



## Relationship between TH1, TH2 Immune Responses and Serum SOD Activity In Scabies

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### ABSTRACT

Scabies is a common ectoparasitic contagious skin infestation globally, particularly in the developing world. Infestation invariably leads to the development of localized cutaneous inflammation, pruritis and skin lesions. The aim of this study was to investigate immunobiochemical response of the host's inflammatory/immune response to scabies during infestation. The present study was conducted on 60 scabietic patients who visit Dermatology Outpatient Clinic, Al-Sader Medical City in Najaf governorate/ Iraq during February 2012 till November 2012. Blood samples from 60 scabietic patients (30 early and 30 late scabietic patients) and 28 healthy and non-allergic controls were collected for eosinophils (%) count using Cell-Dyn Ruby; serum was obtained to estimate serum cytokines (IL-1 $\beta$ , TNF- $\alpha$ , IL-4 & IL-5) and IgE using (ELISA) technique, and SOD levels. All the studied parameters were highly significant elevated ( $p < 0.05$ ) in both early and late scabietic patients in comparison to control group. IgE positively correlated with IL-1 $\beta$ , TNF- $\alpha$ , IL-4 and IL-5 in early scabietic patients, whereas it had negative correlation with IL-1 $\beta$ , TNF- $\alpha$  and positive correlation with IL-4 and IL-5 in late scabietic patients. SOD showed negative correlation with IL-1 $\beta$  and TNF- $\alpha$ , whereas positive correlation appeared with IL-4 and IL-5 in both scabietic patients. In early and late scabietic patients, eosinophils (%) positively correlated with all the studied cytokines IL-1 $\beta$ , TNF- $\alpha$ , IL-4 and IL-5. The findings of the current study demonstrated that host immune response in early scabietic patients resembles a TH2 allergic response, whereas in late scabietic patients; It resembles a TH1 cell-mediated protective response.

**Keywords:** Scabies, IL-1 $\beta$ , IL-4, IL-5, IgE, SOD activity.

### 1. Introduction

Scabies is an intensely itchy ectoparasitic infectious dermatosis caused by *Sarcoptes scabiei* var *hominis*. Scabies is a neglected worldwide health problem. It has been estimated that 300 million people develop scabies worldwide annually (Feldmeier & Heukelbach, 2009). The clinical presentation of scabies is characterized by a generalized itching (pruritus) often more intense at night, and coincides with a widespread eruption of inflammatory papules. The pathognomonic lesions of scabies are burrows, which are best seen in the interdigital webs, wrists, elbows, the borders of the hands, the sides of the fingers, feet, particularly the instep and, in males, on the genitalia; and in females on the chest and nipples (Stone et al., 2008;

Burns, 2010). The topographical distribution of scabies lesions in males and females showed that all the patients had lesions on trunk. Whereas axillae and breasts were most commonly affected in females, in males, there were more lesions on hands and genitalia than in females (Al-Musawi et al., 2013). The inflammatory and immune responses seen with scabies are complex. There is a dearth of literature reporting scabies-specific cellular or humoral immunity. The cell-mediated host immune responses to scabies antigens have been primarily identified by histopathological examination of skin biopsies from scabietic lesions which reveals mite burrows surrounded by inflammatory cell infiltrates comprising eosinophils, lymphocytes and macrophages (Walton & Currie, 2007). The predominant lymphocytes in the infiltrates are T lymphocytes (Walton, 2010), which play a central role in the activation and regulation of immune responses by recognizing antigen and inducing cytokine production. Furthermore, keratinocytes are known to produce pro-inflammatory cytokines and the immunomodulatory cytokines, are considered to be responsible for systemic effects (Grone, 2002). Furthermore, biopsy specimens containing both mites and inflammatory papules have been observed to contain IgE deposits in vessel walls in the upper dermis (Cabrera et al., 1993). In skin diseases, the body possesses an array of a potent antioxidant protection such as SOD, which responsible of degradation of peroxides and free radicals (Bickers & Athar, 2006). Recent findings suggest that tissue damage attributed by oxidative stress and reactive oxygen species (ROS) play a key role in pathogenesis in many ectoparasitic and skin diseases (Saleh et al., 2007; Dimri et al., 2008; Camkerten et al., 2009; Dimri et al., 2010). Eosinophils are multifunctional leukocytes implicated in the pathogenesis of numerous inflammatory processes, including parasitic helminth infections and allergic disorders (Rothenberg & Hogan, 2006). They secrete an array of cytokines (IL-2, IL-4, IL-6, IL-10, IL-12) capable of promoting T cell proliferation, activation and TH1/TH2 polarization (Shi et al., 2000; Lacy & Moqbel, 2000). Eosinophils play an important role in the pathogenesis of arthropod induced- allergic skin conditions as in scabies (Khalifa, 2000). The aim of this study was to evaluate some cytokines, total IgE, SOD levels and eosinophils (%) in early and late scabietic patients.

