CASE REPORT / *CAS CLINIQUE*

Surgery / *Chirurgie*

ORAL LESION AS THE INITIAL PRESENTATION IN THE DIAGNOSIS OF HISTIOCYTOSIS X: A CASE REPORT WITH 16-MONTH FOLLOW-UP

Wassim Manhal[1] | Georgio Saad[2] | Michael El Daccache[3] | Charbel Anka[4] | Sophie Abi Raad[5] | Valerie G. Aftimos[6] | Georges Bou Jaoude[2]

Introduction: Langerhans Cell Histiocytosis (LCH) is a rare neoplastic disease that comes from the abnormal proliferation of Langerhans cells (LCs). It presents with a broad clinical spectrum, varying from involvement of a single organ system to multisystem disease, and frequently affects children, although cases in adults have been reported. Oral manifestations of LCH are non-specific and may mimic common periodontal conditions, posing a diagnostic challenge for dental practitioners.

Case Presentation: A 63-year-old female patient presented with complaints of periodontal problems. She was initially diagnosed with periodontitis, but symptoms persisted despite treatment, prompting a referral for further evaluation. Histopathological analysis, supported by immunohistochemistry (CD1a and S100 positivity), confirmed the diagnosis of LCH. She subsequently underwent systemic chemotherapy with vinblastine and corticosteroids.

Conclusion: LCH is a rare yet important differential diagnosis for patients presenting with persistent oral lesions, requiring a multidisciplinary approach for definitive diagnosis. Early recognition and treatment of LCH can prevent disease progression and complications.

Keywords: Langerhans cell histiocytosis, Periodontitis, Oral manifestations, Differential diagnosis, CD1a glycoprotein, Cytoplasmatic protein S100, Immunochemistry, Histology

Corresponding author:
Wassim Al Manhal, e-mail: wassim.manhal@usj.edu.lb

Conflicts of interest:
The authors declare no conflicts of interest.

1. Assistant Professor, Oral Pathology Department, Co-Director of Cranio-Facial Research laboratory. Faculty of Dental Medicine, Saint Joseph University of Beirut, Lebanon. E-mail: wassim.manhal@usj.edu.lb
2. Department of Oral Surgery, Faculty of Dental Medicine, Saint Joseph University of Beirut, Lebanon. E-mail: georgio.saad@net.usj.edu.lb ; georgesboujaoude@hotmail.com
3. Oral Pathology Department, Faculty of Dental Medicine, Saint Joseph University of Beirut, Lebanon. E-mail: michealeldaccache10@gmail.com
4. Clinical Instructor, Department of Periodontology, Faculty of Dental Medicine, Saint Joseph University of Beirut, Lebanon. E-mail: charbelanka.dds@gmail.com
5. 5th year dental student, Faculty of Dental Medicine, Saint Joseph University of Beirut, Lebanon. E-mail: sophie.abiraad@net.usj.edu.lb
6. Institut Nationale de Pathologie, CHU Notre Dame Des Secours. Liban. E-mail: vaftimos@gmail.com

LÉSION BUCCALE COMME PRÉSENTATION INITIALE DANS LE DIAGNOSTIC DE L'HISTIOCYTOSE X : CAS CLINIQUE AVEC SUIVI DE 16 MOIS

Introduction: L'histiocytose à cellules de Langerhans (HCL) est une maladie néoplasique rare caractérisée par une multiplication anormale des cellules de Langerhans (CL). Elle se manifeste par une diversité clinique, pouvant atteindre un seul organe ou évoluer vers une atteinte multisystémique, et affecte fréquemment les enfants, bien que des cas chez l'adulte aient été signalés. Les manifestations orales de l'HCL sont non spécifiques et peuvent imiter des parodontites, ce qui pose un problème de diagnostic aux dentistes.

Présentation du cas: Une patiente de 63 ans s'est présentée avec des problèmes parodontaux. Un diagnostic initial de parodontite a été posé, mais les symptômes ont persisté malgré le traitement, ce qui a mené une évaluation plus approfondie. L'analyse histopathologique, appuyée par l'immunohistochimie (positivité du CD1a et du S100), a confirmé le diagnostic d'HCL. La patiente a ensuite subi une chimiothérapie systémique à base de vinblastine et de corticostéroïdes.

Conclusion: L'HCL est un diagnostic différentiel rare mais important chez les patients ayant des lésions orales récurrentes, nécessitant une approche multidisciplinaire pour un diagnostic définitif. L'identification et la prise en charge anticipée de l'HCL permettent de limiter l'évolution de la maladie et de réduire le risque de complications.

