Estimation of total IgE, blood eosinophils and phagocytic activity in human scabies

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ABSTRACT

Background: Scabies is a contagious disease. It can be easily misdiagnosed with many other dermatological conditions.

Objective: This study was designed to evaluate some laboratory methods, (e.g. IgE,NBT), which might be useful in confirming the diagnosis of scabies, and to follow up the response of the patients after treatment.

Patients and method: This study was conducted on 103 patients from Al-Zenjelli Custody (jail) in Mosul City (Iraqi) over a period of 6 months (Oct. 2001 to April 2002). Blood samples from 103 scabetic patients and 40 control subjects were analysed by using various tests including total white blood cell count, eosinophil count and nitroblue tetrazolium (NBT) positive neutrophils count. Plasma total IgE levels were measured for only 70 patients and 20 control subjects by using Enzyme Linked Immunosorbert Assay (ELISA).

Results: Plasma total IgE level showed a significant raise in patients with scabies in comparison to control group. (p<0.05) The NBT test revealed a significant raise in both treated and non-treated patients in comparison to control group. (p<0.05) Also, the untreated patients showed a higher NBT positive neutrophils than the treated ones. Eosinophil counts were higher in patients with scabies than the control subjects in the first few postinfection days. (p<0.001) Total white blood cell counts revealed no significant variation between patients and control groups.

Conclusion: The results indicate that nitroblue tetrazolium (NBT) test was the best in comparison to other tests. It was used to study the role of phagocytosis in scabies. Its increase reflected an efficient innate immunity. This test might explain to us some immunopathological aspects of this disease and its symptoms which might help in diagnosis and follow up of patients with scabies.

Key words: Scabies, IgE, NBT
Scabies is a contagious skin disease caused by mites. Sarcoptes scabiei lives in the superficial epidermal layers, where it feeds. It is transmitted from person to person, often through close personal contact and in adults sexual transmission is common. In Iraq, studies on scabies among various groups including prisoners have been previously conducted. Most of these studies provided information regarding the frequency and distribution of the disease in relation to various epidemiological parameters. Both humoral and cell mediated immune responses were studied. In scabies patients, Types I and IV reactions associated with elevation of IgE and eosinophils were seen. Very heavy infections may develop in immunocompromised individuals. Among patients who are unable to care adequately for themselves.

This study was conducted to evaluate some immunological and haematological parameters during scabies infestation which could aid in the laboratory diagnosis of scabies, and also could be used as a follow up test to evaluate the efficacy of treatment in scabetic patients. However, the IgE levels in patients with scabies have been described before (17,20), but to the best of our knowledge, the association between scabies and nitro-blue tetrazolium test (NBT) has not been mentioned in previous medical literature.

Materials and methods
Subjects: One hundred and three patients including 100 males and 3 females, their ages varied from 16 to 66 years (mean age 32.8 ± 12 years) from Al-Zanjili Custody (jail) in Mosul city were investigated for scabies. The study was conducted over a period of six months from October 2001 to April 2002. A questionnaire form was used which included information about age, sex, residence, occupation, size of the family, sequence of the disease, educational status, marital status and possible source(s) of infestation. All patients were examined clinically by the Jail’s physicians. The clinical diagnosis was based on distribution of skin rash, duration of infection, severity of nocturnal itching, history of contact with cases, characteristics of skin lesions, and finding of burrows. As a control group 40 apparently healthy individuals, their ages also ranged from 15 to 48 years (mean age 28.6 ± 8.7 years) were randomly selected from workers and university students. None of these volunteers had any past or present history suggesting allergic disease and/or helminth infections. Venous blood samples were collected from both patients and controls. Sampling of blood was made by antecubital venipuncture using a sterilized disposable needle. The venous blood were collected by using EDTA tubes. The following parameters were studied:

1. Nitro-blue tetrazolium (NBT) test: A yellow water soluble nitro-blue tetrazolium (NBT) dye (from BDH biochemical company, England) was used to determine the phagocytic activity of neutrophils. A modification of a method proposed by Perk (21) was used. A solution of 0.2% of NBT was prepared. This solution is stable for more than a year if frozen at -20°C. Then two equal volumes of NBT solution and phosphate buffer saline were mixed together in a test tube and the fresh blood was added in an equal volume to the NBT buffer mixture and mixed gently. The mixture was incubated at 37°C for 30-40 minutes. Next a routine blood film was done, and stained with Leishman's stain. Two hundred neutrophils were counted, and the percentage of neutrophils with a
2. Total white blood cell count and eosinophil count: A white blood cell (WBC) count was done using the improved Neubauer Chamber, and the numbers were expressed as $x \times 10^9$ / L. Routine blood film was prepared and stained with Leishman’s stain. The total white blood cell count was calculated and the percentage of eosinophils was determined from the 200 counted cells.

3. Serum total IgE (S TgIE) determination: The levels of S TgIE of patients and controls were measured by using Enzyme Linked Immunosorbent Assay (ELISA) kit (Euroimmun - Germany), which provides a quantitative in vitro assay for human IgE antibodies. The test kit contains microtiter strips each with eight break-off reagent wells coated with polyclonal antibodies against human IgE.

The procedure for the determination of total IgE concentration in serum was, as according to the recommendations of the manufacturers. In the first reaction step, diluted patient samples were incubated in the wells. IgE included in the sample would bind to the antibodies. A second incubation was carried out using an enzyme – labeled rabbit IgE (enzyme conjugate). The determination of the IgE concentration in sera was measured by means of a calibration curve using the calibration sera 1 to 4 containing different concentrations of IgE (0, 10, 100, 500 IU / ml).

The total IgE was expressed in IU / ml. The Log$_{10}$ mean (geometric mean) ± standard deviation was used for S TgIE.

4. Parasitological examination for S. scapularis: The adhesive cellulose (Graham’s Scotch tape) method was used as a diagnostic test of choice for identification of E. vermicularis (23). In the present study, a modification of this technique was used to diagnose itch mite. This was done by scratching the skin of study subjects with the sharp edge of the slide to identify the parasite. Potassium hydroxide (KOH) in a form of spray was used to dissolve the skin parts attached to the adhesive tape. Mites or mite parts such as legs, eggs or even faecal pellets could be demonstrated under the microscope.

5. Stool examination: Faeces were collected from subjects. A direct stool examination (wet mount) was applied just to exclude any helminthic infections that might affect the measuring parameters. Sodium acetate-acetic acid-formalin (SAP) solution was used as a preservative that prevents the cysts and ova from lysis in stool samples (22). Stool samples were examined by a direct method, and if negative, a concentration method by zinc sulphate was used (23). Any subject with a positive result for parasitic infection was excluded from the study. Patients infested with lice or had allergic or atopic diseases (like asthma, eczema, allergic rhinitis... etc.) were also excluded. A total of 20 cases were subsequently excluded despite being infested with scabies.

<table>
<thead>
<tr>
<th>Table (1): Sites of scabies infestation on the body</th>
<th>No. of patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>All over the body</td>
<td>41</td>
</tr>
<tr>
<td>Thigs, hips and groin</td>
<td>29</td>
</tr>
<tr>
<td>Trunk</td>
<td>21</td>
</tr>
<tr>
<td>Shoulder, hands and neck</td>
<td>11</td>
</tr>
<tr>
<td>Genital</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>103</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table (2): Scabies profile according to various tests in this study</th>
<th>Test</th>
<th>Subjects</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nitro-blue tetrazium</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total white blood cell count</td>
<td>4+</td>
<td>0+</td>
<td>0+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Eosinophil</td>
<td>4+</td>
<td>0+</td>
<td>0+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IgE</td>
<td>4+</td>
<td>0+</td>
<td>0+</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

A. Acute untreated patients, B. Chronically infected (1-2 months) and/or inadequately treated C. Chronically infected (> 2 months), D. Patients with allergic manifestation, E. Control group.
Results

The site of infestation with scabies on the body surface of study subjects is shown in Table 1. The infestation of scabies all over the body was demonstrated in 41 patients (39.8%), while in 24 patients (28.1%) the sites of infestation were found on thighs, legs, and groin. Other sites of the body showed low infestation rates.