## **2. Materials and Methods**

**Subjects:** 60 scabietic patients who did not have allergic diseases, helminthic infections, secondary infection, previous attack with scabies, and/or getting any antihistamines drugs were included for cytokines assays, estimation of IgE and SOD activity assay. These scabietic patients were divided according to onset of symptoms (or duration of scabies symptoms) into: early scabietic patients (30 patients), who had scabies for less than 1 month; and late scabietic patients (30 patients), who had scabies for more than 1 month. Control group consists of 28 generally healthy and non-allergic volunteers. Informed consent was obtained from all participants in the study. The numbers of males and females of the scabietic patients and the control were chosen nearly equal; to diminish the variations between the two groups as shown Table (1). The same was done in age groups of the studied groups (Table 2).

Table (1): Distribution of the studied groups by gender.

| Gender  | Duration of symptoms |          | Control No. |
|---------|----------------------|----------|-------------|
|         | Early No.            | late No. |             |
| Males   | 18                   | 17       | 14          |
| Females | 12                   | 13       | 14          |
| Total   | 30                   | 30       | 28          |

Table (2): Distribution of the studied groups by age.

| Age (yr) | Duration of symptoms |          | Control No. |
|----------|----------------------|----------|-------------|
|          | Early No.            | Late No. |             |
| 10-19    | 12                   | 12       | 9           |
| 20-29    | 6                    | 6        | 11          |
| 30 -39   | 7                    | 6        | 4           |
| 40-65    | 5                    | 6        | 4           |
| Total    | 30                   | 30       | 28          |

## 2.1 Methods

### 1. Routine Stool Examination

Routine stool analysis was performed to the subjects who included in cytokines, IgE and SOD activity assays. Any subject had a positive result for parasitic infection was excluded from the study.

### 2. Collection of Blood Samples

Approximately 5 ml of venous blood was taken from the subjects (60 scabietic patients and 28 controls) using sterile syringes, divided into two parts: 1 ml in EDTA tubes for eosinophils (%), the rest was transferred to serum tube and left (for 30-60 minutes) to clot. Serum was separated by centrifugation at 3000 rpm for 5 minutes. Sera were obtained and divided into 6 parts using Eppendorf tubes and stored frozen at -20° C (it is important to avoid freeze-thaw cycles) till used for cytokines, IgE and SOD activity assays.

### 3. Haematological Assessments

The haematological parameters were performed using Cell-Dyn Ruby (Abbott Diagnostics, USA) instrument in haematology laboratory of Al-Sader Medical City. Ruby is a fully automated haematology analyzer performing complete blood count (CBC). EDTA blood sample was placed in the aspirator of the instrument. Then start key on the instrument was pressed and the blood sample was aspirated. Result was provided within 1 minute on the LCD display and printed out.

#### 4. Cytokines Assay

Cytokines level was quantitatively determined in 60 sera from patients with scabies and 28 sera from controls. These cytokines were human IL-1 $\beta$ , TNF- $\alpha$ , IL-4, IL-5 and using commercially available kits (RayBiotech Inc., USA), through Enzyme-Linked Immunosorbant Assay (ELISA) technique, using bio Elisa reader ELx 800 in virology laboratory of Al-Sader Medical City.

The principle of cytokine assays involved an antibody specific for human IL-x coated on 96-well plate. Standards and samples are pipetted into the wells and IL-x present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human IL-x antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells, then washed again. TMB substrate solution is added to the wells and color develops in proportion to the amount of IL-x bound. The Stop Solution changes the color from blue to yellow, the intensity of the color is measured at 450 nm. Cytokine concentrations were determined by the graph plotted from standards provided with each kit.

#### Assay Procedures

All cytokines assays were performed according to manufacturers' instructions. All the reagents and samples were brought to room temperature before use. One hundred  $\mu$ l of standards and samples were added into appropriate wells using a multi-channel pipette. The wells were covered with a sealing tape and incubated for 2.5 hours at room temperature. The microplate was washed four times with 300  $\mu$ l of Wash Solution using bio Elisa washer ELx50. After the last wash, the microplate was inverted against clean paper towels. One hundred  $\mu$ l of prepared biotinylated antibody were added to each well, and incubated for 1 hour at room temperature with gentle shaking. The solution was discarded. Washing step was repeated as described above. One hundred  $\mu$ l of prepared Streptavidin solution were added to each well, and incubated for 45 minutes at room temperature with gentle shaking. The solution was discarded. Washing step was repeated as described above. One hundred  $\mu$ l of TMB One-Step Substrate reagent were added to each well, incubated for 30 minutes at room temperature in the dark with gentle shaking. Fifty  $\mu$ l of Stop Solution were added to each well, then absorbance was read in bio Elisa reader ELx800 at a wavelength of 450 nm immediately.

