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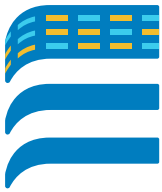

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Summary

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Analysis of the epidemiological profile of men who have sex with men as blood donors

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Abstract

Introduction: In May 2020, the Brazilian Supreme Court ruled that men who have sex with men (MSM) should be eligible to donate blood. Objectives: This study aims to describe the epidemiological, sociodemographic and serological profile of MSM donors in the hemotherapy center of a university hospital. Methodology and Resources: A cross-sectional study was conducted between June 2020 and June 2023, after approval by the Ethics Review Board of the Pedro Ernesto University Hospital. Results: Most MSM donors were young, single, and had higher education (complete or incomplete). In terms of gender specification, 46 (92%) identified as cis. In the sample, 64% (32) of candidates had taken at least one rapid test in the previous 12-month period. Most of the individuals analyzed were donor-eligible (90%) and non-reactive to the serology of interest in the research (91.3%). Discussion: Most MSM donors showed a homosexual orientation and reported regular use of condoms. Despite the restrictions prior to Resolution No. 399/2020, some MSM donors had donated blood in the past. Reactivity for syphilis (6%) among the volunteer donors is below expectations, while above forecast for hepatitis B (2%), based on the indicator presented by the state blood bank network. No HIV reactivity was found. Conclusions: The importance of using risky sexual behavior, rather than sexual orientation, as a criterion for donor eligibility was noted. This change would promote the inclusion of minorities in the blood donation process.

Keywords: Men who have sex with men; Bloodborne infections; Gender dysphoria; Gender identity; Blood donors.

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Introduction

Before April 2020, in Brazil men who had sex with other men (MSM) were considered temporarily unfit to donate blood for a period of 12 months after the sexual encounter with the same-sex partner, since this sexual practice was considered risky. In July 2020, Board Review No. 399 of 7 July 2020 was published,[1] which authorized national blood centers to receive blood donations from the MSM population, regardless of the time elapsed since the last sexual encounter with a person of the same sex. This decision was implemented by a judicial order issued by the Federal Supreme Court in May 2020.

In terms of the history of hemotherapy and MSM donations in Brazil, the pre-existing policy of permanent deferral of donation was changed in 2004 to one of temporary deferral of 12 months since the last sexual encounter with a homosexual partner.[2]

The decision of the Federal Supreme Court in 2020 was influenced by the impact of the COVID-19 pandemic, which led to a reduction in blood supply to blood centers because of social distancing measures and guidelines on staying at home. In addition, the support of social groups, such as pressure from the media and the help of human activist organizations, contributed to the strengthening of the arguments in favor of this change by the judiciary.[3]

Following the adoption of a new blood donor screening protocol, an assessment of individual risk based on risk behaviors that may place blood recipients at risk of transfusion-transmissible infections (TTIs) — regardless of sexual orientation — seemed to be a sensible step, so as to ensure the continuity of transfusion safety for both recipients and donors.[4] Therefore, a study from an epidemiological, sociodemographic, and serological point of view of the inclusion of MSM donors in a hemotherapy center at a university hospital in the State of Rio de Janeiro, Brazil, was conducted.

Methodology

A cross-sectional study of 50 blood donors who identified themselves as MSM was conducted between June 2020 and June 2023. The study was undertaken at the Herbert de Souza Hemotherapy Service of the Pedro Ernesto University Hospital (HUPE) of the State University of Rio de Janeiro (UERJ). Voluntary blood donors identified as MSM were included in the research using convenience sampling. Candidates were approached during the clinical screening for blood donation and then requested to answer a structured questionnaire (APPENDIX 1) and to sign a free and informed consent form (APPENDIX 2). The variables analyzed were socio-demographic, epidemiological, hematological and serological profiles, eligibility for clinical screening and sexual orientation. Secondary data extracted from the hemotherapy system used by the service (Hemote Plus® by SOFIS Ltda.) were used to collect data on the serological profile. The data collected were analyzed in an Excel® spreadsheet. The study was approved by the Ethics Review Board of HUPE under the opinion number 42979421.1.0000.5282. This study complies with the provisions of Resolution No. 466/2012 of the National Health Council5 on the ethics of research involving human subjects.

Results

The 50 participants predominantly comprised young, single men with complete or incomplete higher education. Table 1 shows the socio-demographic profile, including age, skin color, marital status, and education.

Figure 1 shows the distribution of sexual orientation among respondents, with the majority identifying as homosexual. In addition, 46 (92%) identified as cis, 2 (4%) as trans and 2 (4%) as another gender specification. In terms of partnership status, 27 (54%) of respondents had a single partner, 11 (22%) had up to two steady partners, 10 (20%) had multiple partners and 2 (4%) had no sexual partners in the previous 12 months. Regular condom use was reported by 34 (68%), while 9 (18%) never used condoms and 7 (14%) rarely used condoms. In the last 12 months, 27 (54%) had up to two rapid tests for sexually transmitted infections (STIs), 18 (36%) had no tests and 5 (10%) had more than two tests. For 19 (38%), this was their first blood donation; 14 (28%)

had donated at least four times; 12 (24%) had donated once; and 5 (10%) had donated two to three times. Most (80%) of the donations were spontaneous. With regard to the period before the passage of Resolution No. 399/2020[1], which first allowed MSMs to donate blood, 17 (34%) of the candidates stated that they had previously donated blood despite the provisions to the contrary of that law.

Table 1. Sociodemographic profile of MSM candidates for donation, Herbert Souza Hemotherapy Service, HUPE/UERJ, Rio de Janeiro - RJ, Brazil

Variables	N	%
Age group	ASD / gluten free	38
18 to 29 years old	34	68
30 to 39 years old	10	20
40 to 49 years old	5	10
50 to 59 years old	1	2
Skin color		
Yellow	2	4
White	21	42
Brown	17	34
Black	10	20
Marital status		
Married	2	4
Separated/Divorced	1	2
Single	43	86
Civil union	4	8
Educational level		
Primary school complete	1	2
High school complete	8	16
High school incomplete	2	4
Higher education complete	19	38
Higher education incomplete	20	40
Total	50	100

Source: Analysis of the responses to the forms completed by blood donation candidates (2023).

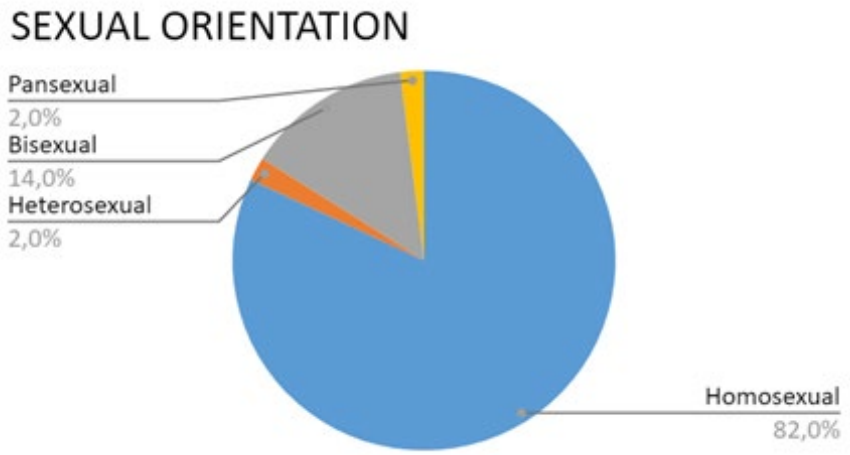

Figure 1. Sexual orientation of MSM blood donors, Herbert Souza Hemotherapy Service, HUPE/UERJ, Rio de Janeiro - RJ, Brazil

Source: Analysis of the responses to the forms completed by blood donation candidates (2023).

Table 2 shows the epidemiological and serological profile of the MSM donors, obtained from clinical and laboratory screening. Most of the individuals analyzed were considered eligible to donate, were non-reactive for the serology of interest to blood donation and, among those with a positive reaction, most tested positive for syphilis. Data that are unavailable due to problems with the computerized system or because of withdrawal from collection are classified as Not Available (NA).

Table 2. Epidemiological and serological profile of MSM donors, Herbert Souza Hemotherapy Service, HUPE/UERJ, Rio de Janeiro - RJ, Brazil

Variables	N	%
Aptitude		
Eligible to donate	45	90
Not eligible to donate	5	10
Serological tests		
Reactive	4	8
Not Reactive	41	82
NA	5	10
Not serologically suitable		
SYPHILIS	3	6
HBV	1	2
Total	**50**	**100**

Legend: HBV (hepatitis B virus); NA: not available.

Source: HEMOTE plus System (2023), consulted by the authors.

The Herbert de Souza Hemotherapy Service, also known as the Hemotherapy Center of the Pedro Ernesto University Hospital, is associated with the State University of Rio de Janeiro (UERJ), and has a history of receiving blood donations mainly from older donors. This characteristic provides an interesting contrast to the profile of young people and students found in the sample of volunteers of the present study.

Consistent with this finding, a survey of the Profile of Brazilian Blood Donors, conducted in national blood centers, found that the propensity to donate blood at any time in life was highest among younger people. However, despite being healthier and having greater access to information, this age group may have yet to acquire awareness and maturity about the importance of consistent donation6.

Self-identification as cisgender was predominant in this sample. According to Reynolds et al.[7], self-identification as trans or some other gender specification provides an opportunity to include a minority that until recently did not have an appropriate gender expression choice in blood donation questionnaires.

The fact that more than two-thirds of donors in this sample have completed and/or are pursuing higher education may be related to their awareness of the importance of blood donation and commitment to this cause, driven by the inclusion of LGBTQIAP+ donors, or to the greater visibility in donor recruitment campaigns targeted at this age group. Indeed, high levels of education are associated with greater opportunities for access to education, and understanding the importance of blood donation may also be motivated by access to education.[8]

Single marital status, as well as being male and young, is associated with a greater tendency to have multiple partners.[9] However, less than one third of donors reported having multiple partners in this study.

Despite the previous restriction on blood donation by MSMs, some of them had donated blood. This finding indicates that blood donation took place even under a policy of restriction, and can be considered a risky practice. Prospective donors must be made aware and educated, so that they do not lie or omit relevant information when filling in questionnaires and/or participating in interviews during clinical screening. At the same time, this situation highlights the need to reflect on the state of "sub-citizenship" in which MSM candidates find themselves, when they feel forced to resort to an illegal act in order to gain the right to donate blood.[10]

The reactivity for syphilis (6%) among the participants in this study with positive serologies was lower than expected, while reactivity for hepatitis B (2%) was above target, bearing in mind that the indicator presented by the state blood bank network as a parameter for the year 2021 was 45.56% of reactivity for syphilis and 1.85% for hepatitis B among donors with serological ineligibility.[11]

Based on indicators from the Rio de Janeiro blood bank network for 2022, the profile of the respondents included in this sample lies within the target of less than 18% ineligibility to donate blood as a result of clinical screening, since only 10% were classified as ineligible. Those ineligible on serological grounds, comprising the 8.7% of participants in the study whose tests showed reactive results, exceeded the target of less than 4%.[12] This result calls attention to the small number of individuals included in this sample, which does not validate any extrapolation. A study with a larger sample would be desirable.

No HIV reactivity was found in the study population. A systematic review based on scientific articles whose studies were conducted in developed countries examined the association between MSM blood donors, deferral policies and the risk of TTI. Four of the articles suggested a higher risk of combined TTI in MSM, considering HIV, syphilis and HBV; however, the limitations of these studies call into question their robustness. One of the articles found that the 12-month deferral policy for blood donation by MSM presented no or a lower risk of TTI. No studies were available on assessing the relation between the risk of TTI associated with the 3-month deferral policy and the individual risk assessment of the donor candidate. Research in this area should be encouraged since some hemotherapy services have begun to use these criteria to select potential donors.[13]

Another study of blood donors from the same hemotherapy service found that 36% of candidates gave blood because they wished to receive the result of the serological test after donation.[14] In this sample, 64% of candidates were found to have had at least one rapid test in the previous 12 months at testing and counselling centers (CTAs). However, identification of the reason for testing was not possible since no such guiding question was included in the interview questionnaire. Even if this is not the case for the participants in this study, it is important to emphasize that some candidates donate blood at blood centers in order to be tested for STIs, a practice engaged in by both MSMs and the heterosexual population. CTAs are available for these purposes, and donors should be encouraged to seek this service and should be offered guidance on the basic conditions of donation, in addition to having their awareness raised about the immunological window period, namely after using pre- and post-exposure prophylaxis.[10]

In a previous study carried out in the period at the same center before Resolution No. 399/2020, a similar profile was found in a study of MSM donors, with differences in sexual orientation and prevalence of condom use. Among MSM donors, the prevalence of homosexual orientation and the regular use of condoms during sexual intercourse were noted.

This study faces limitations due to the inability to inquire about sexual orientation during clinical screening, necessitating individual assessments to avoid bias. The small sample size is an obstacle to any generalization, but the framework of this study can potentially be replicated in other blood centers. In conclusion, MSM donors do not appear to alter the profiles of the hemotherapy service significantly. The relevance of sexual behavior in donor vulnerability classification is highlighted. Future research should involve larger MSM cohorts, so as to provide inputs to blood donation policies. The promotion of education and raising awareness about MSM blood donor vulnerability is recommended in the design of broader campaigns for encouragement of blood donation.

Conflicts of Interest

There are no conflicts of interest for any of the authors.

References

1. Ministério da Saúde. Resolução da Diretoria Colegiada - RDC No 399 [internet], 2020 [cited 2023 June 10]; 1–1. Available from: https://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2020/rdc0399_7_07_2020.pdf.
2. Zucoloto ML, Gonçalez TT, McFarland W et al. Blood donation deferral policies among men who have sex with men in Brazil. Hematol Transfus Cell Ther [internet]. 2019 [cited 2023 June 10]; 41: 164–8. Available from: https://www.scielo.br/j/htct/a/HP86ZqrL6xdRJfCqS8dQxnc/abstract/?lang=en#.
3. Park C, Gellman C, O'Brien M et al. Blood donation and COVID-19: Reconsidering the 3-month deferral policy for gay, bisexual, transgender, and other men who have sex with men. Am J Public Health [internet]. 2021 [cited 2023 June 10]; 111: 247–52. Available from: https://pubmed.ncbi.nlm.nih.gov/33211588/.
4. Hahn B, Quee F, Prinsze FJ et al. Balancing non-discriminatory donor selection and blood safety in the Netherlands: Evaluation of an individual risk assessment of sexual behavior. Transfusion [internet]. 2022 [cited 2023 June 25]; 62: 1241–50. Available from: https://pubmed.ncbi.nlm.nih.gov/35502143/.
5. Conselho Nacional de Saúde. RESOLUÇÃO No 466 [internet]. 2012 [cited 2023 June 25]. Available from: https://conselho.saude.gov.br/resolucoes/2012/Reso466.pdf.
6. Zago A, Silveira MF, Dumith SC. Prevalência de doação de sangue e fatores associados, Pelotas, RS. Rev Saude Publica [internet]. 2010 [cited 2023 June 25]; 44: 112–20. Available from: https://doi.org/10.1590/S0034-89102010000100012.
7. Reynolds V, Van Buren NL, Gorlin JB. Confronting the challenge of gender nonconforming donors: A blood center's three-year experience. Transfusion [internet]. 2023 [cited 2023 Aug 05]; 63: 1284-89. Available from: https://doi.org/10.1111/trf.17446.
8. Silva RMG, Kupek E, Peres KG. Prevalência de doação de sangue e fatores associados em Florianópolis, Sul do Brasil: Estudo de base populacional. Cad Saude Publica [internet]. 2013 [cited 2023 Aug 05]; 29: 2008–16. Available from: https://doi.org/10.1590/0102-311X00174312.
9. Patavino GM, Almeida-Neto C, Liu J et al. NHLBI Retrovirus Epidemiology Study-II (REDS-II), International Component. Number of recent sexual partners among blood donors in Brazil: associations with donor demographics, donation characteristics, and infectious disease markers. Transfusion [internet]. 2012 [cited 2023 Aug 05]; 52: 151-9. Available from: https://doi.org/10.1111/j.1537-2995.2011.03248.x.
10. Martinez EZ, Galdino G, Zucoloto ML. Perceptions and practices of the Brazilian LGBT+ population toward blood donation. Transfus Apher Sci [internet]. 2023 [cited 2023 Aug 11]; 62: 103578. Available from: https://doi.org/10.1016/j.transci.2022.103578.
11. Hemorrede do estado do Rio de Janeiro. Hemorio Indicadores 2021 [internet]. 2021 [cited 2023 June 10]. Available from: http://www.hemorio.rj.gov.br/Html/Apresentacoes/Indicadores_Hemorio_2021/Dados.htm#.
12. Hemorrede do estado do Rio de Janeiro. Hemorio Indicadores 2022 [internet]. 2022 [cited 2023 June 10]. Available from: http://www.hemorio.rj.gov.br/Html/Apresentacoes/Indicadores_Hemorio_2022/Dados.htm#1 .
13. Schroyens N, Borra V, Compernolle V et al. Men who have sex with men and risk for transfusion-transmissible infections in blood donors in Western countries: A systematic review update. Vox Sang [internet]. 2023 [cited 2023 Sept 10]; 118: 709–20. Available from: https://doi.org/10.1111/vox.13482.
14. Costa LSL, Eleuterio TA, Menezes RA et al. A Triagem clínica e a experiência na doação na perspectiva do doador de sangue. Global Academic Nursing Journal [internet]. 2020 [cited 2023 Sept 10]. Available from: https://doi.org/10.5935/2675-5602.20200038.
15. Costa LSL, Eleuterio TA, Pereira SS et al. Perfil epidemiológico do doador voluntário de sangue em um hospital universitário no Rio de Janeiro. Saúde (Santa Maria) [internet]. 2020 [cited 2023 Sept 10]. Available from: https://doi.org/10.5902/2236583443186.

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Teeth arrangements for denture esthetics: perceptions of dentulous and edentulous individuals

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Abstract

While bearing in mind that differing opinions on esthetic outcomes can emerge among dentists, patients and observers during the rehabilitation of edentulous patients, this study compares esthetic perceptions of and preferences for various anterior teeth arrangements for complete dentures among both dentulous and edentulous individuals. Two edentulous individuals were selected, and four maxillary anterior teeth arrangements ("Classic", "Supernormal", "Senile", "Youthful") were proposed. Photographs were evaluated by the patients, prosthodontists and dentistry predoctoral students, as well as edentulous and dentulous laypeople. In the cases of both patients, most respondents did perceive differences among the arrangements, with no differences among groups (male patient, P=0.353; female patient, P=0.387). Considering all respondents, the "Youthful" and "Classic" arrangements were understood to be the most attractive (33/48%), natural (34/51%), least artificial (34/50%), and the preferred (34/56%) smile for the male and female patient, respectively. Within each rater group, the "Classic" arrangement was rated as the least artificial (P=0.03) for the female patient. The "Senile" and "Youthful" arrangements were favored for the male patient, who preferred the "Senile" one. For the female patient, the "Classic" and "Supernormal" were favored, and she chose the "Supernormal" smile. The results highlight that esthetic perceptions and preferences can differ among dentulous and edentulous laypeople, dental professionals and patients. In addition, the same observer may have different opinions regarding tooth arrangements for different patients. Therefore, the patient´s opinion must be taken into consideration when determining the esthetic standards of smiles, and professionals should refrain from imposing their own esthetic preferences on patients.

Keywords: Artificial tooth; Complete denture; Edentulous mouth; Dental esthetics; Patient preference.

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Introduction

An esthetically pleasing smile is crucial for any restorative dental procedure, since this aspect of human appearance directly affects facial harmony, oral health, quality of life, communication, and business relationships.[1-4] To restore oral functions, dental prostheses must ensure harmony

in the shape and color of the teeth, gums, lips, and face. This can be challenging when all maxillary anterior teeth are absent.[5]

The literature presents numerous methods for determining esthetic parameters for anterior teeth. Williams[6] argues that the shape of the face resembles that of the maxillary central incisors, classifying them as square, triangular, or ovoid. Building on this idea, Nelson's "Esthetic Triangle"[7] incorporated the shape of dental arches into the aforementioned concept. Frush and Fischer suggest that factors such as sex,[8] personality,[9] and age[10] influence various tooth parameters, including outlines of incisal angles, size and arrangements, as well as the presence of incisal wear and gingival recessions. Rufenacht's morphopsychology theory[11] links the maxillary central incisors to personality traits such as strength, energy, authoritarianism, magnetism, apathy, or retraction. Meanwhile, the maxillary lateral incisors have been associated with an artistic, emotional, or intellectual personality. Therefore, different dental arrangements have the potential to significantly alter a patient's image.

The requirements for esthetics in restorative procedures have evolved alongside the current trend of increased recourse to anti-aging treatments.[5] In terms of rehabilitation through complete dentures, various concepts — the "natural", "supernormal", and "denture" looks — have been discussed in the literature.[1,2,12] These concepts encompass factors such as the size and proportion of teeth, gingival characterization, occlusal plane, use of diastema, different arrangements of maxillary anterior teeth, and the degree of their exposure, among others. The maxillary central incisors play a significant role in non-verbal communication due to their prominent position in the mouth.[13] The pursuit of younger-looking smile patterns has led to the positioning of maxillary anterior teeth at a more incisal level relative to lateral incisors and canines, with the objective of enhancing their visibility.[14] However, literature that documents the application of this youthful model in the rehabilitation of edentulous elderly patients using complete dentures is lacking.

Ideally, the esthetic parameters employed should align with the demands of patients,[6] given the complex and subjective nature of the concept of beauty.[15] Acknowledging the significance of esthetics in achieving successful treatment outcomes, this study aims to assess how various arrangements of maxillary anterior teeth impact the perceptions and preferences of prosthodontists, predoctoral students in dentistry, dentulous individuals and wearers of complete dentures. The null hypothesis was that the perceptions and preferences of respondents regarding the four esthetic concepts tested would not significantly differ.

