

THE CONCENTRATION OF IL-1 β IN SALIVA OF CHILDREN WITH ORAL LESIONS ASSOCIATED TO HISTIOCYTOSIS

Carolina Benchuya¹, Verónica Paván¹, Ariel Gualtieri², Virginia Fernández de Preliasco¹

¹ Department of Comprehensive Children's Dentistry.

² Department of Biophysics, School of Dentistry, University of Buenos Aires, Buenos Aires, Argentina

ABSTRACT

Langerhans Cell Histiocytosis (LCH) is a disease whose etiology and pathogenesis are still unknown. It affects several organs and tissues, producing lesions of different severity. Its histopathology and clinical picture suggest the participation of cytokines in its pathogenesis. IL-1 β might have an important role in its development.

The purpose of this study was to determine the concentrations of IL-1 β in saliva of pediatric patients diagnosed with LCH, with and without oral manifestations (Groups 1 and 2 respectively) compared to a Control Group (Group 3) of pediatric patients without medical antecedents or oral lesions. The saliva of twenty patients with LCH was studied and compared to a Control Group consisting of eleven pediatric patients without medical antecedents. The children with histiocytosis, aged four months to sixteen years, were referred by the Oncohaematology Service at Garrahan Hospital and

Hospital de Clínicas, to the Department of Comprehensive Children's Dentistry, School of Dentistry, University of Buenos Aires (UBA).

The concentrations of IL-1 β in the different groups were determined using the Enzyme Immune Assay Kit (Cayman MI, USA) and expressed in pg/ml.

Results were analyzed by the Kruskal Wallis test. Significant differences between the three cohorts were found, ($H = 20.36$, $P < 0.001$). Dunn's multiple comparison analysis was performed, which showed significant differences between Groups 1 and 2, and between Groups 1 and 3 ($P < 0.05$). Higher values of IL-1 β were found in the patients with histiocytosis with oral manifestations (Group 1) than in patients without manifestations (Group 2) and patients in the Control Group (Group 3).

Key words: Saliva, Histiocytosis, Langerhans cells, Interleukin, Ibeta, mouth disease, child.

CONCENTRACION DE IL-1 β EN SALIVA DE NIÑOS CON LESIONES BUCALES ASOCIADAS A HISTIOCITOSIS

RESUMEN

La Histiocitosis de células de Langerhans (HCL) es una enfermedad de etiología y patogenia aún desconocidas. Afecta diferentes órganos y tejidos en los que produce lesiones de distinta gravedad. La histopatología de las lesiones y la clínica sugieren la participación de citoquinas en su patogenia. La IL-1 β podría tener un rol importante en el desarrollo de la enfermedad.

El objetivo de este estudio fue determinar las concentraciones de IL-1 β de las salivas de pacientes pediátricos con diagnóstico de Histiocitosis de Célula de Langerhans con y sin manifestaciones bucales (grupos 1 y 2 respectivamente), en relación a un grupo control (grupo 3), de pacientes pediátricos que no presentaron antecedentes médicos ni lesiones bucales.

Fueron estudiadas las salivas de 20 pacientes con la enfermedad de HCL, en relación a un grupo control de 11 pacientes pediátricos que no presentaron antecedentes médicos. Los niños con Histiocitosis cuyas edades oscilaban entre 4 meses y 16 años fueron derivados del servicio de Oncohematología del

Hospital Garrahan y Hospital de Clínicas, a la Cátedra de Odontología Integral Niños de la Facultad de Odontología de la Universidad de Buenos Aires.

Se determinaron las concentraciones de IL-1 β en los diferentes grupos, y se utilizó el Enzyme Immune Assay Kit (Cayman, MI, USA), se expresó en pg/ml.