#### 5. Total Human IgE Test

Sixty sera from patients with scabies and 28 sera from controls were involved in total human IgE quantitative test kit (Diagnostic automation, Inc., USA) through Enzyme-Linked Immunosorbant Assay (ELISA) technique, using bio Elisa reader ELx 800 in virology laboratory of Al-Sader Medical City. Principle of the test is based on solid phase enzyme-linked immunosorbent assay. The assay system utilizes one anti-IgE antibody for solid phase (microtiter wells) immobilization and another anti-IgE antibody in the antibody-enzyme

(horseradish peroxidase) conjugate solution. Serum is added to the IgE antibody coated microtiter wells and incubated with the Zero Buffer. If human IgE is present in the serum, it will combine with the antibody on the well. The well is then washed to remove any residual test specimen, and IgE antibody labelled with horseradish peroxidase (conjugate) are added. The conjugate will bind immunologically to the IgE on the well, resulting in the IgE molecules being sandwiched between the solid phase and enzyme-linked antibodies. After an incubation at room temperature, the wells are washed with water to remove unbound labelled antibodies. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue colour. The colour development is stopped with the addition of 2N HCl, and the color is changed to yellow then absorbance is read at 450 nm. The concentration of IgE is directly proportional to the color intensity of the test sample. Total IgE concentrations were determined by the graph plotted from standards provided with the kit.

#### Assay Procedures

All reagents and samples were brought to room temperature before use. Twenty  $\mu\text{l}$  of standards and samples were dispensed into appropriate wells. One hundred  $\mu\text{l}$  of Zero Buffer were dispensed into each well and thoroughly was mixed for 10 seconds, then the wells were incubated at room temperature for 30 minutes. The incubation mixture was removed and microtiter wells was washed 5 times with washing buffer using bio Elisa washer EL x50. After the last wash, the microtiter wells was stroked sharply onto absorbent paper to remove all residual water droplets. One hundred and fifty  $\mu\text{l}$  of Enzyme Conjugate Reagent were dispensed into each well, then the wells were incubated at room temperature for 30 minutes. The incubation mixture was removed, the microtiter wells was washed as described above. One hundred  $\mu\text{l}$  TMB solution were dispensed into each well, with gentle mix for 5 seconds and incubated at room temperature in the dark for 20 minutes. The reaction was stopped by adding 100 $\mu\text{l}$  of Stop Solution to each well, with gentle mix for 30 seconds. Absorbance was read at 450 nm with a microtiter reader.

#### 6. Superoxide Dismutase (SOD) Activity Assay

Forty sera from scabietic patients (20 sera from early scabietics, 20 sera from late scabietics) and 20 sera from healthy controls were involved in the measurement of SOD activity as a marker of oxidative stress-antioxidant defence. Principle of the assay is based on the ability of SOD to inhibit the reduction of nitro-blue tetrazolium (NBT) to NBT-diformazan via superoxide radicals (Beyer & Fridovich, 1987). SOD reduces the superoxide radical concentration and thereby lowers the rate of NBT-diformazan formation, which absorbs light at 560 nm.

The percent inhibition of the formation of NBT-diformazan by SOD is represented by the activity of SOD. The reaction velocity depends largely on somewhat variable assay conditions such as light intensity and reaction temperature (Liocher & Fridovich, 2007).

#### Preparation of SOD Activity Assay Solutions

1. Solution 1 (Working Phosphate Buffer): This solution was prepared by using two solutions:

A. Solution A (Dipotassium phosphate solution ( $\text{K}_2\text{HPO}_4$ )): prepared by dissolving 8.709 g of  $\text{K}_2\text{HPO}_4$  in 250 ml of distilled water, then the volume was completed to one liter.

B. Solution B (Potassium dihydrogen phosphate solution ( $\text{KH}_2\text{PO}_4$ )): prepared by dissolving 6.805 g of  $\text{KH}_2\text{PO}_4$  in 250 ml of distilled water, then the volume was completed to one liter. Then 800 ml of solution A were mixed with 200 ml of solution B; then, 0.0373 g of EDTA and 0.25 ml of Triton x-100 were added. The pH was adjusted to 7.8 by using 1N of NaOH.

