A Comparative Study of Clinicopathological and Immunohistochemical Expression of CD1a, RANK and RANKL in Langerhans Cell Histiocytosis of Jaw and Skull Lesions

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ABSTRACT

Background: Langerhans' cell histiocytosis (LCH) is a group of conditions affecting the reticuloendothelial system. It includes Letterer-Siwe disease, Hand-Schuller-Christian disease and eosinophilic granuloma and most often presents in childhood.

Materials and methods: Twenty-five cases of LCH were diagnosed histologically and confirmed by CD1a antibody and assessed immunohistochemically using anti-RANKL and anti-RANK antibodies to evaluate osteoclastogenic mechanism.

Results: Regarding jaw cases, there was a significant correlation between CD1a and RANK (P=0.016). While in the skull, highly significant correlation existed between RANK and RANKL (p=0.001). Among the sites, there was no statistically significant difference found for each the immunohistochemical markers used.

Conclusion: LCH of jaws and skull bear similar osteoclastogenic mechanism when quantified with RANK and RANKL immunostaining respectively. With a significant correlation between CD1a and RANK for jaw cases, while in the skull lesions, there was a high significant correlation between RANK and RANKL.

Keywords: Langerhans cell histiocytosis (LCH), receptor activator of nuclear factor kappa-B ligand(RANKL), receptor activator of nuclear factor kappa-B (RANK),cluster of differentiation(CD1a), Langerhans cells(LCs). (J Bagh Coll Dentistry 2016; 28(1):78-83).

INTRODUCTION

Langerhans cell histiocytosis (LCH) is a clonal proliferation of Langerhans cells (LCs) occurring as an isolated lesion or as a part of systemic (multifocal) proliferation. It affects children as well as adults, presenting with a heterogeneous clinical picture ranging from involvement of a single organ system, primarily skin or bone, to multiple organ systems complicated by organ dysfunction [1]. Radiologically, LCH is characterized by destructive osteolytic lesion, edges of which may be beveled, scalloped or confluent (geographic), or show a "button sequestrum" [2].

CD1a, is a specific marker for LCs, it used in the histological comparison of jaw and skull lesions of LCH. RANKL is a potent osteoclastogenic factor that, exists as a type II homotrimeric protein and is expressed as a membrane-bound protein on the surface of osteoblasts, osteocytes and marrow stromal cells. In addition, activated T cells secrete RANKL as a soluble molecule. RANKL binds to its receptor RANK, present at the surface of osteoclast precursors and mature osteoclasts, inducing osteoclast formation and activation [3].

Studies concerning immunehistochemical expression of RANK and RANKL as markers for osteoclastogenesis of bone in LCH are very limited for this reason the present research is aiming to assess histological behavior difference of LCH in the craniofacial region in relation to RANK and RANKL based on CD1a labeling index.

MATERIALS AND METHODS

25 LCH specimens, including 13 cases in the jaws, 9 were in the mandible, 4 in the maxilla, 1 case was in both jaws and 11cases were in the skull. Monoclonal mouse anti-human CD1a [7A7 abcam] antibody was used to confirm the diagnosis. Monoclonal mouse anti-RANK antibody [64C1385 abcam] and Monoclonal mouse anti-RANKL antibody [12A668 abcam] used to assess the osteoclastogenic mechanism.

Immunohistochemistry: Paraffin sections were reacted with CD1a (1:1000), RANKL (1:115), and RANK antibodies (1:100) dilution. To evaluate RANKL, RANK, and CD1a staining, tumor cells exhibiting positive staining on cell membranes and in cytoplasm were counted in at least 5 representative fields (400Xmagnification) and the mean percentage of positive tumor cells was calculated.

CD1a: Labelling index (LI) = (number of positive cells/1,000) × 100. Labeling index of

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those fields were considered to be the labeling index for the case [4].

RANKL: 0 (≤10%), 1(11–50%), 2 (51–75%) and 3 (>75%) immunostained cells [5].
RANK: Cases in which the proportion of positive cancer cells was (≥50%) were positive, and those containing (<50%) positive cells were negative [6].

