Local immune response among Iraqi patients infected with scabies

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Abstract:

Background:- Scabies is a neglected tropical skin disease caused by the tiny parasitic mite *Sarcoptes scabiei*. Scabies affects 300 million people worldwide per years. The disease stimulates cell- mediated immune response, which characterized by inflammatory cells infiltrate typical of delayed sensitivity cell- mediated immunoreaction.
Aims: The current study was design to evaluate the local immune response in skin tissue of Iraqi patients infected with scabies compared with some skin diseases
Material and methods: this study was conducted in Diyala province .The marker T-bet, Gata3, CD8 cell, CD68 and Th17 as a marker for Th1, Th2., cytotoxicity, macrophages and Th17 respectively using immunohistochemical technique (Envision) (NovolinkTM Detection System) which is used at first time in Iraq and (Avidin-Biotin Complex) techniques were used to investigate the immune cells in tissues infected with scabies and some dermal diseases including Chronic eczema, Lichen planus, Schamberg's disease, Urticaria and Cutaneous Leishmaniasis .
Results: The current study showed an increasing in numbers of Th1 in 28.571% of scabies patients, while Th2 response was showed in 42.857% of scabies patients but in low number. For cytotoxicity cells, 28.571% of patients with scabies showed a marked increase in the number of these cells, while the rest patients showed a weak response or no response with these immune cells. The study showed that 28.571% of scabies patients showed an increase in macrophage cell numbers while the rest showed a few macrophages or no response to these cells. All patients of scabies no or weak response to Th17 cells in tissue sections in the current study. It shows that Th1 cells were presented in all dermal diseases were studied (that used for comparison) except lichen disease and one of the patients with leishmaniasis. Also, the study indicated that there was no response to Th2 among patients with dermal diseases except in one of the leishmaniasis patients, it showed a clear increase of Th2 cells. The study showed the presence of high numbers of cytotoxicity cells in all patients with dermal diseases especially in patients with leishmaniasis. Macrophages cells appeared in all patients with dermal diseases patients but in low numbers except in patients with leishmaniasis and patient with lichen (they showed that there was a high increasing in the number of macrophages). A clear response for cytotoxicity cells, while Th17 cells showed a clear response in skin leishmaniasis patients and eczema patients exclusively while there was no response to this immune indicator among those with other dermal diseases

Conclusion: The present study concluded that the presence of high scores CD68, CD8 and T bet positive cells in group of scabies patients indicating that macrophages, cytotoxic T and T helper 1 cells are playing a major role in defense mechanism in scabies infection.

Keywords: Scabies, Immunohistochemical technique, T- bet, Gata3, CD8, CD68. IL-17

I. INTRODUCTION

Scabies is a neglected tropical skin disease caused by the tiny parasitic mite *Sarcoptes scabiei* (1). Scabies affects 300 million people worldwide per years (2,3).The disease is prevalent in overcrowded living conditions, with the highest disease burden's seen in young and children (4). There are two main clinical feature of scabies the first is the Ordinary scabies (classical) and crusted scabies (Norwegian) which is less common than classical (5). *S. scabiei* produces antigenic molecules which modulate the function of the host's immune cells (6), so the disease stimulate cell mediated immune response(7), which characterized by inflammatory cells infiltrate typical of delayed sensitivity cell-mediated immune reaction(8). The infestation with this ectoparasite of the skin causes localized cutaneous inflammation immune reaction, pruritus, skin lesions resulting from mechanical burrowing, and allergic and inflammatory responses are mounted by the host against mite and its products (6). In spite of some available information from few immunological studies that have been conducted previously which suggesting the importance of humeral and cellular responses (2), the immune response to sarcoptic infestation in human remains complex and poorly understood (1). This study was designed to study the local immune response by using T bet, Gata 3, CD8, CD68 and IL-17 as indicator of Th1, Th2, cytotoxic, and lL-17 cells responded

II. MATERAILS AND METHODS

Patients: This study included 7paitents with scabies and 7 patients with dermal diseases (Leishmaniasis, chronic eczema and Urticaria Lichen planus, Shambrag's) as a positive controls. Samples were subjected to immunohistochemichal technique (Envision and Novolink detection system) by using markers (Tbet,Gata3, CD8,CD68 and IL-17) to investigating for immune cells in tissues.