2. Solution 2 (L-Methionine solution): prepared by dissolving 0.3 g of methionine in 10 ml of distilled water.

3. Solution 3 (Nitro-Blue Tetrazolium (NBT)): prepared by dissolving 14.1 mg of NBT in 10 ml of distilled water.

4. Solution 4 (Triton x-100): prepared by mixing 1 ml of triton to small amount of distilled water, then the volume was completed to 100 ml.

5. Solution 5 (Reaction Mixture Solution): prepared by well mixing 117 ml of solution 1, 1.5 ml of solution 2, 1 ml of solution 3, and 0.75 ml of solution 4.

6. Solution 6 (Sodium Cyanide Solution): prepared by dissolving 11 mg of sodium cyanide in 10 ml of distilled water.

7. Solution 7 (Riboflavin Solution): prepared by dissolving 1.1 mg of riboflavin in 10 ml of distilled water.

#### Assay Procedures

Three ml of Solution 5 were added to 39  $\mu\text{l}$  of Solution 6 in clean test tubes according to the number of samples used, including the control tube (Blank). (0.2) ml of serum was added to the test tubes (except Blank) and 0.2 ml of Solution 1 to the Blank. Then 0.523 ml of Solution 1 and 37.8  $\mu\text{l}$  of Solution 7 were added to all test tubes (including Blank). Each test tube contents were mixed using vortex. The absorbance was immediately read spectrophotometrically at 560 nm wavelength. All test tubes were placed in a light box providing uniform light intensity (A foil-lined box with an internally mounted fluorescent bulb). The tubes were incubated for 10 minutes, then absorbance was read again by the same method and wavelength above, the difference between two values (which represented percent inhibition) was calculated (Beyer & Fridovich, 1987).

### 3. Statistical Analysis

Data were statistically analyzed by using Statistical Package for Social Sciences (SPSS version 15). Data were expressed as mean $\pm$  standard deviation (SD). For comparison, Duncan's multiple range test was used for several means. Pearson Correlation ( $r$ ) was performed to determine the correlation between criteria.  $p$ -value  $\leq 0.05$  was considered statistically significant;  $p$ -value  $\leq 0.01$  was considered highly statistically significant.

### 4. Results

Cytokines, total IgE level, SOD activity and eosinophils (%) were estimated in 60 scabietic patients (30 early and 30 late scabietic patients). They were 35 males and 25 females, their age ranging from 10-65 years with a mean age  $25.2 \pm 11.7$  (mean $\pm$  SD). As well as 28 healthy controls (14 M & 14 F), their age ranging from 12 to 54 years with a mean age  $25.17 \pm 10.1$  (mean $\pm$  SD).

The results of present study showed a highly significant elevation in IL-1 $\beta$ , TNF- $\alpha$ , IL-4, IL-5, IgE level, SOD activity and eosinophils (%) in the early and late scabietic patients as compared with healthy control group ( $p < 0.05$ ), as illustrated in Table (3).

Table (3): Studied parameters in scabietic patient and control groups.

| Parameters       | IL-1 $\beta$         | TNF- $\alpha$          | IL-4                  | IL-5                  | IgE                     | SOD                    | Eosinophils (%)      |
|------------------|----------------------|------------------------|-----------------------|-----------------------|-------------------------|------------------------|----------------------|
| Studied group    |                      |                        |                       |                       |                         |                        |                      |
| Early scabietics | 8.12 $\pm$ 1.41      | 61.13 $\pm$ 10.02<br>B | 35.42 $\pm$ 9.23<br>A | 40.19 $\pm$ 4.33<br>A | 521.63 $\pm$ 62.51<br>A | 0.31 $\pm$ 0.11<br>A   | 6.0 $\pm$ 1.08<br>A  |
| Late scabietics  | 9.09 $\pm$ 1.64<br>A | 69.6 $\pm$ 14.26<br>A  | 12.0 $\pm$ 4.25<br>B  | 13.03 $\pm$ 1.82<br>B | 61.28 $\pm$ 346.36<br>B | 0.03 $\pm$ 0.07<br>B   | 4.20 $\pm$ 0.85<br>B |
| Control          | 1.95 $\pm$ 0.77<br>C | 32.64 $\pm$ 5.18<br>C  | 5.06 $\pm$ 0.98<br>C  | 6.17 $\pm$ 1.08<br>C  | 98.43 $\pm$ 15.37<br>C  | 0.016 $\pm$ 0.008<br>C | 2.93 $\pm$ 0.58<br>C |
| Probability      | $\leq$ 0.0001        | $\leq$ 0.0001          | $\leq$ 0.0001         | $\leq$ 0.0001         | $\leq$ 0.0001           | $\leq$ 0.0001          | $\leq$ 0.0001        |

- Data are expressed as mean  $\pm$  standard deviation (SD)
- The different letters in the same column indicate significant difference between groups based on Duncan's Multiple Range Test.

