The use of *Sarcoptes scabiei* crude extract as allergen: An attempt to prepare a vaccine

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**ABSTRACT**

Scabies stands for a skin parasitic infestation as a result of mite *Sarcoptes scabiei*. It is a neglected international health problem; about 300 million cases develop scabies worldwide annually. *Sarcoptes scabiei* mite proteins are extracted, then heat-stable mite proteins concentration has been established by Bradford’s method. SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) analysis was used. The activity of mite allergens was assayed through skin prick test (SPT) in scabietic patients with 1.2 \( \mu g/ml \) and 2.4 \( \mu g/ml \). House dust mite (HDM) antigens were skin prick tested in patients with scabies and controls. The results revealed that the SDS-PAGE profile of the parasite heat-stable proteins consisted of protein bands with molecular weights ranged from less than 10 to over than 100 kDa. Skin test demonstrated that 70% and 80% of scabietic patients had a positivity against 1.2 \( \mu g/ml \) and 2.4 \( \mu g/ml \) of sarcoptic mite extracts, respectively when prick tested. HDM extract was found to be positive in 40% of scabietic patients; while controls revealed a negative result. Sarcoptic proteins contain heat-stable allergens which able to cause immediate type-1 hypersensitivity when 1.2\( \mu g/ml \) of mite protein is skin prick tested, and there is cross-reactivity between *Sarcoptes scabiei* and HDM allergens.

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The predominant scabies appearances are arbitrated via stirring and allergic-like reactions to mite products, causing cutaneous inflammation and forcefully pruritic skin injuries (*Stone et al., 2008*). While scabies itself is not a deadly or dangerous condition, it can be critical and persistent, causing debilitation and anxiety, hopelessness, and secondary skin harms (*Organization, 2005*).

The studies report a primary increase in *S. sca-
The possibility of developing a vaccine in contradiction of scabies mite antigens (Tarigan, 2003, 2006; Hejduk et al., 2010). There were several investigations dealt with the extraction of S. scabiei proteins with the intention of demonstrating and identifying immune responses in contradiction of scabies mite antigens (Tarigan, 2003, 2006; Hejduk et al., 2011).

The allergenicity of the extract was determined by skin tests for an attempt to prepare an anti-scabies vaccine. Developing anti-ectoparasite vaccine is a demanding and laborious task, representing identification and purification of the parasite-protein components that will induce protection when immunized into certain animals. Purification of the protective proteins requires a large amount of parasite as a starting material for biochemical fractionation and a large number of animals for vaccine-challenge trials (Willadsen, 1997). Obtaining sufficient S. scabies material is difficult; for the reason that the parasite burden is usually highly low, lack of in vitro culture system, and collecting mites from infested animals is difficult because the mites are microscopic and live in burrows they make in host’s skin (Walton and Currie, 2007).

The possibility of developing a vaccine in contradiction of scabies has previously discussed (Kemp et al., 2002), the viability of this objective is confirmed by researches where immunization by crude house dust mite extracts lessened the scabies mite burden in rabbits (Arlian et al., 1995). It is potential that immunization with a mixture of the scabies mite homologs of house dust mite allergens could impede the mites; henceforth defend against a scabies infestation. Furthermore, scabies mites becoming extinct in burrows might emit these molecules in a form available to the resistant system and henceforth might develop the immune response (Holt et al., 2003). There is evidence of cross-reactivity between S. scabiei and HDM antigens. This cross-reactivity may partially clarifies the perseverance of pruritus in several appropriately treated scabietic patients (Falk and Bolle, 1980).

Aims of the Study
The current study has to the following aims:

1. Preparation of Sarcoptic mite heat-stable allergen.
2. Detection of hypersensitivity in scabetic patients when skin prick tested (SPT) with sarcoptic mite crude protein extract.

MATERIALS AND METHODS
The current study has achieved in Najaf province during the period from February 2012 till November 2013 in Dermatology Outpatient Clinic in Al-Sader Medical City and Faculty of Science in the University of Kufa.

Mite Samples. Sarcoptes mites were harvested from prisoners who had had scabies according to the dermatologists. The skin lesions were deeply scraped using a blunt blade. The skin scrapings were placed in a Petri dish (Alasaad et al., 2009). In the lab, a beam of light has concentrated to the center of the dish and mites migrating toward the center of the dish, attracting by the light, then they were collected by vacuum aspiration under the dissecting microscope, by means of a Pasteur pipette (Tarigan, 2005).

Subjects. Ten persons (4 males, 6 females): 5 who have active scabies and 5 who had been successfully treated for scabies for (6-10) weeks previous to the test were skin analyzed. Five healthy volunteers who had never had a medical condition from scabies or atopic infection served as controls. There was no subject via oral corticosteroids or antihistamines in either patient or control groups. Permission has obtained from every participant in the study.

