J. Oral Diagn. 2024;09:e257

ORIGINAL ARTICLE

Rubens Oliveira Signoretti-Silva[1]
Ricardo Santiago Gomez[1]
Marina Gonçalves Diniz[2]
Silvana Spíndola de Miranda[3]
Renato Santana Aguiar[4]
Lida Jouca de Assis Figueredo[3]
Marcelo Araújo Buzelin[5]
Thaís Lima Araújo[1]
Vanessa Fátima Bernardes[2]*

Mycobacterium tuberculosis bacilli in oral biopsies containing granulomatous inflammation with caseous necrosis

Abstract

Objetive: This cross-sectional and retrospective study aimed to investigate the presence of *Mycobacterium tuberculosis* bacillus in formalin-fixed paraffin-embedded (FFPE) oral samples that contained granulomas with caseous necrosis. **Methods:** FFPE biopsies that showed granulomas with caseous necrosis, suggestive of the diagnosis of tuberculosis, were selected. *M. tuberculosis* was searched by Ziehl-Neelsen staining (ZN), immunohistochemistry (IHC), nested-PCR, and GeneXpert® MTB/RIF assays. **Results:** Nine samples showing granulomas with caseous necrosis were selected. The study showed a male predominance, with a ratio of 2.5:1, with a mean age of 50 (19-89) years, and the tongue was the most affected anatomical site (n=4). The ZN technique did not detect bacilli in any sample, and IHC staining showed a coarse granular pattern staining, suggestive of *M. tuberculosis*, in three of them. Nested-PCR and the GeneXpert® MTB/RIF assays were positive in two and three of the samples, respectively. **Conclusion:** Molecular tests and IHC may be useful auxiliary methods for suspected cases of oral tuberculosis.

Keywords: Oral tuberculosis; Clinical laboratory techniques; Immunohistochemistry; Nested-PCR; Real-time polymerase chain reaction.

INTRODUCTION

Tuberculosis (TB) is a transmissible infectious disease caused by *Mycobacterium tuberculosis*, described by Dr. Robert Koch in 1882[1]. Globally, an estimated 10.6 million people contracted tuberculosis, and more than 1.5 million people died from tuberculosis in 2021. These figures are particularly concerning, as they reflect a COVID-19-related setback in the progress made by 2019 in disease prevention and treatment[2].

Statement of Clinical Significance
This study highlights a key challenge for oral pathologists, since the lack of clinical information, combined with the paucibacillary characteristic of the manifestation of the disease in the oral cavity, makes the conclusive diagnosis quite challenging. Consequently, there is a real need to combine clinical information with histological characteristics, using more than one test for the diagnosis.

Pulmonary tuberculosis (PTB) is the most prevalent form of TB infection, with an estimated quarter of the global population infected by *M. tuberculosis*. However, most individuals will not develop the disease, and some will clear the infection[3]. Other body sites, such as the lymph nodes, peritoneum, central nervous system, and muscles, can also be affected[2]. Though rare, the oral cavity may be involved as a primary or secondary site of infection PTB. Lesions are most commonly found on the tongue, palate, and tonsils[4]. In cases of oral TB secondary to pulmonary involvement, infection can occur through autoinoculation from infected sputum or hematogenous dissemination[5].

Oral lesions caused by extra pulmonary tuberculosis (EPTB) typically present as ulcers with a granulomatous center and a whitish halo, commonly on the

[1]Department of Oral Surgery and Pathology, School of Dentistry, Universidade Federal de Minas Gerais (UFMG) – Belo Horizonte (MG), Brazil.
[2]Department of Pathology, Biological Sciences Institute, Universidade Federal de Minas Gerais (UFMG) – Belo Horizonte (MG), Brazil.
[3]Department of Internal Medicine, Faculty of Medicine, Universidade Federal de Minas Gerais (UFMG) – Belo Horizonte (MG), Brazil.
[4]Laboratory of Integrative Biology, Department of Genetics, Ecology and Evolution, Institute of Biological Sciences, Universidade Federal de Minas Gerais (UFMG) – Belo Horizonte (MG), Brazil.
[5]Laboratory Center Specialized in Histotechnology – Belo Horizonte (MG), Brazil.
***Correspondence to:** Email: bernardesvf@hotmail.com
Received on August 27, 2024. Accepted on September 18, 2024. Preprint on May 30, 2024 (https://doi.org/10.21203/rs.3.rs-4397345/v1).
https://doi.org/10.5327/2525-5711.257

dorsal tongue. TB symptoms may include ulcers, nodules, fissures, tuberculomas, or granulomas. Ulcers can be painful or painless, with an irregular, hardened surface[6].

