

## Evaluation of some interleukins and immunomodulatory factors in Iraqi scabies patients

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### Abstract

Scabies is a contagious skin infection, caused by *Sarcoptes scabiei*. It is one of a neglected parasitic disease. It causes complications that lead to inflammatory and allergic immune response. This study was designed to obtain the role of some cytokines in scabies patients and compare their levels with dermal diseases patients and control (healthy).

The study included one hundred and three patients infected with scabies, seven dermal disease patients (positive controls) as well as 34 healthy individual as control group. The blood samples were collected from scabies and dermal disease patients as well as the control groups. Enzyme linked immunosorbent assay (ELISA) was used to measure Interleukine-4 (IL-4), Interleukin -8 (IL-8), Interleukin-17A (IL-17A), Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), Interferon- $\gamma$  (IFN- $\gamma$ ), Monocyte Chemotactic Protein-1 (MCP-1) and Macrophage Inflammatory Protein-1- $\alpha$  (MIP-1- $\alpha$ ) in the serum of scabies patients, dermal disease patients and healthy individuals.

The results showed that IL-4, IL-8, IL-17A and TNF- $\alpha$  was higher in scabies patients than in other groups (positive control and healthy), with no significant differences. While both of MIP- $\alpha$  and MCP-1 were higher in scabies patients compared with healthy group, with significant differences. MIP- $\alpha$  was higher in dermal patients individuals than in scabies patients. TNF- $\alpha$  was lower in scabies patients than in healthy group but higher than in dermal disease patient group. There was positive correlation between IL-17A and each of IL-8, TNF- $\alpha$ , MCP-1 and between IL-8 and both of IL-17A and MCP-1, while there was negative correlation between MIP-1- $\alpha$  and both of IL-4 and MCP-1. The results suggested that the scabies infection may induce the systemic and inflammatory immune response.

**Keywords:** Scabies, IL-4, IL-8, IL-17A, TNF- $\alpha$ , IFN- $\gamma$ , MCP-1, MIP-1- $\alpha$

### 1. Introduction:

Scabies is a disease affecting both sexes at different ages for all ethnic and socio-economic levels without exception (W.H.O., 2005). It remains a certain health

problem causes a serious economic loss for cattle breeders and farm animals (Jordan & Verma, 2014). It is cutaneous infestation caused by a tiny obligate parasite belong to scabies mites (*Sarcoptes scabiei*) affects various kinds of domestic and wild animals (Pence & Veckermann, 2002; Walton et al., 2004).

Despite of *Sarcoptes scabiei* worldwide spread and infects over than 40 kinds of mammals including human, it still considers a neglected parasitic disease. The cold weather and high population density are a crucial factors for increase prevalence of disease (Daown et al., 1999; Poulat & Nasirian, 2007). Low temperatures and high relative humidity are suitable environment conditions for increase mites activity and infection (Arlan, 1989; White, 2009).

Scabies has been found to be more prevalent in both developing and developed countries and has high incidence in crowded and poor populations, such as prisons and civil institutions, nursing and orphans centers as well as army and migrant and immigrant camps (Routh et al., 1994). Individuals with scabies suffer from severe itching mediated through hypersensitivity reaction caused by mite's antigens and its secretion (Walton, et al., 2004). Liu, et al., (2014) confirmed that mites may induce an inflammatory and cell-mediated immune responses in its host. Other studies indicated that Scabies induces immune allergic response and keratinocytes and lead to secretion of its some cytokines (Arlan et al., 2003; Walton, et al., 2010) in the other hand, Al-Musawi (2014) referred that the disease stimulates both humoral and cellular immune responses.

A numerous studies focus on the humoral and cellular immune response, most of these studies conducted on laboratory animals (mice, rabbits, pigs), either exposed to the parasite antigens or infected with parasite itself (Smets & Vercruyse, 2000; Mounsey, et al., 2015). Despite the availability of a lot of information on the immunology of scabies in human, there is a dearth of studies that address the immunological changes that occur in human systemic immune response. Previously literature obtained that most common cells in the site of lesion are inflammatory cells (eosinophils, lymphocytes and macrophages), while the most predominant cells are T-lymphocytes which play a main role in the activation and regulation of immune responses by inducing cytokine production (Bhat et al., 2017). Keratinocytes may also produce pro-inflammatory and immunomodulatory cytokines and they consider to be responsible for systemic effects (Al-Musawi et al., 2014). The present study was conducted to determine IL-4, IL-17A, IL-8, TNF- $\alpha$ , IFN- $\gamma$ , MCP-1, MIP-1- $\alpha$  levels in scabies patients, dermal disease patients and healthy (control) in Diyala province and to compare cytokines levels in all studied groups.