Methods: Antigen retrieval was obtained through high temperature (600W microwave for 25 minutes) and appropriate buffers, Tris/EDTA (pH 9.0) for CD8, CD68, Tbet and

Gata 3. The primary antibody was applied for one hour at RT and visualized with Envision kit (Dako) While Th17 producing cells stained by Avidin-biotin system. NovalinkTM detection system (this technique is used at first time in Iraq): Briefly, heat induced epitope retrieval in Tris- citrate EDTA buffer (pH 9.0) was performed prior to application of the primary antibody. Peroxidase and protein block reagents were used before the primary antibody. The primary mouse anti-human T-bet, Gata3 (Santa Cruz, California, USA) , CD8 and CD68 (Dako, Holland) and goat anti-IL-17 (R&D system) antibodies were used for 60 minutes in a humid chamber at room temperature followed by post primary block and the secondary Novolink polymer anti-mouse antibody peroxidase-conjugated (HRP) at the same conditions. The peroxidase reaction was development using the diaminobenzidine (DAB) chromogen substrate for 10 minutes. All steps of the procedure were preceded by washes with PBS buffer. Avidin-biotin system was used to detect IL-17 and heat induced retrieval in citrite buffer pH 6.0 was used prior to application of anti-IL-17. All data are presented as the percentage of positive cells and the percantage was calculated by using the number of density of positive cells for used markers.

III. RESULTS:

In details, the results of the current study showed that 28.571% of scabetic patients (2 out of 7) had an increase of Th1 cells expression of T-bet marker (that used to indicate these cells) , While 57.142% of scabetic patients showed low numbers of Th1 cells presence in dermis (Figure 1 and Figure 2).

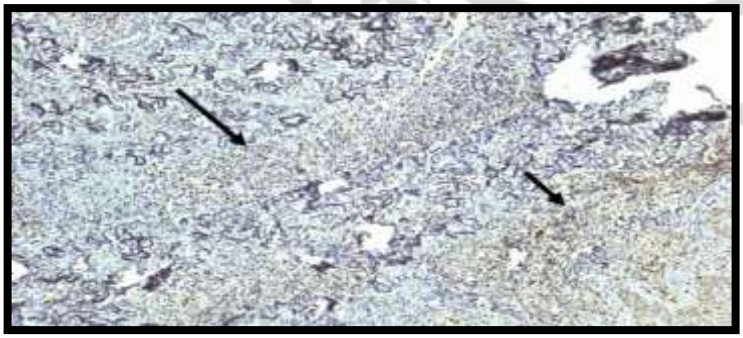

Fig.1. Histological section of T-bet factor with heavy infiltration of Th1 more than 90 cells in dermis region (X10

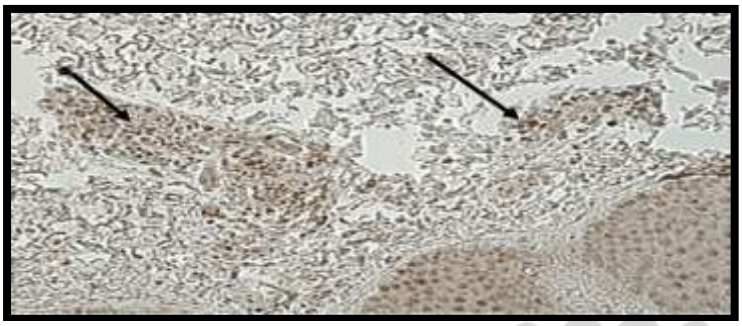

Fig.2. Histological section of T-bet factors appear low numbers of Th1 cells more than 20 cells infiltration in dermis lesion (X20).

In the other hand, 42.857% of scabetic patients appeared low number of Th2 cells using Gata3 as indicator for their presence (Figure 3)., while 57.142% of patients didn't responded for this factor. Highly increasing of cytotoxic T cells were appeared in 28.571% (2 out of 7) patients with scabies, while 57.142% of scabies patients showed low numbers of cytotoxic T cells numbers and these cells didn't appear in 14.285% of them (Figure 4 and Figure5).

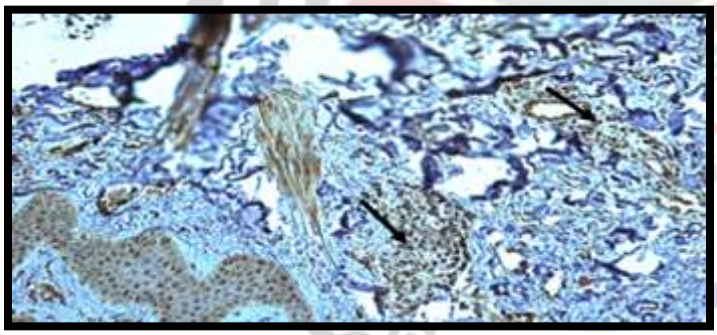

Fig.3. Histological suction of Gata3 with Th2 infiltration up to 20 cells in dermis region (X20).

