor in chief or by an invited contributor at their request.

Letters to the editor: space for readers to talk about recently published papers. Each letter must have up to 200 words (excluding references), five references and one image or table. It must be submitted no later than six months after the publication of the relevant paper. Letters non-related to papers published by BJHBS are limited to 500 words (excluding references), five references, and one image or table. Authors of letters will be required to provide their details, as well as contact information and possible conflicts of interest. The decision about the publication of a letter is made by the editor in chief.

On-line submission

Papers and other types of material must be sent to submission.bjhbs@hupe.uerj.br, along with the introduction letter. The subject of the e-mail must be: "Type of paper [original paper, case report, literature review]" or "Letter to the editor" -- title" + last name of its main author in UPPER CASE.

All subsequent communication must happen through responses to the original e-mail.

The editorial committee will analyze the material according to the editorial policies of BJHBS and will answer regarding acceptance for peer review as soon as possible. If it's considered fit for publication, it will be processed and proceed to editing, proofreading and layout.

After a paper's acceptance, the term of copyright transfer and the statement of conflicts of interest must be sent as soon as possible.

The final layout will be forwarded to the authors for final approval in .pdf format. This approval must be given according to a deadline defined by the editorial team.

Papers and other texts that do not conform to the specifications of these guidelines will be returned without any analysis by the editorial board of BJHBS. Such material must be re-submitted for new analysis once specifications are followed.

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