Patients were divided into 3 groups according to the duration of infestation with scabies. The first group with less than 1 month, the second group with 1-2 months and third group with > 2 months of infestation. The data of immunological and haematological tests were correlated with the duration of scabies and with the treatment.

Figure (3): STiGE level of patients (treated and untreated) and control showed:
- Significant variation between control and patients (both treated and untreated).
- No significant changes between treated and untreated patients.
- Bars with different letters mean significant difference at P < 0.05

Figure (4): NBT positive neutrophils (Mean ± SD of NBT percent) in relation with the duration of infestation.
- Bars with different letters (a, b and c) mean significant difference at P < 0.05.
Serum total IgE: The level of serum total IgE (STIgE) of seventy patients showed no significant changes with the duration of infestation. It staged approximately at the same level since the infestation with S. scabiei was started and stayed to some extent stable as long as there is a contact with the mite and / or its product (Fig 2).

The STIgE levels showed a significant difference (p < 0.05) in patient’s group (both treated and non-treated) versus the control group (20 subjects) as indicated in Fig 3. Also, STIgE levels revealed no significant difference between treated and non-treated groups of patients Z-value = 0.05 and p-value = 0.96, N.S. The geometric means ± S.D of STIgE of both treated (n = 26) and non-treated (n=44) patients were 1.93 ± 0.52 and 1.90 ± 0.51 respectively. The odds ratio was calculated for STIgE between patients and control groups and was found to be 0.4.

Nitra-blue tetrazolium test (NBT): The phagocytic activity of neutrophils was measured by NBT. The results showed significant changes (p < 0.05) between patient’s groups depending on the duration of infestation as shown in (Fig 4). It showed that the NBT-positive neutrophils (Fig 5, stimulated neutrophils) declined in numbers with the increase in the duration of infestation. This figure also shows a...
significant association between patients versus control groups. Patients group showed a higher level of NTB than control group.

The phagocytic activity showed a significant difference (p < 0.05) among treated and untreated groups (Fig. 6). The highest level of NTB-positive neutrophils was found in the untreated group. The treated group showed, to some extent, a lower number of active neutrophils. Both treated and untreated groups had a very significant elevation in active neutrophils versus control group as is shown in Fig. 6.

Total white blood cell count (T.W.B.C). The T.W.B.C showed no significant changes during the progress of the disease. Also, the number of white cells showed no significant changes between the patients and control groups. The mean ± S.D of T.W.B.C in treated (n = 36), and untreated (n=67) were 7986.8 ± 2998.8/mm³ and 9215.8 ± 3065.2/mm³ respectively, with no statistically significant difference (Z-value = 0.41, p-value = 0.68, N.S).

Eosinophils: The differential white blood cell count was concerned only with the estimation of eosinophils. Patients group had significantly higher eosinophils count (p < 0.001) than control groups. Also, there was a significant difference of eosinophil count in patients group during the period of infection, as shown in Fig. 7. The highest rate was demonstrated during the first month of infection, while during the second month eosinophil count of both treated and untreated declined and approximated that of the control group, but during the third month of infection with scabies, eosinophil counts started to rise again.