RESULTS

Clinical Description

The age range of the patients was between 2.5-50 years with the mean of (23.61±12.17) in the jaw bones. While in the skull, was between 2-35 years with the mean of (11.32±10.28), there was a high significant difference according to age between the two sites. According to gender distribution, the jaw cases comprising 12 males, and 2 females, to give a total male/female ratio of (6:1). While the skull cases comprising 6 males, and 5 females to give (1.2:1), there was no significant difference according to genders between the two sites (table 1).

Histopathological Findings

Histologic examination of HandE stained slides showed numerous histiocytic cells. These histiocytes were large cells with elongated, irregular nuclei, prominent nuclear grooves, giving them typical "coffee been" appearance having a moderate amount of homogeneous, pink, granular cytoplasm and distinct cell margins. The background showed lymphocytes, giant cells and a variable numbers of eosinophils. Mitotic figures, were observed in 8 of total 14 cases of LCH in jaws and in 4 of total 11cases in skull lesions. Spearman's correlation showed that there was no significant correlation between CD1a, RANK, RANKL and Mitoses in each group (Table 2).

Immunohistochemical Findings:

CD1a immunoreactivity was recognized in all 25 cases of LCH. In the jaws, the mean of labeling index was (37.21±19.60), while in the skull(38.64±17.33). Comparatively, using Mann-Whitney U test, there was no statistically significant difference of the expression of CD1a between the two sites of LCH (table 3).

In the skull, all cases were positive for RANKL, with the mean (56.36±14.85).While in the jaws, 13cases was positive, with the mean (51.79±21.45).Comparatively, there was no statistically significant difference of RANKL expression between the two sites (table 4).

In the jaws, 10 cases of LCH were positive for RANK, with the mean (60±16.98).While in the skull, 10 cases were positive, with the mean (67.27±10.34). Comparatively, there was no statistically significant difference between RANK positivity between the two sites (table 5).

Correlations among Immunohistochemical Markers

Using Spearman's correlation; for the jaws, there was a significant correlation between CD1a with RANK (P=0.016). While in the skull, a high significant correlation between RANK with RANKL (p=0.001) as shown in table (6).

Table 1: Frequency Distribution and Percentage of Age and Genders

Age group	Jaws	Skull	Genders	
Frequency	Frequency	Frequency	Males	Females
<10	1	6	12	6
10-19	5	4	2	5
20-29	5	0	Male/Female ratio	
30-39	1	1	6:01	1.2:1
40-49	1	-	X^2	2.968
50-	1	-	Continuity correction	1.624
			d.f.	1
			p-value	0.203 (NS)
No. of cases	Jaws	Skull	Total	
	14	11	25	
Mean	23.61	11.32	18.2	
S.D.	12.17	10.28	12.77	
Min.	2.5	2	2	
Max.	50	35	50	
Mann-Whitney U test	-2.606			
p-value	0.009 **			

Table 2: Relation between the Variables in CD1a, RANKL and RANK in the Jaws and Skull

Markers	Site	% of cells	No. of mitoses
CD1a	Jaws	r	-0.44
		p-value	0.11 (NS)
	Skull	r	-0.11
		p-value	0.74 (NS)
RANKL	Jaws	r	-0.11
		p-value	0.72 (NS)
	Skull	r	-0.03
		p-value	0.94 (NS)
RANK	Jaws	r	0.36
		p-value	0.21 (NS)
	Skull	r	0
		p-value	1 (NS)

Table 3: Descriptive Statistics and Site Comparison in CD1a Marker

Variables	Site	Descriptive Statistics			Site Comparison		
		Mean	S.D.	S.E.	Mann- Whitney U test	p-value	Sig.
% of cells for CD1a	Jaw	37.21	19.60	5.24	-0.44	0.66	NS
	Skull	38.64	17.33	5.23			

Table 4: Descriptive Statistics and Site Comparison in RANKL Marker

Variables	Site	Descriptive Statistics			Site Comparison		
		Mean	S.D.	S.E.	Mann- Whitney U test	p-value	Sig.
% of cells for RANKL	Jaw	51.79	21.45	5.73	-0.33	0.741	NS
	Skull	56.36	14.85	4.48			

Table 5: Descriptive Statistics and Site Comparison in RANK Marker

Variables	Site	Descriptive Statistics			Site Comparison		
		Mean	S.D.	S.E.	Mann- Whitney U test	p-value	Sig.
% of cells for RANK	Jaw	60	16.98	4.54	-0.81	0.419	NS
	Skull	67.27	10.34	3.12			