El análisis de los resultados se realizó según el test de Kruskal Wallis, se obtuvieron diferencias significativas entre los tres grupos ($H = 20,36$; $P < 0,001$). Luego se realizó el análisis de comparaciones múltiples de Dunn que mostró diferencias estadísticamente significativas entre los grupos 1 y 2 y entre los grupos 1 y 3 ($p < 0,05$). Se observaron valores más elevados de IL-1 β en los pacientes con Histiocitosis con manifestaciones bucales (grupo 1), en relación con el grupo sin manifestaciones bucales (grupo 2) y con el grupo control (grupo 3).

Palabras clave: saliva, Histiocitosis, células de Langerhans, interleuquina Ibeta, lesiones bucales-niños.

INTRODUCTION

Langerhans Cell Histiocytosis (LCH) is a disease whose etiology and pathogenesis are still unknown. It is characterized by a proliferation of histiocytes,

lymphocytes and eosinophilic cells that form clusters producing several types of lesions^{1,2}.

Different organs and tissues are affected, mainly skin, bones, lungs and liver. Bone tissue is affected

in 72% of pediatric patients³. The most compromised parts are cranium (27%), femur (13%), jaws (11%), pelvis (10%), vertebral bodies (8%), ribs (8%), humerus (5%), and tibia (3%)^{4,5}. Its presentation may vary from a single bone lytic lesion with possible self-resolution without therapeutic intervention, to a disseminated form requiring medical treatment, corticoid administration, and/or chemotherapy⁶⁻⁸.

In addition, lesions may appear within the oral cavity. The most frequent are early dental eruption, dental loss, gingivitis, periodontitis, gingival enlargements and gingival bleeding⁹⁻¹¹.

The anatomical-pathological study of histiocytic lesions shows presence of Langerhans cells, which are gigantic multinucleated histiocytes similar to osteoclasts. Electron microscope images show intra-cytoplasmic inclusions: tennis racket shaped granules, called Birbeck granules¹²⁻¹⁴, which are specific to pathological Langerhans cells. Their presence confirms diagnosis of LCH^{15,16}.

The diagnosis is completed through histochemical analysis to mark proteins CD1a and S 100 for these cells⁴. The etiology and pathogenesis of this disease are still unknown; however, there are numerous hypotheses about its origin, including infectious^{17,18}, immunological^{3,19}, genetic²⁰ and oncologic^{13,17,21}.

The immunological theory is one of the most convincing, because histologically the disease shows similarities to diseases of immune-reactive nature, and patients have diminished suppressing lymphocytes (CD8). In addition, the immunological alteration may be regulated by lymphokines and other growth factors that might modify the migration and maturation of Langerhans cells²².

The pathogenesis of LCH is unknown and there is controversy regarding whether it is a reactive or a neoplastic process²³.

LCH is not recognized as a malignancy. Despite the existence of cell cloning, the disease does not spread through the lymphatic system, and no metastasis occurs^{5,24-26}.

After an indeterminate period of time, reactivations (new isolated lesions) appear in most patients. The reactivations may or may not differ from the initial lesion, and may even involve different tissues²⁵.

Despite the unknown origin of LCH, what is known is that many immunological mechanisms are involved in its pathogenesis, with the clinical picture and lesion histopathology suggesting that cytokines may participate in it^{10,27}.

Classification

Lichtenstein (1853) proposed the term *Histiocytosis X* to group three clinical entities:

Eosinophilic granuloma, Hand – Schüller – Christian's disease, and Letterer – Siwe's disease, adding the letter X to them because he did not know their etiology.²⁸

In 1973, Neselof et al. proposed Langerhans cells (LC) as responsible for the disease, which they called Langerhans Cell Histiocytosis (LCH)²⁹.

To systematize the disease according to the organs involved and further treatment, in 1985 the *Histiocyte Society*, an international scientific entity, proposed the present classification^{9,12,30,31}, as follows: Unifocal single-system histiocytosis: involves a single organ or tissue, usually bone or skin, and has a single location (focus). Corresponds to eosinophilic granuloma in the former classification.

