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 (Iraq) over a period of 6 months (Oct. 2001 to April 2002). Blood samples from 103 scabietic 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 Immunosorbent 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 subjects 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

الخلاصـــة

الهدف: يعتبر مرض الجرب من الأمراض المنتشرة نسبياً، و من السهولة الخطأ بتشخيص المرض و تفريقه في الكثير من الأمراض الجلدية الأخرى.

مكان إجراء الدراسة و الإطار الزمني لها: أجريت هذه الدراسة على عيّنة من المرضى من سجن الزنجيلي في مدينة الموصل (التسفيرات) و على مدى سبعة أشهر من شهر تشرين الأول في العام 2001 و لغاية نيسان من العام 2002 لغرض تقييم طريقة مختبرية مختلفة أو أكثر مثل فحص الامينوكلوبيولين المناعي (IgE) والفعالية البلعمية (NBT) كاختبار مساعد في عملية تشخيص و متابعة مرض الجرب.

المشاركون و طرائق العمل: تم أخذ عيّنات من الدم من 103 مريض من السجن و 40 عيّنة دم من أشخاص عاديين كعيّنة ضابطة و تقييم حالتهم باستخدام اختبارات مختلفة و المتضمنة التعداد الكلي لكريات الدم البيض، تعداد الحمضات و تعداد الخلايا العدلة الموجبة صبغة النايتروبلوتترازوليوم.

تم قياس المستوى الكلي للغلوبين المناعي صنف – إي – لـ 70 مريض فقط و 20 شخص كعيّنة ضابطة باستعمال طريقة (إليزا). تم أخذ عيّنات من الغائط لكلا المجموعتين المرضى و العيّنة الضابطة لاستبعاد أي إصابة بالديدان المعوية.

النتائج: المستوى الكلي للغلوبين المناعي صنف – إي – أظهر اختلافاً واضحاً بين عيّنة المرضى و العيّنة الضابطة عند مستوى معنوية 0.05، و اظهر اختبار النايتروبلوتترازوليوم اختلافاً واضحاً بين المرضى المتلقين للعلاج و غير المتلقين للعلاج عند مستوى معنوية 0.05، و أظهر أيضاً اختلافاً كبيراً بين مجموعة المرضى ككل مقارنة بمجموعة العيّنة الضابطة عند نفس مستوى المعنوية.

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عند الأسابيع الأولى من الإصابة بالمرض أظهر اختبار تعداد الحمضات اختلافاً واضحاً بين المرضى و العيّنة الضابطة عند مستوى معنوية 0.001، أما تعداد الكريات الدم البيضاء الكلي فلم يظهر أي اختلاف معنوي بين مجموع المرضى و العيّنة الضابطة.

الاستنتاج: أظهرت النتائج أن اختبار النايتروبلوتترازوليوم كان أفضل اختبار بالمقارنة مع الاختبارات الأخرى لدراسة دور البلعمة في هذا المرض وان زيادتها تعني أن الجهاز المناعي غير المتخصص يعمل بكفاءة ومن ثم يفسر لنا ذالك جزءا من ال (immunopathology) للمرض والذي ينعكس على أعراضه ومسيرته مما يساعد على تشخيص ومتابعة المصابين بمرض الجرب .

Scabies is a contagious skin disease caused by burrowing of the itch-mite, *Sarcoptes scabiei* into the superficial epidermal layers, where it lives and reproduces. It is characterized by a papulovesicular eruption associated with mite burrows[1]. Scabies affects man and domestic animals. It is present all over the world, and is endemic in many countries especially the developing ones[2-6]. The global prevalence is about 300 million cases[7,8]. Scabies infestations are more prevalent during times of war, in poor living conditions and in institutionalized people[9]. It is transmitted by close personal contact, and in adults sexual transmission is common[9,10]. In Iraq, studies on scabies among various groups including prisoners have previously been conducted[10-13]. 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 investigated in scabietic patients[14,15]. Type I reaction is associated with elevation of IgE[16,17]. Type IV reaction is manifested by superficial and deep perivascular infiltrate of mixed inflammatory cells (lymphocyte, histiocytes and eosinophils)[18]. Very heavy infections may develop in immunocompromised individuals or in people who are unable to care adequately for themselves[19].