Discussion
Infection with scabies was found among prisoners, and this may reflect poor hygienic standards and overcrowding. Similar findings were reported from other regions of Iraq.21,28,29 Transmission of the mites is accomplished by direct contact with infected persons or with their clothing or bedding. The spread of infection to different parts of the body as seen in the present study occurs through scratching and manual transfer of the mite by the infected individual. Humoral and cell-mediated immunity play an important role in the pathogenesis of scabies.30,31 Infestation with scabies seems to elevate IgG, IgM and IgE.32,33 Similar findings were noticed in this study regarding IgE level, which was significantly higher in scabetic patients than in controls. However, there were no significant differences in IgE level between treated and untreated patients. Other reports have also demonstrated a significant increase in IgE levels among patients.34,35 This study explains the type I reaction in scabetic patients which is responsible for expelling the mite and its products from burrows by the intense scratching which lead to sudden reduction of parasite density at a time when itching started.36 The sensation of the itch to the mite and its products probably plays an important role in the pathogenesis of the disease.37

The TWBC were tested in order to evaluate the inflammatory response stimulation in scabetic patients, and to correlate this stimulation with the duration of infection. The TWBC showed no significant changes between patients and controls. This is in accordance with the study of other investigators who estimated TWBC in 02 scabetic patients and found 59 patients with normal range. The efficiency of neutrophils as phagocytes is measured by NTB test.26 The nitro-blue tetrazolium dye is converted by the reduction occurring during phagocytosis and forms fomazines deposits.21,29,32 The percentages of NTB positive neutrophils (with fomazine deposits) in normal individuals was recorded to vary between 3% to 11%.23,29 An increase in the positive NTB1% was reported in the majority of bacterial and parasitic infections.25,26,28 This increase may reach up to 75% in acute phase of infection and then decline gradually until return to normal values. Patients with chronic infection of scabies and patients who have an important role in the non-specific (reteate) immunity of the body27 its importance means that this kind of immunity is efficient.

The untreated patients with primary exposure (no previous infection with scabies) showed a higher level of NTB positive neutrophils especially during the first month of exposure to scabies. A decline in NTB count was observed after treatment, but it did not return to normal until a total eradication of the mites from the skin was seen. Patients with chronic infestation of scabies and patients who received inadequate treatment, both showed a significant increase in NTB positive neutrophils count. However, normal individuals with no infection showed a normal value of NTB positive neutrophils count i.e up to 5% of total neutrophils count as shown in Fig. 4 and 5. Therefore, the efficiency of neutrophils for phagocytosis as

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reflected by the NBT test might help in studying some immunopathological aspects of scabies, and the action of immune system against this disease.

This study showed that NBT test could be efficient in the determination of the stage of infestation with scabies, whether molar or chronic, and primary or secondary exposure. NBT test could also be used as a follow-up test of treatment efficacy and patients' compliance. However, other infectious diseases should be excluded which might give false positive reactions in patients with scabies.

In this study, the eosinophil count showed a significant increase during the first month of infestation with scabies. However, the eosinophil count returned to normal range during second and third months of infestation. This result is in accordance with other reports, which showed that there was a correlation between eosinophil count and the duration of infestation, and this count usually returned to normal after successful treatment. Another investigation recorded that about 23.9% of tested patients who were infested with scabies had eosinophilia. The higher eosinophil count could be attributed to allergic response to the irritant chemicals applied for the treatment, i.e., Benzyl benzoate which is a highly irritant lotion.

The second month of infestation showed a lower eosinophil count, but it was still higher in comparison with control. This could be due to tolerance of the patient or due to the decrease in the severity of the disease. The treated patients during the second month of infestation showed approximately same eosinophil counts in comparison with controls, which was expected. The death of S. scabiei mites and the release of its secrets might evoke some allergic responses. The allergic response could also be due to prolonged treatment by topical solutions.

This was an endemic focus for scabies, and many patients were suffering chronically from the disease due to continuous contact with contagious individuals. Once diagnosed, scabies infestations are easy to control, provided the directions of scabicidal treatment are followed.

The suggested scheme (Table 2) for the laboratory diagnosis of scabies provides a flexible and practical choice of tests for better recognition of this dermatological condition.

References


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