Multifocal single-system histiocytosis: involves a single organ or tissue, with multiple foci. Corresponds to Hand – Schüller – Christian's disease in the former classification.

Multisystem histiocytosis: involves multiple organs or tissues, with multiple foci. It is acute and usually occurs in infants from birth. Corresponds to Letterer Siwe's disease in the former classification.

The aim of this study was to determine the concentrations of IL-1 β in saliva of pediatric patients diagnosed with Langerhans Cell Histiocytosis, with and without associated oral histiocytosis lesions.

MATERIALS AND METHODS

The sample consisted of a total 31 pediatric patients, who were divided into three groups:

- Group 1: 10 patients with Langerhans Cell Histiocytosis, with lesions in the oral cavity.
- Group 2: 10 patients with Langerhans Cell Histiocytosis without oral lesions.
- Group 3: 11 patients with neither medical antecedents nor oral lesions.

Patients in Groups 1 and 2 were referred by the Oncohaematologic Service, Garrahan Hospital, diagnosed with Langerhans Cell Histiocytosis. Group 3 consisted of healthy pediatric patients who visited the Department of Comprehensive Children's Dentistry, School of Dentistry, University of Buenos Aires (UBA), for dental assistance.

The inclusion criteria for Groups 1 and 2 were confirmed diagnosis of Histiocytosis, having signs –

symptoms compatible with LCH seen by light microscopy, plus confirmation by electron microscopy of presence of Birbeck's granules and positive immuno- tracing with CD1a in the lesion cells (Table 1).

Patients taking any medication were excluded from all three Groups (Table1).

Methodology

1. Parent's informed consent for performing this study was obtained. The study was accepted by The Ethics Committee of the School of Dentistry.
2. Dental files and history of the disease in each child were made.
3. Lesions of the oral mucosa were located and diagnosed through visual examination and palpation. Biopsies were sent to Department of Pathological Anatomy, School of Dentistry, Buenos Aires University.
4. Periapical and panoramic radiographs were taken of each child in order to detect osteolytic lesions.

5. Samples of non-stimulated saliva from the three groups were collected in sterile tubes, covered, and sent to the Department of Pharmacology at the same School in order to establish the concentration of IL-1 β . The samples were stored in a freezer at -80° until they were analyzed.

All the procedures used are specified in the protocol for diagnosis of IL-1 β .

RESULTS

Values for Group 1 (with lesions in the mouth) ranged from 2,625.26 to 749 pg/ml, most of them higher than 1,000 pg/ml.

Values for Group 2 (without oral lesions) ranged from 53.84 to 503.44 pg/ml.

Values for Group 3 (control) ranged from 10 to 496.78 pg/ml.

Medians were 1,309.11 for Group 1; 243.54 for Group 2, and 139.00 for Group 3 (Control) (Table 2).

Table 1: Inclusion criteria.

Groups	Inclusion	Exclusion
1	Signs-symptoms compatible with Langerhans Cells Hystiocytosis through Light microscopy. Confirmation of Birbeck's granules through Electronic microscopy	Ingestion of medicines
2	Positive inmuno tracing with monoclonal CD1a in the lesional cells	
3	With neither medical antecedents nor oral lesions	

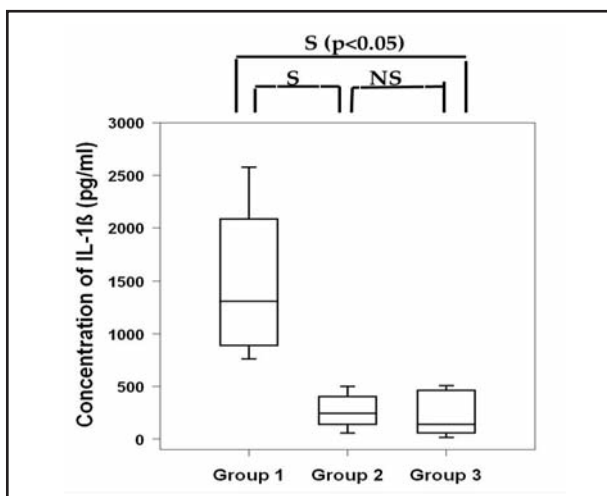


Fig. 1: Dunn's multiple comparisons analysis (graphic). s: significant differences ns: non-significant differences.