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 scabietic 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 literatures.

Materials and methods

Subjects: One hundred and 3 patients including 100 males and 3 females, their ages varied from 18 to 66 years (mean age 32.8 ± 12 years) from Al- Zanjelli 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 19 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 helminthic infections. Venous blood samples were collected from both patients and controls. Sampling of blood was made by antecubital venipuncture using a sterile disposable syringe. Two-three mls. of 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 Park (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

dark blue formazine deposit(s) was determined.

2. Total white blood cell count and eosinophil count:

A routine total white blood cell (WBC) count was done using the improved Neubauer Chamber, and the numbers were expressed as $\times 10^9$ / 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 TIgE) determination:

The levels of S TIgE 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 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 anti-human 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 STIgE.

4. Parasitological examination for S. scabiei :

The adhesive cellophane (Graham's Scotch tape) method is used as a diagnostic test of choice for identification of *E. vermicularis* (22). 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 (SAF) 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 (1): *Sites of scabies infestation on the body.*

Site of infestation	No of patient	%
All over the body	41	39.8
Thighs, legs and groin	29	28.2
Trunk	21	20.4
Shoulders, hands and neck	7	6.8
Sporadic	5	4.8
Total	103	100

Table (2): *Scabies profile according to various tests in this study.*

Test	Subjects				
	A	B	C	D	E
Nitro-blue tetrazelium	+ + +	+ +	+ +	+	0
Total white blood cell count	+ +	0/+	0	0	0
Eosinophil	+ +	+	0	+	0
IgE	+/+ +	+	+	0/+	0

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

Figure (1): *Scabies on back and shoulders of a male patient.*

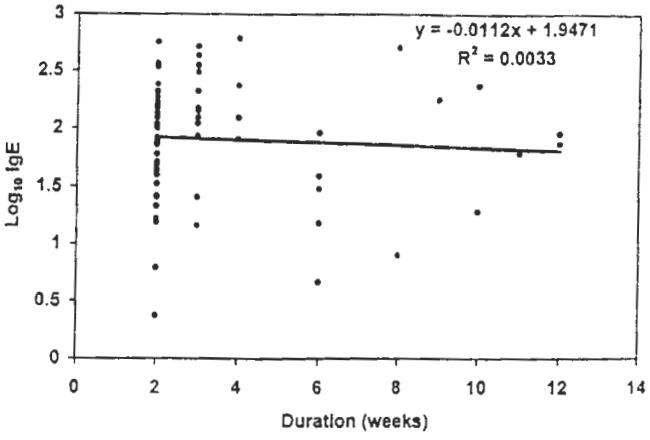

Figure (2): *STIgE of patients and control in correlation with the duration of infestation showed no significant correlation.*

Results

The site of infestation with scabies on the body surface of study subjects is shown in Table 1. and Fig.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.

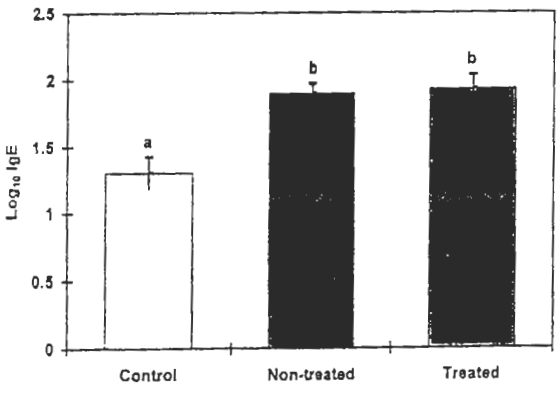

Figure (3): *STIgE level of patients (treated and untreated) and control showed:*
a. Significant variation between control and patients (both treated and untreated).
b. No significant changes between treated and untreated patients.
- *Bars with different letters mean significant difference at P <0.05*