Methodology

Following approval by the Institutional Ethics Committee of the local university (#627.841), two completely edentulous patients who were seeking new full dentures were selected for this observational study. They were both aged 60, and one was male (Patient 1) and the other female (Patient 2). Both patients demonstrated good receptiveness and cognitive ability, and no pre-prosthetic interventions were required. The study's nature and all its details were thoroughly explained, and informed consent was obtained.

Complete dentures were fabricated according to the following technique:[16,17] preliminary impressions were taken using stainless steel stock trays, previously modified by the application of peripheral wax to their edges (Asfer Indústria Química Ltda, SP, Brazil), using irreversible hydrocolloid (Dental News Com. Imp. e Exp Ltda, PR, Brazil) as the impression

material. Preliminary casts were obtained using type III plaster (Chaves F/A Mineração e Indústria, CE, Brazil).

Individual trays were then fabricated using colorless acrylic resin. Clinical adjustments were made to the individual trays, and secondary impressions were taken by border molding using a low-fusion stick compound (Nova DFL, RJ, Brazil) and by molding the supporting surface with zinc oxide–eugenol paste (Technew, RJ, Brazil). Working casts were made using type IV plaster (Pasom, Gold Star Brasil Indústria e Comércio Ltda, SP, Brazil).

Record bases with wax rims were created on these casts. The wax rims were individualized, and maxillomandibular relationship recordings were performed. The working casts were transferred to a semi-adjustable articulator by means of a facebow. Adjustments were made to intercondylar distance, condylar guide, and Bennet angles (30 and 15 degrees, respectively).

The artificial teeth (Vipi Dent Plus, VIPI Indústria, Comércio, Exportação e Importação de Produtos Odontológicos Ltda, SP, Brazil) were chosen by three clinical operators based on shape, size, and color. They followed the reference lines marked on the maxillary wax rim and considered each patient's esthetic preferences. An arrangement known as “Classic” for the six maxillary anterior teeth was utilized for placement of the artificial teeth. This arrangement positioned the central incisors and canines at the same incisal level, with the lateral incisors positioned 1mm above [Figure 1a].

Following clinical trials and approval by both participants and operators, three other arrangements were tested on three additional record bases: the “Senile”, which is similar to the “Classic” arrangement, but has worn cuspids and the same level for the central, canine, and lateral incisors edges [Figure 1b]; the “Supernormal”, in which the teeth were 1.0mm longer and 1.0mm wider than the “Classic” arrangement, with the same ordering of incisal levels [Figure 1c]; and the “Youthful”, which is similar to the “Classic” arrangement, except that the incisal edge of the maxillary central incisors was positioned 1mm below the edge of the lateral incisors, and these, in turn, were 1mm below the cuspid of the canines [Figure 1d]. For example, in the case of patient 1, the model of the anterior teeth selected for the “Classic”, “Senile”, and “Youthful” arrangements measured 8mm in width and 10.4mm in length (model # 264). However, for the “Supernormal” arrangement, the width and length were 9.1mm and 11.4mm, respectively (model #38).

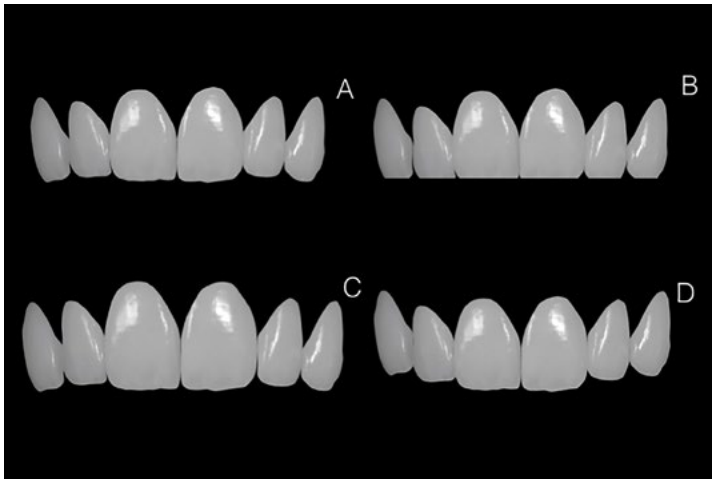

Figure 1. Upper anterior teeth arrangements.

Legend: A: “Classic” arrangement; B: “Senile” arrangement; C: “Supernormal” arrangement; D: “Youthful” arrangement. **Source:** The authors (2020).

After esthetic and functional evaluation and approval of artificial teeth in the “Classic” arrangement, the process aimed to standardize the characteristics for the various teeth assemblies. To achieve this, new record bases and wax rims were obtained using the same working cast mounted on the semi-adjustable articulator. This process maintained the previously established vertical dimension and centric relation. The creation of the four maxillary teeth assemblies was conducted in reference to the same lower record base. These assemblies were executed by a single denture technician who was well-versed in the characteristics of the proposed arrangements.

After approval of the different arrangements, digital camera pictures (sized 15 x 10cm) (Nikon Coolpix P510 Nikon Corp., Tokyo, Japan) were captured and organized into a booklet. Each patient's set of four images was presented on a single page to aid visual comparison. To prevent any influence on the raters' responses, the images were randomly labeled with the letters A, B, C, and D (A – “Classic”, B – “Senile”, C – “Supernormal”, and D – “Youthful”). An illustration featuring the application of the four arrangements on patient 1 is depicted in Figures 2a-d.

Figure 2. Frontal view of the smiles composed by the different teeth arrangements.

Legend: composed by A:“Classic” arrangement; B:“Senile” arrangement; C: “Supernormal” arrangement; D: “Youthful” arrangement.

Source: The authors (2020).

The illustrated booklet was presented to 100 individuals, including prosthodontists, predoctoral dentistry students, edentulous individuals, and dentulous laypeople (25 respondents per group). These participants expressed their esthetic perceptions and preferences by filling in a questionnaire[1,2] specially adapted for this study. The response unit used for comparing groups was the evaluation of the following parameters: Noticeable difference among arrangements (question 1 – “Can you perceive a noticeable difference among the four smiles of this patient?”); Attraction (question 2 – “Which smile do you consider the most attractive?”); Naturalness (question 3 – “Which smile do you consider the most natural?”); Artificiality (question 4 – “Which smile do you consider the least artificial?”); Esthetics (question 5 – “Which smile do you consider the most esthetically pleasing?”); Choice of smile (question 6 – “Which smile would you select for this patient?”).

Each patient conducted a self-evaluation of the smiles featuring different arrangements using the same questionnaire. They selected their preferred dental arrangement, and the dentures

then underwent the curing cycle. Following this, the dentures were delivered, together with instructions regarding their use and maintenance. In addition, follow-up appointments were scheduled after 1, 7, and 14 days. Further appointments were arranged based on the individual needs of each patient.

Differences among groups were examined using Fisher's exact and Chi-square tests. The analyses were conducted at a significance level of 0.05 utilizing statistical software (SPSS 21.0.0; SPSS Inc., Chicago, IL).

Results

The majority of respondents perceived differences among the four dental arrangements (male patient – 92%, female patient – 99%), with no difference between the two groups (male patient, P=0.353; female patient, P=0.387) [Table 1]. In addition, the frequency of "yes" or "no" answers for question 1 in each group was similar, regardless of the patient ("yes", P=0.745; "no", P=0.996) (Table 1).

Table 1. Frequency of responses to question "Can you perceive a noticeable difference among the four smiles of this patient?" (question 1) in each group of respondents concerning their perceptions of the differences among the four dental arrangements.

Group	Do you perceive a difference?		P
	No	Yes	
Male patient			
Dentulous laypeople	3 (12%)	22 (88%)	
Undergraduates	2 (8%)	23 (92%)	
Edentulous	3 (12%)	22 (88%)	0.353
Prosthodontists	0 (0%)	25 (100%)	
Total	8 (8%)	92 (92%)	
Female patient			
Dentulous laypeople	0 (0%)	25 (100%)	
Undergraduates	0 (0%)	25 (100%)	
Edentulous	1 (4%)	24 (96%)	0.387
Prosthodontists	0 (0%)	25 (100%)	
Total	1 (4%)	99 (99%)	
P*	**0.745**	**0.996**	

Legend: *Fisher's exact test. Non-significant differences (P>0.05).
Source: The authors (2020).

For the male patient, similar opinions for each teeth arrangement regarding all evaluated parameters were obtained from the groups of raters (Table 2). Considering the most frequent

responses from all respondents, the "Youthful" arrangement was identified as the most attractive (33%), the most natural (34%), the least artificial (34%), and the preferred (34%) smile. In the case of the female patient, the smile created with the "Classic" arrangement was identified as the most attractive (48%), the most natural (51%), the least artificial (50%), the most esthetic (59%), and the preferred (56%) smile. For this patient, a statistically significant difference of opinion regarding the "artificiality" parameter (P=0.03) was observed within each rater group. The "Classic" arrangement was considered the least artificial by the dentulous (52%), edentulous (48%), and prosthodontists (72%) groups. Undergraduates (36%) selected the "supernormal" arrangement as the least artificial.

Table 3 displays the comparison of preferences and perceptions within each rater group with regard to the patients. Overall, the "Classic" and "Supernormal" arrangements received similar ratings for all questions in both patients. The same trend was noticed for the smiles created with the "Senile" and "Youthful" arrangements. The opinions of the edentulous and prosthodontists groups were aligned — they considered the "Senile" and "Youthful" arrangements

Table 2. Frequency of responses of each rater group for questions 2-6 concerning their perceptions of the differences among the four dental arrangements applied for patients 1 and 2

	Group	Teeth arrangements Male patient				P	Teeth arrangements Female patient				P
		CL	SL	SN	YF		CL	SL	SN	YF	
Most attractive	Dentulous laypeople	7 (28%)	7 (28%)	5 (20%)	6 (24%)	0.943	13 (56%)	1 (4%)	11 (44%)	0 (0%)	0.247
	Undergraduates	8 (32%)	4 (16%)	5 (20%)	8 (32%)		8 (32%)	3 (12%)	11 (44%)	3 (12%)	
	Edentulous	5 (20%)	7 (28%)	4 (16%)	9 (36%)		11 (44%)	3 (12%)	10 (40%)	1 (4%)	
	Prosthodontists	5 (20%)	5 (20%)	5 (20%)	10 (40%)		16 (64%)	1 (4%)	8 (32%)	0 (0%)	
	Total	25 (25%)	23 (23%)	19 (19%)	33 (33%)		48 (48%)	8 (8%)	40 (40%)	4 (4%)	
Most natural	Dentulous laypeople	6 (24%)	4 (16%)	8 (32%)	7 (28%)	0.576	12 (48%)	4 (16%)	5 (20%)	4 (16%)	0.624
	Undergraduates	5 (20%)	4 (16%)	7 (28%)	9 (36%)		10 (40%)	5 (20%)	6 (24%)	4 (16%)	
	Edentulous	4 (16%)	8 (32%)	3 (12%)	10 (40%)		12 (48%)	3 (12%)	8 (32%)	2 (8%)	
	Prosthodontists	3 (12%)	9 (36%)	5 (20%)	8 (32%)		17 (68%)	1 (4%)	5 (20%)	2 (8%)	
	Total	18 (18%)	25 (25%)	23 (23%)	34 (34%)		51 (51%)	13 (13%)	24 (24%)	12 (12%)	
Least artificial	Dentulous laypeople	8 (32%)	3 (12%)	8 (32%)	6 (24%)	0.500	13 (52%) a	1 (4%)	2 (8%)	9 (36%)	0.003*
	Undergraduates	5 (20%)	4 (16%)	6 (24%)	10 (40%)		7 (28%)	5 (20%)	9 (36%) a	4 (16%)	
	Edentulous	5 (20%)	7 (28%)	5 (20%)	8 (32%)		12 (48%) a	1 (4%)	9 (36%)	3 (12%)	
	Prosthodontists	3 (12%)	8 (32%)	4 (16%)	10 (40%)		18 (72%) a	1 (4%)	4 (16%)	2 (8%)	
	Total	21 (21%)	22 (22%)	23 (23%)	34 (34%)		50 (50%)	8 (5%)	24 (24%)	18 (18%)	

Table 2. Frequency of responses of each rater group for questions 2-6 concerning their perceptions of the differences among the four dental arrangements applied for patients 1 and 2 (cont.)

	Group	Teeth arrangements Male patient				P	Teeth arrangements Female patient				P
		CL	SL	SN	YF		CL	SL	SN	YF	
Most esthetic	Dentulous laypeople	6 (24%)	7 (28%)	8 (32%)	4 (16%)		16 (64%)	2 (4%)	7 (28%)	0 (0%)	
	Undergraduates	8 (32%)	7 (28%)	3 (12%)	7 (28%)		11 (44%)	3 (12%)	9 (36%)	2 (4%)	
	Edentulous	5 (20%)	8 (32%)	4 (16%)	8 (32%)	0.367	13 (52%)	2 (4%)	8 (32%)	2 (4%)	0.478
	Prosthodontists	8 (32%)	4 (16%)	6 (24%)	7 (28%)		19 (76%)	1 (2%)	3 (12%)	2 (4%)	
	Total	27 (27%)	26 (26%)	21 (21%)	26 (26%)		59 (59%)	8 (8%)	27 (27%)	6 (6%)	
Smile of choice	Dentulous laypeople	6 (24%)	5 (20%)	8 (32%)	6 (24%)		16 (64%)	1 (4%)	8 (32%)	0 (0%)	
	Undergraduates	6 (24%)	4 (16%)	6 (24%)	9 (36%)		9 (36%)	4 (32%)	9 (36%)	3 (12%)	
	Edentulous	7 (28%)	8 (32%)	2 (8%)	8 (32%)	0.579	12 (48%)	2 (8%)	9 (36%)	2 (8%)	0.175
	Prosthodontists	4 (16%)	5 (20%)	5 (20%)	11 (44%)		19 (76%)	1 (4%)	4 (16%)	1 (4%)	
	Total	23 (23%)	22 (22%)	21 (21%)	34 (34%)		56 (56%)	8 (8%)	30 (30%)	6 (6%)	

Legend: CL - Classic; SN - Supernormal; SL - Senil; YF - Youthful. *Chi-square test; aSignificant differences (P<0.05).
Source: The authors (2020).

more suitable for the male patient; while finding the "Classic" and "Supernormal" arrangements more appropriate for the female patient across all parameters. Similar results were observed for the dentate group, except for the naturalness and artificiality parameters. The undergraduate group was the only one to rate both patients similarly on all parameters.

Table 3. Comparison between both patients within each rater group for questions 2-6.

		P*	Male Patient	Female Patient
Most attractive	Dentulous laypeople	0.002†	CL=SN<SL=YF	CL=SN>SL=YF
	Undergraduates	0.198	ns	ns
	Edentulous	0.005†	CL=SN<SL=YF	CL=SN>SL=YF
	Prosthodontists	0.000†	CL=SN<SL=YF	CL=SN>SL=YF
Most natural	Dentulous laypeople	0.319	ns	ns
	Undergraduates	0.286	ns	ns
	Edentulous	0.003†	CL=SN<SL=YF	CL=SN>SL=YF
	Prosthodontists	0.000†	CL=SN<SL=YF	CL=SN>SL=YF

Table 3. Comparison between both patients within each rater group for questions 2-6 (cont.).

		P*	Male Patient	Female Patient
Least artificial	Dentulous laypeople	0.094	ns	ns
	Undergraduates	0.306	ns	ns
	Edentulous	0.013†	CL=SN<SL=YF	CL=SN>SL=YF
	Prosthodontists	0.000†	CL=SN<SL=YF	CL=SN>SL=YF
Most esthetic	Dentulous laypeople	0.010†	CL=SN<SL=YF	CL=SN>SL=YF
	Undergraduates	0.049†	CL=SN<SL=YF	CL=SN>SL=YF
	Edentulous	0.007†	CL=SN<SL=YF	CL=SN>SL=YF
	Prosthodontists	0.018†	CL=SN<SL=YF	CL=SN>SL=YF
Smile of choice	Dentulous laypeople	0.004†	CL=SN<SL=YF	CL=SN>SL=YF
	Undergraduates	0.241	ns	ns
	Edentulous	0.005†	CL=SN<SL=YF	CL=SN>SL=YF
	Prosthodontists	0.000†	CL=SN<SL=YF	CL=SN>SL=YF

Legend: CL: Classic; SN: Supernormal; SL: Senil; YF: Youthful. *Chi-square test; †Significant differences (P<0.05); ns:– not significant.

Source: The authors (2020).

The male patient preferred the "Senile" smile, considering it the most attractive and natural. The "supernormal" smile was deemed less artificial and more esthetic. In contrast, the female patient found the smile with the "Supernormal" arrangement more attractive, natural, and esthetic, choosing it as their preferred smile. In addition, both patients considered the "Youthful" arrangement to be the least artificial.

Discussion

The primary aim of the current study was to explore the perceptions and preferences of various dentate groups with different levels of dental knowledge, as well as completely edentulous patients wearing complete dentures, with regard to different teeth arrangements during the rehabilitation of edentulous individuals using complete dentures. Although the differences between the smiles in the present study may appear small, 97% of dentulous respondents (dentulous, undergraduates, and prosthodontists groups) and 92% of edentulous individuals perceived distinctions among the four dental arrangements. This suggests that the smiles differ noticeably in the opinion of most observers. Furthermore, this perception within each group was not influenced by the patient's sex. This finding aligns with Waliszewski and cols.[1] who found that 96% of edentulous respondents were able to differentiate between the three proposed dental arrangements ("Natural", "Supernormal", and "Denture look"). In addition, Stockheimer and Waliszewski[2] observed that 96% of both dentulous and edentulous respondents were able to observe differences between such smiles.

In general, significant differences in responses among rater groups for all evaluated parameters were more noticeable in the case of the female patient. Specifically, among edentulous respondents, a majority (48%) preferred the smile with the "Classic" arrangement, aligning with previous findings[1,2] where 55% of edentulous respondents shared this preference. The "Classic" arrangement was also identified as the most attractive (44%), natural (48%), least artificial (48%), and most esthetic (52%). In Waliszewski and cols.2 study, this smile was chosen by 53.5% of edentulous respondents as the most attractive, 56% as the most natural, and 54% as the least artificial. Furthermore, in Stockheimer and Waliszewski study (2012),2 the "Classic" smile was selected by most edentulous respondents as the most attractive (53%), natural (55%), and least artificial (53%). Interestingly, only 12% of respondents considered this smile the most artificial, aligning with previous findings of 9%[1] and 11%[2] in earlier studies.

Furthermore, the "Classic" smile was favored by 64% of dentate laypeople, being considered the most attractive (56%), the most natural (48%), the least artificial (52%), and the most esthetically pleasing (64%) arrangement. In a previous study,2 53% of dentate participants preferred this smile and regarded it as the most attractive (53%) and most natural (55%). The smile featuring the "Supernormal" arrangement ranked second as the most preferred, most attractive, most natural, and most esthetically pleasing by both groups, but was rated as the third most artificial. This finding contrasts with previous studies,[1,2] in which this arrangement was the least preferred, considered the most artificial, least attractive, and least natural by these groups. These differences may have emerged due to variations in the number of respondents and the inclusion of a fourth smile configuration for evaluation in the present study.

When assessing the responses of all raters, notable differences were observed among patients. The female smile typically features rounded outlines of incisal angles, imparting a more delicate appearance.[13] This may explain why the majority of respondents favored the "Classic" arrangement, characterized by a more controlled display of teeth, for the female patient (56%). This was considered the most attractive (48%), the most natural (51%), the least artificial (50%), and the most esthetically pleasing (59%) arrangement.

Conversely, square and straight incisal angles, lending a more robust appearance,[13] appear to be more appealing in the case of the male patient. This could explain why the "Senile" smile was rated as the most attractive, most natural, least artificial, most esthetically pleasing, and the preferred choice for the male patient, compared to the "Classic" and "Supernormal" arrangements (Table 3). Patient 1 favored the "Senile" arrangement in their smile, considering it the most attractive and natural. Patient 2 expressed a preference for more visible teeth, favoring the "Supernormal" arrangement as the most attractive, natural, esthetically pleasing, and their preferred smile.

The smile design referred to as the "Youthful" arrangement in this study was based on parameters identified as ideal for a youthful smile,[14] where the incisal edges of the upper central incisors are positioned below the edges of the lateral incisors, which, in turn, are positioned below the canine cuspid when viewed from the front. Thus, the acceptance of this youthful setting was evaluated when applied to older adult denture wearers, a demographic group not previously explored in the literature. The low acceptance of this arrangement by these patients could likely be attributed to the youthfulness characteristics it imparts to the smile, rendering it incompatible with older patients. According to Pithon and cols.,[18] an esthetic conflict can arise when restoring the smile of an adult with features more suited to a younger individual, which are easily noticed by any observer. For older edentulous persons, a

satisfactory and pleasing smile might be one that mirrors the characteristics of their natural smile, in alignment with their present age.[12]

The results underscore the divergence regarding the esthetic perceptions and preferences observed among the various individuals surveyed and the rehabilitated patients in this study. The level of "dental knowledge" can potentially influence esthetic preferences, since it involves the consideration of theoretical and anatomical aspects during esthetic evaluations. The opinions of laypeople may differ from those of predoctoral dental students and prosthodontists due to this disparity in knowledge. Therefore, the patient's perspective must be taken into account when establishing esthetic standards for smiles. Professionals should refrain from imposing their own esthetic ideals onto patients, since the perception of beauty is entirely subjective and personal. In addition, one must recognize that differences in perceptions of beauty and naturalness exist not only between sexes and ages but also encompass objective aspects, such as dental lines and angles.

The limitations of the present study include that only two patients, both around 60 years old, were evaluated, and that respondents were asked to assess the images only once. Future studies might consider evaluating these arrangements across different age groups (e.g., around 40 or over 70 years old). Increasing the number of respondents could potentially reveal more significant differences between groups. To validate the reliability of participants´ responses, a second set of identical pictures, presented in different sequences, could be used. In addition, demographic characteristics, such as respondents´ gender, age, duration of edentulism, and denture use, could be included in order to achieve a more comprehensive understanding of how these factors influence esthetic perceptions and preferences.