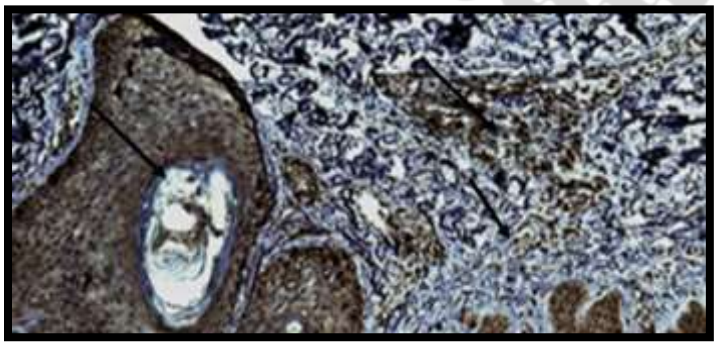

Fig.4. Histological section of CD8, more than 70 cytotoxic T cell infiltrated in dermis lesion with visible appearance of parasite in epidermis (X20).

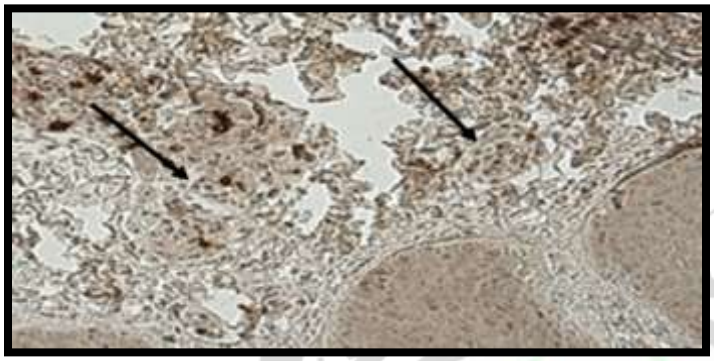

Fig.5. Histological section of CD8 infiltrated more than 20 cytotoxic T cells in dermis (X20)

The results of the present study showed that 57.142% of scabitic patients had simply infiltrated of CD68 cells especially patients who had been infected for one to eight weeks (Figure 6). While 28.571% of patients showed high response to this type of cells especially the patients who had been infected with scabies for more than three months (Figure 7). This cells didn't appear in 14.285% of them. The results had shown that 57.142% of scabies patients didn't respond to IL-17 which indicate to presence of Th17 cells while 42-857% showed simple respond (Figure 8).

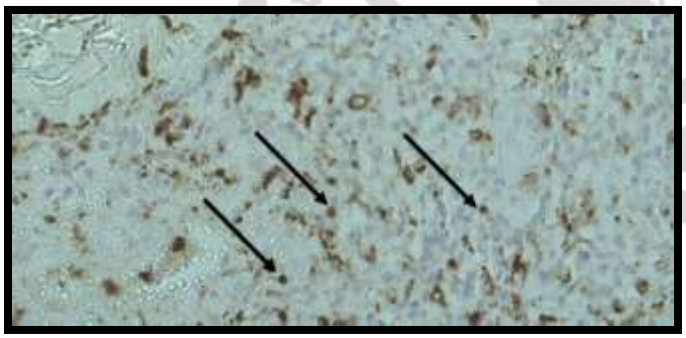

Fig.6. Histological section of CD68 with simply infiltration of cells (5) in dermis (X20).

Fig.7. Histological section of CD68 with heavy infiltration of cells more than 70 in dermis (X20).

Fig.8. Histological section of 1L-17 factor with simple number of Th17 cells more than 20 cells in dermis (XI0).

The curent study reported the immune respose in the dermal diseases patients and the results showed different immune response that Th1 cells were presence in all dermal diseases expect lichen planus and one of Leishmaniasis patient. The other patient with leishmaniasis had very high infiltration of these cells (Figure 9 and Figure10). In the contray , there was no infiltration of Th2 (using transcription factor Gata3) in all dermal diseases patients expect in one of leishmaniasis patients. While there was highly infiltration of CD8 cells in all patients with dermal diseases without expectation (Figure 11, Figure 12).