Table 2: Concentrations of IL-1 β in saliva collected from the study patients.

Group 1	Group 2	Group 3
2625,26	70,11	493,44
2146,92	503,44	10,00
1866,08	153,44	115,67
749,00	237,89	210,11
869,00	225,67	139,00
2075,67	53,84	496,75
885,67	249,19	32,33
1037,67	391,45	66,78
1168,61	467,19	470,02
1449,60	324,92	171,33
		50,30

The Kruskal Wallis test applied to the results showed significant differences among the three groups ($H = 20.36; P < 0.001$).

Dunn's multiple comparison analysis showed statistically significant differences between Groups 1 and 2, and between Groups 1 and 3 ($p < 0.05$) (Fig. 1). Values of IL-1 β were significantly lower in patients presenting lesions outside the mouth, and with no significant difference compared to Group 3 (Control).

DISCUSSION

For over 40 years, saliva has been considered an auxiliary tool in the diagnosis of oral diseases, because organic molecules of proteinic nature have been detected in its composition, mainly specific antigens, proteinic cell particles – receptors, glycoproteins, cytokines, (interleukines and derivatives), which may be linked to oral lesions^{32,33}.

Serum components can also reach saliva through the gingival crevicular fluid, providing potential application in the diagnosis of certain disorders³⁴. The etiology of Langerhans Cell Histiocytosis has not yet been established; nevertheless, it is accepted that it is the expression of an immunological disorder³⁵.

Similarly, the pathogenesis of LCH is enigmatic, although the altered expression of cytokines and cellular adhesion molecules, which are important for migration and homing of the normal Langerhans cells (LC), may play an important role.³⁶⁻³⁷



Fig. 2: Child, eleven years old, with multi-system LCH. Reactivation two years later: an erosive lesion on mucosa, compatible with an aphtha with more than 30 days of evolution is observed. Concentration of IL-1 β in saliva: 1037.67 pg/ml.

Kannourakis et al. (1994) and Egeler (1999) suggested that cytokines participate in the histopathology of the lesions. It has been observed that the production of cytokines plays an essential role in reactivations of the disease, as well as in the inflammatory and immunological processes^{27,38}. Specifically, the IL-1 β are important in the development and evolution of Langerhans cells; and the Langerhans cells also produce them. Interleukines act on osteoclasts linked to bone resorption^{27,38}.

IL – 1 was found in bone granulomas diagnosed as histiocytosis¹⁰.

However, the regulation of cytokine production is still unknown¹⁰.

In other oral diseases, high cytokine values were found in saliva. Kaushik et al. (2011) found high levels of IL-1 β in saliva of patients with untreated chronic periodontal disease (comparable to those found in this study in children with oral lesions), and those levels decreased significantly after basic periodontal therapy³⁹. Katakura et al. (2007) reported a significant increase in cytokines IL-1 β and IL 16 in serum and saliva of patients with oral cancer compared to the healthy Control Group⁴⁰.

However, no previous study analyzing saliva of patients with histiocytosis was found.

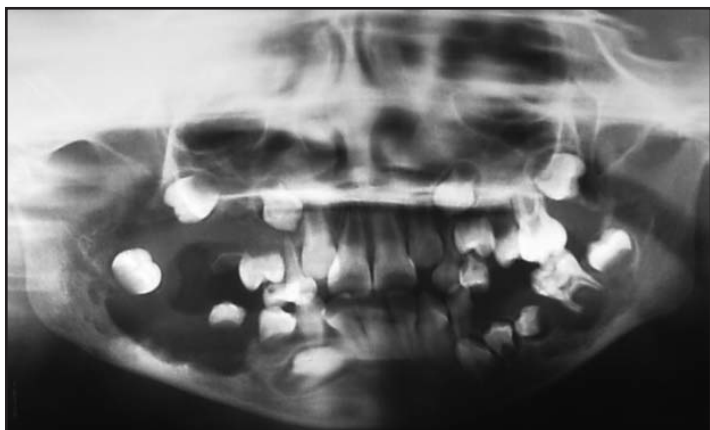
In the present study, among the patients with Langerhans Cell Histiocytosis who showed oral lesions, eight were primary and two were reactivations.