Conclusions

Regardless of the level of dental knowledge or experience with dental prostheses, all groups of evaluators noticed differences between the proposed dental arrangements. With the exception of undergraduates, both "Senile" and "Youthful" arrangements were preferred for the male patient, who selected the "Senile" smile. In the case of the female patient, the preferred arrangements were "Classic" and "Supernormal", and she chose the "Supernormal" smile. Thus, the same observer may have differing opinions when evaluating tooth arrangements proposed for different patients. These results highlight the importance of taking into consideration the patient´s opinion when determining the esthetic standards of a smile, and the need for professionals to avoid imposing their esthetic preferences on patients.

References

1. Waliszewski M, Shor A, Brudvik, J, Raigrodski AJ. A survey of edentulous patient preference among different denture esthetic concepts. J Esthet Restor Dent 2006;18:352-69. doi: 10.1111/j.1708-8240.2006.00048.x.
2. Stockheimer C, Waliszewski MP. A Survey of dentulous and edentulous patient preference among different denture esthetic concepts. J Esthet Restor Dent 2012;24:112-24. 10.1111/j.1708-8240.2011.00449.x.
3. Yamazaki M, Inukai M, Baba K, John MT. Japanese version of the Oral Health Impact Profile. J Oral Rehabil 2007;34:159-68. doi: 10.1111/j.1365-2842.2006.01693.x.
4. Mori C, Shibuya T. Survey of attitudes of workers in a major company concerning the impression of smiles and teeth. J Esthetic Dentist 2007;19:96-101.
5. Ishida Y, Fujimoto K, Higaki N, Goto T, Ichikawa T. End points and assessments in esthetic dental treatment. J Prosthodont Res 2015;59:229-35. doi: 10.1016/j.jpor.2015.05.002.
6. Williams JL. A new classification of human tooth forms with special reference to a new system of artificial teeth. Dent Cosmos 1914;56:62.
7. Nelson AA. The esthetic triangle in the arrangement of teeth: face form, tooth form and alignment form, harmonious or grotesque. National Dent Assoc J 1922;9:392-401.

8. Frush JP, Fisher RD. How dentogenic restorations interpret the sex factor. J Prosthet Dent 1956; 6:160-72.
9. Frush JP, Fisher RD. How dentogenic interprets the personality factor. J Prosthet Dent 1956; 6:441-9.
10. Frush JP, Fisher RD. The age factor in dentogenics. J Prosthet Dent 1957;7:5-8.
11. Rufenacht CR. Fundamentals of Esthetics. Quintessence Publishing Co. Inc., Chicago, Il,1990;4:114.
12. Waliszewski M. Restoring dentate appearance: A literature review for modern complete denture esthetics. J Prosthet Dent 2005;93:386-94. doi: 10.1016/j.prosdent.2005.01.004.
13. Paolucci B, Calamita M, Coachman C, Gürel G, Shayder A, Hallawell P. Visagism: The Art of Dental Composition. QDT 2012;35:187-201.
14. Coachaman C, Calamita M. A Tool for treatment planning and communication in esthetic dentistry. QDT 2012;35:103-11.
15. Cotrim ER, Vasconcelos Júnior ÁV, Haddad AC, Reis SA. Perception of adults smile esthetics among orthodontists, clinicians and laypeople. Dental Press J Orthod 2015;20:40-4. doi: 10.1590/2176-9451.20.1.040-044.oar.
16. Malachias A, Paranhos HFO, Silva-Lovato CH, Muglia VA, Moreto C. Modified functional impression technique for complete dentures. Braz Dent J 2005;16:135-39. doi: 10.1590/s0103-64402005000200009
17. Albuquerque IS, Regis RR, de Souza RF, Gurgel KF, Silva PG, Pinto-Fiamengui LMS, Freitas-Pontes KM. Is a two-step impression mandatory for complete denture fabrication on the severely resorbed mandible? A randomized trial on patient perception and denture quality. J Dent. 2020 Jul;98:103356. doi: 10.1016/j.jdent.2020.103356. doi: 10.1016/j.jdent.2020.103356.
18. Pithon MM, Alves LP, da Costa Prado M, Oliveira RL, Costa MS, da Silva Coqueiro R, et al. Perception of esthetic impact of smile line in complete denture wearers by different age groups. J Prosthodont 2016;25(7):531-35. doi: 10.1111/jopr.12355.

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The dental age estimation by the Demirjian, Willems and AlQahtani methods in a Brazilian population

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Abstract

Introduction: Methods for estimation of dental age are highly reliable and their application plays an important role in the forensic field. Nevertheless, controversies remain regarding the accuracy and applicability of these protocols in specific populations. Objective: Evaluate the accuracy and applicability of the dental age estimation methods recommended by Demirjian, Willems and AlQhatani. Methodology: Two calibrated examiners analyzed 330 panoramic radiographs of a Brazilian population of both sexes, with ages ranging from 6 to 16 years (n = 30), according to each method. The values of the estimated ages were compared with the chronological age (Wilcoxon's test: Demirjian and Willems; Pearson's Chi-square test: AlQahtani; $p<0.05$). Results: For the Demirjian method, the estimated age showed no difference concerning the chronological age for the 9- and 15-years groups ($p=0.758$ and $p=0.510$, respectively), with an overestimate rate up to 1.1 years and an influence due to sex. The Willems method did not show differences for the groups from 11- to 15-years ($p=0.5302$, $p=0.3622$, $p=0.9224$, $p=0.9426$ and $p=0.1024$, respectively), regardless of sex, with an overestimate rate of 0.8 years at maximum. For the AlQahtani method, the highest accuracy rates were found for the 6- and 7-years groups (50% and 57%, respectively), with an overestimate rate up to 0.6 years, without any influence due to sex. Conclusions: The dental age estimated by the Demirjian, Willems and AlQahtani methods in a sample of Brazilian children and adolescents showed excellent match with chronological age, with the Willems method showing the greatest precision in the sample as a whole.

Keywords: Age determination by teeth; Forensic dentistry; Radiography; Panoramic.

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Introduction

The age of an individual can be measured in forensic situations to identify both skeletal remains and bodies in decomposition as well as in the civil field to assist in adoption processes, identification of underage offenders, immigration, asylum procedures, and other purposes.[1,2] Teeth have played a decisive role in cases of forensic investigation since they

are naturally preserved even after the disintegration of tissues and bones.[3] In addition, they have specific identifiable features, which means that each individual has teeth with unique morphological characteristics.[4]

Some authors consider that radiographic methods of age estimation are one of the safest and most reliable for attribution of chronological age since dental radiography is a non-destructive technique and teeth are less susceptible to nutritional, hormonal and pathological changes.[5] Among the various protocols used to measure estimated age, those based on the stages of dental mineralization are more reliable when compared to those that rely on bone development since they suffer less interference from factors such as sex, race, and systemic disease.[6,7] According to several authors,[8,9] the state of the art methods for estimating the dental age of children and adolescents are those of Demirjian and colleagues[10] and Willems and colleagues.[11]

Both the Demirjian and colleagues[10] and the Willems and colleagues[11] methods are based on the maturation stages of the seven left lower permanent teeth, although the Willems method is designed to minimize the estimated divergences of the Demirjian method by adapting statistical data. Despite being popular, the Demirjian method has usually presented age overestimations, while the Willems method has been considered to be the most accurate for the estimation of dental age of young individuals.[8,9,12,13] In addition, AlQahtani and colleagues[14] developed the "London Atlas of Human Tooth Development and Eruption" for age estimation, which uses both dental development and alveolar eruption for individuals from 30 weeks of intrauterine life to 23 years old. The number of analyses made using the AlQahtani method remains low.

Discrepancies in tooth mineralization stages have already been found when comparing populations with different racial features.[9,15-18] Cultural and ethnic differences among populations may explain the discrepancies observed in the estimated dental age and this fact has led to the introduction of new scores and classification criteria for specific populations.[7,9]

Therefore, this study aimed to evaluate the accuracy and applicability of the Demirjian, Willems and AlQahtani methods for estimation of dental age in a population of children and adolescents from the southeastern region of Brazil.

Methodology

Experimental design and sample characterization

This study received ethical approval from the Research Ethics Committee on Human Subjects (CAAE 69505017.8.0000.0064, UNICID, São Paulo-SP, Brazil). It consisted of a cross-sectional, blind observational study in which digital panoramic radiographs ("DVI Radiologia Odontológica" Radiology Clinic database, Ribeirão Preto-SP, Brazil) of children and adolescents with chronological age of 6 to 16 years, of both sexes, residing in the southeastern region of Brazil, were subjected to analysis.

Initially, a total of 352 radiographic images were selected, which comprised a sample number of 176 images for each sex. This selection was based on the statistical data from the study of Frítola and colleagues (2015), which used a sample size of 173 males (43%) and 225 females (57%). After applying exclusion criteria, a total of 330 images were used in the present study, with a sample number of 115 images for each sex, distributed into the 11 age analysis groups (n=15 for each sex, in each age group).

The images were selected according to the following inclusion criteria: digital panoramic radiographs of 6- to 16-year old boys and girls, from southeastern Brazil, taken between 2014 and 2017. The exclusion criteria were: images with low clarity, poor quality and/or distortions, without due record of chronological age or sex, with the presence of agenesis or dental extractions, facial trauma or any other type of anomaly or dental development syndromes (n=30; 11 groups).

Calibration of examiners and evaluation of radiographic images

The radiographic images were analyzed by two previously calibrated examiners, in a dark environment, using a 14-inch monitor, through a specific Windows® image visualization program (Microsoft Office 2010, Windows® 8). The inter- and intra-examiner calibration was calculated by the Intraclass Correlation Coefficient (ICC), using IBM SPSS Statistics v22 x64 for Windows® software, with a Cronbach alpha reliability model and a 95% confidence interval.

Calibration was performed for the three methods of age estimation by analysis of 11 panoramic radiographs that were not included in the sample number. Each examiner evaluated the same radiograph three times, with a 48-hour interval between each evaluation. For the intra-examiner analysis, the estimated dental age in each of the three evaluations was compared, between different analysis times while the estimates of dental age made by the two examiners were compared with each other for inter-examiner analysis.

Application of dental age estimation methods

Three methods of dental age estimation were applied, which are based on the analysis of mineralization and permanent teeth eruption: the Demirjian, Willems and AlQahtani methods.

The Demirjian and colleagues[10] method considers the maturation stages of the seven left lower permanent teeth, classified in categories from "A" to "H", which range from the beginning of the coronal crypt mineralization to the fully closed root apex. Subsequently, a numerical value is assigned to each tooth according to the maturation stage and differentiated by sex. These values are summed, resulting in a total “Maturity Score” value, which in turn, is converted to an estimated dental age.

The Willems and colleagues[11] method uses the same maturation stages "A" to "H". However, the process of estimating age is simplified by new statistical calculations. In this case, the “Maturity Score” table is no longer used. The values of the maturation stages for each tooth are summed and this final result is already the estimated age.

AlQahtani and colleagues[14] established “The London Atlas of Human Tooth Development and Eruption” method for estimation of age, which is based only on the maturation of the right side and involves the measurement of dental eruption in relation to the level of the alveolar ridge. The atlas is used for a direct comparison between the panoramic image and its respective compatible image in the atlas.

Radiographic images were blinded, and the digital image files were identified by number and sex. A maximum of 20 images per day were analyzed in order to avoid visual fatigue and a consequent impairment of the analysis.

Statistical analysis

For the Demirjian and Willems methods, agreement between the estimated ages and the chronological age was evaluated using the Wilcoxon non-parametric test (R Core Team 2017), considering the variable age without normal distribution (Kolmogorov-Smirnov). The comparison was performed by age group and sex. For AlQahtani method, the values of the estimated age interval were compared with the chronological age values for each group, and the accuracy rate of the method was measured. In order to evaluate the influence of sex on age estimation, Pearson's Chi-square (R Core Team 2017) was applied. The significance level was set at 5% in all the tests.

Results

The ICC values presented for intra-examiner 1 were 0.991, 0.991 and 1.000, for the Demirjian, Willems and AlQahtani methods, respectively. For intra-examiner 2, the values were 0.978, 0.978 and 1.000, for the same methods, respectively. The ICC values presented for the inter-examiner calibration were 0.993, 0.993 and 0.991, for the Demirjian, Willems and AlQahtani methods, respectively.

For the Demirjian method, when the mean of the estimated ages was compared with the chronological age of the individuals, the only groups that did not present a statistically significant difference for age correlation were 9- and 15-year groups (Wilcoxon test, p>0.05, Table 1).

Table 1. Mean (±s.d.) and median (±s.d.) values of the estimated age (in years), obtained by the Demirjian method, compared with the chronological age for each age group (n=30, 15 male and 15 female).

Age groups	Estimated age, mean	Chronological age, mean	Estimated age, median	Chronological age, median	p value
6 years	7.6 (±0.5)	6.5 (±0.3)	7.5 (±0.5)	6.5 (±0.3)	p=1.81 x 10-6
7 years	8.1 (±0.4)	7.4 (±0.3)	8.1 (±0.4)	7.5 (±0.3)	p=4.43 x 10-6
8 years	9.0 (±0.9)	8.4 (±0.3)	8.6 (±0.9)	8.4 (±0.3)	p=0.005
9 years	9.7 (±1.2)	9.4 (±0.3)	9.2 (±1.2)	9.5 (±0.3)	**p=0.758***
10 years	10.9 (±1.1)	10.5 (±0.3)	10.9 (±1.1)	10.4 (±0.3)	p=0.038
11 years	12.0 (±1.2)	11.5 (±0.3)	11.9 (±1.2)	11.6 (±0.3)	p=0.037
12 years	13.7 (±1.4)	12.5 (±0.3)	13.5 (±1.4)	12.5 (±0.3)	p=7.35 x 10-5
13 years	14.1 (±1.3)	13.5 (±0.3)	14.4 (±1.3)	13.5 (±0.3)	p=0.010
14 years	15.0 (±1.1)	14.5 (±0.3)	15.2 (±1.1)	14.5 (±0.3)	p=0.026
15 years	15.5 (±0.8)	15.4 (±0.3)	16.0 (±0.8)	15.5 (±0.3)	**p=0.510***
16 years	15.7 (±0.6)	16.4 (±0.3)	16.0 (±0.6)	16.4 (±0.3)	p=1.767 x 10-6

Legend: * Bold values with asterisks did not present significant statistical differences (Wilcoxon test, p>0.05).
Source: Comar LP (2023).

When data were analyzed in relation to the percentage of estimation of the method, an average overestimation rate smaller of up to 1.1 years was observed. Furthermore, the estimated and chronological age values were found to present significant differences for both male and female

individuals (Wilcoxon test, p=5.82×10-10 and p=2.82×10-5, respectively) when the sexes were evaluated separately.

The Willems method showed even greater precision than the Demirjian method (observational data) and was statistically accurate in estimating the age of the population in the 11-, 12-, 13-, 14-, and 15-year groups, regardless of sex (Wilcoxon test, p>0.05, Table 2).

Table 2. Mean (±s.d.) and median (± s.d.) values of the estimated age (in years), obtained by the Willems method, compared with the chronological age for each age group (n=30, 15 male and 15 female).

Age groups	Estimated age, mean	Chronological age, mean	Estimated age, median	Chronological age, median	p value
6 years	7.1 (±0.8)	6.5 (±0.3)	7.0 (±0.8)	6.5 (±0.3)	p=0.0003
7 years	8.0 (±0.6)	7.4 (±0.3)	8.1 (±0.6)	7.5 (±0.3)	p=0.00015
8 years	9.3 (±1.0)	8.4 (±0.3)	9.5 (±1.0)	8.4 (±0.3)	p=0.00017
9 years	9.8 (±0.9)	9.4 (±0.3)	9.7 (±0.9)	9.5 (±0.3)	p=0.0368
10 years	10.9 (±0.8)	10.5 (±0.3)	10.8 (±0.8)	10.4 (±0.3)	p=0.0060
11 years	11.5 (±0.8)	11.5 (±0.3)	11.7 (±0.8)	11.6 (±0.3)	**p=0.5302***
12 years	12.9 (±1.3)	12.5 (±0.3)	12.3 (±1.3)	12.5 (±0.3)	**p=0.3622***
13 years	13.5 (±0.9)	13.5 (±0.3)	13.6 (±0.9)	13.5 (±0.3)	**p=0.9224***
14 years	14.4 (±1.2)	14.5 (±0.3)	14.1 (±1.2)	14.5 (±0.3)	**p=0.9426 ***
15 years	15.1 (±1.1)	15.4 (±0.3)	15.8 (±1.1)	15.5 (±0.3)	**p=0.1024***
16 years	15.2 (±0.9)	16.4 (±0.3)	15.8 (±0.9)	16.4 (±0.3)	p=1.804 x 10-6

Legend: * Bold values with asterisks did not present significant statistical differences (Wilcoxon test, p>0.05).
Source: Comar LP (2023).

In relation to the percentage of estimation of the method, an average overestimate rate of up to 0.8 years was observed, with a statistically significant influence for both sexes (Wilcoxon test, p=0.0006 for males and p=0.5155 for females).

In the case of the AlQahtani method, comparisons were made between the mean interval of estimated age and the mean chronological age, with an accuracy rate that varied from 57% (7-year group) to 27% (15-year group), with the highest accuracy rate found in the cases of individuals aged 6 and 7 years (50% and 57%, respectively, Table 3).

In this method, an average overestimate rate of up to 0.4 years was observed, with no significant difference in relation to sex in general, except for the 16-year group, which presented a 60% accuracy for females, significantly differing from the 6.67% accuracy for males (Pearson's Chi-square, p=0.0067).

Table 3. Accuracy rate (%) of the estimated age interval compared to chronological age (in years), mean (±s.d.), by AlQahtani method, for each age group (n=30, 15 male and 15 female).

Age groups	Minimum estimated age, mean	Maximum estimated age, mean	Chronological age, mean	Accuracy % (n=30)*
6 years	6.4 (±0.8)	7.4 (±0.8)	6.5 (±0.3)	50.00%
7 years	7.2 (±0.8)	8.2 (±0.8)	7.4 (±0.3)	56.67%
8 years	8.5 (±1.0)	9.5 (±1.0)	8.4 (±0.3)	36.67%
9 years	9.3 (±1.0)	10.3 (±1.0)	9.4 (±0.3)	33.33%
10 years	10.5 (±1.0)	11.5 (±1.0)	10.5 (±0.3)	40.00%
11 years	11.0 (±1.0)	12.0 (±1.0)	11.5 (±0.3)	40.00%
12 years	12.3 (±1.1)	13.3 (±1.1)	12.5 (±0.3)	36.67%
13 years	13.5 (±0.9)	14.5 (±0.9)	13.5 (±0.3)	43.33%
14 years	14.4 (±1.1)	15.4 (±1.1)	14.5 (±0.3)	33.33%
15 years	15.0 (±1.2)	16.0 (±1.2)	15.4 (±0.3)	26.67%
16 years	15.8 (±0.8)	16.8 (±0.8)	16.4 (±0.3)	33.33%

Legend: * Simple correlation descriptive data (% of accuracy).
Source: Comar LP (2023).

Discussion

In recent years, the evaluation of the chronological age of individuals has grown in importance in the field Forensic Medicine, and tooth analysis has become a valuable diagnostic tool.[3] In Brazil, the age of 12 represents the legal transition between childhood and adolescence; the age of 14 stands for sexual consent, while 16 is the age for relative incapacity, in which individuals become capable of partially exercising their civil rights.[12,19]

The tooth is the most resistant tissue of the human body and can also withstand high temperatures; therefore, it can often be analyzed when all other tissues, including bones, have been destroyed.[3,20] So, teeth are extremely useful for estimating chronological age and are preferable to skeletal methods, because they are durable and resistant in archaeological contexts, and their development is less influenced by environmental factors than bone growth.[6,7]

Radiographic examination of the stages of development and mineralization of human dentition is one of the most common methods used for estimating age.[21,22] Such methods are non-destructive and provide accurate estimates for both living and dead individuals.[22]

The present study evaluated three methods of dental age estimation in which the stages of teeth mineralization and eruption were considered. In the Demirjian and Willems methods, the stages of mineralization of the seven left lower permanent teeth are evaluated, while the AlQahtani method presents an Atlas of Human Tooth Development and Eruption based on the development of teeth on the right side.

The methods were independently analyzed for accuracy and applicability. The Demirjian method was shown to be statistically accurate only for the 9- and 15-year groups, although the overestimation rates observed can generally be considered acceptable. The highest rates of age overestimation were observed in the 6-, 7-, 8- and 12-year groups, with a variation of up to 1.2 years.

The Willems method proved to be more accurate than the Demirjian one since this method shows no statistical differences in a larger population of individuals (11- to 15-year groups), as well as lower rates of overestimation (up to 0.8 years).

The results of the present study align with published data. Urzel and Bruzek[23] found consistent age overestimation rates for the Demirjian method and greater accuracy and reliability for the Willems method in a French population of children aged 4 to 15 years. Ye and colleagues[24] compared the Demirjian and Willems methods in Chinese children aged 7 to 14 years, and observed that the Demirjian method overestimated age by 1.68 years for boys and 1.28 for girls and that the Willems method showed greater precision, with an overestimation of 0.35 years for boys and underestimation of 0.02 years for girls.

Frítola and colleagues,[8] in a comparison of the Demirjian and Willems methods in a young Brazilian population, observed that the Demirjian method overestimated the chronological age by 0.65 years, whereas the Willems method did not present significant differences between estimated and chronological ages, and was therefore more accurate. Similarly, the Willems method also showed reliable results in a population in southern Brazil, confirming its efficacy and proving its applicability in the context of Brazilian forensics.[12]

In a study of German children aged 6 to 14, the Demirjian method showed overestimates for all ages, especially for boys.[25] After a systematic review and meta-analysis, Sehrawat and Singh[22] concluded that the Willems method provides comparatively lower overestimations in comparison with other methods for estimation of dental age and is accurate and reliable enough to be used for forensic purposes.