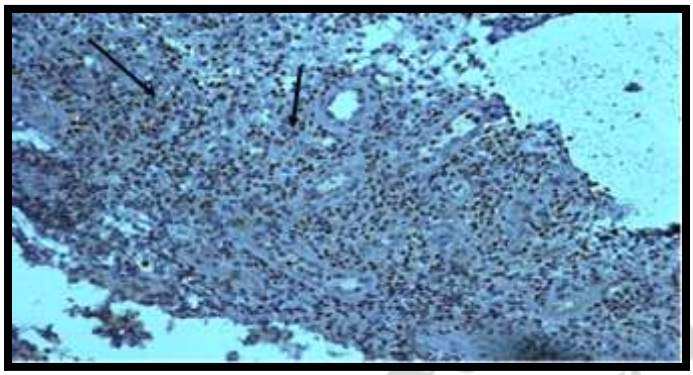

Fig.9. Histological section of T-bet factor with heavy infiltration of Th1 in dermis (X20).

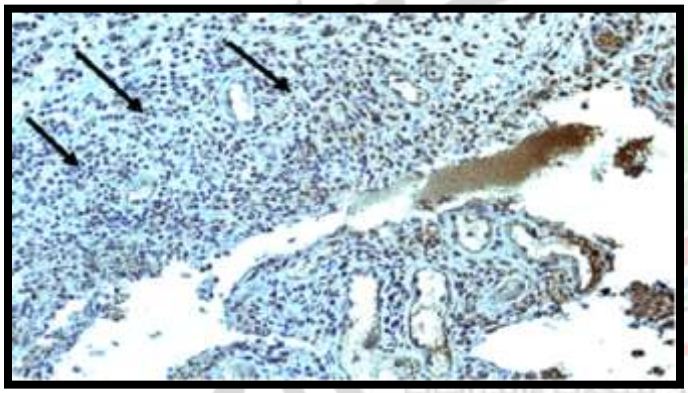

Fig.10. Histological section of Gata3 with heavy infiltration of Th2 in dermis (patient with leishmaniasis) (X20).

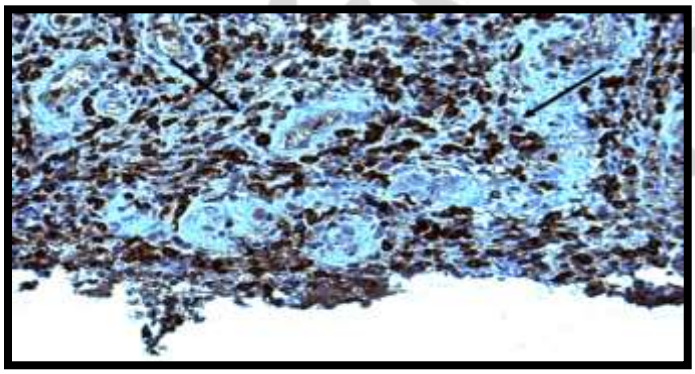

Fig.11. Histological section of CD8 factors with heavy infiltration of cytotoxic T cells in dermis (patient with leishmaniasis) (X20).

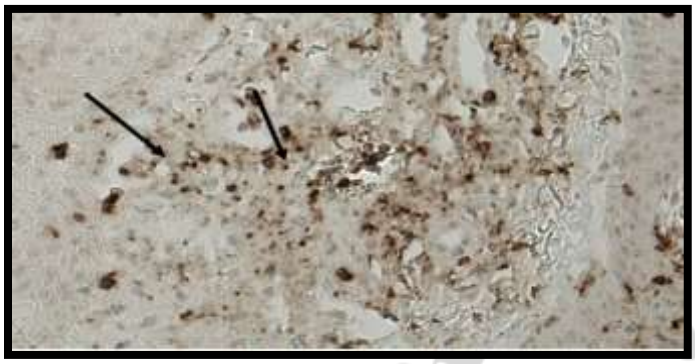

Fig.12. Histological section of CD8 factor with infiltration more than 20 of cytotoxic T cells in upper dermis (patient with Urticaria) (X20).

For CD68 infiltration, there was low positive number in all patients with dermal diseases expect the patient of lichen planus and leishmaniasis who they have high positive cell numbers (Figure 13).