Among the oral manifestations, Hernandez and Juyol et al., found mandibular osteolysis, which can lead to a reduction in mandible height and bone loss linked to inflamed gingiva, looking like “floating teeth”⁴¹. The “floating tooth” is a pathognomonic sign of the disease⁴².

In this study, the lesions in mucosa in Group 1 clinically look like erosions (Fig. 2), and osteolytic lesions of support bone associated to atypical enlargements of oral mucosa were observed (Figs. 3 and 4).

Solitary osteolytic lesions in maxillary bone (Fig. 5), bone reactivations with localized bone loss, similar to periodontal disease (Fig. 6), and bone lesions located in deciduous teeth (“floating tooth”) were observed (Fig. 7).

In this study, open lesions were diagnosed in the oral cavity with inflammatory exudates and closed jaw injuries without any exudation. In all cases, high IL-1 β values were found in saliva.



Figs. 3 and 4: Girl, nine years old, with multi-system LCH. Radiolucent areas compatible with large osteolytic lesions and loss of teeth are observed. Clinically, gingival enlargements are seen. Some teeth presented severe mobility. Concentration of IL-1 β is 2075.67 pg/ml.

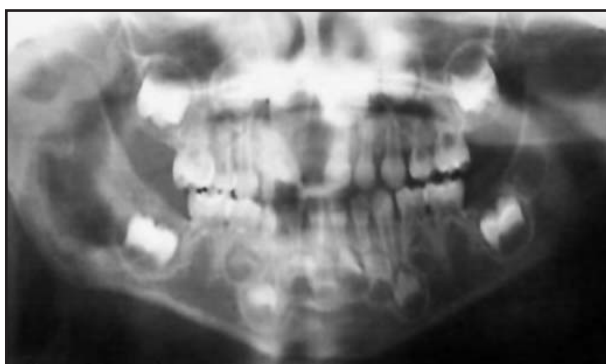


Fig. 5: Panoramic radiograph of a child, four years of age, with single-system unifocal LCH. A single extensive radiolucent area, compatible with an osteolytic lesion, in mandibular ramus. Concentration of IL-1 β : 1866.08 pg/ml.



Fig. 6: Radiograph of child, 13 years old, with multi-system LCH. Tooth 4.5 submerged and a radiolucent area at the apex of the same tooth, compatible with a periodontal lesion are observed. Clinically, it presented a periodontal pocket, 11 mm depth located in 4.5. Concentration of IL-1 β : 1449.60 pg/ml.

Figs. 2 to 7 are clinical examples showing the diversity and aggressiveness of oral lesions associated with LCH.

The panoramic radiograph (orthopantomograph) is the method of choice to study the jaws. It allows observation of the integrity of the cortex, presence of osteolytic lesions in maxilla and mandible that may compromise tooth buds, and comparisons with later radiographs during the follow-up of each patient^{9,10}. The active bony lesions present indefinite borders, and are rounded or elliptic. In contrast, when the lesion is healing, the size and density of the trabecular bone within the lytic areas diminishes, and the thickness of the lesion margin increases, appearing as a radiopaque halo similar to cortex bone⁴³. The high levels of IL-1 β found in saliva of children with histiocytosis lesions in the oral cavity



Fig. 7: Radiograph of child, 2 years of age, with multi-system LCH. A radiolucent area compatible with a localized osteolytic lesion, at support bone of 8.5 "floating tooth" is observed. Concentration of IL-1 β : 2146.92 pg/ml.

allow us to assess or raise the value of saliva as a fluid that could contribute to the diagnosis of LCH. It is concluded that:

- Pediatric patients with Langerhans Cell Histiocytosis with oral manifestations show high values of IL-1 β in saliva, which are higher than those in patients who suffered the disease without oral manifestation, and higher than those in the group of healthy children.