Tuberculosis, whether PTB or EPTB, manifests as characteristic inflammatory granulomatous lesions in response to the bacilli. These lesions, initially microscopic, coalesce and become clinically visible granulomas, known as the tubercles[5]. Although caseous granulomatous inflammatory lesions are characteristic of tuberculosis, they are not exclusive to this disease. These lesions can also be found in conditions such as actinomycosis, leprosy, and leishmaniasis, making histology-based diagnosis a significant challenge for pathologists. Therefore, it is essential to correlate these findings with clinical characteristics to rule out other differential diagnoses[5,7].

Several diagnostic methods for TB exist, with sputum smear microscopy being the oldest and still widely used[2]. Other effective methods include the Ziehl-Neelsen (ZN) staining technique for identifying acid-fast bacilli, and immunohistochemistry (IHC), though ZN has lower sensitivity than IHC and molecular techniques[5,8-10]. Molecular methods include nested Polymerase Chain Reaction (nested-PCR), known for its enhanced sensitivity but with risks of cross-contamination, and the GeneXpert® MTB/RIF assay, recently endorsed by the World Health Organization (WHO) as the fastest method[5,9]. This fully automated test is highly effective in diagnosing TB and detecting rifampicin resistance[11].

The present study aimed to investigate the presence of the *M. tuberculosis* bacillus in formalin-fixed paraffin-embedded (FFPE) oral lesion samples containing granulomas with caseous necrosis through some already well-established diagnostic methods.

MATERIAL AND METHODS

Study design, settings, and ethical issues.

A cross-sectional and retrospective study of a case series, with a population-based approach was conducted at the School of Dentistry, Institute of Biological Sciences, and School of Medicine, all centers of Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Brazil. The guidelines for Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) were followed. All procedures involving human participants followed the ethical standards of the institutional committee and the 1964 Helsinki Declaration, including its amendments. The participants signed informed consent, and anonymity was ensured. The UFMG Human Research Ethics Committee (Protocol No. 5.429.194) approved the study.

Sample characteristics

Eligibility criteria included formalin-fixed paraffin-embedded (FFPE) tissue biopsy samples archived in the Oral and Maxillofacial Pathology Laboratory at UFMG School of Dentistry from 1953 to 2022, which presented granuloma with caseous necrosis. Intraosseous biopsies and samples without sufficient material for analysis were excluded. A sample selection flowchart is presented in Supplementary Figure 1. The clinical diagnostic hypotheses and histological diagnoses of each sample were described in Supplementary Table 1.

Data collection

Sociodemographic and clinical data were obtained, and the information collected included: age, sex, lesion type, lesion color, symptomatology, biopsy type (incisional or excisional), human immunodeficiency virus (HIV) status, location, time of evolution (months), size (mm), and other clinical observations.

Histological examination and immunohistochemistry

Hematoxylin-Eosin (H&E) and ZN staining for acid-fast bacillus (AFB) were performed. Histological criteria compatible with oral TB included the presence of typical granulomas with caseous necrosis, consisting of epithelioid histiocytic, Langhans-type multinucleated giant cells, and an abundance of macrophages and lymphocytes near the granuloma. For the ZN evaluation, the presence of *M. tuberculosis* bacilli was observed based on its ability to bind to the Fuchsine dye under high power magnification (x400). Three observers (R.S.O.S., V.F.B., and R.S.G.) reviewed all H&E and ZN-stained slides.

IHC reactions were conducted using a standard protocol. Slides with 3µm sections were used in adhesive glass slides (StarFrost, Knittel Glass, Germany). The Polyclonal Antibody Derived from Purified Protein (pAbPPD – BIO SB, Santa Barbara, CA, USA), catalogue number BSB 2995, was used at a dilution of 1:500 in Envision + Antibody Diluent (Dako Denmark A/S, Denmark). Dako EnVision® + Dual Link System-HRP (Agilent Technologies, Santa Clara, CA, USA), following the manufacturer's recommended protocol, was used. Negative controls were included by omitting the primary antibody. The IHC results were assessed by three observers (R.S.O.S., V.F.B., and R.S.G.), using a light microscope, evaluating the entire slide for a coarse granular pattern indicative of phagocytized and/or fragmented *M. tuberculosis* bacilli within the granuloma. The fine

grain pattern was also considered. Periodic acid-Schiff (PAS) and Grocott staining were performed to exclude other infectious diseases.