## 2. Material and Methods:

**2-1. Subjects:** The study samples were collected from March to May 2016. One hundred and three patients infected with scabies, (50 males and 53 females), their ages between 1-90 years were including in the present study. The diseases were diagnosed by dermatologist, the study included also seven dermal disease patients (five males and two females). Their ages between 2-54 years as a positive control, as well as the healthy group (control) included 34 persons (21 males and 13 females) their ages between (5-63) years. It has been confirmed that all group individuals did not have allergic diseases, helminthic infections, secondary infection, previous attack with scabies, and/or getting any antihistamines drugs were included for cytokines assays.

**2-2. Blood sample collection:** The blood samples were collected from scabies and dermal disease patients as well as the control groups. Five ml of venous blood was taken and left to clot in room temperature for 30-60 minutes. Serum was separated by centrifuging at 3000 rpm for five minutes. Sera were divided into four parts using Eppendorf tubes (0.5ml per each). The samples were kept at  $-20\text{ C}^{\circ}$  until it uses.

**2-3. Clinical examination:** The clinical examination had been done by the dermatologists in hospital and the scabies and other dermal diseases were diagnosed according to clinical features.

**2-4. The cytokines assay:** The cytokines were quantitatively measured in all groups individuals. These cytokines were human IL-4, IL-8, IL-17A, TNF- $\alpha$ , IFN- $\gamma$ , MCP-1 and MIP-1- $\alpha$  by using enzyme linked immunosorbent assay, according to manufacturers' instruction, PeproTech Com, UK.

**2-5. Statistical Analysis:** The results were statistically analyzed using Statistical Package for Social Sciences (SPSS), version 15. Data were expressed as mean  $\pm$  standard error (SE). Duncan's multiple range test was used for comparison among several means. Pearson Correlation (r) was used to determine the correlation between criteria. P-value  $\leq 0.05$  was considered statistically significant.

## 3. Results:

**3-1. Cytokines levels:** The results of the present study showed high level concentration of cytokine IL-4 in scabies patients ( $84.101 \pm 23.844$  pg/ml) comparing with its levels in dermal diseases patients ( $77.871 \pm 45.242$  pg/ml), and the control group ( $49.106 \pm 16.044$  pg/ml) but without significant differences as shown in Table 1.

**Table (1): Cytokines and chemokines level among individuals infected with scabies, dermal diseases and healthy persons.**

Cytokines and chemokines	Groups	Mean $\pm$ SE pg/ml	$\rho$ - value
IL-4	Scabies patients	84.101 $\pm$ 23.844	0.812(NS)
	Dermal diseases patients	77.871 $\pm$ 45.242	
	Control	49.106 $\pm$ 16.044	
IL-8	Scabies patients	33.746 $\pm$ 9.996	0.755(NS)
	Dermal diseases patients	34.742 $\pm$ 14.642	
	Control	14.143 $\pm$ 4.379	
IL-17A	Scabies patients	49.738 $\pm$ 9.768	0.651(NS)
	Dermal diseases patients	33.285 $\pm$ 14.842	
	Control	44.817 $\pm$ 7.694	
IFN- $\gamma$	Scabies patients	177.864 $\pm$ 10.626	0.042*
	Dermal diseases patients	86.142 $\pm$ 11.244	
	Control	180.705 $\pm$ 19.114	
TNF- $\alpha$	Scabies patients	75.306 $\pm$ 23.321	0.785(NS)
	Dermal diseases patients	49.028 $\pm$ 23.361	
	Control	55.217 $\pm$ 17.990	
MCP-1	Scabies patients	253.466 $\pm$ 18.979	0.01**
	Dermal diseases patients	332.714 $\pm$ 45.531	
	Control	185.750 $\pm$ 32.543	
MIP-1- $\alpha$	Scabies patients	601.194 $\pm$ 140.528	0.023*
	Dermal diseases patients	2426.857 $\pm$ 1352.358	
	Control	491.706 $\pm$ 488.358	

\* Significant differences in  $p < 0.05$ .

\*\* Significant differences in  $p < 0.001$

The results showed elevation in IL-8 concentration in both scabies and dermal diseases serum (33.746  $\pm$  9.996 and 34.742  $\pm$  14.642 pg/ml, respectively) compared with in healthy group (14.143  $\pm$  4.379 pg/ml), while IL-17A was increase in scabies patients serum (49.738  $\pm$  9.768 pg/ml) and healthy group (44.817  $\pm$  7.694 pg/ml) compared with dermal diseases patients (33.285  $\pm$  14.842 pg/ml) as showed in Table 1.

