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Original Article

Comparison of expression of CD1a and CD68 markers in skin leishmaniasis samples with positive and negative Leishman body

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Abstract: Background: Leishmaniasis is one of the most important infectious illnesses around the world. Given the high commonness of this disease, specifically its skin type in Iran and due to the role of the Leishman bodies in diagnosis, the aim of present study was evaluating the expression of two CD1a and CD68 markers in cutaneous leishmaniasis lesions with and without Leishman bodies. Methods: In this case-control study, 70 skin samples of patients with cutaneous leishmaniasis (35 patients with Leishman body as case group and 35 patients without Leishman boy as control group) were investigated during 2018-2019. The expression of CD1a and CD68 markers and immunohistochemistry staining (IHC) were investigated in this study. Results: The expression of CD1a in the group with Leishman body was significantly higher than group without Leishman body (P=0.01), but there was no significant difference between groups as expression of CD68 (P=0.40). The frequency of hyperkeratosis, parakeratosis, exocytosis, acanthosis, spongiosis, hydropic degeneration of basal cell layer, lichenoid reaction, pseudoepitheliomatous hyperplasia, ulcer, thinning of the epidermis, mononuclear cells, and extension of inflammation into lower dermis in the group with Leishman body was higher than group without Leishman body (P<0.05). Conclusion: The expression of CD1a and other morphological findings help to diagnose the difference between leishmaniasis with and without Leishman body.

Keywords: CD1a, CD68, leishmaniasis, immunohistochemistry staining, morphological

Introduction

Leishmaniasis is one of the six most remarkable infectious diseases around the world. Almost 350 million people are exposed to leishmaniasis and every 2 million new cases are being added to it continuously [1-3]. Clinical types observed in Iran include dry-type cutaneous leishmaniasis [4], wet type cutaneous leishmaniasis [5], Lymphadenitis [6] and visceral leishmaniasis [7]. In histopathology of cutaneous leishmaniasis, which is the most prevalent form of skin involvement, the dense and diffuse infiltration of histiocytic with lymphocytes and low plasma cells can be seen. Eosinophils and neutrophils are very low. The cytoplasm of the histiocytic is full of Lyman bodies and when these bodies are abundant, they can also be seen out of the cells [8-10]. In several studies, importance of the immunohistochemistry of inflammatory cells in the cutaneous lesions of different species of leishmaniasis were investigated for diagnosis of cutaneous leishmaniasis [11, 12]. Based on these studies, it has been found that cellular immunity has an important role in the pathogenesis of cutaneous leishmaniasis. T-lymphocytes are the major factor in lymphocytic infiltration and the control of parasite replication in the cutaneous leishmaniasis [13-16].

It has been observed that the molecules of CD1, group one, including human CD1a, CD1b and CD1c, lipid-mycolipids and glycolipids to specialized T-cells. This can assuredly help antimicrobial resistance with the production of interferon-gamma (IFN-γ) [17-19] and the antimicrobial protein of granulation [20, 21].

Therefore, CD1 group I protein probably participates in the protected immune response against complex intracellular pathogens such as Leishman species known as glycolipid antigens. On the other hand, the expression of CD1a, CD1b, CD1c, and CD1d has been seen more in CD68 + cells and these cells can present external antigens to T cells, while the normal arterial samples do not express CD1 molecules [19, 22]. In fact, leishmaniasis parasites interact with the types of host T cells and infect them; CD68 + macrophages and dendritic cell 1a (DC1a) are the most important cells that adjust the outcome of infection. After the first absorption of amastigotes by macrophages into phagosome, subsequent fusion will happen with the lysosome, and parasites must stay alive in this environment. This is probably one of the most controversial environments for many diseases, and the leishmaniasis is one the protozoa that can survive and reproduce under difficult circumstances. Knowing how these organisms can survive and manipulate host cells for the purpose of replication and transfer is quite critical to the design of new drugs or treatment strategies against the disease [23, 24]. In this regard, Taheri and colleagues in their study (2017) showed that chronic cutaneous leishmaniasis had a significant relationship with macrophage CD68 + and dendritic CD1a + cells and macrophage + CD68 and CD1A + increases the duration of chronic leishmaniasis [25]. Correlation of CD1a and CD68 in patients with leishmaniasis was evaluated in the previous studies and indicated expression of these markers could be increase in the chronic leishmaniasis [25-28]. However, in the present study, the difference between leishmaniasis with and without Leishman body was compared for first one in the Iran. Therefore, due to the high prevalence of this disease, the role of the Leishman bodies in diagnosis, considering the assumption that the samples of skin leishmaniasis in the case with or without the Leishman body are similar in immunohistochemistry staining (IHC) staining and have no difference with each other, so it can be utilized instead of polymerase chain reaction (PCR), and in addition to the lack of study in this field, this study examined expression of two CD1a and CD68 markers in cutaneous leishmaniasis lesions with and without Leishman bodies.