Rapid molecular tests by nucleic acid amplification (NAA tests)

Nested-PCR

For DNA extraction, 15 sections of 5µm thickness were cut from the FFPE tissue samples. Deparaffinization was performed using a modified xylene method, involving two washes in 1 mL of xylene and one wash in 1 mL of absolute ethanol. Subsequently, the samples were digested with 20–40 µl proteinase K (QIAGEN, Hilden, Germany) at 56°C overnight. Genomic DNA (gDNA) was extracted from the FFPE tissue samples, using the QIAamp DNA FFPE Tissue kit (QIAGEN, Hilden, Germany) and following manufacturer instructions. For nested-PCR reactions, amplification of the region of interest corresponding to the IS6110 gene (GenBank: NC_000962.3) was performed. The forward (F) and reverse (R) primers for the region of interest (both external and internal primers) were designed based on previous studies (Supplementary Table 2) and are presented in Supplementary Table 3. For both reactions, a final volume of 25µl, 10µM of each primer, and 12.5 µl of MyTaq HS Red Mix, two times (Bioline Reagents Ltd, UK) were used. The temperature protocol involved an initial activation step of heating at 95°C for 3 minutes, followed by 45 cycles for 15s at 95°C (denaturation), 15s at 55°C (annealing), 30s at 72°C (extension), and a final extension at 72°C for 10 minutes. The hold temperature was 4°C. In addition, 1µl of the product from the first reaction was used for amplification in the second reaction. A negative control (a reaction without gDNA) was used at each step.

The amplification of nested-PCR products was confirmed by agarose gel electrophoresis using a 3% agarose gel and a fluorescence band marker (SYBR® Safe DNA gel stain; Invitrogen by Thermo Fisher Scientific). The gels were viewed using the iBright Imaging Systems 750 (Thermo Fisher Scientific, USA). Samples showing positive bands of 92 bp in molecular weight, as compared to the 100 bp ladder standard (Ludwig Biotecnologia LTDA, Alvorada, Brazil), were considered positive.

GeneXpert® MTB/RIF assay

For deparaffinization, 15 sections of 5µm thickness were cut from the FFPE tissue samples. The samples were deparaffinized using 320µl of deparaffinization solution (QIAGEN, Hilden, Germany) at 56°C for 15 minutes. After the formation of the tissue pellet, the deparaffinization solution was removed, and the pellet was washed with 320µl of sterile saline solution (0.9% NaCl). Subsequently, 320µl of sterile saline solution was added, and the tissue was macerated until a liquid consistency had been reached. This step was performed at the Mycobacteria Laboratory of the UFMG School of Medicine, using the GeneXpert® System (Cepheid, Sunnyvale, CA). The Xpert MTB/RIF assay amplifies a specific sequence of the rpoB gene for members of the *M. tuberculosis* complex and probe mutations within the rpoB gene for Rifampicin Resistant Tuberculosis (RR-TB)[12].

RESULTS

Sociodemographic and clinical characteristics

The achieved data were included in text, tables, figures, and supplementary materials. Nine samples were included in the study. The sample consisted of predominantly male (2.5:1), with a mean age of 50 (± 23.08; 19-89) years (Supplementary Table 4). Only one of the individuals had informed positive HIV status (Table 1 and Supplementary Table 5).

Most lesions were located on the tongue (n=4), with ulceration observed in six cases (n=6) with the time of evolution ranging from 0.26 (eight days) to 6 months. The average size of the lesions was 22mm, and two of the individual's presented symptoms (Supplementary Table 4). Table 1 and Supplementary Table 5 provide detailed descriptions of the individuals' clinical and sociodemographic data.