Table 6: Correlations among Immunohistochemical Markers

		Jaws		Skull	
Variables		RANK	RANKL	RANK	RANKL
CD1a	r	0.627	0.056	-0.121	-0.222
	p-value	0.016 (S)	0.850 (NS)	0.723 (NS)	0.512 (NS)
RANK	r		0.070		0.852
	p-value		0.811 (NS)		0.001 (HS)

DISCUSSION

Langerhans cell histiocytosis is by far the commonest of the histiocytoses, is one of the rarest bone tumors representing less than 1% of them. Bone involvement is seen in 80-100% of LCH patients. In this study, there was a male predominance, these findings were in agreement with other studies [7,8], where all showed a slight to pronounced male predominance, while disagrees with others [9,10], all demonstrated that females and males were equally affected, with a female predominance in pulmonary (LCH) cases, with approximately (5:3) ratio.

In this study, the mean age of all cases was lower than those demonstrated by previous researchers [11,12], who described a mean age above 30 years. This study showed that there was no significant correlation in the expression values of CD1a, RANK, RANKL and mitotic figures between jaws and skull lesions. These findings support that the LCH is a locally infiltrative neoplasm with frequent pleomorphism and no abnormal mitotic figures

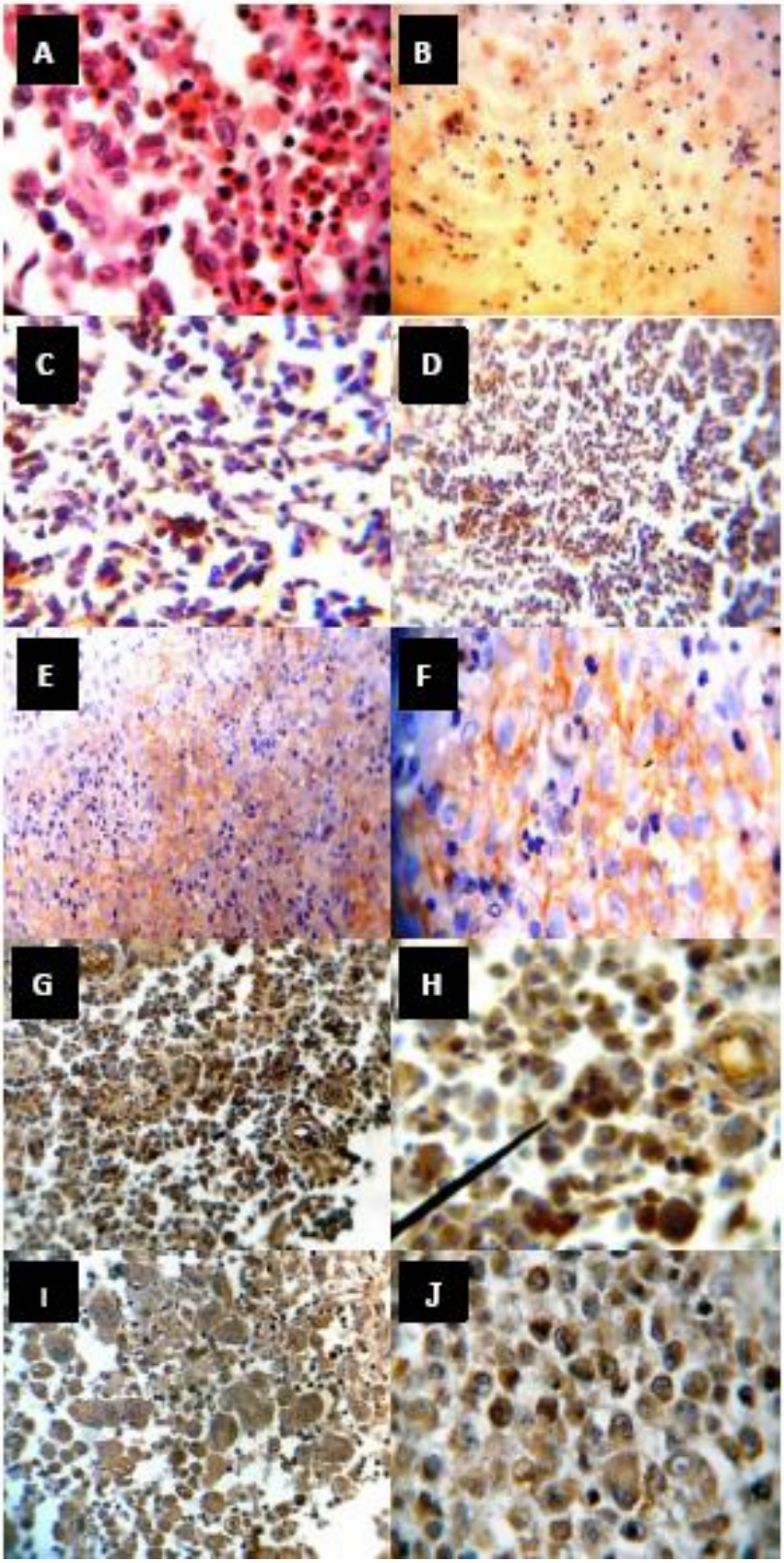

Figure 1: A: H&E Stained Photomicrograph of a Jaw LCH (40x). B: Positive Control ''Normal Brain Tissues'' for CD1a. C&D: Positive Control ''Tonsillar Hyperplesia'' for RANKL&RANK. E&F: CD1a Stain (40x objective) Demonstrating Positive Membrane Staining of Lesional Histiocytes in a Skull LCH (40x&10x).G&H: Positive Cytoplasmic Immunostaining to RANKL Antibody in a Jaw LCH(40x&10x), Pointer Showed a Mitotic Figure. I&J: Positive Cytoplasmic Immunostaining to RANK Antibody in a Skull LCH (40x&10x).

pleomorphism and no abnormal mitotic figures were seen. This results in accordance with ***Bank et al.,*** [13] who observed mitotic figures in 34 of 61 evaluated specimens based upon Ki-67 expression, the presence of mitotic figures indicate that local proliferation contributes to the accumulation of LCs, and a level of Ki-67 expression was lower than that of neoplastic tissue. RANKL expression in this study indicated that the tumor cells acts as a source of this osteoclastogenic factor. This consistently seen in jaw and skull lesions of LCH, which may indicate that RANKL- producing tumor cells had the potential to induce osteoclastogensis that account to aggressive behavior and recurrence of LCH in both sites.