ACKNOWLEDGMENT

This work was supported by a Grant from University of Buenos Aires, UBACyT Program O403

REFERENCES

1. Munn S, Chu AC. Langerhans cell histiocytosis of the skin. *Hematol Oncol Clin North Am* 1998; 12:269-286.
2. Chu T, D'Angio GJ, Favara BE, Ladisch S, Nesbit M, Pritchard J. Histiocytosis syndromes in children. *Lancet* 1987; 2:41-42.
3. Schmitz L, Favara BE. Nosology and pathology of Langerhans cell histiocytosis. *Hematol Oncol Clin North Am* 1998; 12:221-246.
4. Kilpatrick SE, Wenger DE, Gilchrist GS, Shives TC, Wollan PC, Unni KK. Langerhans' cell histiocytosis (histiocytosis X) of bone. A clinicopathologic analysis of 263 pediatric and adult cases. *Cancer* 1995; 76:2471-2484.
5. Huang F, Arceci R. The histiocytosis of infancy. *Semin Perinatol* 1999; 23:319-331.
6. Histiocytosis syndromes in children. Writing Group of the Histiocyte Society. *Lancet* 1987; 1:208-209.
7. Egeler RM, D'Angio GJ. Langerhans cell histiocytosis. *J Pediatr* 1995; 127:1-11.
8. Minkov M, Grois N, Braier J, Rosso D, Aricò M, Broadbent V, Gardner H, Ladisch S, Histiocyte Society. Immunosuppressive treatment for chemotherapy-resistant multisystem Langerhans cell histiocytosis. *Med Pediatr Oncol* 2003; 40:253-256.
9. Fernández de Preliasco V, De la Cal C, Benchuya C, Paván V, Martín A. Actualización en el Diagnóstico de la enfermedad de Histiocitosis de células de Langerhans en niños. *Boletín de la Asociación Argentina de Odontología para Niños* 2007; 36:23-27.
10. Preliasco VF, Benchuya C, Paván V, de la Cal C, Ganzinelli S, Sterin Borda L. IL-1 β and PGE2 levels are increased in the saliva in children with Langerhans cell histiocytosis. *J Oral Pathol Med* 2008; 37:522-527.
11. Fernández de Preliasco V, Benchuya C, Paván V, Lesiones Bucuales asociadas a Histiocitosis de Células de Langerhans. *Revista de la Academia Nacional de Odontología* 2011; 9:12-17.

- A saliva sample, which is a simple, non-invasive procedure, may allow detection of high concentrations of IL-1 β associated to primary or reactivation lesions in the mouths of children with Langerhans Cell Histiocytosis.
- Further studies should be conducted on a larger sample to confirm the association between the oral lesions and the interleukines in saliva from patients with LCH.

CORRESPONDENCE

Dr. Carolina Benchuya
Cátedra de Odontología Integral Niños
Facultad de Odontología
Universidad de Buenos
MT de Alvear 2142 Piso 15
CABA Argentina
carolbenchu@yahoo.com