Materials and methods

In case-control group, 70 skin samples of patients with cutaneous leishmaniasis were entered in study between 2018-2019. The current study was approved in ethical committee of Isfahan University of Medical Sciences (IR.MUI.MED.REC.1398.176) and all patients signed the informed consent to for participation in the study.

Among 70 patients, including 35 cases with Leishman body (seen in their pathology culture in paraffin blocks) were considered case group and 35 cases without Leishman body (not seen in their pathology culture but PCR was confirmed leishmaniasis) were considered control group. Therefore, the present study was conducted on two groups of patients with cutaneous leishmaniasis with and without Leishman body in their skin samples pathology. All patients were selected in Isfahan, Iran, and demographic information of patients including results, age, sex, location, and duration of were collected.

The treatment of the patients with lesions were based on age, duration of disease, stage of disease, and place of lesion, that dermatologist recommended different treatment including [29] cryotherapy, infiltration of sodium stibogluconate at 0.3-0.8 mL, local heat therapy at 40-42°C, and topical paromomycin.

Histopathology changes

The paraffin block cuts of the biopsy, which were fixed with formalin 10% (neutral buffered formalin), were stained with Hematoxylin and eosin and investigated by light microscopy. In addition to topographic descriptions, epidermis and dermis changes were recorded. Epidermis changes may include hyperkeratosis, Parakeratosis, exocytosis, acanthosis, spongiosis, and hydropic degeneration of basal cell layer, lichenoid reaction, pseudoepitheliomatous hyperplasia, psoriasiform epidermal hyperplasia, Ulcer, and thinning of the epidermis. Dermal changes involved severity and extension of inflammation (upper, middle and lower of derma) as well as the type and amount of inflammatory cells including lymphocyte, plasma cell, histiocyte, neutrophils, and also presence or absence of granuloma, necrosis, fibrosis and abscess.

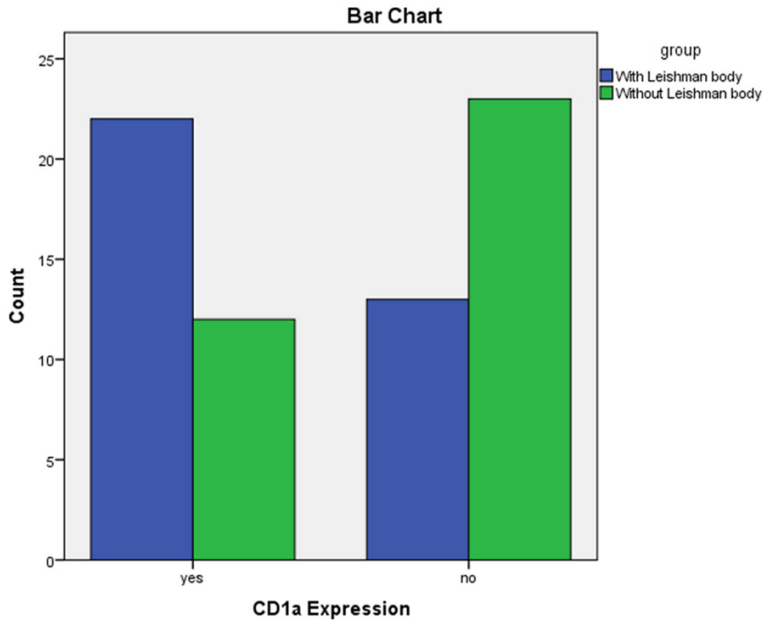

Figure 1. CD1a expression based on groups as with or without Leishman body (Independent T test).

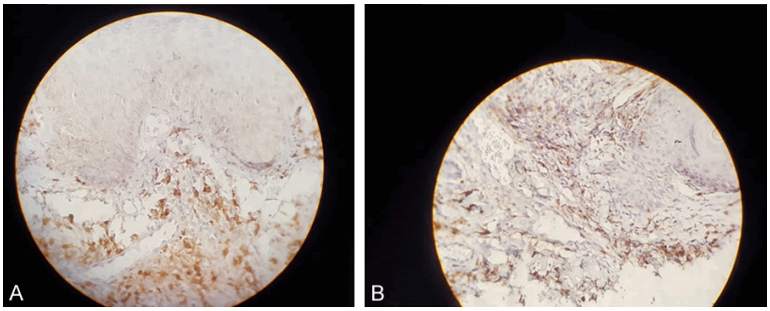

Figure 2. Microscopic findings of CD1a and CD68 in immune cells, A: CD1a in dendritic cells (×40), B: CD68 in macrophage (×40).