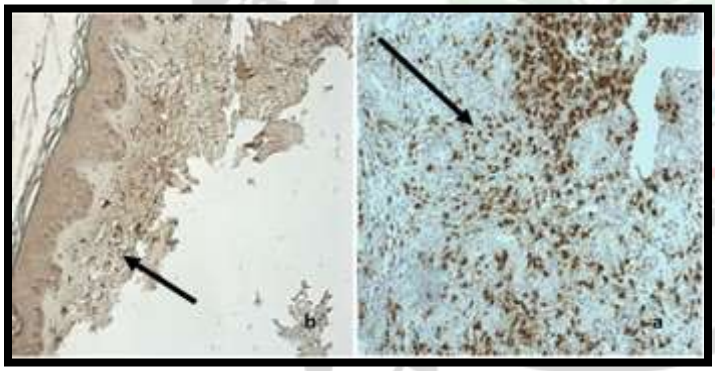

Fig.13. Histological section of CD68 factor with heavy macrophages infiltration in lesihmaniasis patient (a) and lichen planus (b) (X10)

Th17 cells infiltrated with a high number in leishmaniasis patient and chronic eczema (Figure 14 and Figure15).

Fig.14. Histological section of 1L-17 with infiltration of Th17 cells in dermis (patient with leishmaniasis) (X10).

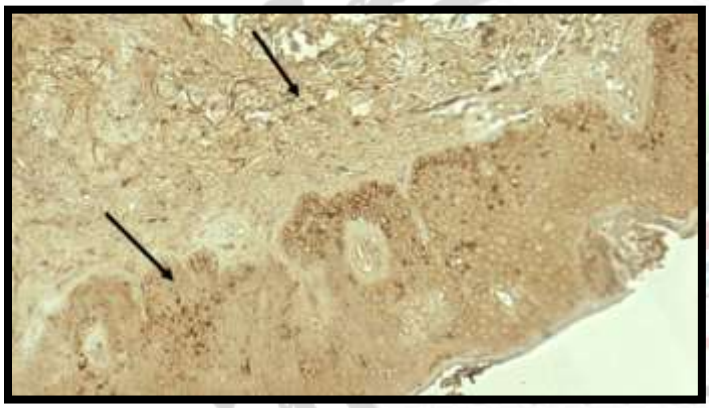

Fig.15. Histological section of IL-17 with simple infiltration of Th17 cells in upper dermis and epidermis (patient with chronic eczema) (X20)

IV Discussion:

The current study showed that just 28.571% of the scabies tissue biopsies (2 out of 7) had high Th1 (used T-bet) cell infiltration, while 57.142% of patients showed the presences of these cells but in low numbers. [9] reported that T-bet is transcription factor for Th1 cells and it is control of their differentiation, growth and stimulation, he referred that the mice lacking T-bet factor failed to differentiate Th1 not Th2 cells, so the presence of T-bet act as evidence for presence of the Th1 cell in any tissue. It is the evidence of their differentiation and activition leading to an autoimmune response and causes destruction of the tissues where these cells found. This is consistent with the current study that

showed the patients with large numbers of Th1 cells suffered from severe symptoms more harmful than those who showed fewer of Thl cells, it was associated with the length of exposure to injury. The current study showed that 42.857% of scabies patients had few numbers of Th2 cells (using Gata3 factor), while 57.142% didn't respond. Gata3 is a factor that controlled the differentiation of Th0 cell to Th2 cells and activates their cytokines secretion [11]. This results agree with [12] who finding that the immune-response in early-stages of scabies is associated with Th2 activity, while the late stages immune response to scabies is associated with Th1 activity. [13] noted that in psoriasis there was a low expression of Gata3 but high expression of T-bet, he and [14,15,13] explained this result due to immune response which tending to Th1 response that stimulate other cells and leading to hyper keratinocytes and high inflammation responses.