Mots Clés: Histiocytose à cellules de Langerhans, Parodontite, Manifestations orales, Diagnostique différentiel, Immunochimie, Histologie

Introduction

Systemic diseases are known to impact the oral cavity, as oral lesions may stem from either a local process or an overall change in the body [1]. Dentists therefore play a crucial role in treating patients through proper diagnosis and recognizing any associated systemic issues [1].

Langerhans cell histiocytosis (LCH), previously referred to as Histiocytosis X, is a neoplastic disorder [2] caused by a dysfunction of myeloid dendritic cells, known as Langerhans cells (LCs), predominantly located in the skin, bone, lymph nodes, ears, gums, and lungs [3]. It mainly affects children and young adults, with a higher prevalence in males [4]. According to reports, this illness affects between 4 and 9 children for every million people and 1 to 2 adults per million per year [5]. The disease can be classified as involving only one organ or multiple organs. Its etiology remains unidentified, and it presents with a highly variable clinical spectrum [4]. It may present with cancer-like behavior, often necessitating management by oncology specialists [6].

Single-system and multisystem LCH are the two currently known subtypes of LCH, which was previously divided into three distinct clinical entities: eosinophilic granuloma, Letterer-Siwe and Hand-Schüller-Christian diseases [5]. In single-system LCH, the bones are the most involved, with the skin, lymph nodes, and lungs also frequently affected [4]. Bone lesions are usually unifocal in nature, most often affecting the skull, ribs, long bones, pelvic bones and vertebrae [4]. LCH can also affect the jaw and skull bones, with the most frequently impacted areas in the maxillomandibular region being the gingiva and hard palate [6].

The oral manifestations related to histiocytosis X lesions can be single or multiple. They can affect bones and cause ulcerated mucosal lesions along with lymph node swelling and periodontal problems often accompanied by common signs such as gingivitis, dental mobility, periodontitis and premature tooth loss [7]. Oral lesions can be the initial sign of LCH, yet their non-specific nature often contributes to diagnostic challenges. Therefore, a comprehensive evaluation and careful consideration of differential diagnoses are crucial for the early identification of LCH [4]. Hence, the challenge for dentists resides in determining whether a lesion represents a local periodontal problem that requires regular treatment or if there is suspicion for a systemic condition warranting a biopsy [8].

This report discusses the case of a 63-year-old female patient diagnosed with LCH, who visited the Saint Joseph's University Dental Care Center in Beirut. She exhibited periodontal problems affecting the gums, along with resorption of the mandibular and maxillary bones. The diagnosis was established using histopathological analysis following tooth extraction, which prompted further evaluation revealing an underlying systemic disorder. The patient's condition showed significant improvement following the treatment. Based on this observation, we provide a concise literature review highlighting the clinical, histological, radiological, therapeutic, and progressive aspects of LCH. This study aims to enhance clinicians' awareness of the unique characteristics that make LCH a diagnostic challenge.

Case Presentation

In this report, we present the case of a 63-year-old female patient with LCH, who visited the Saint Joseph's University Dental Care Center in Beirut. She reported difficulty chewing and pain on the right side of the mandible, which she stated had begun two years earlier. A panoramic radiograph (Figure 1), along with periapical radiographs, revealed resorption of both the mandibular and maxillary bones. Additionally, the patient exhibited periodontal issues affecting the gums. Based on these findings, she was diagnosed with periodontitis, and periodontal treatment was initiated.

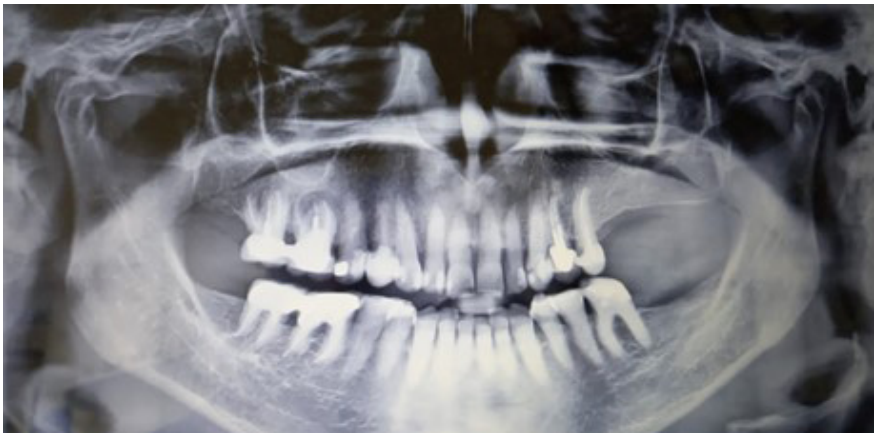

Figure 1. Panoramic radiograph of the patient's mouth before extractions.