Histological characteristics, ZN staining, and Immunohistochemistry

The histopathological features were analyzed through H&E staining, confirming the presence of granuloma with caseous necrosis in all samples (n=9). Langhans-type multinucleated giant cells were present in eight of the samples and epithelioid histiocytic cells, numerous lymphocytes, and macrophages were observed in all samples (n=9), with higher quantities observed next to granulomas. However, the presence of *M. tuberculosis* bacilli observed through the ZN technique was inconclusive in all samples. Finally, IHC staining showed a fine granular pattern in all samples (n=9), as compared to a coarse granular pattern in three of the samples (Figure 1). Special stains by PAS and staining by Grocott proved to be negative in all samples.

Table 1. Clinical-pathological characteristics of each individual.

Sample	Age (years)	Sex	Lesion type	Lesion color	Symptomatology	Biopsy type	HIV status	Location	Time of evolution (months)	Size (mm)	Clinical observations
1	19	Male	Tumor	-	-	-	-	Mandibular alveolar ridge	6	-	-
2*	43	Male	Ulcer	-	-	I	-	Ventral region of the tongue	3	-	-
3*			Ulcer	-		I		Labial commissure		-	
4	49	Female	Tumor	Similar to oral mucosa	-	I	-	Buccal mucosa	2	-	-
5	38	Male	Cystic	-	-	E	HIV+	Bilateral buccal floor	Ind	60	-
6	89	Female	Ulcer	Erythematous	S	I	-	Tongue border	0,26	25	-
7*	41	Male	Ulcer	Erythematous	S	I	-	Tongue border (posterior third)	Ind	10	Patient living on the street Pulmonary findings suggestive of PTB
8*			Ulcer	Erythematous		I		Lateral border of the tongue (apex of the tongue)		10	
9	71	Male	Ulcer	-	AS	-	-	Mandibular alveolar ridge	-	5	-

(-): Not informed. *Samples from the same individual.
Abbreviations: AS: Asymptomatic; E: Excisional; HIV+: Individual living with Human Immunodeficiency Virus; I: Incisional; Ind: Indeterminate; PTB: Pulmonary Tuberculosis; S: Symptomatic.

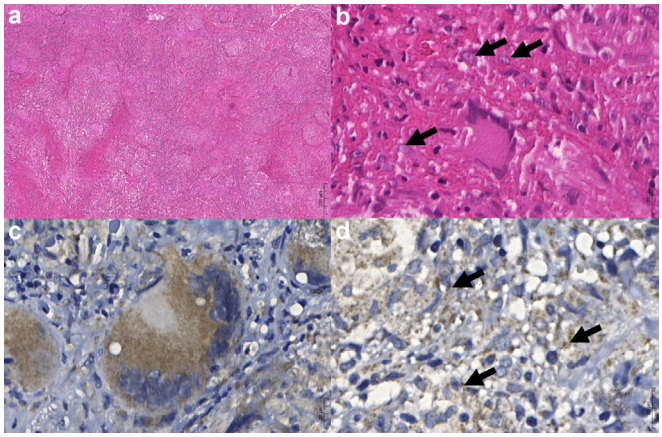

Figure 1. (a, b) H&E staining; a) Presence of granuloma with caseous necrosis (40x); b) High magnification depicting Langhans-type multinucleated giant cell multinucleated giant cells and epithelioid histiocytic cells (arrows) (400x); (c, d) Immunohistochemically staining; c) Presence of cytoplasmic positivity in plasma cells and lymphocytes evidenced by immunostaining, which reveals a brown-stained, fine, and homogeneous cytoplasm, suggesting the presence of fine antigen dust derived from mycobacterial products and homogeneous multinucleated giant cell suggestive of fine granular pattern (400x); d) Cytoplasmic positivity in epithelioid macrophages, with non-homogeneous staining, suggesting the presence of fragmented bacilli observed within or around the necrotizing granuloma (arrows) (400x).

Rapid molecular tests

The positive results for nested-PCR were considered for those samples that showed amplification on agarose gel electrophoresis with bands similar to 92 bp. The results of the GeneXpert® MTB/RIF assay were considered for the presence of MTB and negative for rifampicin resistance, positive for the presence of MTB and negative or indeterminate for rifampicin resistance, or positive for the presence of MTB and positive for rifampicin resistance.

The sample results of AFB staining, IHC, and molecular tests are presented in Table 2.