In early scabietic patients, a correlation between serum IgE and all the studied cytokines has been established. IgE positively correlated with IL-1 $\beta$ , TNF- $\alpha$ , IL-4 and IL-5 ( $r = 0.156$ ,  $r = 0.019$ ,  $r = 0.246$  and  $r = 0.384$  respectively) as shown in Table (4).

Table (4): Correlation of IgE (IU/ml) with other studied cytokines in early scabietic patients.

| Cytokines Level (pg/ml)                                 |                     | IL-1 $\beta$    | TNF- $\alpha$     | IL-4             | IL-5             |
|---|---------------------|-----------------|-------------------|------------------|------------------|
| IgE(IU/ml)<br>62.51 $\pm$ 521.63                        | Mean $\pm$ SD       | 8.12 $\pm$ 1.41 | 61.13 $\pm$ 10.02 | 35.42 $\pm$ 9.23 | 40.19 $\pm$ 4.33 |
|   | Pearson correlation | 0.156           | .0190             | 0.246            | 0.384            |
|   | Sig. (2-tailed)     | .0540           | 0.245             | .8090            | 0.659            |
| Correlation is significant at the 0.05 level (2-tailed) |                     |                 |                   |                  |                  |

In late scabietic patients, IgE had negative correlation with IL-1 $\beta$ , TNF- $\alpha$  ( $r = -0.096$  and  $r = -0.046$ ), whereas it had positive correlation with IL-4 and IL-5 ( $r = 0.214$  and  $r = 0.148$  respectively), Table (5).

Table (5): Correlation of IgE (IU/ml) with other studied cytokines in late scabiectic patients.

| Cytokines Level (pg/ml)                                 |  | IL-1 $\beta$    | TNF- $\alpha$    | IL-4            | IL-5             |
|---|--|-----------------|------------------|-----------------|------------------|
| IgE(IU/ml)<br>$\pm 61.28346.36$                         | Mean $\pm$ SD                          | 9.09 $\pm$ 1.64 | 69.6 $\pm$ 14.26 | 12.0 $\pm$ 4.25 | 13.03 $\pm$ 1.82 |
|   | Pearson correlation<br><i>r</i> -value | -0.096          | -0.046           | 0.214           | 0.148            |
|   | Sig.<br>(2-tailed)                     | .6150           | 0.811            | 0.255           | .4350            |
| Correlation is significant at the 0.05 level (2-tailed) |  |                 |                  |                 |                  |

SOD showed negative correlation with IL-1 $\beta$  and TNF- $\alpha$  ( $r = -0.029$  and  $r = -0.171$  respectively) whereas, positive correlation appeared with IL-4 and IL-5 ( $r = 0.442$  and  $r = 0.255$  respectively) in early scabiectic patients (Table 6).

Table (6): Correlation of SOD with the studied cytokines in early scabiectic patients.

| Cytokines Level (pg/ml)                                 |  | IL-1 $\beta$    | TNF- $\alpha$     | IL-4             | IL-5             |
|---|--|-----------------|-------------------|------------------|------------------|
| SOD<br>$0.31 \pm 0.11$                                  | Mean $\pm$ SD                          | 8.12 $\pm$ 1.41 | 61.13 $\pm$ 10.02 | 35.42 $\pm$ 9.23 | 40.19 $\pm$ 4.33 |
|   | Pearson correlation<br><i>r</i> -value | -0.029          | .171-0            | 0.442            | 0.255            |
|   | Sig.<br>(2-tailed)                     | .9030           | .4710             | .05*0            | .2790            |
| Correlation is significant at the 0.05 level (2-tailed) |  |                 |                   |                  |                  |

Table (7) showed negative correlation of SOD with IL-1 $\beta$  and TNF- $\alpha$  ( $r = -0.039$ ,  $r = -0.073$  respectively). Positive correlation was demonstrated with IL-4 and IL-5 ( $r = 0.169$ ,  $r = 0.064$  respectively) in late scabiectic patients.