Immunohistochemistry changes

IHC, histologic 3-micron slices were put on the special Silanization and paraffin troule was performed with xylenol, ethanol and water. Antigen Retrieval was performed with (pH=9) Tris-EDTA buffer and microwave heat during 10 minutes. The activity of peroxidase with 0.5% methanol solution and H_2O_2 block were performed and incubated with key special antibodies in certain concentration of wet environment and room temperature during 30 minutes.

The characteristics of used monoclonal antibodies were CD1a (DAKO M3571), dilution factor {df}: 1/50 and CD68 (DAKO M0814, df: 1/100). The EnVision was performed to solution that was contains secondary antibody bonded to biotin and streptavidin bonded to peroxidase. DAB chromogene was used to determine the anti-antibody bands and the fields were stained with Hematoxylin (**Figure 2**).

All in all, the collected data was analyzed using Statistical Package for Social Sciences (SPSS) software version 24 (IBM, USA), and data were shown based on mean and standard deviation or frequency and percentage. Also Fisher exact test, chi-square. the independent T-Test and Pearson correlation coefficient were used to compare variables between two groups. The significance level was considered less than 0.05.

Results

Demographical

Patients were categorized into two groups as leishmaniasis with (24 male and 11 female) and without (27 male and 8 female) Leishman body; there was no significant differences between groups in terms of age, gender, region of lesions, and duration of disease (P>0.05).

Expression of markers

The expression of CD1a in the group with Leishman body was significantly higher than group without Leishman body (P=0.01), but there was no significant difference between groups in terms of the expression of CD68 (P=0.40) (**Figure 1**; **Table 1**).

Morphological characteristics

According to morphological characteristics, the frequency of hyperkeratosis, parakeratosis, exocytosis, acanthosis, spongiosis, hydropic

Table 1. Variables of study between two groups

Variables		With Leishman body	Without Leishman body	*P*-value
Age (year) f (mean ± SD)		29.48±10.51	32.74±10.58	0.20
Gender (m/f)		24/11	27/8	0.29
Region	Lower limb	20 (57.1%)	24 (68.6%)	0.40
	Upper limb	12 (34.3%)	7 (20%)	
	Others	3 (8.6%)	4 (11.4%)	
Duration of disease [33] (mean ± SD)		15.51±4.13	15.20±3.04	0.71
Expression of CD1a		22 (62.9%)	12 (34.3%)	0.01
Expression of CD68		19 (54.3%)	17 (48.6%)	0.40

Table 2. Morphological characteristics of lesions in the groups with and without Leishman body

Morphological characteristics			With Leishman body	Without Leishman body	*P*-value*
Hyperkeratosis			33 (94.3%)	15 (42.9%)	<0.001
Parakeratosis			26 (74.3%)	6 (17.1%)	<0.001
Exocytosis			24 (68.6%)	11 (31.4%)	0.002
Acanthosis			22 (62.9 %)	10 (28.6%)	0.004
Spongiosis			26 (74.3%)	8 (22.9%)	<0.001
hydropic degeneration of basal cell layer			34 (97.1%)	13 (37.1%)	<0.001
lichenoid reaction			30 (85.7%)	19 (54.3%)	0.004
pseudoepitheliomatous hyperplasia			28 (80%)	8 (22.9%)	<0.001
Psoriasiform epidermal hyperplasia			18 (51.4%)	13 (37.1%)	0.16
Ulcer			35 (100%)	3 (8.6%)	<0.001
Thinning of the epidermis			35 (100%)	13 (37.1%)	<0.001
Epithelial cell	PMN	<10	11 (31.4%)	6 (17.1%)	0.13
		Absence	24 (68.6%)	29 (82.9%)	
	Eosinophils	<10	6 (17.1%)	3 (8.6%)	0.23
		Absence	29 (82.9%)	32 (91.4%)	
	Mononuclear	>10	28 (80%)	1 (2.9%)	<0.001
		<10	7 (20%)	20 (57.1%)	
		Absence	0	14 (40%)	
The extent of the inflammation	Upper of dermis		1 (2.9%)	18 (51.4%)	<0.001
	Middle of dermis		12 (34.3%)	16 (45.7%)	
	Lower of dermis		22 (62.9%)	1 (2.9%)	
Granuloma			2 (5.7%)	0	0.24
Necrosis			4 (11.4%)	0	0.05
Fibrosis			4 (11.4%)	0	0.05

PMN: Polymorphonuclear neutrophil, *Chi Square.

degeneration of basal cell layer, lichenoid reaction, pseudoepitheliomatous hyperplasia, ulcer, thinning of the epidermis, mononuclear cells, and extension of inflammation into a lower dermis in the group with Leishman body was higher than group without Leishman body (P<0.05); but there was no significant difference between groups in terms of psoriasiform epidermal hyperplasia, PMNs and eosinophils cells, granuloma, necrosis and fibrosis of lesions (P>0.05) (**Table 2**).