DISCUSSION

TB is transmitted through the air, mainly by talking, sneezing, and coughing, making it one of the most transmissible diseases[2,6,13-16]. Men are the most affected by oral TB, as reported in the literature, in a ratio of 1.7:1, in the fifth and sixth decades of life[17], as also observed in this study (2.5:1), in the 5th decade of life in three cases (n=7). The male gender has been identified as a risk factor for TB, along with alcohol and drug abuse, social vulnerability, HIV-positive status, and sociocultural determinants, which are possible factors for infection of the disease[18,19]. The most common site of involvement in these cases is the tongue, especially the lateral edges[16]. Our study also found that the most affected site tends to be the tongue, in an ulcerated, erythematous, and symptomatic manner. Furthermore, these clinical features, despite their relation to oral TB, do

Table 2. Histological and molecular results

Sample	Ziehl-Neelsen	Immunohistochemistry		Molecular tests		
	Bacilli	Granular pattern		*nested*-PCR	GeneXpert MTB/RIF assay	
		Fine	Coarse		*M. tuberculosis* Complex	Rifampicin resistance
1	-	+	-	-	-	-
2	-	+	-	-	-	-
3	-	+	-	-	-	-
4	-	+	-	-	-	-
5	-	+	+	+	+	ind
6	-	+	-	-	-	-
7	-	+	+	+	+	ind
8	-	+	+	-	+	ind
9	-	+	-	-	-	-
Total	0/9 (0%)	9/9 (100%)	3/9 (33.3%)	2/9 (22.2%)	3/9 (33.3%)	0/9 (0%)

Abbreviations: PCR: Polymerase Chain Reaction; ind: indeterminate.

not definitively establish the diagnosis. It is necessary to prove the presence of bacillus through histological, molecular, or culture tests[8,9].

Regarding histological characteristics, Kohli et al.[20] reported that the presence of epithelioid cells, lymphocytes, and Langhans-type multinucleated giant cells were observed in all cases. Likewise, in this present study, all samples showed the presence of epithelioid histiocytic, along with numerous macrophages and lymphocytes surrounding the granuloma. By contrast, Langhans-type multinucleated giant cells were observed in eight samples. These are expected histological characteristics for TB, but they can become a confounding factor with other granulomatous lesions, being mycobacterial or not[21]. In this aspect, Langhans-type multinucleated giant cells are a key feature in granulomatous inflammatory diseases. However, it remains uncertain if the granulomas develop as a body's immune response to bacteria, which can develop a granuloma with caseous necrosis due to this persistence or if the lesions create a favorable environment for the pathogen's survival[22].

Despite being a simple, cheap, and quick technique, ZN staining becomes challenging primarily due to the small quantity of bacilli typically found in this type of sample[8]. In this investigation, no AFB was detected in any of the samples using this technique. However, the absence of detection through ZN staining should not be considered an exclusionary factor for the diagnosis of oral TB in any sample, given the low sensitivity that can be attributed to this type of test[7]. In such cases, the combination of additional information, features, and tests becomes necessary to reach a conclusive diagnosis[20,23].

The IHC technique can identify fragmented bacilli and antigenic residues. This offers a significant improvement in the diagnostic potential of TB, especially in cases of paucibacillary EPTB, when compared to the ZN technique, as it requires a bacillus' intact cell wall. However, the dilution in IHC represents the main challenge in obtaining appropriate staining, as a fine granular background staining may lead to false-positive interpretations, particularly if the pathologist is not familiar with this technique. Nevertheless, when the staining shows positivity as a coarse granular cytoplasmic pattern, stained fragmented bacilli, or stained bacillus forms, the specificity percentage for this technique is significantly increased[24,25].

This correlation of positivity between ZN and IHC is shown in accordance with previous studies. Karimi et al.[25] and Kohli et al.[20] used polyclonal antibody BCG (pAbBCG) in tissue samples. While results from Karimi et al.[25] showed a positivity rate of 100% (23/23) for IHC and 39.1% (9/23) for ZN staining, results from Kohli et al.[20] showed positivity rates of 72% (72/100) and 23% (23/100) for IHC and ZN staining, respectively. Furthermore, Addo et al.[26] and Masoud et al.[27] used the polyclonal antibody anti-*M. tuberculosis*, pAbPPD, similar to that used in the present study. Addo et al.[26] reported a positivity rate of 52.5% (21/40) for IHC in histologically suggestive tissue sections and 0% (0/40) for ZN staining. Masoud et al.[27] found a positivity rate of 100% (12/12), but they did not use the special ZN stain. In the present study, there were wide markings of

staining patterns, mainly of the fine granular pattern in all selected samples. By contrast, the coarse granular pattern was observed in three (n=9) of the samples, showing that the IHC marking in this type of sample can be an aid in the diagnosis when combined with all the clinical and histological characteristics.