More recently, Chandail and colleagues[13] compared the Willems and Demirjian methods in an Indian population aged 7 to 14 years and found that the former showed a lower mean percentage error when compared to the results obtained from the Demirjian method. The authors concluded that the Willems method was an effective method of age estimation and was better and more accurate than the Demirjian technique.

For the AlQahtani method, high accuracy rates were observed in individuals up to 13 years old, with the highest accuracy percentages being observed in 6- and 7- year groups (50% and 57%, respectively) and an overestimate up to 0.6 years. Considering that 57% was the highest accuracy rate found, one might assume that this method presented a low accuracy rate for this specific population. However, few studies about AlQahtani method are available for comparison, especially in Brazilian populations, which may be due to the fact that it is relatively new (2010) and also because methods based on image comparisons can be considered less accurate in heterogeneous populations, such as those of Brazil.

AlQahtani and colleagues[26] observed that Schour & Massler, Ubelaker and the London Atlas underestimated ages in a study with skeletal samples aged up to 23 years, but that the London Atlas was the most accurate, with an underestimation of 0.10 years. These results differed from the present study; however, a comparison between the present study and

AlQahtani and colleagues[26] may be impractical since the current work evaluated a Brazilian population of children and adolescents. More recently, Willmann and colleagues[27] evaluated the accuracy of biological age determination in a multiethnic European sample using the Nolla, Demirjian, and the London Atlas (AlQahtani) methods. The authors observed that the most accurate methods were those of the London Atlas, which showed an average absolute deviation 1.2 years.

All three methods evaluated in the present study were shown to be applicable and presented estimated age results very close to the chronological age. The Demirjian and Willems methods may be preferable when gender differentiation is required and the Willems method demonstrated the greatest accuracy in this specific population. The present results agree with a recent review study, in which the authors demonstrated that the Demirjian, Willems and AlQahtani methods were found to be useful in an Indonesian population of children and adolescents, presenting greater accuracy than other methods.[28]

The search for precise methods to estimate the dental age of individuals in specific populations is also based on the importance of its applicability in the forensic context. Several forensic case reports have demonstrated the importance of applying an adequate and accurate method to estimate the dental age of individuals.[9,28-32]

All three protocols analyzed in the present study were considered easy to apply and no major difficulties were found during their application. The Willems method was considered by the examiners to be the simplest and the AlQahtani the most error-prone, despite the Atlas method being easy to analyse. For this reason, interval values of estimated age were adopted for this method.

One relevant limitation is the heterogeneity of the evaluated population since the individuals were not classified by race and origin since these data were inaccessible to the researchers. One should also bear in mind that the development of an individual can be influenced by several factors, which result in differences in the stages of tooth mineralization among individuals of different sex, biotypes and ethnicities.[20]

Therefore, further research with samples from other Brazilian regions and ethnicities should be conducted, with the aim of confirming the accuracy and applicability of the three methods in specific Brazilian populations. Forensic experts should not restrict themselves to a single method of analysis but should invest in the application of different available methods in order to reproduce, as far as possible, the available data and thus estimate more precisely the chronological age of individuals.

The estimates of dental age using the Demirjian, Willems and AlQahtani methods in a sample of Brazilian children and adolescents showed an excellent match with chronological age, with the Willems method showing the greatest precision in the sample as a whole.

Declaration of Interests

The authors declare they have no conflict of interest.

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References

1. Willems G. A review of the most commonly used dental age estimation techniques. J Forensic Odontostomatol; 2001; 19(1), 9-17.
2. Olze A, Reisinger W, Geserick G, Schmeling A. Age estimation of unaccompanied minors: Part II. Dental aspects. Forensic Sci. Int.; 2006; 159, S65-S67. DOI: 10.1016/j.forsciint.2006.02.018.
3. Hofmann E, Robold M, Proff P, Kirschneck C. Age assessment based on third molar mineralisation. J Orofac Orthop; 2017; 78(2). DOI: 10.1007/s00056-016-0063-z.
4. Meinl A, Huber CD, Tangl S, Gruber GM, Teschler-Nicola M, Watzek G. (2008). Comparison of the validity of three dental methods for the estimation of age at death. Forensic Sci. Int.; 2008; 178(2-3), 96-105. DOI: 10.1016/j.forsciint.2008.02.008.
5. Gruber J, Kameyama MM. The role of radiology in forensic dentistry. Brazil. Oral Res., 15, 263-268; 2001; DOI: 10.1590/S1517-74912001000300014.
6. Rai V, Saha S, Yadav G, Tripathi AM, Grover K. Dental and skeletal maturity-a biological indicator of chronologic age. J Clin. Diagn. Res.; 2014; 8(9), ZC60. 10.7860/JCDR/2014/10079.4862.
7. Kotecha, SD. Dental age estimation in children: A review. Forensic Res Criminol Int J; 2016; 3(1), 264-7.
8. Frítola M, Fujikawa AS, Morais FF, Franco A, Fernandes Â. Estimativa de idade dental em crianças e adolescentes brasileiros comparando os métodos de Demirjian e Willems. Rev Bras Odontoleg; 2015; 2(1). DOI: 10.21117/rbol.v2i1.18.
9. De Donno A, Angrisani C, Mele F, Introna F, Santoro V. Dental age estimation: Demirjian's versus the other methods in different populations. A literature review. Med, Sci and the Law, 61(1_suppl); 2021; 125-129. DOI: 10.1177/0025802420934253.
10. Demirjian A, Goldstein H, Tanner JM. A new system of dental age assessment. Human biology; 1973; 211-227.
11. Willems G, Van Olmen A, Carels C, Spiessens B. Dental age estimation in Belgian children: Demirjian's technique revisited. J Forensic Sci; 2001; 46(4), 893-895.
12. Franco A, Thevissen P, Fieuws S, Souza PHC, Willems, G. Applicability of Willems model for dental age estimations in Brazilian children. Forensic Sci Int; 2013; 231(1-3), 401-e1. DOI: 10.1016/j.forsciint.2013.05.030.
13. Chandail K, Goyal V, Kaul M, Dutt S, Koul T, Misgar BA. Estimation of dental age using Willems method and comparing it with Demirjian's method in 7–14-year-old children of Uttarakhand. J Indian Soc Pedod Prev Dent; 2022; 40(1), 43-47. DOI: 10.4103/jisppd.jisppd_333_21
14. AlQahtani SJ, Hector MP, Liversidge HM. Brief communication: The London atlas of human tooth development and eruption. Am J Phys Anthropol; 2010; 142(3), 481-490. DOI: 10.1002/ajpa.21258.
15. Oliveira OFD, Fernandes MM, Daruge Júnior E, Melani RFH, Paranhos LR. Estimating age using panoramic radiographs. RGO. Rev Gaúcha de Odontol (Online); 2010; 58(2), 203-206.
16. Oliveira FT, Capelozza ALÁ, Lauris JRP, Bullen IRFR. Mineralization of mandibular third molars can estimate chronological age — Brazilian indices. Forensic Sci Internat; 2012; 219(1-3), 147-150. DOI: 10.1016/j.forsciint.2011.12.013.
17. Gundim ADC, Sousa AP, Silva JC, Oliveira RD, Yamamoto-Silva FP, Silva BSDF. Third molars stage of mineralization and its relation to chronological age: Midwest Brazil sample. Rev Odontol UNESP; 2014; 43, 294-298. DOI: 10.1590/rou.2014.047.
18. Tonin LO, Leite NLP, Galo R, Silva RHA. Age estimation based on the stage of mineralization of third molars on orthopantomograms. Biosci. J.(Online); 2016; 805-812.
19. Cidade R, Santos M, Alves TC, Bueno JM, Soares M, Arakelyan M, Junqueira JLC, Franco A. Radiographic dental age estimation applying and comparing Demirjian's seven (1973) and four (1976) teeth methods. Forensic Sci, Med Pathol; 2023; 1-9. DOI: 10.1007/s12024-022-00563-5.
20. Figueira Junior E, Moura LCLD. The importance of the dental arches in human identification. Revi Bras Odontolog; 2014; 71(1), 22-27.
21. Liversidge HM, Lyons F, Hector MP. The accuracy of three methods of age estimation using radiographic measurements of developing teeth. Forensic science international; 2003; 131(1), 22-29. DOI: 10.1016/S0379-0738(02)00373-0.
22. Sehrawat JS, Singh M. Willems method of dental age estimation in children: A systematic review and meta-analysis. J Forensic Leg Med; 2017; 52, 122-129. DOI: 10.1016/j.jflm.2017.08.017.
23. Urzel V, Bruzek J. Dental age assessment in children: a comparison of four methods in a recent French population. J Forensic Sci; 2013; 58(5), 1341-1347. DOI: 10.1111/1556-4029.12221.
24. Ye X, Jiang F, Sheng X, Huang H, Shen X. Dental age assessment in 7–14-year-old Chinese children: Comparison of Demirjian and Willems methods. Forensic Sci Internat; 2014; 244, 36-41. DOI: 10.1016/j.forsciint.2014.07.027.
25. Wolf TG, Briseño-Marroquín B, Callaway A, Patyna M, Müller VT, Willershausen I, Ehlers V, Willershausen B. Dental age assessment in 6-to 14-year old German children: comparison of Cameriere and Demirjian methods. BMC Oral Health; 2016; 16(1), 1-8. DOI: 10.1186/s12903-016-0315-8.
26. AlQahtani SJ, Hector MP, Liversidge HM. Accuracy of dental age estimation charts: Schour and Massler, Ubelaker and the London Atlas. Am J Phys Anthropol; 2014; 154(1), 70-78. DOI: 10.1002/ajpa.22473.
27. Willmann C, Fernandez De Grado G, Kolb C, Raul JS, Musset AM, Gros CI, Offner, D. Accuracy of Age Estimation

Using Three Dental Age Estimation Methods in a Young, Large, and Multiethnic Patient Sample. Dent J; 2023; 11(12), 288. DOI: 10.3390/dj11120288.

28. Kurniawan A, Chusida AN, Atika N, Gianosa TK, Solikhin MD, Margaretha MS, Margaretha MS, Utumo H, Marini MI, Rizky BN, Prakoeswa BFWR, Alias A, Marya A. The applicable dental age estimation methods for children and adolescents in Indonesia. Internat J Dent; 2022. DOI: 10.1155/2022/6761476.

29. Cornélio Neto WL, Conélio GC, Conceição MB. Estimate the age of the third molars through x-rays: relate case. Revista Gaúcha de Odontologia; 2006; 54(3), 230-233.

30. Silva RF, Marinho DEA, Botelho TL, Caria PHF, Bérzin F, Júnior ED. Determination of age by dental and wrist joint radiograph analyses: a forensic case report. Arq Odontol; 2008; 44(2), 45-50.

31. Silva RF, Mendes SDSC, Rosário Junior AF, Dias PEM, Martorell LB. Documental vs. biological evidence for age estimation–forensic case report. ROBRAC; 2013; 21(60), 6-10

32. Silva RF, Franco A, Dias PEM, Gonçalves AS, Paranhos LR. Interrelationship between forensic radiology and forensic odontology — A case report of identified skeletal remains. J Forensic Radiol Imag; 2013; 1(4), 201-206. DOI: 10.1016/j.jofri.2013.06.005.

BJHBS

Association between endodontic conditions and the relationship of upper posterior teeth and the thickening of the maxillary sinus mucosa. A study using cone beam computerized tomography

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Abstract

Introduction: Sinus mucosal thickness and maxillary sinusitis can be caused by several predisposing factors. Some studies indicate that sinusitis can be of odontogenic origin and that this condition is responsible for 10% to 12% of maxillary sinusitis. Objective: To assess the odontogenic conditions of the maxillary posterior teeth and their proximity to the maxillary sinus (SM) and correlate these factors with the mucosal thickness of the SM, using cone beam computed tomography images. Methodology and resources: CBCT images of 393 patients aged between 18 and 75 years were analyzed, noting aspects such as the presence of root canal fillings, periapical lesions, and the contact of the roots of the maxillary posterior teeth with the floor of the SM. Results and discussion: All results are presented as odds ratios (OR) and 95% confidence intervals (CIs). Statistically significant differences ($p<0.001$) were found among the variables studied, and the probability of thickness of the MS mucosa is greater in men (1.73%), 7.27% when periapical lesions are present and 2.03% in the presence of endodontic treatment. Conclusion: We conclude that periapical lesions, root canal filling and proximity of the dental roots to the floor of the maxillary sinus interfere with the appearance of sinus mucosal thickness.

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Introduction

The paranasal sinuses are cavities inside some skull and facial bones caused by pneumatic *diverticula*. These sinuses have several functions, including reducing the weight of the skull, protecting infraorbital and intracranial structures, absorbing impacts and contributing to facial growth. The maxillary sinus (MS) is the largest of the paranasal sinuses and is the pneumatic space contained within the maxilla, a pair of bones that constitute the middle third of the face.[1]

Thickness of the sinus mucosa, greater than 2mm, and maxillary sinusitis are globally prevalent pathologies.[2,3] Inflammation of the Schneiderian membrane can be caused by several predisposing factors, including upper respiratory tract infections, immunodeficiency, asthma and inhalation of foreign bodies.[4] However, some studies indicate that sinusitis can be of odontogenic origin and that this condition corresponds to 10 to 12% of maxillary sinusitis, being more common in adults than in children.[2,3]

The anatomical proximity of the floor of the MSs to the apices of the teeth leads one to assume that dental and sinus pathologies may be closely related (AKSOY, 2016). According to some studies, periapical changes, dental implants, periodontal diseases and endodontic treatments point to such a relationship.[5,6]

Since treatments differ in accordance with the cause of maxillary sinusitis, an accurate diagnosis needs to be made. If the underlying odontogenic conditions are not diagnosed correctly, the treatment will not be successful. However, even today, some scholars disagree with this position and believe that sinus mucosal thickening is totally unrelated to dental pathologies and, therefore, further studies must be conducted.[7]

In search of evidence to prove this association between periapical lesions, intimate contact relationships of dental roots, and endodontic treatments with mucosal thickening and MS opacification, this study using Cone Beam Computed Tomography (CBCT) was conducted to better assess dental and sinus conditions and to evaluate the existence of this relationship in a Brazilian population.

The study's null hypothesis is that periapical lesions, endodontic treatment, and proximity to the root of teeth with MS do not influence its thickening.

Methodology

This is an observational, longitudinal, and retrospective study, which was approved by the Permanent Committee on Ethics in Research Involving Human Beings of the State University of Maringá (CAAE - 03629118.4.00000104). Since it is an observational study, the informed consent form was waived for patients. A total of 750 CBCT scans were evaluated, and referred to the Image Laboratory for Clinical Research (LIPC) of the Health Technology Center (CTS), of the Research Support Center Complex (COMCAP), located in the Department of Dentistry of the Universidade Estadual of Maringá (DOD-UEM), from 2014 to 2019. The study included CBCT scans performed for different purposes, which contained the regions of interest and images of: patients younger than 18 years old; patients with a history of trauma or surgery in the region to be investigated; syndromic patients; patients with some bone pathology (especially osteoporosis); patients whose images showed scattering and insufficient visualization of bone edges; edentulous patients; and patients in whom a mucous retention cyst was present.

All images were obtained by use of i-CAT Next Generation® equipment (Imaging Sciences International, Hatfield, PA, USA), with a volume of 300µ of isometric voxel, FOV (Field of View) of 17 × 23cm, tube tension of 120kVp and tube current 3-8mA. All examinations were performed by the same radiologist as prescribed by the clinician responsible for each patient. The images were analyzed with the tomographer's own program (Xoran version 3.1.62; Xoran Technologies, Ann Arbor, MI, USA) by two previously calibrated examiners, who evaluated the images at two different times, with a minimum time interval of one week between assessments. To avoid eyestrain, only 10 images/day were observed.

Twenty tomographic images were evaluated for calibration purposes. All images used for calibration came from the LIPC database and were subsequently discarded. The calibration lasted 2 weeks, and intra-examiner agreement was evaluated, using Cohen's Kappa test for categorical and discrete variables and Lin's Concordance Correlation Coefficient (CCC) for continuous variables to ensure the reproducibility of the study. A significance level of 5% was adopted.

For image analysis, the work by Aksoy et al. 2019 was used as a reference, where mucosal thickness in the MS at CBCT was measured (at the point of maximum thickness of the MS floor), recorded and classified as: Grade 1 - 0 to ⩽2mm (normal mucosal thickening), Grade 2 - <2 to ⩽10mm (moderate mucosal thickening), and Grade 3 - <10mm (severe mucosal thickening)8.

The presence of the first and second premolars, as well as the first, second and third molars in the upper right and left regions, was registered in the data table. The presence of root canal fillings and periapical lesions of these teeth was also recorded when these had a thickness greater than or equal to 0.5mm. The anatomical relationship between MSs and teeth was determined for each tooth individually and classified as: Type 1: A gap exists between the roots and the MS floor; Type 2: At least one tooth root is in contact with the MS floor; and Type 3: At least one tooth root has entered the floor of the MS. These measurements and classifications were made in the sagittal and coronal reconstructions and entered into a spreadsheet for statistical analysis.

A database containing qualitative and quantitative variables was organized to allow tabulation and statistical analysis. All statistical procedures were performed with the R software version 3.6.0. (R., Auckland, NZL). Descriptive analysis was performed to extract initial information from the data. The chi-square test was used for categorical variables. A multinomial logistic regression model was performed for subgroup analysis. All results are presented as odds ratios (OR) and 95% confidence intervals (CIs) obtained by multinomial regression, while controlling for the risk of thickening in relation to age, sex, periapical lesions, endodontic treatment, and the relationship with the roots and the MS. The Intraclass Correlation Coefficient (ICC) was used to evaluate inter-observer and intra-observer agreement. The chi-square test was applied to compare the distribution of MS mucosal thickness according to the number of cases and their form (unilateral or bilateral), the type of relationship between the tooth roots and MS thickness, and the association between periapical lesions, endodontic treatment, and thickening of the MS mucosa.

The established significance level was p-value ⩽0.05.

Figure 1. Parasagittal reconstruction exemplifying measurements of mucosal thickness in the sinus mucosa.
Legend: A: Grade 1 - 0 to ⩽2mm (normal mucosal thickening), B: Grade 2 - <2 to ⩽10mm (moderate mucosal thickening) and C: Grade 3 - <10mm (severe mucosal thickening)

Figure 2. Parasagittal reconstruction exemplifying the close relationships between the roots of posterior teeth and the floor of the sinus mucosa.
Legend: A: Type 1: A space between the roots and the sinus floor, B: Type 2: At least one tooth root is in contact with the sinus floor, and C: Type 3: At least one tooth root has ente-red the sinus floor from the breast

Results

The sample consisted of 393 individuals, 246 of whom were female (62.59%) and 147 male (37.40%). The mean age was 33.64 years (±12.21), with a maximum of 80 years and a minimum of 18 years. Use of the Intraclass Correlation Coefficient (ICC) led to the conclusion that the null hypothesis — that inter-examiner agreement is purely random — was rejected for all variables under study (p-value <0.001). In other words, inter-examiner and intra-examiner agreements were verified, with coefficients ranging from 0.89 to 0.98 (Landis & Koch, 1977), thus indicating excellent reliability and reproducibility.

Table 1. Demographic characteristics of study subjects

Analyzed groups	Thickness of the MS mucosal (n=284)		Normal mucosa of the MS (n=502)	
	N	%	n	%
Gender				
Male	7.2 (±0.8)	8.2 (±0.8)	7.4 (±0.3)	56.67%
Female	8.5 (±1.0)	9.5 (±1.0)	8.4 (±0.3)	36.67%
Age				
18 to 33	10.5 (±1.0)	11.5 (±1.0)	10.5 (±0.3)	40.00%
34 to 49	11.0 (±1.0)	12.0 (±1.0)	11.5 (±0.3)	40.00%
>49	12.3 (±1.1)	13.3 (±1.1)	12.5 (±0.3)	36.67%

Legend: MS= Maxillary Sinus.

The right MS mucosa presented 250 type 1 (normal), 94 type 2 (moderate), and 49 type 3 (severe) cases. The left MS mucosa was responsible for 252 type 1 (normal), 97 type 2 (moderate), and 44 type 3 (severe) cases. A breakdown of the total of 786 MSs and adjacent teeth shows 736 first premolars, 725 second premolars, 725 first molars, 749 second molars, and 224 third molars, totaling n=3159 teeth and a total of 771 missing teeth. Of the total, 502 MSs were classified with type 1 mucosa (0mm to 2mm-normal), 191 MSs with type 2 mucosa (2.1mm to 10mm-moderate), and 93 MSs with type 3 mucosa (greater than 10mm-severe), resulting in 284 MSs (36.13%) with moderate to severe thickening. Thickening of the MS mucosa greater than 2mm in one or both SMs was observed in 193 (49.11%) patients. This thickening was present only on the right side in 55 patients, of which 44 type 2 and 11 type 3; while 52 showed thickening only on the left side, with 39 type 2 and 13 type 3; and 86 were bilateral.

As shown in Table 2, when analyzing all MSs (n=786), most scans presented grade 1 thickening (normal) and a type 2 relationship with the roots (at least one tooth root in contact with the MS floor). However, grade 2 thickening and type 2 root relationship were significantly elevated (n=135).

Table 2. Association between the types of anatomical relationship of the roots of the teeth and thickening of the maxillary sinus mucosa.

Degree of thickness	Number total	Type 1 relationship all teeth n (%)	Type 2 relationship at least 1 tooth n (%)	Type 3 relationship with at least 1 tooth n (%)	Total n (%)
1	502	100 (12.72)	361 (45.93)	41 (5.22)	502 (63.87)
2	191	14 (1.79)	135 (17.17)	42 (5.34)	191 (24.30)
3	93	4 (0.51)	61 (7.76)	28 (3.56)	93 (11.83)
Total	786	118 (15.02)	557 (70.86)	111 (14.12)	786 (100)

Legend: Degree of thickness = Degree of thickness of the MS mucosa. Total number = Total number of MS. Type 1 relationship all teeth = Number of MSs that presented type 1 anatomical relationship (%) in all teeth. Type 2 relationship with at least 1 tooth = Number of MSs that have type 2 anatomical relationship with at least 1 tooth (%). Type 3 relationship in at least 1 tooth = Number of MSs that presented type 3 anatomical relationship in at least 1 tooth (%).