- Preparation of PBS (Phosphate buffered saline). This solution has organized by dissolving 0.34 g of KH2PO4, 1.21 g of K2HPO4 and 8 g of NaCl in distilled water, pH has regulated to 7.2 then the volume has accomplished to one liter. PBS solution then sterilized by autoclaving (20 minutes, 121°C) and stored at room temperature (Hudson and Hay, 1976; Atlas et al., 1995).

Mite Proteins Extraction. Approximately 100 mites were collected from scabetic patients as previously illustrated, 1 ml of cold PBS were added to a test tube containing the mites, vortexed for 5 minutes and pelleted through centrifugation at 10,000 ×g for 5 minutes. Mites have cleaned with 1 ml of cold PBS with 1% sodium dodecyl sulfate (SDS) to remove host immunoglobulin that might be attached to the surface of mites, then centrifuged at 10,000 ×g for 5 minutes. SDS residue has removed through washing two times with 1 ml of cold PBS. After the washings, the mites were homogenized.
in liquid nitrogen, using a micropestle. Then 1 ml of cold PBS was added to the homogenate, centrifuged at 10,000 × g for 10 minutes (Rampton et al., 2013). The supernatant was collected and heated in a 60°C water bath for 60 minutes; to obtain the heat-stable allergens (Tarigan, 2006). The concentration of the final supernatant has been predictable to be 1:1000 and 1:2000 (volume/volume). Protein concentration was evaluated through Bradford’s method (Bradford, 1976) by means of bovine serum albumin (BSA) as a typical principle. The protein samples were kept at -20°C until used.

Estimation of Mite Proteins Extract Concentration

-Preparation of Solutions Used in Estimation of Protein Concentration

Protein Reagent Solution: This solution was organized by dissolving 0.1g of Coomassie brilliant blue in 50 ml ethanol (95%), then 100 ml of phosphoric acid (85%) were inserted. The subsequent solution was diluted to a final volume of 1 liter (Bradford, 1976).

Bovine Serum Albumin Standard Solutions and Micro protein Assay: BSA standard has organized by dissolving 10 mg of BSA in 1 liter of PBS to prepare 10 µg/ml stock protein standard, then it used to prepare a dilution series. Two hundred µl of stock protein standard was added into a test tube. One hundred µl of PBS were pipetted into 5 new test tubes. Then 100 µl of stock protein standard were transferred into the first test tube to prepare a 5 µg/ml standard solution, then 100 µl of 5 µg/ml standard solution were transferred into the second test tube to form 2.5 µg/ml standard solution. (1.25) µg/ml standard solution was organized by adding 100 µl of 2.5 µg/ml standard solution into the third test tube. Finally, 100 µl of 1.25 µg/ml standard solution were added into the fourth one to prepare 0.63 µg/ml standard solution. Each test tube was thoroughly mixed before the next transfer. Then 1 ml of protein reagent solution has inserted into all test tubes, and the contents were mixed by vortexing. Absorbance at 595 nm has recorded after 2 minutes in 1 ml cuvettes in contradiction of a reagent blank (zero standard 0 µg/ml) organized from 100 µl of PBS and 1ml of protein reagent solution (Bradford, 1976). The standard curve, measures the relationship between absorbance at 595 nm and standards concentrations (µg/ml), was plotted. The results were interpolated using Microsoft Office Excel 2003. The correlation coefficient (r) was 0.9914.

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

-Preparation of SDS-PAGE Stock Solutions

1. Solution A (30% Acrylamid- bisacrylamide solution): organized through dissolving 29.2 g of acrylamide and 0.8 g of bisacrylamide in distilled water, then volume completed to 100 ml.

2. Solution B (1.5 M Tris buffer solution, pH 8.8): organized by dissolving 18.17 g of Tris and 0.4 g of SDS in distilled water, pH was adjusted to 8.8 with HCl, then volume completed to 100 ml.

3. Solution C (0.5 M Tris buffer, pH 6.8): organized through dissolving 6.06 g of Tris and 0.4 g of SDS in distilled water, pH has adjusted to 6.8 with HCl, then volume completed to 100 ml.

4. Solution D (10% ammonium persulfate): organized by adding 1ml of distilled water to 0.1 g of ammonium persulfate. This solution should be organized just in advance of use.

5. 10% SDS solution: organized by means of dissolving 10 g of SDS in 100 ml of distilled water (Dubey, 2009).