Molecular tests may face challenges due to uneven microorganism distribution but remain reliable for detecting *M. tuberculosis* in paucibacillary EPTB, offering rapid and accurate results[9]. Allahyartorkaman et al.[28] evaluated the performance of GeneXpert® MTB/RIF, a WHO-recommended rapid diagnostic test for TB, found a higher sensitivity for lung samples than for extra pulmonary samples (95.5 vs. 76.5%). This can be explained by the possibility that the decontamination step determines a decrease in the bacillary load and, consequently, a decrease in the sensitivity of the test.

In correlating the molecular tests, this investigation detected *M. tuberculosis* in only two samples through the IS6110 gene amplification using nested-PCR. In contrast, three samples tested positive in the GeneXpert® MTB/RIF assay. However, this discrepancy should not undermine the utility of the method; instead, it highlights the need for studies with larger sample sizes. Additionally, this was the first study to use the GeneXpert MTB/RIF assay in samples suggestive of oral TB. The observed differences between tests may be attributed to the higher level of automation in the GeneXpert® MTB/RIF assay, which reduces the need for manual intervention[9,12].

Furthermore, it is important to emphasize that TB is a disease closely associated with poverty, and individuals affected by it often face economic difficulties, vulnerability, marginalization, and stigma[2,19]. In addition, approximately 50% of those co-infected with HIV may develop EPTB[9]. Correlating the demographic characteristics of individuals with the results of IHC and molecular tests, particularly the positive findings, the results supported the fact that the individuals tended to belong to populations that are more vulnerable to TB. For instance, one of the participants was a homeless person, and another was living with HIV. These clinical factors, combined with the positive molecular test results, suggest a higher likelihood of TB infection in these individuals. The combination of these demographic and clinical characteristics with positive test results highlights the reliability of molecular tests and IHC in diagnosing TB. Granulomas alone are insufficient for a definitive diagnosis, as several other conditions may present similar features.

Despite the strengths, this study did present some limitations. First, because it was a retrospective and laboratory-based study, the amount of demographic and clinical data available was limited, and the study sample was small to determine the sensitivity and specificity of the tests. In addition, it was not possible to confirm true positive and true negative cases. In view of all the questions raised, studies with a larger sample size and as much sociodemographic and clinical information on patients as possible, in addition to confirmations of true positive and negative cases to consolidate the accuracy of diagnostic methods in cases of oral TB, are recommended.

This study exemplifies the main difficulty faced by oral pathologists, since the lack of clinical information, combined with the paucibacillary characteristic of the manifestation of the disease in the oral cavity, makes the conclusive diagnosis quite challenging. Consequently, there is a real need to combine clinical information with histological characteristics, using more than one test for the final diagnosis.

CONCLUSION

In conclusion, our study shows that molecular tests and IHC may be useful auxiliary methods for suspected cases of oral tuberculosis, which present granulomas with caseous necrosis. This study provides information on the use and effectiveness of diagnostic methods needed to assist pathologists in the accurate diagnosis of oral TB lesions.

ACKNOWLEDGMENTS

The authors thank the Coordination for the Improvement of Higher Education Personnel (CAPES, Finance Code 001), Brazil. R.O.S.S. is the recipient of fellowships. We acknowledge the Minas Gerais Research Foundation (FAPEMIG) for a fellowship to T.L.A. (PROBIC). RSG is research fellow at Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq/Brazil) (National Council for Scientific and Technological Development). We also acknowledge Leni Verônica de Oliveira and Todd Marshall for provision of writing assistance, Domenico Romano Romani, Josiane Vieira Lopes and Mércia Cristina de Freitas for their technical assistance. The microscopic images shown in this work were obtained using the slide scanner in the Centro de Aquisição e Processamento de Imagens (CAPI-ICB/UFMG).