Table 3 shows that, when analyzing all MSs (n=786), grade 1 MS thickening predominates in all tooth groups.

Table 3. Association between the degree of mucosal thickening in the MS and different groups of teeth

MS thickness degree	Group of teeth					
	1 PM n (%)	2PM n (%)	1M n (%)	2M n (%)	3M n (%)	Total n (%)
1	476 (15.07%)	468 (14.81%)	469 (14.84%)	482 (15.26%)	141 (4.46%)	2036 (64.44%)
2	175 (5.54%)	169 (5.35%)	171 (5.41%)	176 (5.57%)	55 (1.74%)	746 (23.61%)
3	85 (2.69%)	88 (2.79%)	85 (2.70%)	91 (2.88%)	28 (0.89%)	377 (11.95%)
Total	786	118 (15.02)	557 (70.86)	111 (14.12)	786 (100)	

Legend: 1PM: 1 premolar. 2PM-2 premolar. 1M- 1 molar. 2M- 2molar. 3M- 3 molar.

Table 4 shows that the 1M and 2M are the teeth that present the greatest association with MS mucosa thickening (n=786) grades 2 and 3 in type 2 and 3 root relationships with the MS.

Table 4. Association between groups of teeth with MS mucosal thickness and type of relationship between roots and MS.

Groups of teeth	Thickness of the MS mucosa	Relationship of the roots with the MS			
		Type 1 n	Type 2 n	Type 3 n	Total n
1PM	1	466	10	0	476
	2	169	6	0	175
	3	78	7	0	85

Table 4. Association between groups of teeth with MS mucosal thickness and type of relationship between roots and MS (cont.)

Groups of teeth	Thickness of the MS mucosa	Relationship of the roots with the MS			
		Type 1 n	Type 2 n	Type 3 n	Total n
2PM	1	380	83	5	468
	2	126	42	1	169
	3	63	23	2	88
1M	1	163	280	26	469
	2	31	116	24	171
	3	15	54	16	85
2M	1	121	336	25	482
	2	32	124	20	176
	3	9	63	19	91
3M	1	51	84	6	141
	2	12	38	5	55
	3	3	19	6	28
Total		1719	1285	155	3159

Legend: 1PM: 1 premolar. 2PM-2 premolar. 1M- 1 molar. 2M- 2molar. 3M- 3 molar.

Individual analysis of the MSs shows that most of the grade 2 and 3 thickenings occurred unilaterally (n=154 and n=73, respectively), and that these differences are statistically significant (Table 5).

Table 2. Association between the types of anatomical relationship of the roots of the teeth and thickening of the maxillary sinus mucosa

Thickness of the MS mucosa (n=786)	Maxillary Sinus n (%)	Form of involvement n (%)		p value
		Unilateral n (%)	Bilateral n (%)	
1	502 (63.88)	107 (13.61)	395 (50.25)	<0.001*
2	191 (24.30)	154 (19.59)	37 (4.71)	
3	93 (11.83)	73 (9.32)	20 (2.54)	
Total	786 (100)	334 (42.52)	452 (57.5)	

Legend: *p<0.05 statistically significant difference. Chi square test.

Table 6 shows that approximately half of the MSs present some degree of mucosal thickening (n=1122), and that n=585 of them have some relationship with the root of at least one tooth.

Table 6. Type of relationship between tooth roots and MS thickening

Thickness of the MS mucosa	Relationship of the roots with the MS			Total n (%)	P-value
	Type 1 n (%)	Type 2 n (%)	Type 3 n (%)		
1	1181 (37.40)	793 (25.11)	62 (1.96)	2036 (64.47)	<0.001*
2 + 3	537 (17.1)	492 (15.58)	93 (2.94)	1122 (35.62)	
Total	1718 (54.5)	1285 (40.6)	155 (4.9)	3158 (100)	
P-value	0.0002*[3]	0.038*[3]	<0.001*[3]		

Legend: *p<0.05 statistically significant difference. Chi square test.

Table 7 shows that the majority of the 786 MSs did not present any mucosal thickening. However, in cases where the MS presented thickening, both grade 2 and grade 3, an association existed between the thickening and the presence of a periapical lesion or endodontic treatment, and this association was statistically significant (p<0.001).

Table 7. Association values between periapical lesions, root canal treatment and mucosal thickening in the MS.

Degree of thickness of the MS mucosa	Total	Total number of periapical lesions	Mean number of periapical lesions of the MS	P value	Total number of endodontic treatments	Mean number of root treatments per MS	P value
1	502	22	0.04 a	<0.001	81	0.16 a	<0.001*
2	191	43	0.22 b	<0.001	54	0.28 b	0.001*
3	93	28	0.30 c	<0.001	26	0.28 c	<0.001*
Total	786	93	0.12		161	0.20	

Legend: Chi-square test *Statistically significant (p<0.05) Different superscript letters indicate that a significant difference between the datasets pertaining to the presence of lesions and the degree of mucosal thickening, in each vertical column, at the 0.05% level. Values with the same superscript letters were not statistically different, p<0.05 (a vs b, a vs c, and b vs c).

Given the evidence from the statistical tests suggesting a possible association between some variables that could cause thickening of the MS mucosa, a new statistical test was performed. Table 8 presents the risk of MS thickening concerning the presence of a periapical lesion, endodontic treatment, male sex, and type 2 and 3 root relationships of the teeth, with the risks being 7.27, 2.03, 1.73, 1.36, and 3.29, respectively.

Table 8. OR and 95% CIs for risk of MS thickening in terms of sex, age, periapical lesion, endodontic treatment and relationship between tooth roots and MS.

Variables	MS mucosal thickness						
	Present	%	Absent	%	OR	95%CI	p value
Gender							
Male (n=294)	130	16.54	164	20.86	1.73	1.29-2.34	0.0004*
Female (n=492)	154	19.59	338	43.00	1		
Age							
18 to 33 (n=431)	144	18.32	287	36.56	1		
34 to 49 (n=255)	95	12.08	160	20.35	1.18	0.86-2.20	0.34
>49 (n=100)	45	5.72	55	7.00	1.55	1.04-2.53	0.06
Periapical lesion							
Present (n=93)	71	9.03	22	2.8	7.27	4.39-12.04	<0.0001*
Absent (n=693)	213	27.0	480	61.07	1		
Endodontic treatment							
Present (n=161)	80	10.18	81	10.30	2.03	1.43-2.89	<0.0001*
Absent (n=625)	204	25.95	421	53.6	1		
Proximity relationship of roots with MS							
Type 1 (n=1718)	537	17	1181	37.39	1		
Type 2 (n=1285)	492	15.58	793	25.11	1.36	1.17-1.58	<0.0001*
Type 3 (n=155)	93	2.94	62	1.96	3.29	2.35-4.62	<0.0001*

Legend: Chi square test. CI = confidence interval at 95%.

Discussion

Sinusitis is a pathology that affects the population of the entire world and can manifest itself chronically or acutely (Aksoy and Orhan, 2019). The main causes are septum deviation or enlarged nasal turbinates, issues related to bacterial, viral, and fungal infections, as well as allergies and polyps, cystic fibrosis, nasal and sinus tumors, and odontogenic conditions.[9]

Some studies report a prevalence rate of mucosal sinus thickening that varies between 37% and 62%.[6,10–12] In our study, a prevalence of 36.13% was found, which is close to the values found in the literature.

Our results reveal that the teeth most closely related to the MSs were the maxillary second molars, first molars, second premolars, third molars and first premolars respectively. This result is very similar to the findings of several authors,[13–16] except for the inversion of the order of the second premolar with the third molar.[13–16] This inversion may have been caused by the number of missing third molars in our sample.

In the present study, 585 roots were found in close contact with the floor of the MS or within the MS, suggesting that this is an odontogenic factor for possible mucosal thickening. The analysis shows that when there is a relationship between the floor of the MS and the root of the type 3 tooth, the probability of developing mucosal thickening increases (OR= 3.29), while the probability is lower (OR=1.36) in type 2.

In clinical practice, the proximity between adjacent teeth and the MS should be considered, since alterations can result in various complications such as sinusitis, oroantral fistula, displacement of the file, and root or filling materials into the MS.[15]

In our work, we observed that individuals over 49 years of age have an increased chance of having mucus thickening in the MS (OR=1.55). Some studies[3,6,10] indicate that the occurrence of mucosal thickening increases with patient age. According to Aksoy and Orhan 2018, older individuals may be more susceptible to dental disease, which in turn increases the prevalence of maxillary sinusitis.[16]

From the results obtained, we observed a statistically significant difference (p=0.0004) between the presence of mucous thickening and sex. Males are more likely (OR=1.73) to develop this alteration than females. Perhaps this greater involvement can be explained by the fact that some studies show us that men have more endodontic treatments and periapical lesions. In addition, women between 40-59 years of age use regular dental services approximately 40% more than men in the same age group.[17]

With regard to the prevalence of periapical lesions, our results in general show that 11.83% (n=93) of the teeth had periapical lesions and 20.48% (n=161) had undergone endodontic treatment. These values are close to those found by Aksoy and Orhan 2018. Our study found that 71 teeth (76.34%) with periapical lesions and 80 teeth (49.68%) with endodontic treatment were involved with thickening of the MS mucosa.

Most previous studies[2,3,6,18–21] have reported a positive association between periapical lesions and mucosal thickening, although some results show the opposite.[10–12]

The results of this study indicate a positive relationship between the prevalence of sinus mucosal thickening and the presence of periapical lesions (p<0.001). Furthermore, we found that individuals with periapical lesions are more likely (OR=7.27) to show thickening of the MS mucosa. In individuals with endodontic treatment, this chance decreases considerably (OR= 2.03) but even so, it was twice as high as that of an untreated individual.

This can be explained, given that, after the occurrence of pulpal necrosis, bacterial virulence factors promote bacterial invasion and tissue degradation in the periodontal bone.[22] The infection and its by-products originating from the infected tooth can spread to the MSs and irritate the sinus mucosa.[23,24]

A relevant point to be remembered is that mild mucus thickening in the MS is a normal and frequent finding in asymptomatic patients, but sinus mucus thickening greater than 2mm may

be related to sinusitis.[25] On the other hand, mucosal thickening of the MS equal to or greater than 4mm may represent rhinosinusitis, and this individual may suffer from headache or facial pain, daytime cough, nighttime cough, obstruction, and nasal discharge.[26] In cases where treatments have already been performed for these conditions and a recurrence of the case happens, a dental investigation should be carried out.

This research study presents limitations due to the number of participants and would have been greatly enriched by an expansion that would enable us to evaluate patients and verify the clinical signs and symptoms caused by the mucous thickening, identify where patients presented symptoms, what these were, how to treat them and how to help the patient in relation to the treatment.

Conclusion

We can conclude that the presence of periapical lesions, root canal filling, and proximity of the dental roots to the floor of the sinus mucosa may be related to the appearance of sinus mucosa mucosal thickening. Patients with type 3 mucosal thickening can develop maxillary sinusitis of odontogenic origin.

References

1. Sérgio Batista P, Do Rosário Junior AF, Wichnieski C. Contribuição para o estudo do seio maxilar. Revista Portuguesa de Estomatología, Medicina Dentária e Cirugia Maxilofacial. 2011 Oct;52(4):235–9.
2. Kasikcioglu A, Gulsahi A. Relationship between maxillary sinus pathologies and maxillary posterior tooth periapical pathologies. Oral Radiol. 2016 Sep 28;32(3):180–6.
3. Shanbhag S, Karnik P, Shirke P, Shanbhag V. Association between Periapical Lesions and Maxillary Sinus Mucosal Thickening: A Retrospective Cone-beam Computed Tomographic Study. J Endod. 2013 Jul;39(7):853–7.
4. Aksoy U, Kermeoğlu F, Kalender A, Eren H, Kolsuz ME, Orhan K. Cone-beam computed tomography evaluation of palatogingival grooves: a retrospective study with literature review. Oral Radiol. 2017 Sep 3;33(3):193–8.
5. Vallo J, Suominen-Taipale L, Huumonen S, Soikkonen K, Norblad A. Prevalence of mucosal abnormalities of the maxillary sinus and their relationship to dental disease in panoramic radiography: results from the Health 2000 Health Examination Survey. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2010 Mar;109(3):e80–7.
6. Lu Y, Liu Z, Zhang L, Zhou X, Zheng Q, Duan X, et al. Associations between Maxillary Sinus Mucosal Thickening and Apical Periodontitis Using Cone-Beam Computed Tomography Scanning: A Retrospective Study. J Endod. 2012 Aug;38(8):1069–74.
7. Melén I, Lindahl L, Andréasson L, Rundcrantz H. Chronic Maxillary Sinusitis: Definition, Diagnosis and Relation to Dental Infections and Nasal Polyposis. Acta Otolaryngol. 1986 Jan 8;101(3–4):320–7.
8. Aksoy U, Orhan K. Association between odontogenic conditions and maxillary sinus mucosal thickening: a retrospective CBCT study. Clin Oral Investig. 2019 Jan 17;23(1):123–31.
9. De Lima CO, Devito KL, Vasconcelos LRB, Prado M Do, Campos CN. Sinusite odontogênica: uma revisão de literatura. Rev Bras Odontol. 2017 Mar 31;74(1):40.
10. Phothikhun S, Suphanantachat S, Chuenchompoonut V, Nisapakultorn K. Cone-Beam Computed Tomographic Evidence of the Association Between Periodontal Bone Loss and Mucosal Thickening of the Maxillary Sinus. J Periodontol. 2012 May;83(5):557–64.
11. Janner SFM, Caversaccio MD, Dubach P, Sendi P, Buser D, Bornstein MM. Characteristics and dimensions of the Schneiderian membrane: a radiographic analysis using cone beam computed tomography in patients referred for dental implant surgery in the posterior maxilla. Clin Oral Implants Res. 2011 Dec;22(12):1446–53.
12. Rege ICC, Sousa TO, Leles CR, Mendonça EF. Occurrence of maxillary sinus abnormalities detected by cone beam CT in asymptomatic patients. BMC Oral Health. 2012 Dec 10;12(1):30.
13. Eberhardt JA, Torabinejad M, Christiansen EL. A computed tomographic study of the distances between the maxillary sinus floor and the apices of the maxillary posterior teeth. Oral Surgery, Oral Medicine, Oral Pathology. 1992 Mar;73(3):345–7.
14. Kang SH, Kim BS, Kim Y. Proximity of Posterior Teeth to the Maxillary Sinus and Buccal Bone Thickness: A Biometric Assessment Using Cone-beam Computed Tomography. J Endod. 2015 Nov;41(11):1839–46.
15. Tian X mei, Qian L, Xin X zhen, Wei B, Gong Y. An Analysis of the Proximity of Maxillary Posterior Teeth to the Maxillary Sinus Using Cone-beam Computed Tomography. J Endod. 2016 Mar;42(3):371–7.
16. Aksoy U, Aksoy S, Orhan K. A cone-beam computed tomography study of the anatomical relationships between mandibular teeth and the mandibular canal, with a review of the current literature. Microsc Res Tech. 2018

Mar;81(3):308–14.
17. Camargo MBJ, Dumith SC, Barros AJD. Uso regular de serviços odontológicos entre adultos: padrões de utilização e tipos de serviços. Cad Saude Publica. 2009 Sep;25(9):1894–906.
18. Bornstein MM, Wasmer J, Sendi P, Janner SFM, Buser D, von Arx T. Characteristics and Dimensions of the Schneiderian Membrane and Apical Bone in Maxillary Molars Referred for Apical Surgery: A Comparative Radiographic Analysis Using Limited Cone Beam Computed Tomography. J Endod. 2012 Jan;38(1):51–7.
19. Goller-Bulut D, Sekerci AE, Kose E, Sisman Y. Cone beam computed tomographic analysis of maxillary premolars and molars to detect the relationship between periapical and marginal bone loss and mucosal thickness of maxillary sinus. Med Oral Patol Oral Cir Bucal. 2015;e572–9.
20. Nunes CABCM, Guedes OA, Alencar AHG, Peters OA, Estrela CRA, Estrela C. Evaluation of Periapical Lesions and Their Association with Maxillary Sinus Abnormalities on Cone-beam Computed Tomographic Images. J Endod. 2016 Jan;42(1):42–6.
21. Yildirim E, Ciftci ME, Kamak G, Aktan AM. Evaluation of the relationship between maxillary sinus floor position and maxillary sinusitis using cone beam computed tomography. Oral Radiol. 2017 Jan 11;33(1):16–22.
22. Hauman CHJ, Chandler NP, Tong DC. Endodontic implications of the maxillary sinus: a review. Int Endod J. 2002 Feb;35(2):127–41.
23. Longhini AB, Ferguson BJ. Clinical aspects of odontogenic maxillary sinusitis: a case series. Int Forum Allergy Rhinol. 2011 Sep;1(5):409–15.
24. Nurbakhsh B, Friedman S, Kulkarni G V., Basrani B, Lam E. Resolution of Maxillary Sinus Mucositis after Endodontic Treatment of Maxillary Teeth with Apical Periodontitis: A Cone-Beam Computed Tomography Pilot Study. J Endod. 2011 Nov;37(11):1504–11.
25. Maillet M, Bowles WR, McClanahan SL, John MT, Ahmad M. Cone-beam Computed Tomography Evaluation of Maxillary Sinusitis. J Endod. 2011 Jun;37(6):753–7.
26. Baracat ECE, Araújo-Neto SA de. Clinical progression of incidental tomographic findings in paranasal sinuses of asymptomatic individuals: cohort study. J Pediatr (Rio J). 2011 Sep 30.

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Venous thromboembolism during air travel in pregnant women

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Abstract

Introduction. Air travel (AT) has become an integral part of modern life, including for pregnant women who may need to travel for a variety of reasons. While the overall risk of venous thromboembolism (VTE) during AT is low, specific populations, such as pregnant women, face significantly higher risks. This study explores the relation between air travel and VTE in pregnant women, by examining the risk factors, epidemiology, and preventive strategies. Objectives. To analyze the incidence of VTE in pregnant women during air travel, identify associated risk factors, and evaluate prophylactic measures to mitigate these risks. Methodology and Resources. A comprehensive review of existing literature was conducted, focusing on studies related to the incidence of VTE in air travel, especially in pregnant women. Data from epidemiological and review studies were reviewed to assess the risk factors and the efficacy of prophylactic measures, including mechanical and pharmacological interventions. Results and Discussion. The review indicates that pregnant women are at an elevated risk for VTE, especially during flights that last longer than 6 hours. Contributing factors include venous stasis, hypercoagulability, and hypoxia. Prophylactic measures, such as the use of graduated compression stockings and low molecular weight heparin (LMWH), are recommended for high-risk individuals, although routine pharmacological prophylaxis is not generally indicated. Conclusion. The study highlights the importance of a personalized risk assessment for pregnant women who plan to travel by air. While general preventive measures should be universally recommended, pharmacological prophylaxis should be reserved for high risk travelers. Further research is needed to refine the guidelines for pregnant travelers and ensure their safety.

Keywords: thrombosis; pregnancy; anticoagulants; air travel.

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Introduction

Air travel (AT) has become increasingly common and accessible in recent decades and has become part of the lives of women, including pregnant ones, who need to travel for work, leisure, or even as part of their employment in the airline industry.[1] In 2015, approximately 3.5 billion people were estimated to have traveled by air worldwide, a 6.8% increase compared to the previous year.[2] In Brazil, over the past 10 years, with the exception of 2016, the number

of passengers on commercial flights has followed the global growth trend. According to the National Civil Aviation Agency (ANAC), in 2016, around 115 million Brazilians were transported on domestic and international flights.[3] The increase in the number of passengers has been accompanied by an increase in medical issues related to AT, especially in long-haul or long-distance flights. Overall, the most frequently reported medical issues have been hypoxia, infectious diseases, jet lag, anxiety crises, and venous thromboembolism (VTE).[4] Generally, the risk of VTE during air travel is not high; however, it can be significant in some specific groups, such as pregnant women.[5-7]

Epidemiology

Defining which VTE episodes are directly related to AT can be difficult, since there is no general consensus about the maximum time interval between the landing of the aircraft and the diagnosis of VTE that characterizes such an association.[2-4] Initial studies were based on isolated clinical data with no laboratory or ultrasound confirmation, and the definition of what constituted a long-distance flight varied greatly.[4,7]

Another factor complicating a more consistent epidemiological analysis is that studies tend to be rather specific, with some exclusively reporting the incidence of deep vein thrombosis (DVT) and others focus only on pulmonary embolism (PE), while a smaller number encompasses both factors.

The incidence and prevalence of VTE also vary according to the method used for diagnosis, and also whether it was measured through active search, for example, in asymptomatic patients, or if it was conducted only in symptomatic patients who sought medical assistance after a flight. Nevertheless, a direct relation appears to exist between the duration of the flight and VTE, which mostly manifests itself within the first two weeks after landing, within four days on average, and with the risk persisting for up to four weeks.[3,8]

A meta-analysis of 14 studies involving 4,055 episodes of DVT, published in 2009, showed that the pooled overall relative risk is 2.8 times higher in long AT. Furthermore, the absolute risk of symptomatic DVT in the 8 weeks following a long-haul flight is one episode in 4,500 flights.[8] Such data, therefore, do not suggest universal and widespread practices of VTE prophylaxis during AT, although, risks can increase significantly in some passenger profiles.