Egeler et al., [14] showed that the environment in which the mononuclear cells are present determines their differentiation into the various mononuclear phagocyte system-derived cells. In this study, there was a statistically significant relationship between CD1a and RANK, concerning jaw bones alone, that means the expression of RANK would be increased with increased number of tumor cells.

Specifically CD1a+ve LCs which led to local infiltrative activity of LCH lesions and bone resorption. *Egeler et al.,* [14] had extended the analysis of cytokines to those specifically involved in the induction of osteoclast differentiation. In 24 LCH lesions studied for RANKL expression, 17 were found to be positive.

The majority of CD1a + LCs expressed RANKL. Thus, both the CD1a+ LCH cells and T cells contribute to osteoclast-togenesis through up-regulated RANKL, thus, provide a mechanism for the potentiation of osteoclast formation and bone resorption in LCH lesions. In this study, the expression of RANK, in the tumor cells of both sites indicate that it play a role in the local bone resorption. Similarly, there was a statistically significant relationship between RANK and RANKL, concerning skull bones alone, which indicated that RANK and its ligand had play a role in osteoclastognesis process of LCH lesions.

Egeler et al., [14] showed that the one key feature of osteoclast differentiation is the interaction between RANKL and its receptor. The expression of RANK by CD1a+ cells as well as the presence of its ligand by activated T cells in LCH lesions is also important, as this interaction is known to induce a survival signal to dendritic cells (DCs) [15]. *Senechal et al.,* [16] found that LCs from LCH granulomas expressed RANK and RANKL [17]. The present study improves the way of understanding the mechanism of osteoclast activation in LCH of the jaws and skull.

In summery, Langerhans cell histiocytosis is a locally destructive neoplasm with similar biological behaviour in the jaws and the skull, according to immunohistochemical expression of the studied markers which showed a positive correlation between CD1a and RANK in the jaws, RANK and RANKL in the skull, indicated that RANK and RANKL contributed to the osteoclastogenesis process of LCH in both sites.

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