Table (7): Correlation of SOD with the studied cytokines in late scabiectic patients.

| Cytokines Level (pg/ml) |  | IL-1 $\beta$    | TNF- $\alpha$    | IL-4            | IL-5             |
|-------------------------|--|-----------------|------------------|-----------------|------------------|
| SOD<br>$\pm 0.030.07$   | Mean $\pm$ SD                          | 9.09 $\pm$ 1.64 | 69.6 $\pm$ 14.26 | 12.0 $\pm$ 4.25 | 13.03 $\pm$ 1.82 |
|                         | Pearson correlation<br><i>r</i> -value | -0.039          | .073-0           | .1690           | 0.064            |



|   |                    |       |       |       |       |
|---|--------------------|-------|-------|-------|-------|
|   | Sig.<br>(2-tailed) | .8820 | .7810 | .5180 | 0.807 |
| Correlation is significant at the 0.05 level (2-tailed) |                    |       |       |       |       |

In early scabietic patients, eosinophils (%) positively correlated with all the studied cytokines IL-1 $\beta$ , TNF- $\alpha$ , IL-4 and IL-5 ( $r = 0.034$ ,  $r = 0.177$ ,  $r = 0.225$  and  $r = 0.284$  respectively) as illustrated in Table (8).

Table (8): Correlation of eosinophils (%) with the studied cytokines in early scabietic patients.

| Cytokines<br>Level (pg/ml)                              |   | IL-1 $\beta$    | TNF- $\alpha$     | IL-4             | IL-5             |
|---|---|-----------------|-------------------|------------------|------------------|
| Eosinophils (%)<br>6.0 $\pm$ 1.08                       | Mean $\pm$ SD                             | 8.12 $\pm$ 1.41 | 61.13 $\pm$ 10.02 | 35.42 $\pm$ 9.23 | 40.19 $\pm$ 4.33 |
|   | Pearson<br>correlation<br><i>r</i> -value | .0340           | .1770             | 0.225            | .2840            |
|   | Sig.<br>(2-tailed)                        | .8590           | .1390             | 0.231            | .3310            |
| Correlation is significant at the 0.05 level (2-tailed) |   |                 |                   |                  |                  |

As shown in Table (9), eosinophils (%) correlated positively with IL-1 $\beta$ , TNF- $\alpha$ , IL-4 and IL-5 ( $r = 0.272$ ,  $r = 0.250$ ,  $r = 0.019$  and  $r = 0.175$ ) in late scabietic patients.

Table (9): Correlation of eosinophils (%) with the studied cytokines in late scabietic patients.

| Cytokines<br>Level (pg/ml)                              |   | IL-1 $\beta$    | TNF- $\alpha$    | IL-4            | IL-5             |
|---|---|-----------------|------------------|-----------------|------------------|
| Eosinophils (%)<br>4.20 $\pm$ 0.85                      | Mean $\pm$ SD                             | 9.09 $\pm$ 1.64 | 69.6 $\pm$ 14.26 | 12.0 $\pm$ 4.25 | 13.03 $\pm$ 1.82 |
|   | Pearson<br>correlation<br><i>r</i> -value | .2720           | 0.250            | .0190           | 0.175            |
|   | Sig.<br>(2-tailed)                        | .1460           | .1830            | .9210           | .3560            |
| Correlation is significant at the 0.05 level (2-tailed) |   |                 |                  |                 |                  |

## 5. Discussion

The present study demonstrated that IL-1 $\beta$  level had highly significant difference in early and late scabietic patients as compared with control. Proinflammatory cytokine production appears to be the effector key in the pathogenesis of scabies. Previous studies showed that IL-1 $\beta$  can be generated from the inflammation of the skin caused by physical stimulation of the burrowing mites (Portugal et al., 2007). Similarly, *S. scabiei* extract also induces up-regulated secretion of IL-1 $\beta$ , IL-6, IL-8, and TNF- $\alpha$  from peripheral blood mononuclear cells from