Following a recurrence of periodontal disease, and despite the initial diagnosis, the persistence of symptoms prompted further investigation. The patient was referred to the Department of Oral Pathology at Saint Joseph's University of Beirut for clinical and diagnostic evaluation.

The patient had good overall physical condition, reporting no bone pain or alterations in daily activity. The patient had hypertension, which was well controlled, high cholesterol levels and a glucose level of 116.5 mg/dL (milligrams per deciliter).

Extraoral examinations revealed no signs of adenopathy and palpation of the submandibular, sublingual and parotid lymph nodes were

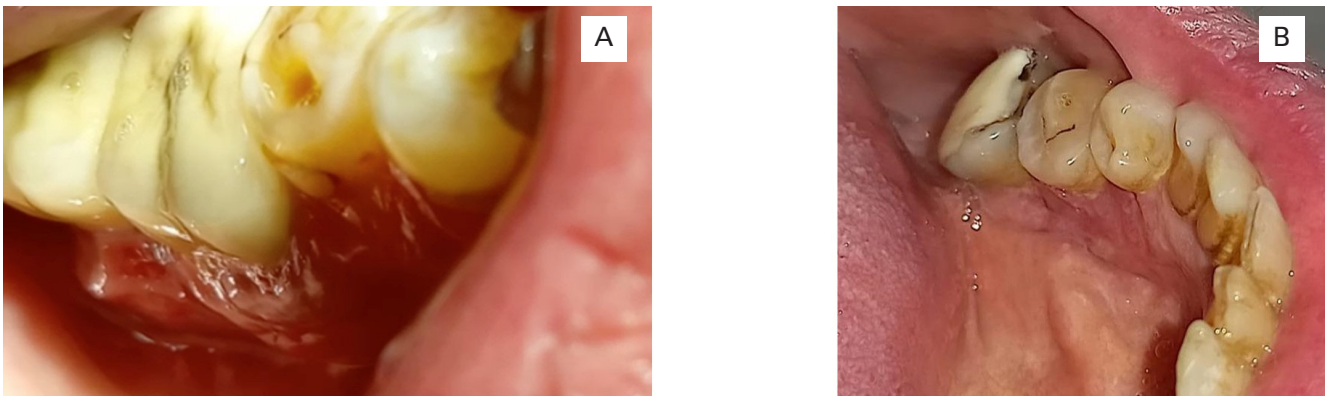

Figure 2. A- Buccal view of the gingiva around teeth #46 and #47 B- lingual view of the gingiva around teeth #34, #35 and #36.

painless. Intraoral exams showed generalized gingival hyperplasia, which was most prominent in the lower right molar region. The gingival inflammation was sessile, exhibited a soft texture, and appeared red in color. It was localized on the buccal side of the first and second lower right molars (Figure 2A), the lingual side of the first and second lower left premolars (Figure 2B) as well as the second upper right molar. Furthermore, clinical findings included gingival recessions, periodontal pockets, bleeding on probing, and halitosis were recorded. Furcation involvement was observed with significant tooth mobility in the first and second lower right molars (Figure 3).

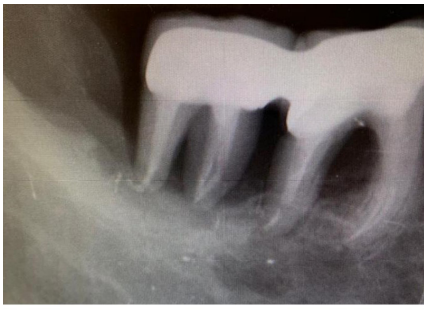

Figure3. Periapical radiograph of teeth #46 and #47.

Following extraction of the tooth #16, periodontal therapy was initiated, involving scaling and root planing, combined with maintaining good oral hygiene practices to prevent disease. The differential diagnosis considered aggressive periodontitis among other conditions. Despite these interventions, no noticeable improvement was observed in the periodontal health of the remaining teeth, nor was there a reduction in the severity of the oral lesions (Figure 4-A). Subsequent radiological assessment using cone beam computed tomography (CBCT) showed multiple periapical radiolucency with inadequately defined and invasive margins (Figure 4-B). Additionally, a new gingival lesion appeared in the region of the lower first and second right molars; an excisional biopsy was obtained from them after their extraction (Figure 5).