IFN- $\gamma$  was decrease in scabies patients and dermal patients diseases comparing to healthy group (177.864  $\pm$  10.626 pg/ml, 86.142  $\pm$  11.244 pg/ml, 180.705  $\pm$  19.114 pg/ml, respectively). The present study showed increase in TNF- $\alpha$  levels in scabies patients comparing with dermal diseases patients and healthy group (75.306  $\pm$  23.321 pg/ml, 49.028  $\pm$  23.361 pg/ml, 55.217  $\pm$  14.990 pg/ml, respectively)

The current study showed increase in both of MCP-1 and MIP-1- $\alpha$  scabies and dermal diseases patients serum comparing with healthy group (253.466  $\pm$  18.979 pg/ml, 332.714  $\pm$  45.531 pg/ml and 185.750  $\pm$  32.543 pg/ml for MCP-1 and 601.194  $\pm$

140.528pg/ml,  $2426.857 \pm 1351.358$ pg/ml and  $601.194 \pm 140.528$  pg/ml, respectively)

### 3-2. Correlation of cytokines in scabies patients:

The current study showed that there was positive correlation between IL-17A and each of IL-8, TNF- $\alpha$ , MCP-1 and between IL-8 and both of IL-17A and MCP-1, while there was negative correlation between MIP-1- $\alpha$  and both of IL-4 and MCP-1 as showed in table (2):

**Table (2): Cytokines correlation among 103 scabies patients**

	IL-4	IL-8	IL-17A	IFN- $\gamma$	TNF- $\alpha$	MCP-1	MIP-1- $\alpha$
IL-4	1						
IL-8	0.339**	1					
IL-17A	0.011	0.731**	1				
IFN- $\gamma$	0.285**	0.128	1.142	1			
TNF- $\alpha$	0.070	0.093	0.288**	0.110	1		
MCP-1	0.096	0.386**	0.294**	0.132	0.039	1	
MIP-1- $\alpha$	0.019-	0.050	0.025	0.056	0.351**	0.008-	1

**\*\*Correlation in P- value 0.01**

### 4. Discussion:

The current study showed increase in the level of IL-4 in scabies patients serum comparing with dermal diseases patients and healthy group (control). This results agree with Karthikeyan&Ragunatha (2011) , Al-Musawi, et al., (2014) and Mounsey, et al., (2015). They mentioned that there is an increase in IL-4 levels in scabies patients comparing with the healthy group while Arlian et al., (2006) did not record this cytokine in scabies patients. In the other hand Walton, et al., (2010) showed there is no significant differences in the levels of IL-4 between the scabies patients and healthy groups. The increase of this cytokine (IL-4) (which is one of the cytokines expression of Th2 ) indicates that Th2 cells stimulate in scabies infestation. This cytokine regulates the production of IgE and control the production of mast cell and eosinophils which are stimulating in hypersensitivity reaction (as shown in histological changes obtained in previous study for Almusawi et al, 2018). In addition the stimulating mast cell and eosinophils produce IL-4 (Zamorano et al, 2003). On the other hand, IL-4 play important roles as chemotactic immune response in skin lesion. Many other histological studies use human and animal models had been detected mast cells and basophils in skin lesion of human and animals infected with scabies (Amer et al ., 1995; Ito et al.,2011; Nimmervoll et al., 2013; Mounsey et al.,2015 ). Activated mast cells and basophils rapidly produce some cytokines

(including Th2 cytokines IL-4) which are the main molecules as well as that the cytotoxicity against keratinocytes mostly release cytokines responsible for amplify the allergic Th2-type inflammatory response (Bhat et al., 2017).

The present study obtained that the levels of IL-8 was increase in scabies and dermal disease patients comparing with healthy group, this results agree with Morgan & Arlian (2010) who showed that monocytes secrete IL-8 in high levels after adding *Sarcoptes scabiei* antigen to the culture media or when exposed skin cells culture media (EpiDerm EFT-400 full-thickness Human Skin Equivalents) to extract of parasites. IL-8 secretes from skin cells (keratinocytes, fibroblasts, and macrophages), and the secretion of IL-8 increase in dermal diseases (Coondoo, 2012).