A cohort study involving 7,592 employees of large corporations who traveled regularly for about 4.4 years showed that the risk of VTE can increase up to 20 times in passengers who have recently undergone surgery, and up to 18 times in those diagnosed with an active cancer.[8]

The incidence of VTE after flights lasting longer than 4 hours is of one event in 4,656 flights,[5,9] and around 0.5% after those lasting over 8 hours, in low and medium-risk passengers. Severe episodes of PE immediately after flights of up to 8 hours are extremely rare. On flights lasting longer than 12 hours, this figure is five episodes per million.[10]

Air travel and venous thromboembolism

The association between AT and VTE has been recognized for more than 70 years. In the 1970s, the increase in the number of flights that transported passengers in positions that often require prolonged immobility, led to the coining of the term “economy class syndrome”. However, VTE can affect passengers in any aircraft seating class.[11] At least eight risk factors can be related to

VTE during AT: flight duration exceeding 6 hours; age higher than 40; use of oral contraceptives (OC) or hormone replacement therapy (HRT); gravidic-puerperal cycle; chronic venous disease; obesity (body mass index [BMI] >30kg/m^2); acquired or genetic thrombophilias; and extremes of stature.[12,13] Combinations of these factors increase the risk; for example, an overweight woman using OC has a risk of VTE after a long flight 60 times higher than a woman without these factors.[6]

Aircraft-related VTE risk factors

Hypoxia

For financial reasons, most commercial flights maintain an atmospheric pressure (AP) inside the aircraft similar to an altitude between 1,800 and 2,400 meters above sea level, since maintaining higher AP requires greater fuel consumption due to increased aircraft weight.[4] Prolonged passenger exposure to this hypoxia can trigger the activation of the extrinsic coagulation pathway through microparticles carrying tissue factor[14,15] and raise serum concentrations of factor VIII and plasminogen activator inhibitors (PAI) 1.[16] Nevertheless, the clinical impact seems to be restricted to passengers with associated risk factors, such as thrombophilias or OC users.[15]

Position during travel

In addition to causing compression of the popliteal vein for long periods, venous stasis caused by sitting in a position with limited space between rows that do not allow regular movement of the feet and lower limbs can trigger a coagulation cascade during AT.[4,14,17,18] This factor is more evident in people taller than 1.90 meters, since they remain in more restricted positions, and in those shorter than 1.60 meters, in whom compression of the popliteal vein occurs because their feet do not touch the ground and remain in a hanging position.[17,18]

Dehydration

During AT, the cabin humidity is generally low. In a study evaluating 18 flights, the relative average air humidity in the cabin was 10.3%. For purposes of comparison, the relative humidity ranges between 40 and 60% indoors at sea level, and between 70 and 90% outdoors.[19] Moreover, dehydration can be intensified by regular consumption of alcoholic beverages, coffee, and teas, which induce diuresis.[4] Based on these data, passengers are advised to increase their fluid intake to prevent VTE.[19,20] Contradicting these findings, some studies do not support the hypothesis that, in isolation, fluid loss during AT contributes to VTE formation.[15,19]

Flight duration

Although there is no consensus regarding the exact duration for a flight to be considered long-haul, a clear relationship exists between VTE and flights lasting longer than 6 hours. The risk of VTE increases 2.3 times in long flights compared to short flights, and rises by 26% for every two hours of flight.[2,4] The estimated risk of fatal PE is 0.5/106 for flights over 3 hours and 1.3/106 for flights over 8 hours.[1]

Class type and seat location

Although the term “economy class syndrome” is systematically used as a synonym for AT-related VTE, the risk factors for passengers traveling in any class — economy, business or first

— remain basically equal.[4,22] However, the risk of VTE doubles for passengers seated by the window compared to those seated in the aisle, especially obese passengers, for whom the risk increases sixfold.[6,22]

Passenger-associated risk factors

Gender

No statistical differences in VTE between males and females were found in several studies.[23] However, Lapostolle et al. published a cohort study that indicates that the female sex constitutes an independent risk factor for VTE after long-duration AT that is three times higher than in males (0.61 vs. 0.20 per 1 million passengers).[24]

Others

Recent surgery, cancer, acquired and genetic thrombophilias, oral contraceptives, and HRT are considered additional risk factors for passengers engaging in long-distance AT.[4,5,8,22,25-27]

Pregnancy

Pregnant women present the three etiopathogenic components of Virchow's triad: a) venous stasis, due to compression of the inferior vena cava and left common iliac vein by the gravid uterus and decreased venous tone due to the myorelaxant action of progesterone; b) hypercoagulability, secondary to the induction of hepatic synthesis of coagulation FVII, FVIII, and FX by placental estriol, increased fibrinogen and PAI I and II, and decreased protein S synthesis; and c) endothelial injury, which occurs during nidation, endovascular remodeling of the uteroplacental arteries, and with the delivery.[28,29] These changes, which are important to protect women from bleeding during childbirth, predispose to VTE.[29,30]

Recent data showed that approximately 44% of women have traveled during pregnancy, among which around 66% sought some form of counseling.[31]

In the absence of obstetric complications or comorbidities, AT is generally safe for pregnant women.[31] Most airlines allow boarding up to the 36th week of pregnancy, although some restrict international flights during the early stages due to the risk of spontaneous abortion.[32]

Nausea and hyperemesis gravidarum, common complications in the first trimester, can exacerbate dehydration due to fluid loss and the inability to replenish fluids, as well as limit ambulation, all of which favor the development of VTE.[31]

In the third trimester, the risk of VTE may increase due to excess weight or obesity, restricted mobility, preeclampsia, or even the onset of premature labor during AT.[31]

The frequency of VTE specifically in pregnant women who are traveling is still unknown.[6,30] The MEGA 5 study showed that the risk of VTE during the first two trimesters of pregnancy is slightly elevated. However, this risk increases in the third trimester by 9 times and in the first six weeks postpartum by 20 to 80 times.[5,33]

In any case, the estimated risk of VTE of 0.3 to 1/1,000 for pregnant women, means that pharmacological prophylaxis is not routinely recommended, although general measures should be encouraged.[6,28,31]

VTE prophylaxis during AT in pregnant women

The risk level of each passenger should be individually defined.31 Preventing VTE during pregnancy, based on guidelines that take into account risk factors, and subsequently instituting mechanical and/or pharmacological prophylaxis is the best strategy to reduce this complication.[12,28-31]

General measures

All pregnant and postpartum women should be encouraged to adopt a range of measures to mitigate the risk of VTE in flights that exceed 4 hours. These include: choosing aisle seats to facilitate movement; regular ambulation; plantar dorsiflexion exercises every 30 minutes while seated; wearing comfortable and non-restrictive clothing; maintaining good fluid intake; avoiding diuretic beverages; and keeping the space under the seat free for better foot movement.[10,12,23,28,31-34]

Graduated compression elastic stockings (GCES)

GCES, up to knee height and with a compression between 14 and 30mmHg at the ankle, reduce venous stasis and the incidence of VTE in intermediate or high-risk patients, including pregnant women. These stockings are especially useful for those with contraindications to anticoagulants and/or a high risk of bleeding.[36] Contraindications for using GCES include: substantial lower limb edema; severe peripheral arterial disease or neuropathy; dermatitis; significant lower limb deformities; and congestive heart failure.[38]

Pharmacological prophylaxis

The pharmacological prophylaxis of VTE during AT has been a subject of debate, both regarding which medications to use (and their doses) and the patient profile that should receive them.[11,12,39] No indication exists for routine use in pregnancy, and only some profiles, considered to be of high risk, may benefit from this measure.[10,39]

Acetylsalicylic acid (ASA)

The effectiveness of ASA in VTE prophylaxis is limited, and its side effects, such as bleeding, can be severe. Furthermore, ASA crosses the placental barrier and, therefore, should not be recommended.[12,23]

The LONFLIT III study showed that passengers with high-risk of VTE who used 400mg of ASA 12 hours before the flight and for three days after landing did not have protection against VTE, whereas the use of enoxaparin (1mg/kg, 2 to 4 hours before flight) reduced the prevalence of VTE (evaluated by Doppler ultrasound) from 4.8 to 0%.[11]

Low molecular weight heparin (LMWH)

LMWH is used in the prophylaxis and treatment of VTE during the gravidic-puerperal cycle, since it does not cross the placental barrier and possesses an excellent efficacy-to-safety ratio. LMWH is, therefore, the drug of choice in pregnant women at high risk of VTE during long-distance AT. Enoxaparin (0.5mg/kg, subcutaneously before the flight) is the most widely used substance due to its practicality, rapid onset of action, and protection for at least 16 hours.[6,12,34-39]

Direct oral anticoagulants (DOACs)

DOACs have gradually replaced warfarin and LMWH in the prophylaxis and treatment of VTE due to their posological advantages and excellent efficacy-to-safety profile. In addition, they do not require routine laboratory monitoring.[40] The rapid onset of action and short half-life of DOACs are characteristics that would be relevant to preventing VTE during AT.[2,39] Since there are no studies corroborating this indication, however, DOACs are still considered off-label.[2,39,40] Moreover, they are contraindicated throughout the gravidic-puerperal cycle and during breast-feeding, because they cross the placental barrier and are excreted in breast milk.[40]

Final message

Air travel, especially flights lasting more than 6 hours, poses various risks for pregnant women, one of the most feared of which is venous thromboembolism. All pregnant women who plan to take a long-distance flight should seek medical advice on the risks to safety and health both for themselves and their fetuses, including VTE prevention through general measures and, potentially, pharmacological prophylaxis.

References

1. Sammour RN, Bahous R, Grupper M, et al. Pregnancy course and outcome in women traveling to developing countries. J Travel Med. 2012; 19:289-93.
2. Clark SL, Onida S, Davies A. Long-haul and venous thrombosis: what is the evidence? Phlebology. 2018;33(5):295-7.
3. ANAC [site na internet]. Dados estatísticos [atualizado 2016 abr 19; citado 2017 out. 19]. http://www.anac.gov.br/assuntos/dados-e-estatisticas/dados-estatisticos/dados-estatisticos.
4. Gavish I, Brenner B. Air travel and the risk of thromboembolism. Intern Emerg Med. 2011;6(2):113-6.
5. Cannegieter SC, Doggen CJM, van Houwelingen HC, Rosendaal FR. Travel-related venous thrombosis: results from a large population-based case control study (MEGA study). Plos medicine. 2006; 3(8): e307.
6. Cannegieter SC, Rosendaal FR. Pregnancy and travel-related thromboembolism. Thrombosis Research. 2003; 131(S1): S55-S58.
7. Schreijer AJM, Cannegieter SC, Meijers JCM, Middeldorp S, Buller HR, Rosendaal FR. Activation of coagulation system during air travel: a crossover study. Lancet. 2006; 367:832-38.
8. Kuipers S, Venemans A, Middeldorp S, Büller HR, Cannegieter SC, Rosendaal FR. The risk of venous thrombosis after air travel: contribution of clinical risk factors. Br J Haematol. 2014; 165(3):4123.
9. Ringwald J, Schifferdecker C, Raemsch C, Strobel J, Eckstein R. Travelers´ thrombosis – A state of practice in Germany. J Travel Med. 2011; 18(1): 44-52.
10. Watson HG, Baglin TP. Guidelines on travel-based venous thrombosis. Br J Haematol. 2011; 152(1):31-4.
11. Brenner B. Prophylaxis for travel-related thrombosis? Yes. J Thromb Haemost. 2004; 2(12):2089-91.
12. Brenner B. Prophylaxis of travel-related thrombosis in women. Thromb Res. 2009; 123(S3): S26-9.
13. Martinelli I, Taioli E, Battaglioli T, et al. Risk of venous thromboembolism after air travel: interaction with thrombophilia and oral contraceptives. Arch Intern Med. 2003; 163:2771-4.
14. Schut AM, Venemans-Jellema A, Meijers JCM, et al. Coagulation activation during air travel is not initiated via the extrinsic pathway. Br J Haematol. 2015;169(6):903-5.
15. Venemans-Jellema A, Schreijer AJM, Le Cessie S, Emmerich J, Cannegieter SC. No effect of isolated long-term supine immobilization or profound prolonged hypoxia on blood coagulation. J Thromb Haemost. 2014; 12:902-909.
16. Schreijer AJ, Hoylaerts MF, Meijers JC, et al. Explanations for coagulation activation after air travel. J Thromb Haemost. 2010; 8:971-8.
17. Flinterman LE, Vlieg AH, Rosendaal FR, Cannegieter CS. Body height, mobility, and risk of first and recurrent venous thrombosis. J Thromb Haemost. 2015; 13:548-554.
18. Hitos K, Cannon M, Cannon S, Garth S, Fletcher JP. Effect of leg exercises on popliteal venous blood flow during prolonged immobility of seated subjects: implications for prevention of travel-related deep vein thrombosis. J Thromb and Haemost, 2007; 5:1890-1895.
19. Schreijer AJM, Cannegieter SC, Caramella M, et al. Fluid loss does not explain coagulation activation during air travel. Thromb Haemost. 2008; 99:1053-1059.
20. Chee YL, Watson HG. Air travel and thrombosis. Br J Haematol 2005; 130: 671-680.
21. Al-Hameed FM, Al-Dorzi HM, Abdelaal MA, et al. The Saudi clinical practice guideline for the prophylaxis of venous thromboembolism in long-distance travelers. Saudi Med J. 2017;38(1):101-7.
22. Schreijer AJ, Cannegieter SC, Doggen CJM, Rosendaal FR. The effect of flight-related behavior on the risk of venous thrombosis after air travel. Br J Haematol. 2009;144(3):425-9.
23. Aryal KR, Al-khaffaf H. Venous thromboembolic complications following air travel: what´s the quantitative risk? A literature review. Eur J Vasc Endovasc Surg. 2006; 31:187-199.

24. Lapostolle F, Le Toumellin P, Chassery M, et al. Gender as risk factor for pulmonary embolism after air travel. Thromb Haemost. 2009;102(6):1165-1168.
25. Martinelli I, Taioli E, Battaglioli T, et al. Risk of venous thromboembolism after air travel. Interaction with thrombophilia and oral contraceptives. Arch Intern Med. 2003; 163:2771-2774.
26. Nunn KP, Bridgett MR, Walters MR, Walker I. All I want for coagulation. Scott Med J. 2011;56(4):183-7.
27. Co M, Ng J, Kwong A. Air Travel Safety in Postoperative Breast Cancer Patients: A Systematic Review. Clin Breast Cancer. 2018; 18(5): e813-e817.
28. Izadi M, Alemzadeh-Ansari MJ, Kazemisaleh D, Moshkani-Farahani M, Hafiee AS. Do pregnant women have a higher risk for venous thromboembolism following air travel? Adv Biomed Res. 2015; 4:60.
29. Oliveira ALML, Marques MA. Profilaxia de tromboembolismo venoso na gestação. J Vasc Bras. 2016;15(4):293-301.
30. Antony KM, Ehrenthal D, Evensen A, Iruretagoyena JI. Travel during pregnancy: considerations for the obstetric provider. Obstet Gynecol Surv. 2017; 72(2):97-115.
31. Fernando M, Giles ML, Krishnaswamy S, Cole S. The pregnant traveler: an overview of general travel advice. Aust N Z J Obstet Gynaecol. 2023;63;638-642.
32. Greer IA. Thrombosis in pregnancy: Updates in diagnosis and management. Hematology Am Soc Hematol Educ Program. 2012; 2012:203-7.
33. Pomp ER, Lesenlink AM, Rosendaal FR, Doggen CJM. Pregnancy, the postpartum period and prothrombotic defects: Risk of venous thrombosis in the MEGA study. J Thromb Haemost. 2008; 6(8):632-637.
34. ACOG Committee Opinion No. 746: Air travel during pregnancy. Obstet Gynecol. 2018;132(2): e64-e66.
35. Schobersberger W, Toff WD, Eklöf B, et al. Traveler´s thrombosis: international consensus statement. VASA. 2008; 37:311-317.
36. Nunn KP, Bridgett MR, Walters MR, Walker I. All I want for coagulation. Scott Med J. 2011;56(4):183-7.
37. Cesarone MR, Belcaro G, Errichi BM et al. The LONFLIT4 - Concorde deep venous thrombosis and edema study: prevention with travel stockings. Angiology. 2003; 54(2):143-154.
38. Scottish Intercollegiate Guidelines Network (SIGN). Publication No. 62: Prophylaxis of Venous Thromboembolism. October 2002.
39. Lubetsky A. Prophylaxis for travel-related thrombosis? No. J Thromb Haemost. 2004; 2(12):2092-3.
40. Ringwald J, Grauer M, Eckstein R, Jelinek T. The place of new oral anticoagulants in travel medicine. Travel Med Infect Dis. 2014; 12(1):7-19.

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The advent of Paracoccidioidomycosis Ceti and new views on Lobomycosis (Jorge Lobo's disease)

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Abstract

The disease caused by the fungus *Paracoccidioides lobogeorgii* – called lobomycosis – is an uncommon morbid condition that produces skin lesions in humans. Since its formal description in the 1930s, the disease has continued to be the subject of research, especially concerning a better characterization of its etiological, diagnostic, and therapeutic aspects. In addition to a series of questions about the disease's pathology that have persisted over the decades, the possible involvement of cetaceans, which have lesions similar to those described in Homo sapiens, must also be investigated. The debate about the etiology of the proliferative verrucous cutaneous lesions caused by non-cultivable yeast that has been reported in dolphins has been almost completely resolved in recent years, as all the evidence points to the etiological agent belonging to the genus Paracoccidioides. There is no molecular evidence of infection by *Paracoccidioides lobogeorgii* in cetaceans. Based on these preliminary considerations, the objectives of the present article are (1) to review the main etiological, pathogenic, clinical, diagnostic, therapeutic, and ecoepidemiological findings of human lobomycosis and (2) to present the most important aspects of the ceti PCM of aquatic mammals.

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Introduction

Jorge Lobo's disease (JLD) or lobomycosis is a non-debilitating chronic skin disease that affects human beings – mainly in the epidermis and dermis as well as, more rarely, in the hypodermis – in the tropical area of Central and South America. It is caused by the fungus *Paracoccidioides lobogeorgii*.[1] Pathogen access to Homo sapiens tissues often occurs after traumatic injury.[2]

The first reports of the disease, dating from 1915, refer to the involvement of the Kaiabi Indians, in Mato Grosso, Central Brazil, a region in which the disease was called "miriaip" or "piriaip", i.e. that which "burns".[3] Formally, however, the first description of mycosis was made in 1931, by the Brazilian physician Jorge Lobo, professor of dermatology at the Recife Faculty of Medicine (Pernambuco, Brazil), who examined a 52-year-old rubber tapper from the Amazon basin. The patient had confluent nodular lesions on the skin over the lumbosacral region for 19 years, which the biopsy revealed to be a yeast-like microorganism, currently called *Paracoccidioides lobogeorgii*[1], a causative agent of chronic localized cutaneous paracoccidioidomycosis – both of which are microorganisms belonging to the order of the Onygenales.[4]

An interesting aspect of lobomycosis – and reason for much debate over the years – concerns the involvement of cetaceans through a disease like the morbid condition produced by *Paracoccidioides lobogeorgii*.[1,5,6] This observation generated significant doubts about whether such attacks – in humans, and cetaceans – are the same nosological entity, or if they represent different morbid conditions. More recent studies have allowed the differentiation between the disease of *H. sapiens*, lobomycosis, and the disease described in non-human animals, which, as will be seen below, became known as *paracoccidioidomycosis ceti* (*PCM ceti*).[7] It is important to highlight the existence of paracoccidioidomycosis, a disease caused by *Paracoccidioides spp*. in humans, which has been described in other mammals (e.g., armadillos), but not in cetaceans.[8]

Furthermore, researchers have been attempting to define the exact genus and species responsible for Jorge Lobo's disease for more than 90 years (Table 1). Initially described by Jorge Lobo in 1931, the disease's causative agent was surrounded by taxonomic uncertainties, particularly due to its non-cultivable nature. Over the years, various names were proposed for this elusive pathogen, including *Loboa loboi* and *Lacazia loboi*.[2,6,9] However, molecular advances have clarified these ambiguities. Recent DNA sequencing and phylogenetic studies have conclusively determined that the etiologic agent in humans is *Paracoccidioides lobogeorgii*, which places this causative agent firmly within the *Paracoccidioides genus* that also includes species known to cause systemic infections, such as *Paracoccidioides brasiliensis*.

Based on those brief notes, this article presents the main aspects of JLD, with emphasis on: (i) the etiology and its controversial points; (ii) the pathogenesis and pathological findings; (iii) the clinic, diagnosis, treatment; and (iv) ecoepidemiology – addressing the findings in humans (JLD) and related conditions in aquatic mammals (*PCM ceti*).