human donors (Arlian et al., 2004). Mites burrowing into the epidermis on the surface of HSEs model induced secretion of significantly increased amounts of IL-1 $\alpha$ , IL-1 $\beta$ , and interferon- $\gamma$  (IFN- $\gamma$ ) onto the skin-equivalent surface (Arlian et al., 1996; Lalli et al., 2004; Morgan & Arlian, 2010). Because IL-1 $\beta$  is one of the potent inducers of inflammation (proinflammatory), this cytokine is key initiator of the inflammatory/immune reaction against scabies. TNF- $\alpha$  level showed a highly significant increased in both scabietic patient groups as compared with the control group. These results were similar to that reported by (Morsy et al., 1995). *S. scabiei* extract induces up-regulated secretion of IL-1 $\beta$ , IL-6, IL-8, and TNF- $\alpha$  from peripheral blood mononuclear cells from human donors (Arlian et al., 2004). Portugal et al. (2007) showed that TNF- $\alpha$  can be generated due to physical stimulation of the burrowing mites from the inflammation of the skin. TNF- $\alpha$  level tended to be higher in late scabietic patients than its level in early scabietic patients; that may be explained by the capability of SOD to reduce the emission and expression of TNF- $\alpha$  (Diehl et al., 2009), since SOD level was higher in early scabietic patients than its level in late patients. Serum levels IL-4 were significantly elevated in both groups of scabietic patients in comparison to the healthy controls. IL-4 triggering in early scabietic patients was 3-fold more than its level in late scabietic patients. The elevated level of IL-4 suggests a preferential activation of TH2 cells in the scabietic patients. IL-4 regulates IgE production, controls production and recruitment of mast cells and eosinophils. Mast cells and activated eosinophils are also able to produce IL-4 that can thus amplify the inflammatory response (Zamorano et al., 2003). Walton et al., (2004) demonstrated a statistically significant elevation of IL-4 in scabietic patients as compared in controls. The present study showed that there was a highly significant elevation in serum level of IL-5 in two groups of scabietic patients as compared with controls. The same was suggested by Elmaraghy & El Meghawry (2011) who found that serum IL-5 was a highly significant elevation in the scabies group compared to the healthy normal controls. Another study showed no significant difference in IL-5 levels between the ordinary scabietic patients and control groups, but there was significant difference in IL-5 levels between the crusted scabietics and the controls (Walton et al. 2010). IgE level in both scabietic patient groups showed a significant elevation compared to its level in control group. This explains type I hypersensitivity reaction in scabietic patients which is responsible for expelling the parasite and products from the borrows by the intense itching and scratching, which in turn leads to sudden reduction in parasite density at time when itching started (Al-Rawi, 2000). The sensitization of the host to the mites and its products may be has an important role in pathogenesis of scabies (Van Nest, 1986). This findings agreed with other works (Arlian et al., 1994; Morsy et al., 1995; Senol et al., 1997; Al-Dabbag & Al-Dabbag, 2006; Najem et al., 2009; Elmaraghy & El Meghawry, 2011). Ibrahim et al. (2012) revealed that IgE concentration was lower than its cutoff concentration in 50% of scabietic patients, whereas the rest scabietic patients had elevated concentration of IgE. While no significant differences were reported between scabietic patients and control group in study done by Nassef et al. (1991). In the present study, IgE level was higher in early scabietic patients than its level in late scabietics. The same was suggested by Galosi et al. (1982), who found that 93.3% of the early scabietic patients had elevated IgE concentration while only 30% of late scabietic patients showed increased IgE concentrations. Another study demonstrated that no significant correlation was found between IgE level and the duration of scabies infestation (Al-Dabbag

& Al-Dabbag, 2006). The present study showed significant increase in SOD in scabietic patients versus control group. Triggering of proinflammatory cytokines can lead to excessive generation of the reactive oxidants, free radicals which include reactive oxygen (Bickers & Athar, 2006), steady state is maintained immediately by SOD and other antioxidant scavenging systems (Kumar et al., 2010). The increased levels of SOD in early scabietic patients may indicate an enhancement of this antioxidant to cope with free radical generation and to protect tissues from damage. Although this homeostatic defense is highly effective, but it has limited capacity and can be depleted leading to increased oxidative stress (Bickers & Athar, 2006). The highest level of SOD refers to exposure to severe oxidative stress. This stress factor may have a role in the pathogenesis of scabies. Decreased SOD level in late scabietic patients may be consumed as free radical scavengers during the oxidative process when the duration of scabies infestation increased. Therefore, it can be hypothesized that SOD formation is enhanced in early phase of scabies and begins to decline when the duration of scabies is persisted. A study done by Dimri et al. (2010) demonstrated decreased SOD in sheep with psoroptic mange. Another study observed increased SOD in dogs with demodicosis (Dimri et al., 2008). SOD levels were higher in mild naturally infested camels with *Sarcoptes scabiei* and lower in moderate and severely infested camels compared to controls (Saleh et al., 2007). Eosinophils were significantly increased in both groups of scabietic patients as compared to controls. Increased eosinophils in scabietic patients could be attributed to allergic response against the mites and their products. Eosinophils have an important role in non-specific (innate) immunity. Its elevation means that this kind of immunity is efficient. Significant increase in eosinophils (%) in the scabies group compared to the healthy controls in the current work agrees with results of previous studies (Najem et al., 2009; Elmaraghy & El Meghawry, 2011). Early scabietic patients were higher eosinophils (%), whereas in late ones, eosinophils (%) declined but it was still higher in comparison with controls. As IL-5 promotes the maturation of eosinophils and it is a key factor for eosinophilia, the early scabietic patients had higher eosinophils than the late ones. Similar result was obtained by Al-Dabbag & Al-Dabbag (2006). This could be due to tolerance of the patients or to the decrease in scabies severity.