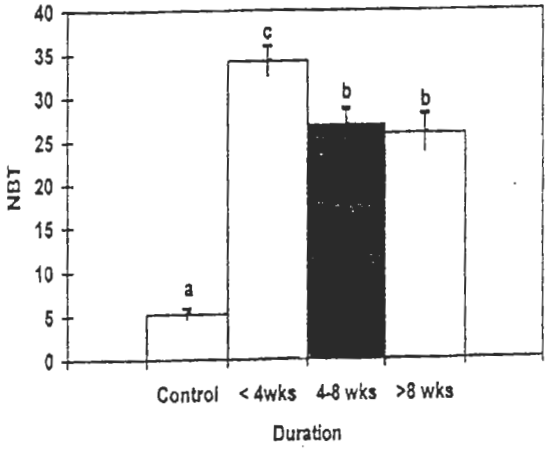

Figure (4): *NBT positive neutrophils (Mean ± SD of NBT percent) in relation with the duration of infestation.*
- *Bars with different letters (a, b and c) mean significant difference at P <0.05.*

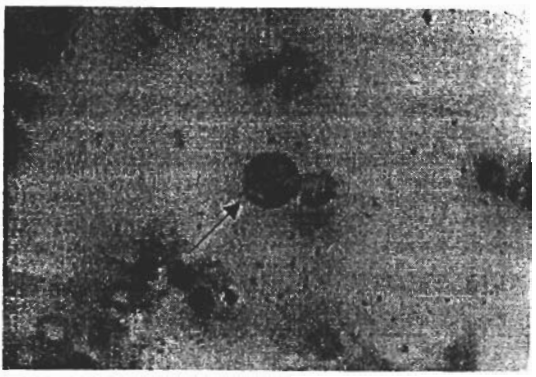

Figure (5): *NBT test. Positive neutrophil (with blue formazine deposit) of patient with scabies.(see arrow).*

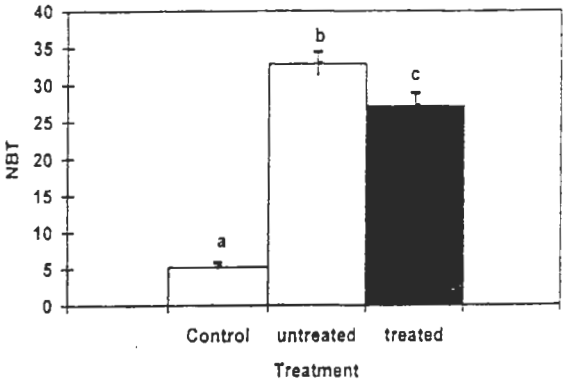

Figure (6): *NBT positive neutrophils (Mean ± SD of NBT percent) of patients (treated and untreated) and control*
Bars with different letters (a, b and c) mean significant difference at P <0.05.

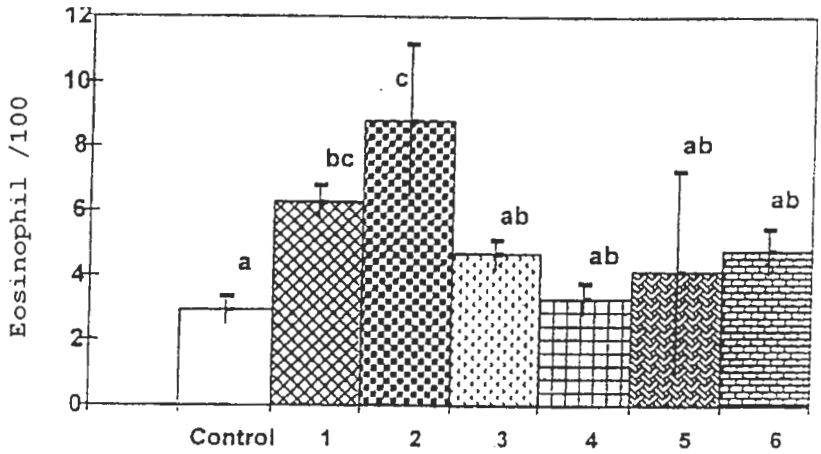

Figure (7): *Eosinphils count (mean ± SD) in control versus patients groups, and in patients according to the duration of infestation and treatment.*
1: *Untreated patients in the 1st month of infection.*
2: *Treated patients in the 1st month of infection.*
3: *Untreated patients in the 2nd month of infection.*
4: *Treated patients in the 2nd month of infection.*
5: *Untreated patients in the 3rd month of infection.*
6: *Treated patients in the 3rd month of infect*
- *Bars with different letters mean significant difference at P <0.001*

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 odd ratio was calculated for STIgE between patients and control groups and was found to be 5.4.