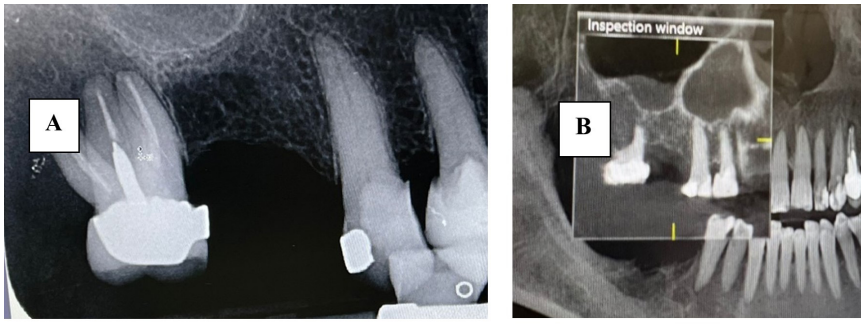

Figure 4. A- Periapical radiograph of tooth #17 B-CBCT of tooth #17.

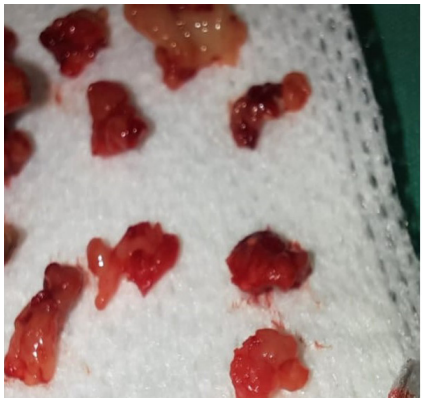

Figure 5. Extracted teeth #46 and #47 along with associated oral lesions.

Several fragments of tissue, associated with two molars, were received in the pathology laboratory, the largest measuring 0.6x0.5cm, submitted and examined entirely. After inclusion of the tissue, cut sections with a Hematoxylin and Eosin (H&E) standard stain (Figure 6-A) and with a Giemsa staining (Figure 6-B) were obtained. Microscopic examination under H&E stain revealed a dense proliferation of mononuclear cells with irregular nuclear contours which suggest a histiocytic appearance. They were mixed with polymorphic leukocytes including numerous eosinophils, an appearance compatible with Langerhans histiocytosis. The background exhibits scattered lymphocytes and multinucleated giant cells. The Giemsa stain enhanced nuclear details, making it useful for identifying histiocytes, eosinophils, and other leukocytes. Cells were predominantly blue to purple, which suggested high nuclear content. The scattered eosinophils, seen as cells with dark granules, were a key feature of LCH.

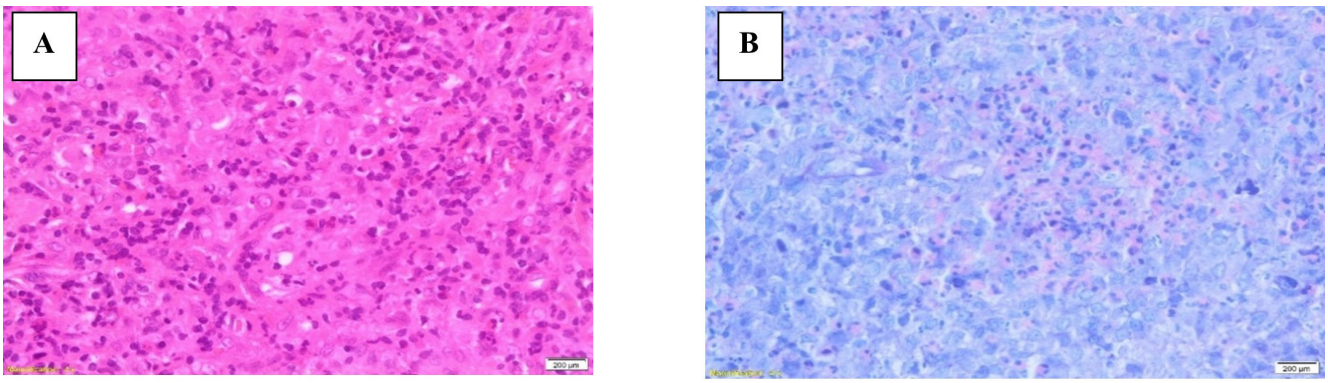

Figure 6. A-Fragment of the lesion stained with H&E B- Giemsa lent (x40).