## **5.1 Correlation between Criteria**

### **5.1.1 Correlation of IgE with Cytokines**

Data of the present study found that IgE positively correlated with IL-1 $\beta$ , TNF- $\alpha$ , IL-4 and IL-5 in early scabietic patients (Table 4), whereas IgE had negative correlation with IL-1 $\beta$ , TNF- $\alpha$ , and positive correlation with IL-4 and IL-5 in late scabietic patients as demonstrated in (Table 5). All allergic diseases are characterized by a specific pattern of inflammation that is largely driven by IgE-dependent mechanisms. This would result in an increase in the number of TH2 cells and the cytokines IL-4 and IL-5 (Mate's *et al.*, 2000a). IL-4 has marked inhibitory effects on the expression and release of the proinflammatory cytokines, whereas TH2 immune response and IgE are activated. This could be the probable reason of positive correlation was observed between the serum IgE and cytokines in early scabietic patients and negative correlation with IL-1 $\beta$ , TNF- $\alpha$  in late scabietic patients. This finding demonstrated that host immune response in early scabietics resembles a nonprotective TH2 allergic

response, whereas in late scabietic patients; It resembles a TH1 cell-mediated protective response.

### 5.1.2 Correlation of SOD with Cytokines

SOD showed negative correlation with IL-1 $\beta$ , TNF- $\alpha$ , whereas positive correlation with IL-4, IL-5 in early and late scabietic patients (Table 6 and Table 7 respectively). Symptoms of scabies onset when histamine (and other mediators) released from mast cells due to ROS production. The production of ROS has been stimulated by proinflammatory cytokines such as IL-1 $\beta$ , TNF- $\alpha$  and IL-2. SOD has a down-regulatory effect on proinflammatory cytokines. Therefore the enhancement of SOD activities could act as a defence mechanism against ROS and histamine release is blocked (Mate's *et al.*, 2000b). Many previous studies have demonstrated that there is an efficacy of SOD in reducing the emission and expression of numerous proinflammatory cytokines (IL-1 $\beta$ , TNF- $\alpha$ ). At the same time, IL-4 and IL-5 are secreted by TH2 lymphocytes and mast cells (Kouro & Takatsu, 2009). These findings suggest the negative relations of SOD with IL-1 $\beta$  and TNF- $\alpha$ , meanwhile, positive relations of SOD with IL-4 and IL-5 in early and late scabietic patients.

### 5.1.3 Correlation of Eosinophils (%) with Cytokines

In early scabietic patients, eosinophils (%) positively correlated with all the studied cytokines IL-1 $\beta$ , TNF- $\alpha$ , IL-4 and IL-5 as illustrated in Table (8) as well as in late scabietic patients (Table 9). Eosinophils (%) correlated positively with TNF- $\alpha$ . As TNF- $\alpha$  is known to activate eosinophils by inducing self GM-CSF autocrine stimulatory effect (Levi-Schaffer *et al.*, 1998), the elevation of TNF- $\alpha$  is probably activate eosinophilic responses among the scabietic patients. In allergic circumstances, the cytokines secreted by TH2 cells, including IL-4, control production and recruitment of mast cells and eosinophils. Mast cells and activated eosinophils are also able to produce IL-4 that can thus amplify the inflammatory response (Zamorano *et al.*, 2003). Eosinophils (%) correlated positively with IL-5. Secretion of IL-5 has been closely linked with eosinophil recruitment. Over-expression of IL-5 significantly increases eosinophil numbers and antibody levels *in vivo* (Kouro & Takatsu, 2009). IL-5 induces terminal maturation of eosinophils, prolongs eosinophil survival by delaying apoptotic death, possesses eosinophil chemotactic activity, increases eosinophil adhesion to endothelial cells and enhances eosinophil effector functions (Kouro & Takatsu, 2009).

## 6. Conclusions

Understanding the immunology of scabies is still in its infancy. The findings of the current study demonstrated that host immune response in early scabietic patients resembles a nonprotective TH2 allergic response, whereas in late scabietic patients; It resembles a TH1 cell-mediated protective response.

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