AUTHORS' CONTRIBUTIONS

ROSS: data curation, formal analysis, investigation, methodology project administration, validation, writing – original draft, writing – review & editing. RSG: conceptualization, data curation, formal analysis, funding acquisition, project administration, supervision. MGD: data curation, methodology, project administration, supervision, writing – review & editing. SSM: data curation, formal analysis, funding acquisition, supervision, writing – review & editing. RSA: data curation, funding acquisition, supervision, writing – review & editing. LJAF: data curation, methodology, validation. MAB: methodology, validation, visualization. TLA: data curation, investigation, writing – original draft. VFB: conceptualization, data curation, formal analysis, methodology, project administration, supervision, writing – review & editing, validation.

CONFLICT OF INTEREST STATEMENT

Funding: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001 (research fellow R.O.S.S.).

Competing interests: The authors have no relevant financial or non-financial interests to disclose.

Ethics approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The UFMG Human Research Ethics Committee (Protocol No. 5.429.194) approved this study.

REFERENCES

1. Sakula A. Robert Koch: centenary of the discovery of the tubercle bacillus, 1882. Thorax. 1982;37(4):246-51. https://doi.org/10.1136/thx.37.4.246
2. Bagcchi S. WHO's global tuberculosis report 2022. Lancet Microbe. 2023;4(1):e20. https://doi.org/10.1016/S2666-5247(22)00359-7
3. Emery JC, Richards AS, Dale KD, McQuaid CF, White RG, Denholm JT, et al. Self-clearance of *Mycobacterium tuberculosis* infection: Implications for lifetime risk and population at-risk of tuberculosis disease. Proc Biol Sci. 2021;288(1943):20201635. https://doi.org/10.1098/rspb.2020.1635
4. Sharma AB, Laishram DK, Sarma B. Primary tuberculosis of tongue. Indian J Pathol Microbiol. 2008;51(1):65-6. https://doi.org/10.4103/0377-4929.40402
5. Natarajan A, Beena PM, Devnikar AV, Mali S. A systemic review on tuberculosis. Indian J Tuberc. 2020;67(3):295-311. https://doi.org/10.1016/j.ijtb.2020.02.005
6. Kumar S, Sen R, Rawal A, Dahiya RS, Dalal N, Kaushik S. Primary lingual tuberculosis in immunocompetent patient: a case report. Head Neck Pathol. 2010;4(2):178-80. https://doi.org/10.1007/s12105-010-0180-8
7. Zumla A, James DG. Granulomatous infections: etiology and classification. Clin Infect Dis. 1996;23(1):146-58. https://doi.org/10.1093/clinids/23.1.146
8. Purohit M, Mustafa T. Laboratory diagnosis of extra-pulmonary tuberculosis (EPTB) in resource-constrained setting: state of the art, challenges and the need. J Clin Diagn Res. 2015;9(4):EE01-6. https://doi.org/10.7860/JCDR/2015/12422.5792
9. Mehta PK, Raj A, Singh N, Khuller GK. Diagnosis of extrapulmonary tuberculosis by PCR. FEMS Immunol Med Microbiol. 2012;66(1):20-36. https://doi.org/10.1111/j.1574-695X.2012.00987.x
10. Masoud S, Mihan P, Hamed M, Mehdi M, Mohamad RM. The presence of mycobacterial antigens in sarcoidosis associated granulomas. Sarcoidosis Vasc Diffuse Lung Dis. 2017;34(3):236-41. https://doi.org/10.36141/svdld.v34i3.5739
11. World Health Organization. Xpert MTB/RIF implementation manual: technical and operational 'how-to'; practical considerations [Internet]. Geneva: World Health Organization; 2014 [cited 2023 Mar 28]. Available from: https://apps.who.int/iris/bitstream/handle/10665/112469/9789241506700_eng.pdf?sequence=1&isAllowed=y
12. Helb D, Jones M, Story E, Boehme C, Wallace E, Ho K, et al. Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol. 2010;48(1):229-37. https://doi.org/10.1128/JCM.01463-09
13. Lewinsohn DM, Leonard MK, LoBue PA, Cohn DL, Daley CL, Desmond E, et al. Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: diagnosis of tuberculosis in adults and children. Clin Infect Dis. 2017;64(2):111-5. https://doi.org/10.1093/cid/ciw778
14. Vaid S, Lee YYP, Rawat S, Luthra A, Shah D, Ahuja AT. Tuberculosis in the head and neck--a forgotten differential diagnosis. Clin Radiol. 2010;65(1):73-81. https://doi.org/10.1016/j.crad.2009.09.004
15. Razem B, El Hamid S, Salissou I, Raiteb M, Slimani F. Lingual primary tuberculosis mimicking malignancy. Ann Med Surg (Lond). 2021;67:102525. https://doi.org/10.1016/j.amsu.2021.102525
16. Rout P, Modipalle V, Hedge SS, Patel N, Uppala S, Shetty PK. Prevalence of oral lesions in tuberculosis: a cross sectional study. J Family Med Prim Care. 2019;8(12):3821-5. https://doi.org/10.4103/jfmpc.jfmpc_714_19
17. Gabriel AF, Kirschnick LB, Só BB, Schuch LF, Silveira FM, Martins MAT, et al. Oral and maxillofacial tuberculosis: a systematic review. Oral Dis. 2023;29(7):2483-92. https://doi.org/10.1111/odi.14290
18. Narasimhan P, Wood J, MacIntyre CR, Mathai D. Risk factors for tuberculosis. Pulm Med. 2013;2013:828939. https://doi.org/10.1155/2013/828939
19. Silva DR, Rabahi MF, Sant'Anna CC, Silva-Junior JLR, Capone D, Bombarda S, et al. Diagnosis of tuberculosis: a consensus statement from the Brazilian Thoracic Association. J Bras Pneumol. 2021;47(02):e20210054. https://doi.org/10.36416/1806-3756/e20210054
20. Kohli R, Punia RS, Kaushik R, Kundu R, Mohan H. Relative value of immunohistochemistry in detection of mycobacterial antigen in suspected cases of tuberculosis in tissue sections. Indian J Pathol Microbiol. 2014;57(4):574-8. https://doi.org/10.4103/0377-4929.142667