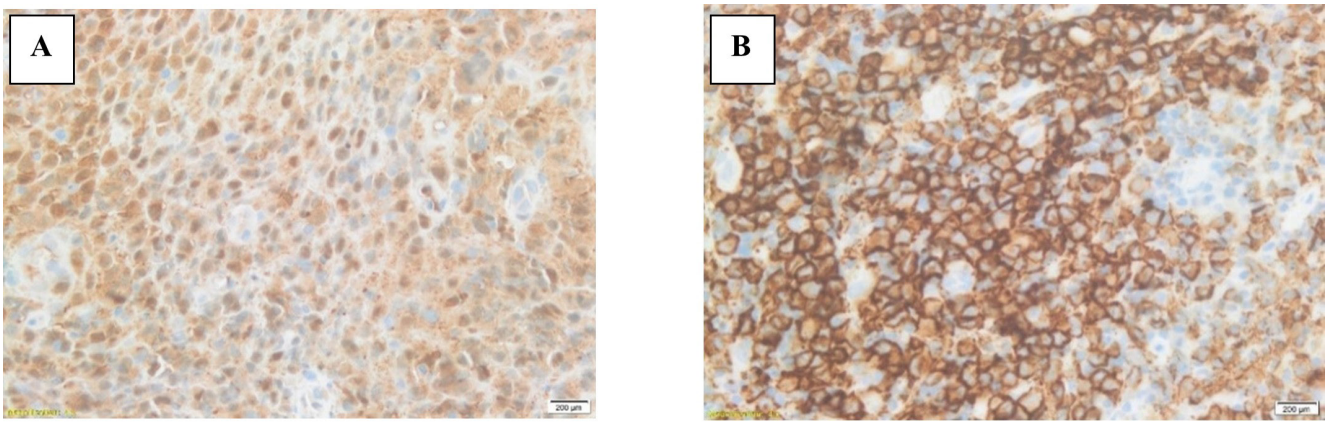

Figure 7. A- Immunolabelling within the tumor cells with anti-S100 antibody B- CD1a antibody (x40).

Langerhans-like cells appeared as large cells with indented or grooved nuclei (coffee bean appearance).

Immunolabelling with anti-S100 antibody (Figure 7-A) and CD1a antibody (Figure 7-B) demonstrated high expression of both markers within the tumor cells. The CD1a stain (Figure 7-B) exhibited strong membranous staining, with intense brown outlining the cell borders, a defining feature of LCs that distinguishes LCH from other histiocytic disorders. In contrast, the S100 protein stain (Figure 7-A) displayed diffuse cytoplasmic and nuclear staining, appearing as lighter brown staining throughout the cells. The combined immunophenotype and morphological findings confirmed the diagnosis of LCH. To further investigate the underlying molecular alterations, a tumor DNA molecular analysis was performed on a biopsy sample from the mandibular lesion. The analysis revealed the presence of an oncogenic BRAF mutation.

The patient was submitted to a medical center for additional evaluation. Laboratory evaluations, including blood tests, liver function analysis, and urine examination, did not provide any significant findings. Ground-glass nodules were identified in the upper left lung lobe through conventional chest radiography (Figure 8); however, computed tomography (CT) scans and positron emission tomography (PET) did not indicate any lung involvement. Moreover, a bone marrow biopsy confirmed the absence of infiltration. Based on the combined clinical and radiological assessments, the diagnosis was confirmed as Langerhans Cell Histiocytosis with unifocal involvement.

Systemic chemotherapy with vinca alkaloids (vinblastine 6 mg/m^2 every 21 days) and steroids (prednisolone 40 mg/m^2 per day) were administered to the patient, who continued regular dental check-ups follow-ups with the surgery and oral pathology departments. Routine scintigraphy examinations were also performed to monitor disease progression and assess systemic involvement.

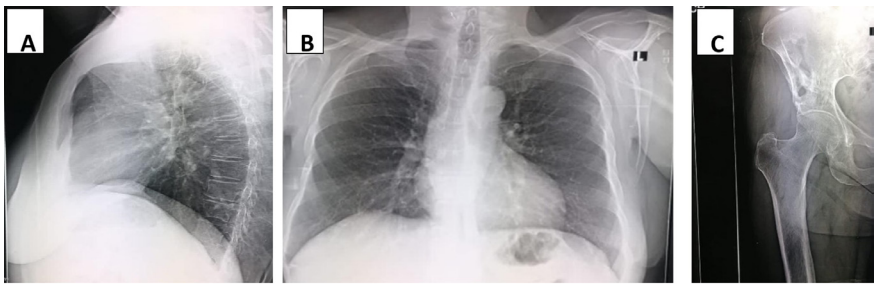

Figure 8. Radiographic images of the patient A- Side lung B- Frontal view of the lungs C-Frontal view of the right hip.

Clinical and radiological examinations after one year of chemotherapy revealed a significant decrease in gingival enlargement and a reduction in the remaining teeth mobility with no progression in alveolar bone loss. However, after one year and four months, the patient's most recent scintigraphy report revealed the presence of two small areas of tracer hyperfixation at the mandibular level bilaterally, with the more intense on the left, likely associated with a dental problem.

A panoramic (Figure 9) and periapical radiograph (Figure 10) revealed a well-defined radiolucent lesion in the left mandibular posterior region, associated with a granulomatous gingival tissue in the same area (Figure 11-A). Notably, this region was unrelated to any teeth or recent extractions. Following the oncologist's consultation and approval, a biopsy was performed by excising a small fragment of gingival tissue and conducting a curettage of the alveolus (Figure 11-B). Histopathological analysis of the biopsy confirmed that the lesion was infiltrated by Langerhans cells.