Discussion

According to the results of this study, the expression of CD1a was significantly higher in patients with Leishman body, but there was no difference between patients with and without Leishman bodies in CD68 expression. Therefore, CD1a marker as a marker in determining the Leishman body can be effective; on the other hand, patients who had a Leishman body and patients without it were different in mor-

phological characteristics. In terms of the frequency of hyperkeratosis, parakeratosis, exocytosis, acanthosis, spongiosis, hydropic degeneration of basal cell layer, lichenoid reaction, pseudoepitheliomatous hyperplasia, and ulcer, thinning of the epidermis, mononuclear cells, and extension of inflammation in patients with Leishman body was significantly more compared with patients without Leishman body.

In this regard, Taheri and colleagues (2017) showed that chronic cutaneous leishmaniasis had a significant relationship with macrophage CD68 + and dendritic CD1a + cells and macrophage + CD68 and CD1A + increases the duration of chronic leishmaniasis [25].

Karram et al. in 2012, showed how the intra-epidermal amastigotes express CD1a. They examined the removal of epidermal leishmaniasis in 60 of 212 biopsy samples from cutaneous leishmaniasis. In all cases, the positive CD1a in the intra-epidermal amastigotes was determined. In this study, authors indicated that low level CD1a was associated high risk of leishmaniasis. Karram and his colleagues provided two plausible explanations for this abnormal safety phenotype of amastigotes [1]: the amastigotes had gained CD1a after leaving the dendritic cells that expressed this marker with the exocytosis process; Or [2]: the leishmaniasis level has shown a mutual reaction with CD1a antibodies (which is less than the authors' opinion [26]. Jabbour et al. (2015) also examined 11 skin biopsy samples from 33 patients with L tropica or major L and added new data about the process of acquiring CD1a by leishmaniasis. The cultured amastigotes were CD1a. Therefore, the amastigotes will gain CD1a during the host infection [27]. In the present study, there was a significant relationship between expression of CD1a and leishmaniasis, which was higher in patients with Leishman body.

Based on Karram et al. and the report of Jabbour et al., the authors studied the items created by Tropica L and Major L. None of these species is found in our area, whereas the cases of leishmaniasis are created exclusively by the infantum L. However, there are some major differences between the 3-species genome, including the genome size, guanine and cytosine content, and the number of genes.

A recent study by Dias-Polak et al. in 2017 showed that properties of skin lesions in leishmaniasis could be varied. They also reported that the presence of Leishman body or absence of it might influence these varieties [28]. Furthermore, in another study by Meymandi and others in 2009, it was shown that the presence of Leishman body could make differences in histological and immunological details related to the disease including CD1a [12]. These results are in line with the findings of our study. Herein we showed that expression of CD1a is significantly different among skin samples with or without Leishman body. Our results highlighted higher expression of CD1a in patients with Leishman body in their skin samples.

Another study was carried out by Tabrizchi et al. and they focused on histopathological properties of skin lesions in leishmaniasis and assessed these features by the means of IHC and reported that CD1a has higher expression rate in patients with Leishman body. They also showed no significant differences between samples concerning on other markers including CD68 [30]. The results of Tabrizchi study was in line with our results.

Moreover, histological and morphological evaluation of leishmaniasis lesions was performed by Asgari and others in 2007 in Iran. They noticed increased frequency of characteristics including exocytosis, acanthosis, spongiosis, hydropic degeneration of basal cell layer and lichenoid reaction in patients with Leishman body in their skin lesions [31]. These results are also in line with the findings of Oryan and colleagues in 2008 [32] and also in line with our findings.

As it was mentioned before, we depicted that histological characteristics such as hyperkeratosis, parakeratosis, exocytosis, acanthosis, spongiosis, hydropic degeneration of basal cell layer, lichenoid reaction, pseudoepitheliomatous hyperplasia, ulcer, thinning of the epidermis, mononuclear cells, and extension of inflammation were more common in patients who had Leishman body in their skin samples. Actually, these results could be helpful in diagnosis and treatments of patients with leishmaniasis. Utilization of immunological markers and their associations with pathogenesis of the

disease could be helpful in further novel treatments.

Disclosure of conflict of interest

None.

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