21. Kim SY, Byun JS, Choi JK, Jung JK. A case report of a tongue ulcer presented as the first sign of occult tuberculosis. BMC Oral Health. 2019;19(1):67. https://doi.org/10.1186/s12903-019-0764-y
22. Ahmadzadeh K, Vanoppen M, Rose CD, Matthys P, Wouters CH. Multinucleated giant cells: current insights in phenotype, biological activities, and mechanism of formation. Front Cell Dev Biol. 2022;10:873226. https://doi.org/10.3389/fcell.2022.873226
23. Mustafa T, Wiker HG, Mfinanga SGM, Mørkve O, Sviland L. Immunohistochemistry using a *Mycobacterium tuberculosis* complex specific antibody for improved diagnosis of tuberculous lymphadenitis. Mod Pathol. 2006;19(12):1606-14. https://doi.org/10.1038/modpathol.3800697
24. Goel MM, Budhwar P. Immunohistochemical localization of *Mycobacterium tuberculosis* complex antigen with antibody to 38 kDa antigen versus Ziehl Neelsen staining in tissue granulomas of extrapulmonary tuberculosis. Indian J Tuberc. 2007;54(1):24-9. PMID: 17455420.
25. Karimi S, Shamaei M, Pourabdollah M, Sadr M, Karbasi M, Kiani A, et al. Histopathological findings in immunohistological staining of the granulomatous tissue reaction associated with tuberculosis. Tuberc Res Treat. 2014;2014:858396. https://doi.org/10.1155/2014/858396
26. Addo SO, Abrahams AOD, Mensah GI, Mawuli BA, Mosi L, Wiredu EK, et al. Utility of anti- *Mycobacterium tuberculosis* antibody (ab905) for detection of mycobacterial antigens in formalin-fixed paraffin-embedded tissues from clinically and histologically suggestive extrapulmonary tuberculosis cases. Heliyon. 2022;8(12):e12370. https://doi.org/10.1016/j.heliyon.2022.e12370
27. Masoud S, Mihan P, Hamed M, Mehdi M, Mohamad RM. The presence of mycobacterial antigens in sarcoidosis associated granulomas. Sarcoidosis Vasc Diffuse Lung Dis. 2017;34(3):236-41. https://doi.org/10.36141/svdld.v34i3.5739
28. Allahyartorkaman M, Mirsaeidi M, Hamzehloo G, Amini S, Zakiloo M, Nasiri MJ. Low diagnostic accuracy of Xpert MTB/RIF assay for extrapulmonary tuberculosis: a multicenter surveillance. Sci Rep. 2019;9(1):18515. https://doi.org/10.1038/s41598-019-55112-y