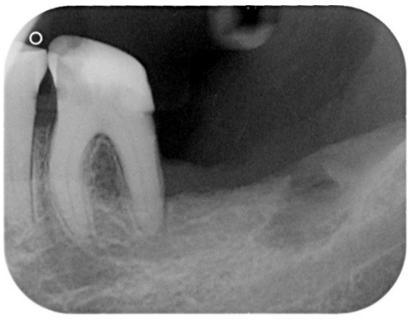

Figure 10. Periapical radiograph showing a radiolucent lesion posterior to tooth #36 in the left mandibular region.

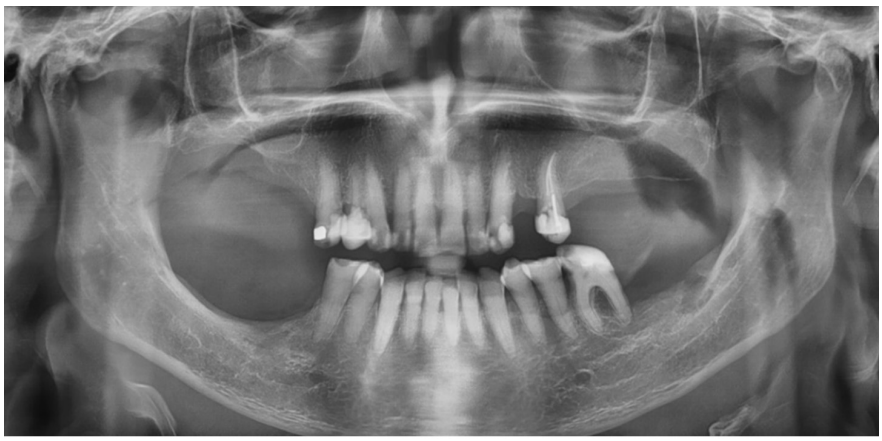

Figure 9. Panoramic radiograph showing a radiolucent lesion in the left posterior mandible

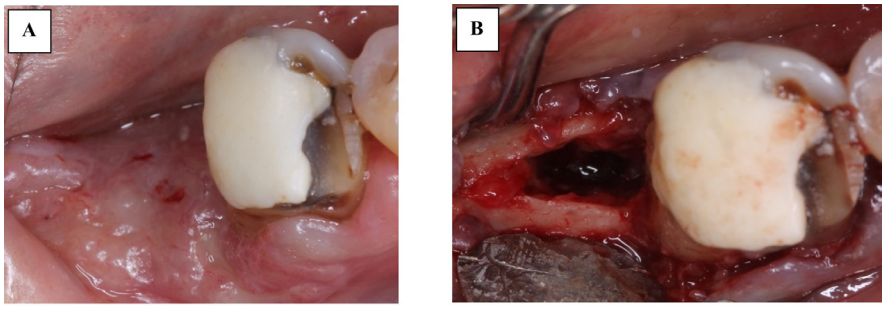

Figure 11. A- Clinical photograph showing gingival tissue posterior to tooth #36 in the left mandibular region B- a clinical photograph showing the alveolar bone during the curettage in the left mandibular region.

Discussion

Unknown in origin, Langerhans cell histiocytosis is a rare reticuloendothelial system disorder [9]. However, it has been associated with factors such as infections (Epstein–Barr virus), environmental influences, immunological factors, genetic mutations, smoking (particularly in cases of isolated pulmonary LCH), and neoplastic processes [7]. This is an abnormal proliferation of myeloid dendritic cells that can affect people of any age, although it primarily impacts young children, typically between the ages of 1 and 5 years [10]. The diagnosis of LCH is established through histopathological and immunohistochemical analyses, following a comprehensive assessment of the patient's clinical presentation and radiographic findings [5]. The histological results in this medical condition are centered on the invasion of eosinophilic granulocytes, Langerhans cells, lymphocytes, and multinucleated cells causing tissue destruction [11]. Langerin (CD207), S100 protein, and CD1a expression are among the markers that define LCH. Cytoplasmic Birbeck granules are seen as elongated, zipper-like structures under electron microscopy [12].