The present study showed that 28.571% (2 out of 7) of scabies patients had high positive number of cytotoxic T cells while 57.142% had low numbers, and 14.285% had not any response to this cells. The increasing in CD8 cells agree with the study carried out on pigs by [16] and with the study of [17] which conducted on scabies patient and some others dermal diseases and with [18,19] who demonstrated the predominant of CD8 and showed the absence of B cells in Australians people who infected with crusted scabies, this led to conclusion that cytotoxic T cells (CD8) contribute to an imbalance in immune response and absence of B cells leads to a failure of immunity and failed in control of the parasite growth and reproduction, So it can survival. The current study indicated that the number of CD8 cells was a rise in patients suffered from scabies for long time so they had severe injury. while the patients who had CD8 but with a few numbers (in the present study) agree with [20] who indicated that CD8 numbers were lower in classical scabies comparing with crusted [21] Suggested that classical scabies might develop into crusted scabies but rarely. [1] Suggested that crusted scabies is the severing form disease which associated with weak immunity, [23] indicated that stimulation and activation of CD8 lead to apoptosis of keratinocytes, which lead to skin hyperkeratination, So CD8 activity increases with the duration of patient exposure (table 1). This study showed that 57.142% of scabies patients had few number infiltration of CD68 cells especially in patients who had been infected one to eight weeks while 28.571% showed a high infiltration of these cell in patients who had been infected with scabies for more than three months. 14.285% of patients had no response of these cells. CD68 indicated to infiltration of macrophages in the local of infection in scabies patients skin, and agree with [23] who referred that the goat with scabies had CD68 in the local of infection. They

explained their results by the presence of CD68 cell in large numbers related with the severity of disease and duration of exposure which confirm with the present study as there were high cells numbers in cases with prolonged exposure to disease and the patients suffer from severity symptoms. The present study showed that 57.142% of scabies patients had no IL-17 cell infiltration while 42.857% had few infiltration of IL-17 cells in patients who suffered from scabies for 12 weeks and those who suffered infection for 16 weeks, this results agree with [1] who indicating that the increasing in Th17 was observed at the fifteenth week of infection in pigs which referred to the development of the disease and occurrence of inflammation. In general, 1L-17 which considered as a pro-inflammatory cytokine and associated with allergic and inflammatory diseases it usually excreted by CD8 cells and other cells [1 ,16]. [16] and [24] showed that the maturation of Th17 cells and 1L-17 secretion was stimulated by IL-23 secretion from dendritic cells Macrophages and keratinocytes ,all of these cells occurrence in scabies infection [24] . The results of present study showed that Th1 cells were presence in all dermal diseases expect lichen planus and one of Leishmaniasis patient. The other patient with leishmaniasis had very high infiltration of these cells. In the contrary, there was no infiltration of Th2 (using transcription factor Gata3) in Dermal diseases patient's. The presence of Th1 cells, confirms the role of Th1 cell immune response in many dermal diseases whether it was caused by bacterial, parasitic, auto immune or dermatitis disease [25, 26]. The presence of T-bet in most patients with dermal diseases has been documented in previous studies and suggested that T-bet plays an important role in the differentiation of Th0 to Th1 cells by regulates natural killer cells and controlling the differentiation of CD8 cells [25, 26]. The current study indicated that there was no transcription factor Gata3 except in one of Leishmaniasis patients. This agree with [25] which indicated a high expression of T-bet and Gata3 in patient with Leishmaniasis viannia and there was a negative correlation between Gata3 and the pro- inflammatory immune response. The same study [25] obtained that there was a rise in T-bet and decreasing in Gata3 in patients with acute and chronic leishmaniasis that referred to that both of these factors (T bet and Gata3) may play a role in regulation of the immune response in leishmaniasis. While [25,27,28] noted that Gata3 may play as a regulator of inflammation response and proliferation of keratinocytes. The study showed the presence of CD8 in all dermal diseases patients without exception with high levels in leishmaniasis patient. This agree with studies that indicated to mediate of CD8 cells in immune responses as an important factor in pathogenesis and they are responsible of keratinocytes damage [29,30]. The current study referred to occurrence of CD68 in all dermal

diseases patients at low levels, but with high levels in lichen planus and Leishmaniasis patients, This agree with [31,32] who demonstrated the presence of CD68 in many dermal diseases which may play a role in cellular immune response and infiltration of CD68 in cutaneous Leishmaniasis may be due to invade macrophages by the parasite and proliferation inside these cells resulting in rupture of these cells. Th17 cells founded exclusively in Leishmaniasis and chronic eczema in the present study. [32] referred to the role of Th17 cells in inflammatory immune response in some dermal diseases, this is due to the function of these cells as the secretion of IL-17 (pro inflammatory cytokine). It is play an important role in acute and chronic diseases [33,34] while [35] showed the role of these cells in defense mechanism against *Leishmania tropica*. The present study concluded that the presence of high scores CD68, CD8 and T bet positive cells in group of scabies patients indicating that macrophages, cytotoxic T andT helper 1 cells are playing a major role in defense mechanism in scabies infection.

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