Etiology

For many years, the taxonomic classification of the etiological agent of human lobomycosis and the corresponding disease in cetaceans was a major scientific challenge since the pathogens involved are fungi that, although abundant in the lesions, are not cultivable.[4,10,11]

Under microscopy, whether direct examination using potassium hydroxide (KOH) or evaluation of histological sections stained by Grocott, the pathogen morphology involved in lobomycosis can be identified: globular and multinucleated cells. The microorganism has a clear, well-refringing double membrane, thicker than that seen in *Paracoccidioides brasiliensis*, with an inner membrane free from irregularities or folds. In tissues, the etiologic agent presents a globoid shape with a thick wall. The size corresponds to approximately 8 to 12μm in diameter. The multiplication of this fungus occurs through continuous budding, whose structures are identified in numerous ways in the tissues. The formation of chains of several elements joined

characteristically, like a rosary, is common.[12] The proliferation of *L. loboi* is related to a decrease in mediated immunity due to the high expression of transforming growth factor cytokines (TGF-β); this leads to reduced macrophage activity, which contributes to the non-elimination of the pathogen.[13]

In 1971, Migaki et al.[14] described a lacaziosis-like disease identified in H. sapiens in a bottlenose dolphin, Tursiops truncates, off the coast of Florida.[14] Since then, similar cases have been reported, with the first record of the disease in dolphins in Brazil, also in *T. truncatus*, being described by Simões-Lopes et al,[15] in 1993, off the coast of Santa Catarina.[15] Due to the similarities in the macroscopic and microscopic characteristics of the disease in humans and dolphins — in addition to the inability to cultivate both etiologic agents responsible for each of the morbid conditions — some believed that *L. loboi*, the etiologic agent of lobomycosis in humans, also affects these animals.[10,11] This perception is also due to the existence of several human pathogens – such as *P. brasiliensis*, *Paracoccidioides lutzii* e *L. loboi* – which are grouped in the order Onygenales and are responsible for diseases that may present cutaneous manifestations similar to the disease described in the species *T. truncatus*.[10,11,16]

The morbid condition – very similar to lobomycosis – that affects cetaceans has been named by some authors as lacaziosis-like disease (LLD = lacaziosis-like disease or lobomycosis-like disease).[9,10]

However, preliminary phylogenetic analyses have already shown a greater proximity between LLD agents and *Paracoccidioides spp.*, when compared with the fungus *L. loboi*.[10] Therefore, the hypothesis that *Lacazia loboi* would be the fungus responsible for the disease, both in humans and in dolphins, was recently challenged following the DNA sequencing of the pathogen, which identified a fungus distinct from *L. loboi* named *Paracoccidioides lobogeorgii*.[6,11,17]

From these current findings of phylogenetic and/or molecular associations, the classification used to name the disease in dolphins is no longer sustainable. Thus, and also to minimize further divergences and confusion, the name *Paracoccidioidomycosis ceti* (*PCM ceti*) has been proposed for the disease present in cetaceans – compatible lesions have already been identified in *T. truncatus* (bottle nose dolphins), *Tursiops aduncus* (Indo-Pacific bottlenose dolphins), *Sotalia guianensis* (Grey dolphins) and *Lagenorhynchus obliquidens* (Pacific white-backed dolphins) – ,[18,19,9,20,11] which is triggered by a non-cultivable fungus of the genus *Paracoccidioides*,[11,17] for which the name *Paracoccidioides brasiliensis var. ceti*16 has been suggested.

One theory, based on phylogenetic data, associates the emergence of *PCM ceti* with infections in dolphins that swim along the coastal areas of South America. It is caused by an ancestor of *P. brasiliensis* (which, as mentioned above, is a cultivable fungus). The fact that *P. brasiliensis* var. ceti cannot be cultured suggests two hypotheses of/for infection: (i) through infected animals transmitting the pathogen by direct contact with uninfected dolphins; and/or (ii) through routine dolphin contact with the pathogen located in South American river estuaries.[21]

Despite comparable clinical manifestations, important distinctions exist between the human pathogen and the similar disease found in dolphins. Initially, dolphin disease was thought to be caused by the same agent as in humans. However, modern molecular techniques have revealed that the dolphin pathogen, now named Paracoccidioides ceti, forms a distinct species within the same genus. This species was also found to be non-cultivable, but its genetic material shares significant similarities with *P. lobogeorgii*. This confirms their close evolutionary relationship while also highlighting the differences between the human and dolphin variants of the disease.[22,23]

The identification of these species, despite their inability to grow in culture, was made possible through the extraction of DNA from infected tissues and the subsequent genetic sequencing. By comparing specific genetic markers, such as the Gp43 gene and other coding regions, researchers could place *P. lobogeorgii* and *P. ceti* within the *Paracoccidioides genus*. The construction of phylogenetic trees showed that these non-cultivable pathogens cluster closely with other cultivable Paracoccidioides species, thus solidifying their taxonomic placement (Table 1).[22,23]

In summary, the recent taxonomic review has resolved the long-standing confusion surrounding the causative agents of Jorge Lobo's disease by confirming that *Paracoccidioides lobogeorgii* is responsible for the human form, while *Paracoccidioides ceti* affects dolphins. This distinction underscores the unique evolutionary paths of these organisms, despite their shared genus and similar disease presentation.[22,23]

Table 1. Controversial points of the name of the agents that caused Jorge Lobo´s disease over the years

PROPOSED NAME	YEAR OF PROPOSAL	AUTHOR(S)	REASON FOR REJECTION
Glenosporella loboi	1940	Fonseca OF, Leão AE.[24]	Later identified as *Paracoccidioides brasiliensis* due to contamination with a typical isolate. Not the true agent of Jorge Lobo's disease (JLD).
Glenosporopsis amazonica	1943	Fonseca OF.[25]	Identified as *Aspergillus penicillioides*, an environmental contaminant. Incorrect original identification.
Paracoccidioides loboi	1948-1949	Almeida F, Lacaz C.[26]	Molecular studies showed this was not a variety of *Paracoccidioides brasiliensis* but a different species. Name corrected to *Paracoccidioides lobogeorgii*.
Blastomyces loboi	1952	Langeron M, Vanbreuseghem R.[27]	Proposed as a new name but based on erroneous phenotypic comparisons with other Blastomyces species.
Loboa loboi	1956	Ciferri R.[28]	Later identified as a synonym of *Paracoccidioides brasiliensis* due to contamination. Rejected for not describing the true pathogen.
Lobomyces loboi	1958	Borelli D.[29]	Name did not follow botanical nomenclature rules and lacked a Latin description. Later deemed invalid.
Lacazia loboi	1999	Taborda PR, Taborda VA, McGinnis MR.[30]	Widely accepted for a time, but molecular analyses showed it was related to *Paracoccidioides genus*. Renamed as *Paracoccidioides lobogeorgii*.
Candida loboi	2015	Costa PF.[31]	Identified as *Candida tropicalis*, a contaminant, not the true etiologic agent of Jorge Lobo's disease.
Paracoccidioides lobogeorgii	2021	Vilela, de Hoog, Bagagli, and Mendoza.[22]	Confirmed by molecular analyses as the true etiologic agent of Jorge Lobo's disease in humans. The recent taxonomic review has resolved the longstanding confusion surrounding the causative agents of Jorge Lobo's disease, confirming that *Paracoccidioides lobogeorgii* is responsible for the human form, while *Paracoccidioides ceti* affects dolphins. This distinction underscores the unique evolutionary paths of these organisms, despite their shared genus and similar disease presentation.

Source: The authors (2024).

The disease in humans: pathogenesis, clinical aspects, diagnosis and treatment

Pathogenesis

After inoculation of the fungus in the skin, the appearance of the first symptoms and signs usually takes between three months and two years. The lesions' evolution generally occurs very slowly; such changes may be unique and localized or disseminated over large areas of the tegument.[12] Multiple lesions are presumed to arise from autoinoculation. The hypothesis of hematogenous dissemination, as in leishmaniasis, is considered for more distant manifestations[32] and is supported by observation of the fungus in an intravascular environment.[33] Lymphatic involvement is quite uncommon,[4] although it has been described in the Kaiabi Indians[33] and in cases from the 1970s.[34]

The histopathology of lobomycosis has enough typical characteristics to distinguish it from other lesions of paracoccidioidomycosis. The following aspects should be highlighted in the examination: richness of pathogens; intense histiocytosis, without the formation of individualized granulomas; and absence of yeasts with the typical “rudder wheel” morphology.[35] The epidermis normally shows atrophy, although vegetating and hyperkeratotic epithelial hyperplasia can be seen. In the dermis, a thin layer of subepithelial collagen, usually unharmed, like the “Unna band” of lepromatous leprosy, is present. The remainder of the dermis is filled along its entire length by a diffuse inflammatory granuloma with a fibrous but never necrotizing evolution. Histochemical analyses demonstrate a polysaccharide impregnation of the inflammatory granuloma, which usually extends to the contours of the hypodermis, but does not jeopardize it.[32]

No B lymphocytes and few T lymphocytes are found in lobomycosis granulomatous lesions.[32,36] Infiltrative-looking masses are formed, which contain granulomas composed of giant foreign body-type cells, each harboring numerous phagocytosed fungal elements in addition to quiescent macrophages in different proportions.[12,36] The characteristics of the tissues of the affected lymph nodes are similar to those described in granulomas.[34,37] In the intracytoplasmic region of some giant cells, asteroid-shaped bodies – of unknown nature – are observed. They have the appearance of solitary eosinophilic intracytoplasmic structures surrounded by a lipid layer. Under the electron microscope, these asteroid bodies consist of branches of dense, filamentous material, as well as myelin structures, which make the assembly resemble the image of asteroid bodies in sarcoidosis.[13,38] This appearance can be confused with the sporotrichosis findings. The fungus *L. loboi* (now known as *Paracoccidioides lobogeorgii*[22]) can also be found in the extracellular environment surrounded by histiocytes.[39,40] The viscera are not usually affected because of the poor thermotolerance of the fungus, which is confirmed by the presence of lesions mainly on the skin of the coldest regions of the human body.[12]

The possibility of malignant transformation of lesions, which have a chronic evolution to squamous cell carcinoma, also exists. This fact was verified in two Kaiabi Indians, one of whom had disseminated metastases.[3,33] Other cases of carcinomatous degeneration were also described in a Colombian indigenous person;[41] a 64-year-old farmer from the state of Pará;[42] an 83-year-old indigenous person residing in the state of Amazonas;[40] and an 87-year-old rubber tapper from the banks of the Purus River, Amazonas.[5]

Clinical aspects

Lobomycosis is a cutaneous mycosis (whose presence is almost entirely restricted to the skin; only one case on the labial mucosa has been reported),[43] that is, without visceral involvement (one reported case of testicular involvement).[44] Satellite adenopathy is not described, except in scarce circumstances. Most of the patients come from the Amazon Basin, and their general condition is satisfactory despite the long evolution of the process, since the disease can affect the individual for decades and presents a slow clinical course.[12,32]

Commonly reported symptoms include burning and itching near the lesions. The disease is characterized by dermal-epidermal nodules of various sizes or plaques resulting from the confluence of nodules. The lesion that most affects patients – and that gives rise to the diagnosis – is keloidiform nodular alteration. Plaques are formed, which can occupy a considerable area of the body and present polycyclic contours with prominent edges.[12]

In some patients, multiple lesions of different shapes are described, with numerous types of manifestations in the epidermis, such as macules, papules, infiltrated plaques, gums, keloid-like nodules, and even ulcerated lesions.[12,45] Atrophic skin lesions, which resemble tuberculoid leprosy in their clinical form, have also been reported.[32] Another form that has been detected includes verruciform disorders.[12,32] The appearance of the lesions generally varies with their topography: the points of support are unharmed, while the dorsum of the foot has hardened, polylobed, non-fistula-shaped vegetations with a shell-on-rock appearance. In addition to the other lesions mentioned above, those of the pseudokeloid type can be observed in the limbs and in the rest of the body.[33]

The habit of carrying straw and wood on their shoulders to build tents, as well as chestnuts and flour.[32] Manifestations in the distal extremities of the upper and lower limbs and in the gluteal region are also common; however, other parts of the body can also be affected, such as shoulders, back and abdomen (Figure 1).[12,16] In more severe cases, large areas of skin may be affected by dispersion or by clustered and confluent nodules. Disseminated skin lesions have been described in an HIV-infected patient.[46]

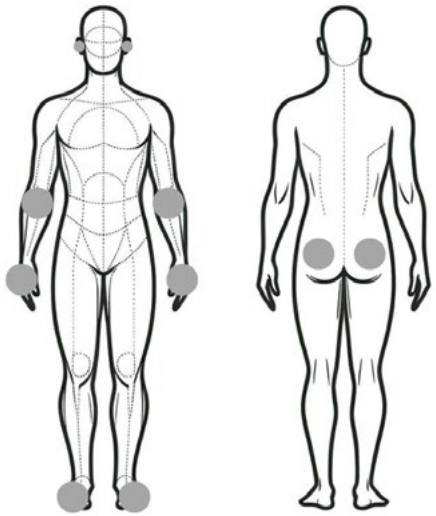

Figure 1. Regions of the human body, marked in gray circles, where skin changes. They are most commonly in cases of Jorge Lobo's disease

Source: The authors (2024).

Complications include possible restrictions of movement, significant cosmetic damage, secondary infections, ulcerations, and carcinomatous degeneration.[14,19,5] Squamous cell carcinoma infiltration in long-term lobomycotic lesions has been described in some patients,[33,40,41,42,47,48] including cases of death.[40,47]

Differential diagnoses that must be established include: paracoccidioidomycosis, in its nodular and vegetative cutaneous form; vegetative chromomycosis;[3,41] histoplasmosis, in its African and American forms;[3] sporotrichosis;[49] cutaneous leishmaniasis, in its pseudo-lepromatous form;[3,32] keloid scar; dermatofibrosarcoma protuberans; fibroma and neurofibroma;[50] *lupus vulgaris*, in verrucoid forms;[51] nodular or reactional tuberculoid leprosy, especially ear lesions;[32] squamous cell epithelioma; skin metastases from deep squamous cell carcinomas;[3,32] Kaposi's sarcoma;[49] sarcoidosis; and lymphomas, mainly mycosis fungoides.[46] Cases of co-infection with *L. loboi* (now, known as *Paracoccidioides lobogeorgii*[22]) and *P. brasiliensis*, an association already described in the literature,[4] may also occur.

Diagnosis

Laboratory diagnosis can be performed by analysis of the material collected from the skin lesion (whether ulcerated or not) or the pus from gummy lesions, diluted in saline solution and examined between the slide and coverslip under an optical microscope. The material can be stained with hematoxylin-eosin (H&E) or Gomori's methenamine silver (GMS), which is specific for fungi.[12] The result of the analysis can be confirmed by the finding of characteristic globular cells in the shape of a lemon, alone or grouped in small chains.[1]

Treatment

The recommended therapy is conservative surgical extirpation of non-extensive or disseminated lesions, with clinical follow-up of up to three years.[52] Cryosurgery is an alternative and presents good results for this type of injury.[12,53]

Lobomycosis is normally resistant to antimicrobial treatment; in rare cases, clinical improvement is observed with the use of azole derivatives – such as ketoconazole (at a dosage of 400mg/day) –, but the fungi always persists.[32,33,54] Due to its anti-inflammatory action, another therapeutic option that has been put forward involves the use of clofazimine (with or without itraconazole) at a dosage of 200mg/day for three months, followed by 100mg/day for a variable period. Furthermore, a recent study conducted in Acre (Brazil) showed that the treatment used in leprosy (clofazimine, dapsone, and rifampicin) can also be relatively effective in treating lobomycosis. Pharmacological treatment is appropriate, especially for extensive and disseminated forms of the disease.[12,32,33,49,53]

One possible treatment option is posaconazole, which has shown good results – in an individual who had lesions on the face, trunk, and limbs since childhood – by generating a reduction in keloids and even scarring. Although this is the first report in the literature of treatment with posaconazole in the extensive form of the disease, the results were satisfactory and may indicate a possible therapeutic alternative.[55]

The early diagnosis and treatment of lobomycosis are hampered by many factors, such as the similarity in the pattern of lesions with other diseases already mentioned, resulting in the need to exclude differential diagnoses, in addition to the long incubation period of the disease.[56] The general health of individuals affected by lobomycosis is not usually impacted; how-

ever, late or incorrect diagnoses can worsen the disease and/or leave the patient susceptible to co-infections.[56]

The disease in aquatic mammals (Pcm Ceti): clinical aspects, diagnosis and treatment

Pathogenesis

The fact that the alterations caused by *P. brasiliensis var. ceti* in dolphins are confined to subcutaneous tissues suggests that the pathogens are most likely introduced in this region by traumatic implantation of fungal elements. Upon reaching the subcutaneous tissues *P. brasiliensis var. ceti* changes to a yeast-like form and successfully establishes itself inside the host.[16]

After entering the animal's organism, the pathogens increase in size very slowly and may take months or even years to produce large parakeloid granulomatous lesions, with rare dissemination to other organs. Yeast cells trigger a slowing of immunity, via a Th2 response pattern, when they proliferate inside inflammatory cells and affect macrophages. Transforming growth factor β1 (TGF-β1) is released, blocking the release of nitric oxide and inhibiting the production of interferon gamma (IFN-γ). Adaptive immunity is compromised due to a dramatic decrease in the number of circulating helper B and T cells. Antibodies against *P. brasiliensis var. ceti* cross-reacted with the antigens of cultivable P. brasiliensis isolates.[16,44]

The presence of melanin in the cell wall of *P. brasiliensis var. ceti* is believed to protect pathogens from the host's immune response. The viability of yeast cells is reduced to about 40% of viable cells in the tissues of the infected host.[57] Subcutaneous granulomas, present in infected dolphins, show numerous branched yeast cells of uniform size, arranged in chains and surrounded by inflammatory cells and significant fibrosis44. The proliferation of CD8 T cells believed to be promoted by TGF-β1 is also responsible for producing immunoglobulins and other factors that promote the fibrosis process, causing the paracheloid external appearance of skin lesions.[16]

Clinical aspects

The PCM ceti lesions described in dolphins are generally similar to the changes produced by lobomycosis in humans. The disease usually has a chronic course and takes time to develop into large, characteristic monomorphic or multimorphic lesions.[16] In fact, nodular, ulcerous-crusted, verrucous skin lesions with colors ranging from white to pink are identified. The changes described in dolphins may be more pronounced and reach the lymph nodes, but without the involvement of internal organs.[14,51,58] Lesions in these animals can cover large somatic areas, predominantly in the cephalic region and with a possibility of extension to the back and tail. In addition, they may be associated with scarring from previous injuries, such as collisions and shark bites.[59]

The dolphins affected by the disease are older and usually show evidence of immune deficiency. In fact, these animals are often found to be very debilitated and/or carry multiple associated comorbidities.[28,60,61] Furthermore, in dolphins, a substantial decrease in CD4+ helper T lymphocytes, B cells, CD19+ and CD21+ associated with the morbid condition has been described.[39] Indications also exist that the presence of the fungus predisposes to co-infections,

as occurred in a bottlenose dolphin in Kinko-wan, Japan, which presented bronchopneumonia with the isolation in culture of *Enterobacter cloacae*, *Klebsiella pneumoniae* and *Aeromonas hydrophila*. Indeed, areas where reports of the disease are more frequent may harbor populations of immunocompromised dolphins.[21,58]

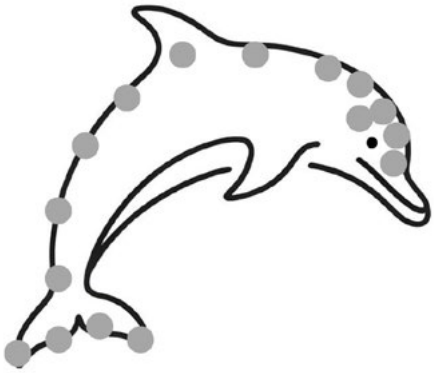

Figure 2. The regions of the dolphin's body, marked by gray circles, where skin changes most commonly appear in cases of Paracoccidioidomycosis ceti

Source: The authors (2024).

Diagnosis

The investigation of this disease in cetaceans can be a genuine challenge since they are protected species and the collection of material from them for histopathology is strictly regulated. For a long time, cases were diagnosed only by photographs and macroscopy, without histopathological, serological, or molecular data.[62] Subsequently, a presumptive diagnosis was made from the observation of the yeast structures detected in the lesions of these animals by means of histopathology, including procedures of yeast staining with Gomori's methenamine silver (GMS). This process revealed the presence of numerous dark ovals, 2 to 10µm wide, connected by short isthmuses. Such findings were considered a diagnosis of cases of the disease in dolphins.[16,63] Currently, detection of the disease is based on molecular techniques, as well as on observation of the yeast cells, which are characteristic of the disease, either by cytology or histopathology.[64]

Many of the cases reported as being LLD – "lobomycosis-like disease", currently called *PCM ceti* – were classified as such without pathological examinations or without evidence of fungal bodies. This classification was based solely on external observations of skin lesions that resemble those described in humans.[7]

Treatment

The paucity of experiences in capturing dolphins for treatment is the reason for the lack of data on the effectiveness of drugs in the literature. When these animals are treated with strategies used in humans, the responses obtained are usually similar.[61]

So far, in attempts to treat *PCM ceti*, no antifungal agent has been proven effective, except for one case of apparent improvement reported by Dudok Van Heel,[35] based on the use of miconazole and a supposed remission of the disease in a dolphin of the *T. truncatus* species.[9,35]

Esperón et al.[63] reported ineffective treatment of a dolphin with topical itraconazole and ketoconazole. Subsequently, the same animal received 2.5mg/kg of oral itraconazole and 2.0mg/kg of terbinafine. The skin lesions were reduced to small nodules, and subsequently disappeared and did not recur. Surgical removal of small lesions may be an option; however, surgery in large or multicentric lesions is not recommended.[16]

Ecoepidemiology: lobomycosis and *Pcm Ceti*

Up to 1996, 418 cases of the disease had been reported in humans, of which 255 were in Brazil and 50 in Colombia.[32] In 2020, after epidemiological studies of the Kaiabi tribes, Florian et al.[32] identified 63 occurrences of lobomycosis between the years 1965 to 2019, of which 60 had already been diagnosed and three were new cases. Among the 63 patients, 39 (61.9%) were male and 24 (38.1%) were female. Most of the reports originated from Central and South America, mainly in the Amazon region;[56] in Brazil, all cases came from the Amazon, which is an endemic area for *P. lobogeorgii*. Six Colombian soldiers who served in the Amazon region had nodular and cheilodian lesions on the face, trunk, and limbs, which took two to 15 years to become noticeable.[36,65] Sporadic and isolated cases of the disease have been reported in Europe, the United States, and Canada. All such patients reported previous travel to Central or South America. Recently, two cases of lobomycosis were reported in South Africa.[49] The geographic distribution extends between the Tropic of Cancer and the Tropic of Capricorn, especially near the Equator. The climate and regions conducive to the development of the fungus are tropical and subtropical humid forests, with an average annual temperature of 24°C and rainfall greater than 2000mm per year.[3,33,51]