The clinical presentation of LCH can vary significantly, ranging from a single, localized lesion to a widespread systemic disease affecting multiple organ systems [10]. Historically, LCH was categorized into three distinct conditions; however, the current classification is based on the site and the number of the lesions, and the extent of organ involvement, dividing cases into two main categories. Localized (single-system disease) LCH affecting one organ at one or multiple sites, and disseminated (multisystem disease) LCH where two or more organs may be affected [13]. The single-system variant, previously referred to as "eosinophilic granuloma," is considered to be the benign form of LCH. The multisystem LCH is subdivided into low- and high-risk categories, depending on whether high-risk organs such as lungs, spleen, liver, or hematopoietic system are involved [3]. The typical sites of involvement vary by age, with children mainly affected in areas like the skull, femur, ribs, vertebrae, and humerus, while adults more frequently present with

lesions in the jaw, skull, vertebrae, pelvis, extremities, and ribs. In adults, the symptoms of LCH depend on the specific organs affected [4]. The most frequent symptoms are localized pain, weight loss, and fever. Diabetes insipidus, which results from pituitary gland involvement, is a key indicator of LCH [14] [15].

Bone involvement, fever, skin rash, and lymphadenopathy are among the most often reported systemic symptoms [12]. Oral manifestations of LCH include cheek, palate, or tongue mucosal ulcers, as well as underlying bone lesions [7]. For dental practitioners, differentiating LCH from other medical conditions of the oral cavity can be challenging since some of the LCH's symptoms overlap with those of more frequent conditions such as periodontal disease, tumoral, granulomatous and ulcerative lesions [7]. LCH remains a disease with no distinct pathognomonic features. Its differential diagnosis includes periodontitis, cystic lesions, periapical pathologies, and even malignancies. A definitive diagnosis relies on histopathological analysis through biopsy, complemented by clinical evaluation, radiographic imaging, and immunohistochemical testing [16] [17].

In this case, the main diagnosis of aggressive localized periodontitis was based on clinical and radiological findings. However, the dentists were able to avoid misdiagnosing the condition and prevent inappropriate therapy through a thorough reevaluation and histological investigation. The differential diagnosis of LCH includes other types of histiocytosis, bone metastases, and lymphomas. A biopsy of the lesion is essential for diagnosis, with the gold standard being the positive identification of CD1a, CD207 (Langerin) and S100 markers through immunohistochemistry (IHC) [18]. However, in this case, immunolabeling for CD207 (Langerin) was not performed, even though current diagnostic guidelines recommend its use [10].

Chemotherapy, radiation therapy, surgical excision, or a mix of these treatments are used to treat LCH [1]. The patient was treated with systemic chemotherapy using vinblastine, which, along with steroids (prednisolone), constitutes the first-line treatment for LCH in both children and adults [4].

However, Abdellatif Tazi et al. (2017), have reported comparable treatment responses, with approximately 70% of adults with LCH showing significant improvement following similar therapeutic regimens used when treating children. Moreover, after long-term follow-up, this study also demonstrated disease remission in 40% of LCH patients, highlighting the potential for durable responses in a subset of cases [19].

Despite its efficacy, studies have indicated that adults may not tolerate vinblastine as well as children, leading to the consideration of alternative treatment options such as cytosine arabinoside (ARA-C) [20]. The study by Maria A. Cantu et al. (2012) found that 84% of adult patients treated with a combination of vinblastine and prednisone either failed to respond or relapsed within a year, whereas only 21% of patients treated with ARA-C experienced treatment failure. Furthermore, vinblastine/prednisone was associated with higher neurotoxicity compared to ARA-C. Given these findings, ARA-C is recognized as an effective and less toxic treatment option for LCH bone lesions in adults, offering a potential alternative for patients with vinblastine intolerance [20].

This case revealed the presence of an oncogenic BRAF mutation. It is known to activate a signaling pathway, leading to uncontrolled proliferation of Langerhans cells, which plays a central role in LCH pathogenesis. The discovery of this mutation has established LCH as a hematopoietic neoplasm, paving the way for the use of targeted therapies such as BRAF inhibitors [10].

In this case the patient presented with localized LCH affecting the bone and gingival tissue. The oral lesions were managed locally through extraction of the affected teeth, followed by curettage of the surrounding lesions. Additionally, the patient underwent both local dental treatment and systemic chemotherapy, which together contributed to an overall improvement in health. A good treatment plan can only be implemented when a definitive diagnosis has been made, which requires prompt and accurate assessment of symptoms and lesions [5].

The prognosis of LCH is intimately tied to the age at which it first manifests, its category, and the severity of the organ involvement; early identification is thus critical. This is especially noteworthy considering the possibility that the oral cavity may exhibit the first signs of LCH [2]. Single system LCH showed a better prognosis than multi system LCH [2].

It has been shown that early detection and appropriate treatment of (LCH) could not only stop the disease from getting more serious but also prevent other problems like growth retardation in children, chronic pulmonary failure, liver cirrhosis, hearing loss, diabetes insipidus, skin damage, and neuropsychiatric conditions [2].