12. Favara BE, Feller AC, Pauli M, Jaffe ES, Weiss LM, Aricò M, et al. Contemporary classification of histiocytic disorders. The WHO Committee On Histiocytic/Reticulum Cell Proliferations. Reclassification Working Group of the Histiocyte Society. *Med Pediatr Oncol* 1997; 29:157-166.
13. Vernon ML, Fountain L, Krebs HM, Horta-Barbosa L, Fuccillo DA, Server JL. Birbeck granules (Langerhans cell granules) in human lymph nodes. *Am J Clin Pathol* 1973; 60:771-779.
14. Betts DR, Leibundgut KE, Felges A, Plüss HJ, Niggli FK. Cytogenetic abnormalities in Langerhans cell histiocytosis. *Br J Cancer* 1998; 77:552-555.
15. Scappaticci S, Danesino C, Rossi E, Klersy C, Fiori GM, Clementi R, Russotto VS, Bossi G et al. Cytogenetic abnormalities in PHA-stimulated lymphocytes from patients with Langerhans cell histiocytosis. AIEOP-Istiocitosi Group. *Br J Haematol* 2000; 111:258-262.
16. Aricò M, Egeler RM. *Hematol Oncol Clin North Am* 1998; 12:247-258.
17. Fadeel B, Henter JI. Langerhans-cell histiocytosis: neoplasia or unbridled inflammation? *Trends Immunol* 2003; 24:409-410.
18. Leahy MA, Krejci SM, Friednash M, Stockert SS, Wilson H, Huff JC, Weston WL, Brice SL. Human herpes virus 6 is present in lesions of Langerhans cell histiocytosis. *J Invest Dermatol* 1993; 101:642-645.
19. Madrigal-Martínez-Pereda C, Guerrero-Rodríguez V, Guisado-Moya B, Meniz-García C. Langerhans cell histiocytosis: Literature review and descriptive analysis of oral manifestations. *Med Oral Patol Oral Cir Bucal* 2009; 14:E 222-228.
20. Larralde M, Abad ME, Gomar B. Histiocitosis de células de Langerhans en menores de un año. *Arch Argent Pediatr* 2008; 106:269-272.
21. Laman JD, Leenen PJ, Annels NE, Hogendoorn PC, Egeler RM. Langerhans cell histiocytosis 'insight into DC biology'. *Trends Immunol* 2003; 24:190-196.