The humans most commonly affected are aged between 21 and 40 years. No single ethnic group predominates, since all populations appear to be equally susceptible.[49] Men are affected in 92% of cases, which can be explained by their carrying out of professional activities in a rural environment, as is the case of rubber tappers, foresters, prospectors, hunters and farmers. Early exposure in a hyperendemic environment, probably associated with occupational activities, could clarify this occurrence of cases. The Kaiabi Indian tribe, who inhabited Mato Grosso, Central-West Brazil, constitutes a different epidemiological model for this disease. In a span of 30 years, the tribe accounts for 32% of Brazilian cases and 21% of the total number of cases.[32] In this setting, the affected group is aged between 10 and 25 years. Furthermore, among the Kaiabi, agricultural activities are more developed than in other tribes in the locality. No reports of cases of lobomycosis in adjacent villages have been filed.[47,52] No new cases of lobomycosis among the Kaiabi have appeared since they migrated to the Xingu National Park (southeastern Pará), which is in the Amazon.[3,33]

Possible reservoirs and transmission routes are still not well clarified. The etiological agent was detected in the soil of Argentina and Venezuela, and also in vegetables in Brazil; while speculation about an aquatic reservoir is based on the existence of a similar disease (*PCM ceti*) in cetaceans. The entry point of the fungus is probably the skin, and local trauma usually precedes the skin injury. A wound over a pre-existing lesion can cause a local recurrence.[3,33] Interhuman transmission has never been confirmed, but two cases stand out as possible instances of non-human animals participating in the disease of *H. sapiens*.[33,66,67,68,69]

The first case was reported in Europe in 1983, when a granuloma, as well as supratrochlear lymphadenitis, was observed on the skin of one hand of an aquarium attendant three months after occupational contact with a bottlenose dolphin (*T. truncatus*). This specimen had been

captured in the Bay of Biscay and showed the presence of skin granulomas, whereas biopsied specimens showed morphologically indiscriminate organisms of *L. loboi*[66] (now, known as *Paracoccidioides lobogeorgii*[22]). Analogous pathogens were also present in the patient's skin lesions and in the supratrochlear lymph nodes66. Based on these findings, one cannot conclude with certainty that the microorganism infected the aquarium attendant. The second case was described in São Paulo, Brazil – an area where the disease is not endemic – and involved a veterinarian who handled mice in a laboratory, which had been experimentally infected with *L. loboi* (now, known as *Paracoccidioides lobogeorgii*).[22] Thus, the transmission of lobomycosis from a non-human animal to a human has been documented.[68]

Despite scant evidence of zoonotic behavior – from lobomycosis? from *PCM ceti*? –, the possibility of such an occurrence raises concern in areas that are endemic of the disease in cetaceans, especially in those cases where such animals are used for recreational purposes, such as, for example, in the Indian River Lagoon, Florida.[9,19,59,69]

Until the present moment, no registered cases of paraccidioidomycosis ceti in porpoises exist. All the confirmed reports relate to Delphinidae. The disease in cetaceans has been diagnosed in: (1) *T. truncatus* (bottle-nosed dolphins), in Brazil, in Cuba, on the east coast of the United States (histologically and microbiologically indistinguishable from JLD), in Europe and Japan;[10,11] (2) *T. aduncus* (Indo-Pacific bottlenose dolphins), in the Indian Ocean, between Mozambique and Madagascar;[9,18] (3) *Sotalia guianensis* (Guiana dolphin - Delphinidae), in Suriname;[19] and (4) *Lagenorhynchus obliquidens* (Pacific white-backed dolphin), in Japan.[11,20] Similar cases of *PCM ceti* have not been described in the boto, *Inia geoffrensis*, and the tucuxi, Sotalia fluviatilis, which inhabit the Amazon and Orinoco rivers.[19,59]

Dolphins are more sensitive to environmental changes, whether of natural or anthropogenic origins. Therefore, the increased incidence of disease in these animals may also be related to responses to environmental changes, such as exposure to pesticides and other contaminants or variations in temperature, salinity, vegetation, and even increased survival of the pathogen in the marine environment.[69] Furthermore, the molecular confirmation of the disease in *L. obliquidens*, in Japan, suggests a possible geographic expansion of the etiologic agent, in addition to raising the hypothesis that other cetacean species may be infected. In this context, the possible spread of the pathogen by ballast water from ships or other floating media (oil platforms, for example) is a subject of discussion; meanwhile, the disease could be expanding.[17]

Final considerations

After almost one hundred years of its recognition among the Kaiabi Indians and seventy years of its more systematic clinical description, lobomycosis continues to raise questions for clinicians and mycologists. Similarly, many gaps remain in our understanding of *PCM ceti* in aquatic mammals, a disease once confused with lobomycosis. Scientific advances have increased our knowledge of the areas of molecular biology and genetic engineering of etiological agents and the respective diseases in humans and cetaceans. However, further research is required into aspects of etiopathogenesis, transmission, diagnosis (including the possibility of cultivation in the laboratory) and prevention. Similarly, more effective treatments may be proposed, which can lead to improvements in the quality of life for the different animal species affected by these diseases as well as psychosocial gains.

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References

1. Carvalho KA, Floriano MC, Enokihara MM, Mascarenhas MR. Jorge Lobo's disease. An Bras Dermatol. 2015;90(4):586-588.
2. Symmers WS. A possible case of Lobo's disease acquired in Europe from a bottle-nosed dolphin (Tursiops truncatus). Bull Soc Pathol Exot. 1983;76:777-784.
3. Baruzzi RG, Lacaz SC, Souza FAA. Natural history of Jorge Lobo's disease: Occurrence among the Caiabi (Central Brazil). Rev Inst Med Trop Sao Paulo. 1971;13:71-77.
4. Heleine M, Blaizot R, Cissé H, Labaudinière A, Guerin M, Demar M, Couppie P. A case of disseminated paracoccidioidomycosis associated with cutaneous lobomycosis. J Eur Acad Dermatol Venereol. 2020;34(1).
5. Nogueira L, Rodrigues L, Rodrigues CAC, Santos M, Talhari S, Talhari C. Lobomycosis and squamous cell carcinoma. An Bras Dermatol. 2013;88(2):293-295.
6. Teixeira MM, Theodoro RC, de Carvalho MJP, Fernandes L, Paes HC, Hahn RC, et al. Paracoccidioides species: Genetic diversity, ecology, and the role of animal transmission. Fungal Genet Biol. 2017;94:39-56. doi:10.1016/j.fgb.2016.11.007.
7. Vilela R, Bossart GD, Leger JAS, Dalton LM, Reif JS, Schaefer AM, Mendoza L. Cutaneous granulomas in dolphins caused by novel uncultivated Paracoccidioides brasiliensis. Emerg Infect Dis. 2016;22(12):2063. doi:10.3201/eid2212.160860.
8. Kluyber D, Desbiez ALJ, Attias N, Massocato GF, Gennari SM, Soares HS, et al. Zoonotic parasites infecting free-living armadillos from Brazil. Transbound Emerg Dis. 2021;68(3):1639-1651. doi:10.1111/tbed.13839.
9. Paniz-Mondolfi AE, Talhari C, Sander Hoffmann L, Connor DL, Talhari S, Bermudez-Villapol L, Van Bressem MF. Lobomycosis: an emerging disease in humans and delphinidae. Mycoses. 2012;55(4):298-309. doi:10.1111/j.1439-0507.2012.02184.x.
10. Sacristán C, Esperón F, Ewbank AC, Kolesnikovas CKM, Catão-Dias JL. Paracoccidioidomycosis ceti in an Atlantic bottlenose dolphin (Tursiops truncatus), Brazil. Transbound Emerg Dis. 2018;65(2):585-587. doi:10.1111/tbed.12781.
11. Sacristán C, Esperón F, Ewbank AC, Kolesnikovas CKM, Catão-Dias JL. Lacaziosis in an Atlantic bottlenose dolphin (Tursiops truncatus) from Brazil. Transbound Emerg Dis. 2017;65:585-587. doi:10.1111/tbed.12781.
12. Arju R, Kothadia JP, Kaminski M, Abraham S, Giashuddin S. Jorge Lobo's disease: a case of keloidal blastomycosis (lobomycosis) in a nonendemic area. Ther Adv Infect Dis. 2014;2(3-4):91-6.
13. Hornberger MM, Farnsworth GS, Vilela R, Mendoza L, Bandino JP. Extensive, multifocal lobomycosis. J Cutan Pathol. 2021. doi:10.1111/cup.14114.
14. Migaki G, Valerio MG, Irvine B, Gardner FM. Lobo's disease in an Atlantic bottle-nosed dolphin. J Am Vet Med Assoc. 1971;159:578-82.
15. Shumoto G, Ueda K, Yamaguchi S, Kaneshima T, Konno T, Terashima Y, Sano A. Immunohistochemical cross-reactivity between Paracoccidioides sp. from dolphins and Histoplasma capsulatum. Mycopathologia. 2018;183(5):793-803. doi:10.1007/s11046-018-0295-0.
16. Vilela R, de Hoog S, Bensch K, Bagagli E, Mendoza L. A taxonomic review of the genus Paracoccidioides, with focus on the uncultivable species. PLoS Negl Trop Dis. 2023;17(4). https://doi.org/10.1371/journal.pntd.0011220.
17. Van Bressem MF, Simões-Lopes PC, Félix F, Kiszka JJ, Daura-Jorge FG, Avila IC, Van Waerebeek K. Epidemiology of lobomycosis-like disease in bottlenose dolphins (Tursiops spp.) from South America and southern Africa. Dis Aquat Organ. 2015;117(1):59-75. doi:10.3354/dao02932.
18. Kiszka J, Van Bressem MF, Pusineri C. Lobomycosis-like disease and other skin conditions in Indo-Pacific bottlenose dolphins (Tursiops aduncus) from the Indian Ocean. Dis Aquat Organ. 2009;84:151-7.
19. Paniz-Mondolfi AE, Sander-Hoffmann L. Lobomycosis in in-shore and estuarine dolphins. Emerg Infect Dis. 2009;15:672–3. doi:10.3201/eid1504.080955.
20. Caldwell DK, Caldwell MC, Woodard JC, Ajello L, Kaplan W, McClure HM. Lobomycosis as a disease of the Atlantic bottle-nosed dolphin (Tursiops truncates Montagu, 1821). Am J Trop Med Hyg. 1975;24(1):105-114.
21. Bossart GD, Schaefer AM, McCulloch S, Goldstein J, Fair PSA, Reif JS. Mucocutaneous lesions in free-ranging Atlantic bottlenose dolphins (Tursiops truncates) from the southeastern USA. Dis Aquat Organ. 2015;115:175-184.
22. Vilani-Moreno FR, Opromolla DV. Determination of the viability of Paracoccidioides loboi in biopsies from patients with Jorge Lobo's disease. An Bras Dermatol. 1997;72:433–437.
23. Vilela R, Huebner M, Vilela C, et al. The taxonomy of two uncultivated fungal mammalian pathogens is revealed through phylogeny and population genetic analyses. Sci Rep. 2021;11:18119. doi:10.1038/s41598-021-97429-7.
24. Fonseca OF, Leão AE. Contribution to the knowledge of blastomycoid granulomatoses: The etiological agent of Jorge Lobo's disease. Rev Med Cir Bras. 1940;48(3):147–58.
25. Fonseca OF. Medical Parasitology: Parasites and Parasitic Diseases of Humans. Vol. I. Rio de Janeiro, Brazil: Guanabara, Waissman, Koogan Ltda; 1943. p. 703–26.
26. Almeida F, Lacaz C. Blastomycosis "Jorge Lobo type." Ann Fac Med São Paulo. 1948/1949;24:5–37.
27. Langeron M, Vanbreuseghem R. Cheloid blastomycosis or Lobo's disease. In: Précis de Mycologie. Mycologie Générale. Mycologie Humaine et Animale Techniques.

1952. p. 490–1.
28. Ciferri R. Reference of the genus Paracoccidioides to Entomophthorales Imperfecti (Entomophthorales Coccidioidaceae). Mycopathologia. 1951;6:42.
29. Borelli D. Aspergillus surprises in mycopathology. Dermatol Venezol. 1958;1:290.
30. Symmers WS. A possible case of Lobo's disease acquired in Europe from a bottle-nosed dolphin (Tursiops truncatus). Bull Soc Pathol Exot. 1983;76:777-784.
31. Costa PF. In vitro isolation and cultivation of the etiological agent of Jorge Lobo's disease: Morphology, physiology, and complete genome of Candida loboi sp. nov. [Thesis]. University of Pará, Brazil; 2015. p. 120.
32. Florian MC, Rodrigues DA, de Mendonça SB, Colombo AL, Tomimori J. Epidemiologic and Clinical Progression of Lobomycosis among Kaiabi Indians, Brazil, 1965-2019. Emerg Infect Dis. 2020;26(5):930-936.
33. Lacaz CS, Baruzzi RG, Berthe MC. Jorge Lobo's disease. Rev Inst Med Trop Sao Paulo. 1986;258:1-91.
34. Azulay RD, Carneiro JA, Cunha MDGS, Reis LT. Keloidal blastomycosis (Lobo's disease) with lymphatic involvement: a case report. Int J Dermatol. 1976;15(1):40-42.
35. Dudok van Heel WH. Succesful treatment in a case of lobomycosis (Lobo's disease) in Tursiops truncatus (Mont.) at the dolfinarium, Harderwijk. Aquat Mamm. 1977;5:8-15.
36. Arrese EJ, Rurangirwa A, Pierard GE. Lobomycosis. Ann Pathol. 1988;8:325-827.
37. Gadelha SQ, de Almeida GE, dos Reis Gadelha A, de Souza MLG, Figueiras VV. Keloid-like lesions in a farmer from Amazonas. JAAD Case Rep. 2021;10:57-59. doi:10.1016/j.jdcr.2021.01.028.
38. Rodriguez-Toro G, Barrera GP. The asteroid body of lobomycosis. Mycopathologia. 1996;136(2):71-74.
39. Sacristán C, Réssio R, Castilho P, Fernandes N, Costa-Silva S, Esperón F, Catão-Dias J. Lacaziosis-like disease in Tursiops truncatus from Brazil: a histopathological and immunohistochemical approach. Dis Aquat Organ. 2016;117(3):229–235. doi:10.3354/dao02954.
40. Lima WC, Gadelha SQ, Souza MLG, Figueiras VV. Lobomycosis: exuberant presentation with malignant transformation. An Bras Dermatol. 2021;96:762-764. doi:10.1016/j.abd.2021.05.004.
41. Rodriguez-Toro G, Téllez N. Lobomycosis in Colombian Amerindian patients. Mycopathologia. 1992;120:5-9.
42. Brito AC, Bittencourt MDJS, Gonçalves TDS, Cavalcante RH. Jorge Lobo's disease with malignant degeneration to squamous cell carcinoma: case report. An Bras Dermatol. 2022;97(1):93-95. doi:10.1016/j.abd.2021.08.001.
43. Loureiro AA, de Brito AC, Silva D. Jorge Lobo's mycosis of unusual location. An Bras Dermatol. 1971;46:1-6.
44. Francesconi VA, Klein AP, Santos AP, Ramasawmy R, Francesconi F. Lobomycosis: epidemiology, clinical presentation, and management options. Ther Clin Risk Manag. 2014;10:851-860.
45. Siqueira-Batista R, Gomes AP. Antimicrobianos: guia prático. 3rd ed. Rio de Janeiro: Rubio; 2021.
46. Ramos-e-Silva M, Vilela FAS, de Brito AC, Carneiro SC. Lobomycosis: Literature Review and Future Perspectives. Actas Dermosifiliogr. 2009;100 Suppl 1:92-100.
47. Baruzzi RG, Rodriguez-Toro DA, Michalany NS, Salomao R. Squamous-cell carcinoma and lobomycosis (Jorge Lobo's disease). Int J Dermatol. 1989;28:183-185.
48. Nogueira L, Rodrigues L, Rodrigues CAC, Santos M, Talhari S, Talhari C. Lobomycosis and squamous cell carcinoma. An Bras Dermatol. 2013;88(2):293-295.
49. Taborda PR, Taborda VA, McGinnis MR. Lacazia loboi gen. nov., comb. nov., the etiologic agent of lobomycosis. J Clin Microbiol. 1999;37:2031–3. doi:10.1128/JCM.37.7.2031-2033.1999.
50. Nalovic K, Pradinaud R. Anergic cutaneous leishmaniasis versus lobomycosis. Int J Dermatol. 1996;35:438-440.
51. Rodriguez-Toro G. Lobomycosis. Int J Dermatol. 1993;32(5):324-332.
52. Haubold EM, Aronson JF, Cowan DF, McGinnis MR, Cooper CR. Isolation of fungal rDNA from bottlenose dolphin skin infected with Loboa loboi. Med Mycol. 1998;36(5):263-267.
53. Araújo MG, Cirilo NS, Santos SNMB, Aguilar CR, Guedes ACM. Lobomycosis: a therapeutic challenge. An Bras Dermatol. 2018;93(2):279-281.
54. Simões-Lopes PC, Paula GS, Both MC, Xavier FM, Scaramello AC. First case of lobomycosis in a bottlenose dolphin from Southern Brazil. Mar Mamm Sci. 1993;9:329-31.
55. Pasqualotto AC, Jaskulski Filho SD, de Sena MG, dos Santos AF, Severo MMS. Posaconazole for lobomycosis. Braz J Infect Dis. 2021;25(2):101576.
56. Tajima Y, Sasaki K, Kashiwagi N, Yamada TK. A case of stranded Indo-Pacific bottlenose dolphin (Tursiops aduncus) with lobomycosis-like skin lesions in Kinko-wan, Kagoshima, Japan. J Vet Med Sci. 2015;77(8):989-992.
57. Ueda K, Sano A, Yamate J, Itano EN, Kuwamura M, Izawa T, et al. Two cases of lacaziosis in bottlenose dolphins (Tursiops truncatus) in Japan. Case Rep Vet Med. 2013. doi:10.1155/2013/318548.
58. Floriano MC. Jorge Lobo's disease among the Kaiabi Indians: epidemiology. Histopathological and immunohistochemical analysis in different clinical presentations. [Dissertation]. São Paulo, Brazil: Universidade de São Paulo; 2014. xvii, 152f.
59. Reif JS, Mazzoil MS, McCulloch SD, Varela RA, Goldstein JD, Fair PA, Bossart GD. Lobomycosis in Atlantic bottlenose dolphins from the Indian River Lagoon, Florida. J Am Vet Med Assoc. 2006;228:104-8. doi:10.2460/javma.228.1.104.
60. Durden WN, Leger JS, Stolen M, Mazza T, Londono C. Lacaziosis in bottlenose dolphins (Tursiops truncatus) in the Indian River Lagoon, Florida, USA. J Wildl Dis. 2009;45(3):849-856.
61. Minakawa T, Ueda K, Tanaka M, Tanaka N, Kuwamura M, Izawa T, et al. Detection of multiple budding yeast cells and partial sequence of 43-Kda glycoprotein coding gene of Paracoccidioides brasiliensis from a case of lacaziosis in a female Pacific white-sided dolphin (Lagenorhyncus obliquidens). Mycopathologia. 2016;181:523–529.
62. Teixeira IM, Almeida CG, Ávila GP, Corrêa NB, Rodrigues RJV, de Moraes Damasceno ÁÁ, Figueira LF. Case report: Lacaziosis (Jorge Lobo's disease) in a patient from the Legal Amazon. Braz J Health Rev. 2020;3(4):9819-9826. doi:10.34119/bjhrv3n4-213.
63. Esperón F, García-Párraga D, Bellière EN, Sánchez-Vizcaíno JM. Molecular diagnosis of lobomycosis-like disease in a bottlenose dolphin in captivity. Med Mycol.

2012;50(1):106-109. doi:10.3109/13693786.2011.594100.

64. Sesso A, de Faria FP, Baruzzi RG. The causative agent of Jorge Lobo's disease, the fungus P. loboi (= Loboa loboi) may be seen extracellularly tightly encircled by histiocytes. Observations at the transmission and scanning electron microscopes. Rev Inst Med Trop Sao Paulo. 1993;35:305-306.

65. Arenas CM, Rodriguez-Toro G, Ortiz-Florez A, Serrato I. Lobomycosis in Soldiers, Colombia. Emerg Infect Dis. 2019;25(4):654-660.

66. Sousa PP, Schettini APM, Rodrigues CAC, Westphal DC. Lacaziosis – unusual clinical presentation. An Bras Dermatol. 2015;90(2):268-269.

67. Belone AFF, Madeira S, Rosa PS, Opromolla DVA. Experimental reproduction of Jorge Lobo's disease in BALB/c mice inoculated with Lacazia loboi obtained from a previously infected mouse. Mycopathologia. 2001;155:191-194.

68. Rosa PS, Soares CT, Belone AFF, Vilela R, Ura S, Cury Filho M, Mendoza L. Accidental Jorge Lobo's disease in a worker dealing with Lacazia loboi infected mice: a case report. J Med Case Rep. 2009;3:67. doi:10.1186/1752-1947-3-67.

69. Reif JS, Schaefer AM, Bossart GD. Lobomycosis: risk of zoonotic transmission from dolphins to humans. Vector Borne Zoonotic Dis. 2013;13(10):689-693. doi:10.1089/vbz.2012.1280.

Brazilian Journal of Health and Biomedical Sciences
Paper submission

Brazilian Journal of Health and Biomedical Sciences (BJHBS), formerly titled HUPE Journal, publishes new articles about several themes all related to health and biomedical sciences, since provided that they we're not in simultaneous analysis for publication in any other journal.

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BJHBS features dedicated sections to original research, literature reviews, case studies, and letters to the editor. Papers must be submitted in only one language: English. The submission process comprises the following steps:

Fees and charges: BJHBS does not charge any Article Publication Charges (APC), as it aims to publish and disseminate quality research in the fields of health and biomedical sciences aligned with the terms of the Budapest Open Access Initiative.

Peer review: papers are reviewed by at least two reviewers (specialists). Accepted papers will be edited according to the publishing standards of BJBHS, to improve readability and minimize redundancy, without loss of original meaning. The final edited version will be sent to authors for approval.

Copyright/conflicts of interest agreement: after the final approval, authors must send the copyright transfer agreement signed by the first author representing each additional author. In this agreement, it must be stated any conflicts of interest.

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;

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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.

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

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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.

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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:

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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.

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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..."

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