Conclusion

LCH is a rare disease and has multiple systemic manifestations. An interdisciplinary approach is required for a definitive diagnosis and successful treatment. Clinicians can help reduce the morbidity and mortality linked to this severe disease by being aware of the oral signs of LCH and maintain a high degree of suspicion [10].

References

1. Aruna D, Pushpalatha G, Galgali S, Prashanthy. Langerhans cell histiocytosis. J Indian Soc Periodontol. 2011;15(3):276.
2. Tillotson CV, Reynolds SB, Patel BC. Langerhans Cell Histiocytosis. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2024.
3. Allen CE, Merad M, McClain KL. Langerhans-Cell Histiocytosis. N Engl J Med. 2018 Aug 30;379(9):856–68.
4. Rodriguez-Galindo C, Allen CE. Langerhans cell histiocytosis. Blood. 2020 Apr 16;135(16):1319–31.
5. Gulati N, Allen CE. Langerhans Cell Histiocytosis: Version 2021. Hematol Oncol. 2021 Jun;39(Suppl 1):15–23.
6. El Ouali Z, Khoubila N, Cherkaoui S, Rachid M, Lamchahab M, Qachouh M, et al. Langerhans cell histiocytosis in children: a case report and brief review of the literature. PAMJ-CM. 2019
7. Faustino ISP, Fernandes PM, Pontes HAR, Mosqueda-Taylor A, Santos-Silva AR, Vargas PA, et al. Langerhans cell histiocytosis in the oral and maxillofacial region: An update. J Oral Pathol Med. 2021;50(6):565–71.
8. Neves-Silva R, Fernandes DT, Fonseca FP, Rebelo Pontes HA, Brasileiro BF, Santos-Silva AR, et al. Oral manifestations of Langerhans cell histiocytosis: A case series. Spec Care Dent Off Publ Am Assoc Hosp Dent Acad Dent Handicap Am Soc Geriatr Dent. 2018 Nov;38(6):426–33.
9. Lian C, Lu Y, Shen S. Langerhans cell histiocytosis in adults: a case report and review of the literature. Oncotarget. 2016 Mar 3;7(14):18678–83.
10. Goyal G, Tazi A, Go RS, Rech KL, Picarsic JL, Vassallo R, et al. International expert consensus recommendations for the diagnosis and treatment of Langerhans cell histiocytosis in adults. Blood. 2022 Apr 28;139(17):2601–21.
11. Munkhdelger J, Vatanasapt P, Pientong C, Keelawat S, Bychkov A. Epstein-Barr Virus-Associated Langerhans Cell Histiocytosis of the Thyroid Gland. Head and Neck Pathol. 2021 Sep;15(3):1054–8.
12. Emile JF, Abla O, Fraitag S, Horne A, Haroche J, Donadieu J, et al. Revised classification of histiocytoses and neoplasms of the macrophage-dendritic cell lineages. Blood. 2016 Jun 2;127(22):2672–81.
13. Jezierska M, Stefanowicz J, Romanowicz G, Kosiak W, Lange M. Langerhans cell histiocytosis in children – a disease with many faces. Recent advances in pathogenesis, diagnostic examinations and treatment. Adv Dermatol Allergol Dermatol Alergol. 2018;35(1):6–17.
14. Safaei A, Bagheri M, Shahryari J, Noori S, Esmailzade E. Langerhans Cell Histiocytosis Followed by Hodgkin Lymphoma: A Case Report. Iran J Med Sci. 2015 May;40(3):282–6.
15. Ji W, Ladner J, Rambie A, Boyer K. Multisystem Langerhans Cell Histiocytosis in an infant. Radiol Case Rep. 2021 May 6;16(7):1798.
16. Aruna DR, Pushpalatha G, Galgali S, Prashanthy. Langerhans cell histiocytosis. J Indian Soc Periodontol. 2011 Sep;15(3):276.
17. Lavaee F, Nazhvani AD, Afshari A. A case report of adult Langerhans cell histiocytosis and review of the literature. Clin Case Rep. 2023 Feb 10;11(2):e6927.
18. Kamal AF, Luthfi APWY. Diagnosis and treatment of Langerhans Cell Histiocytosis with bone lesion in pediatric patient: A case report. Ann Med Surg. 2019 Sep;45:102–9.
19. Tazi A, Lorillon G, Haroche J, Neel A, Dominique S, Aouba A, et al. Vinblastine chemotherapy in adult patients with langerhans cell histiocytosis: a multicenter retrospective study. Orphanet J Rare Dis. 2017 May 22;12(1):95.
20. Cantu MA, Lupo PJ, Bilgi M, Hicks MJ, Allen CE, McClain KL. Optimal Therapy for Adults with Langerhans Cell Histiocytosis Bone Lesions. PLOS ONE. 2012 Aug 15;7(8):e43257.