22. García-Ortega FP, Carcasés Ortiz MJ, Martínez Reig S, Beviá González MC, Durán R, Malluguiza Calvo JR. Langerhans's cell histiocytosis in otorhinolaryngology. *Acta Otorrinolaringol Esp* 2001; 52:351-354.
23. Chirino CN, Schwartz RJ., Musitani O I. Diagnósticos diferenciales de la Histiocitosis a células de Langerhans. *Rev Argent Dermatol* 2007; 88:108-120.
24. Glotzbecker MP, Carpentieri DF, Dormans JP. Langerhans cell histiocytosis: a primary viral infection of bone? Human herpes virus 6 latent protein detected in lymphocytes from tissue of children *J Pediatr Orthop.* 2004; 24:123-129.
25. Braier J, Pollono D, Rey G, Latella A, De Socio S, Tomarchio S, Rosso D, Goldberg J. Reactivations in Langerhans cell histiocytosis. (LCH) Histiocyte Society. 18 th Annual Meeting Porto, Portugal Sept 2002. *Med Pediatr Oncol* 2003; 40:180. (Abstr).
26. Braier J, Chantada G, Rosso D, Bernaldez P, Amaral D, Latella A, Balancini B, Masautis A, Goldberg J. Langerhans cell histiocytosis: retrospective evaluation of 123 patients at a single institution. *Pediatr Hematol Oncol* 1999; 16:377-385.
27. Kannourakis G, Abbas A. The role of cytokines in the pathogenesis of Langerhans cell histiocytosis. *Br J Cancer* 1994; 23:S37-40.
28. Bonet HB, Boente MC, Lavado G, Avila S, Salman J. Histiocitosis X: lesiones óseas craneo-vertebrales y manifestaciones neurológicas. *Arch. argent. Pediatr* 1994;92:80-7. URL:<http://bases.bireme.br/cgi-bin/wxislind.exe/iah/online/?IsisScript=iah/iah.xis&src=google&base=LILACS&lang=p&nextAction=lnk&exprSearch=256538&indexSearch=ID>
29. Nezelof C, Basset F, Rousseau MF. Histiocytosis X histogenetic arguments for a Langerhans cell origin. *Biomedicine.* 1973; 18:365-371.
30. Meyer JS, Harty MP, Mahboubi S, Heyman S, Zimmerman RA, Womer RB, Dormans JP, D'Angio GJ. Langerhans cell histiocytosis: presentation and evolution of radiologic findings with clinical correlation. *Radiographics* 1995; 15: 1135-1146.
31. Svarch E, Arteaga R, Morán, González Otero. Las histiocitosis *Rev. Cubana Hematol Inmunol Hemoter* 2001; 17:151-63. URL: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0864-02892001000300001&lng=es.
32. Taboada Vega M, Chuquihuaccha Granda V. Rol de la saliva como marcador biológico en patología bucal *Odontol. Sanmarquina,* 2006; 9:38-40. URL:<http://revistasinvestigacion.unmsm.edu.pe/index.php/odont/article/view/5353>
33. Aguilar Gonzalez FI, Romero Sanchez M. La saliva: revisión sobre composición, función, usos diagnósticos: primera parte *Univ. Odontol,* 2003; 23:18-24; URL: <http://bases.bireme.br/cgi-bin/wxislind.exe/iah/online/?IsisScript=iah/iah.xis&src=google&base=LILACS&lang=p&nextAction=lnk&exprSearch=348898&indexSearch=ID>
34. Haeckel R, Hänecke P. The application of saliva, sweat and tear fluid for diagnostic purposes. *Ann Biol Clin.* 1993; 51:903-910.
35. Axiotis CA, Merino MJ, Duray PH. Langerhans cell histiocytosis of the female genital tract. *Cancer.* 1991; 67:1650-1660.
36. Geissmann F, Lepelletier Y, Fraitag S, Valladeau J, Bodemer C, Debré M, Leborgne M, Saeland S. Differentiation of Langerhans cells in Langerhans cell histiocytosis. *Blood,* 2001; 97:1241-1248.
37. Arenzana-Seisdedos F, Barbey S, Virelizier JL, Kornprobst M, Nezelof C. Histiocytosis X: purified (T6 1) cells from bone granuloma produce interleukin 1 and prostaglandin E2 in culture. *J Clin Invest.* 1986; 77:326-329.
38. Egeler, R M, Favara B, van Meurs M, Laman, J & Classen E. Differential in situ cytokine profiles of Langerhans-like cells and T cells in Langerhans cell histiocytosis: abundant expression of cytokines relevant to disease and treatment. *Blood,* 1999; 94:4195-4201.
39. Kaushik R, Yeltiwar RK, Pushpanshu K. Salivary Interleukin-1 β Levels in Patients With Chronic Periodontitis Before and After Periodontal Phase I Therapy and Healthy Controls: A Case-Control Study *J Periodontol.* 2011; 82:1353-1359.
40. Katakura A, Kamiyama I, Takano N, Shibahara T, Muramatsu T, Ishihara K, Takagi R, Shouno T. Comparison of Salivary Cytokines levels in Oral Cancer Patients and Healthy Subjects. *Bull Tokyo Dent Coll* 2007; 48:199-203.
41. Hernández Juyol M, Boj Quesada JR, Gallego Melcon S. Oral manifestations of Langerhans cell histiocytosis. Case study of a two-year-old boy. *Med Oral* 2003; 8:19-25.
42. Rios C, Gonzalez Diaz M, Bello Rodriguez R, Torrens de la Nuez R. Granulomatosis de células de Langerhans. Presentación de un caso. *Rev. Cubana Estomatológica* 1998; 35:124-6. URL: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0034-75071998000300010&lng=es.
43. Dagenis M; Pharoach MJ; Sikorski PA. The Radiographic characteristics of histiocitosis X. A study of 29 cases that involve the jaws. *Oral Surg Med Oral Pathol.* 1992;74: 230-236.