The increase in IL-17A level in scabies patients in the present study agrees with Liu, et al. (2014) and Mounsey, et al. (2015) who reported that IL-17-A was increased in pigs infested with scabies. IL-17A is pro-inflammatory cytokine related with many hypersensitivity, host defense and inflammation diseases (Jin and Dong, 2013). Its secreted from mast cells and Th17 cells. Arlian et al., 2007, Martin et al., (2014) and Mounsey et al., (2015) referred that this cytokine related with IL-23 secreted by dendritic cells, macrophages and keratinocytes, all these cells are recorded in scabies cases, supporting an IL-17 environment (Arlian et al., 2007; Martin et al., 2014). The present study showed that INF- $\gamma$  was decrease in scabies patients comparing with healthy and patients with dermal diseases groups. This results agree with Zamorano, Walton et al. (2003, 2010) who obtained that there was a clear decreased of IFN- $\gamma$  production was observed in scabies patients as a response to parasite cysteine – proteinase. Arlian et al., (2007) referred that expose mice to live mites lead to decrease the expression of IFN- $\gamma$  and suggest that the mite produce molecules reduce expression of immune cytokines and chemokine including IFN- $\gamma$ . In the other hand, Arican, et al. (2005) showed that the cells production of this cytokine downregulates in the blood of dermal disease (such as psoriasis) and lead to aggravation of disease, in the contrary, some studies reported that there was increase in INF- $\gamma$  in dermal diseases combined with macrophage (Hua, et al., 2006; Huard, et al., 2017).

In the present study TNF- $\alpha$  level was increase in scabies patients serum and decrease among dermal diseases patients. This result agree with Arlian et al (2004) Morsy, et al., (1995) who demonstrated that TNF- $\alpha$  was increased in monocyte culture media when exposing to culture to scabies mite extract which indicate the ability of the molecules in the extract's molecules to modulate the monocytes and dendritic cells functions. Likewise Portugal, et al. (2007), Al-Musawi, et al. (2014) and Abd El-Aal, et al. (2016) indicated a higher levels of TNF- $\alpha$  among individuals infested with scabies and suggested that there was an important role of TNF- $\alpha$  in human scabies

control . While Portugal, et al. (2007) Levi – Schaffer, et al., (1998) showed that TNF- $\alpha$  production may be due to the physical stimulation by parasite's burrowing in the skin that lead to inflammatory response., Levi – Schaffer, et al., (1998) Mulline, et al . (2009) Morgan, et al., (2013) and Al-Musawi, et al., (2014) suggested that TNF- $\alpha$  associated with presence of eosinophils and its ability to active this cells in scabies patients.

The present results showed increase in MCP-1 level in scabies patient serum. This result agrees with Morgan & Arlian (2010) who indicated that MCP-1 increase in the culture media using (human skin equivalent) human skin cells was that exposed to mite extract and suggested that this result may be due to the physical stimulation resulting from extract's molecules borrowing by the mites which lead to produce this cytokine . Another study observed that salivary secretions and mite's antigen stimulate MCP-1 which in turn attracts lymphocytes, monocytes and dendritic cells to lesion areas result in inflammatory reactions (Kobets, et al., 2012).

This study showed increasing in MIP-1 $\alpha$  among dermal diseases patient and scabies patients. These This results agree with Morgam et al, (2013) Kobets, et al. (2012) who suggested that in vivovitro, the interplay between cell media culture and the antigens of parasite is responsible for increase of this some chemokines also including MIP-1 $\alpha$ , re MIP-2 $\alpha$  and M3P-1 $\alpha$  and suggested that the antigen of the parasite stimulates inflammatory immune response. In the other hand ,Kobets, et al. (2012) suggest that the increase of MIP-1 $\alpha$  related with increase of TNF-  $\alpha$  . MIP-1 $\alpha$  was increase in cutaneous leishmaniasis patients this agree with Al-Saadi (2014) who showed increasing this cytokine among individual infected with cutaneous leishmaniasis and related with skin lesion numbers. MIP-1  $\alpha$  produce by macrophages and dendritic cells where Leishmaniatropica proliferate and increasing numbers of this parasite leading to rupture of these cells and release their contents (including (CC13) MIP-1  $\alpha$ ) . (Kobets, et al.,2012).

The current study showed that there was positive correlation between IL-17A and each of IL-8, TNF-  $\alpha$ , MCP-1 and between IL-8 and both of IL-17A and MCP-1, while there was negative correlation between MIP-1-  $\alpha$  and both of IL-4 and MCP-1 . These may reflect the complex associate relation in immune response and help explain the delayed inflammatory reaction to infestation with *S. scabiei* which needs further investigation and study .

## Conclusion

The study concluded that the scabies infection may induce the systemic and inflammatory immune response.

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