Nitro-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 NBT than control group.

The phagocytic activity showed a significant difference ($p < 0.05$) among treated, untreated and control groups (Fig. 6). The highest level of NBT-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 7966.5 ± 2998.8/mm^3 and 8215.8 ± 3065.2/mm^3 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 infestation as is shown in Fig. 7. The highest rate was demonstrated during the first month of infestation, while during the second month eosinophil count of both treated and untreated groups declined and approximated that of the control group, but during the third month of infestation with scabies, eosinophil counts started to rise again.

Discussion

Infestation with scabies was found among prisoners,and this may reflect poor hygienic standards and overcrowding. Similar findings were reported from other regions of Iraq[10,11,13]. Transmission of the mites is accomplished by direct contact with infected persons or with their clothing or bedding. The spread of infestation to different parts of the body as seen in the present study occurs through scratching and manual transfer of the mite by the infested individual.

Humoral and cell-mediated immunity play an important role in the pathogenesis of scabies[15,24]. Infestation with scabies seems to elevate IgG, IgM and IgE[14,15, 20,24]. Similar findings were noticed in this study regarding IgE level, which was significantly higher in scabietic patients than in controls. However, there were no significant differences in STIgE level between treated and untreated patients. Other reports have also demonstrated a significant increase in STIgE levels among patients with scabies[10,17,25-27]. This explains the type I reaction in scabietic patients which is responsible for expelling the parasite and its products from the burrows by the intense scratching which lead to sudden reduction of parasite density at time when itching started[20]. The sensitization of the host to the mite and its products probably plays an important role in the pathogenesis of the disease[24].

The TWBC were tested in order to evaluate the inflammatory response stimulation in scabietic 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[28] who estimated TWBC in 62 scabietic patients and found 59 patients with normal range.

The efficiency of neutrophils as phagocytes is measured by NBT test[29]. The nitro-blue tetrazolium dye is converted by the reduction occurring during phagocytosis to an insoluble blue formazine deposit[21,29]. The percentages of NBT positive neutrophils (with formazine deposits) in normal individuals was reported to vary between 3% to 11%[21,29]. An increase in the positive NBT% was reported in the majority of bacterial and parasitic infections[21,29,30]. This increase may reach up to 75% in acute phase of infection and then decline gradually until return to normal values within 4-6 weeks[31]. These cells have an important role in the non-specific (innate) immunity of the body[31]. Its increase means that this kind of immunity is efficient.

The untreated patients with primary exposure (no previous infestation with scabies) showed a highly raised NBT positive neutrophils specially during the first month of exposure to scabies. A decline in NBT 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 NBT positive neutrophils count. However, normal individuals with no infection showed a normal value of NBT 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

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 acute or chronic, and primary or secondary exposure. NBT test could also be used as follow up test to evaluate the degree of treatment efficacy and patients' compliance. However, other infectious diseases should be excluded which might give false positive results if present in patients with scabies.

In this study, the eosinophils count showed a significant increase during the first month of infestation with scabies. However, the eosinophils count returned to normal range during second and third months of infestation. This result is in accordance with other reports[26], 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[32] 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 against the irritant chemicals applied for the treatment, i.e., Benzyl benzoate which is a highly irritant lotion[33]. 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 to the decrease in the severity of the disease. The treated patients during the second month of infestation showed approximately same eosinophils counts as the control groups. This result was in accordance with others[26]. The higher level of eosinophils count during the third month of infestation in comparison to eosinophils level during second month could be attributed to the development of atopic dermatitis due to a prolonged infestation with *sarcoptis scabiei* mites. The death of *S.scabiei* mites and the release of its secreta might evoke some allergic response[34]. The allergic response could also be due to prolonged treatment by topical lotions[33].

The prison was an endemic focus for scabies and many patients were suffering chronically from the disease due to continuous contact with contagious persons. Once diagnosed, scabies infestations are easy to control, provided the directions of scabicide 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 dermal condition.

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