-Preparation of SDS-PAGE Buffers

1. Sample buffer: organized by adding 20 µl of Tris buffer pH 6.8 (stock solution C) to 2.5 ml of glycerin, 0.2 g of SDS, 0.01 g of bromophenol blue, 0.5 ml of 1% 2-mercaptoethanol.

2. Electrophoresis buffer: organized by means of adding 10 ml of 10% SDS solution to 3 g of Tris and 14.4 g of glycine, volume completed to 1 liter with distilled water (Dubey, 2009).

-Preparation of Electrophoresis Gel

1. Separating gel (12%): organized by mixing 7.5 ml of solution A, 4.5 ml of solution B, 0.07 ml of solution D, 0.01 ml of TEMED, and 6 ml of distilled water.

2. Stacking gel (4%): organized by mixing 0.9 ml of solution A, 1.5 ml of solution C, 0.018 ml of solution D, 0.01 ml of TEMED and 3.6 ml of distilled water (Dubey, 2009).

-Preparation of Staining and Destaining Solutions

1. Staining solution: organized by adding 25 ml of Coomassie brilliant blue, 500 ml of methanol and 100 ml of glacial acetic acid for making up 1 liter.

2. Destaining solution: organized by adding distilled water to 250 ml of methanol and 70 ml of glacial acetic acid to make up 1 liter (Dubey, 2009).

- SDS-PAGE Separation. SDS-PAGE was set up on a 12% separating gel and 4% stacking gel, according to (Laemmli, 1970) with slightly modification. Briefly, 4 volumes of Sarcoptes mite protein extract was mixed with 1 volume of sample buffer. Then
the mixture were heated in a water bath at 95°C for 5 minutes before loading [9]. The wells were filled with 15 μl of the mixture and 10 μl of prestained molecular weight protein marker (Bio Basic Inc., Canada). Electrophoresis was performed at 100 volts. This was normally taken about 3 hours (Tari-gan, 2006).

- Staining and Destaining Steps. Reagents that required for Coomassie brilliant blue staining were previously described. After removing the gel from SDS-PAGE, it was washed in distilled water several times to remove all the SDS. Then the gel has positioned in staining solution on a rotary shaker for 30 minutes. Finally, the gel was transferred into a destaining solution on a rotary shaker overnight. The destaining step was repeated until the background became clear.

**Skin Test.** Prick test was carried out on the lower forearm of 15 subjects (their ages between 18 and 51 years with a mean age 37.4 ± 10.26) as described above. The skin was cleaned and dried, sites of injection were marked. Each subject was prick tested with scabies mite extract (1.2 μg/ml and 2.4 μg/ml), HDM commercially available aqueous allergen extract (Allergy Laboratories, Baghdad); the concentration used was 1/10000 (w/v). Histamine (Alyostal, France) and normal saline have used as a positive and negative control, correspondingly. A small drop of each of the prick tested materials was put in its site on the skin, then disposable pins (Stal-lergenes, France) were used to prick the skin. The reaction was examined and photographed after 20 minutes (Li, 2002; Damin and Peebles, 2006).

**RESULTS AND DISCUSSION**

**SDS-PAGE:** The result of SDS-PAGE analysis showed that the crude heat-stable sarcoptic mites protein extract consisted of a variety of protein bands with molecular weights ranged from less than 10 to over than 100 kDa (Figure 1).

The result of the SDS-PAGE profile was in agreement with a study done by (Tari-gan, 2006). In previous studies, it was demonstrated that the mite extract contained more than 10 allergens based on their reactivity with specific IgE (Tari-gan, 2004; Tari-gan and Huntley, 2005).

HDM (*Dermatophagoides pteronyssinus* and *D. farinae*) have four allergens, apolipoprotein, paramyosin, glutathione S-transferase and serine proteases, that have been identified their homologues in *S. scabiei* cDNA libraries (Fischer et al., 2003; Harumal et al., 2003). The molecular weights of these allergens have been predicted to be 160, 83, 26 and 25 kDa, respectively (Kawamoto et al., 2002). The approach used in the present study did not detect proteins with molecular weights similar to those proteins. The probable reason why some of those proteins failed to be identified in the current study was these proteins are heat labile, which affected due to heat-treated. Whereas, proteins which less than 10 kDa are probably haptenic allergens derived from the breakdown of high molecular weight proteins. Similar results regarding these low molecule allergens have been documented by (Tari-gan, 2006).

- Skin Test: Ten scabietic patients were skin prick tested. Positive in 2 out of 10 (20%) of scabietic patients; while controls revealed a negative result when prick tested (Table 1).

In scabietic patients and control group, seven out of 10 (70%) and eight out of 10 (80%) of scabietic patients had immediate type-1 hypersensitivity was characterized by rapid formation (20 minutes) of wheal, and erythema or flare (Figure 2). This reaction was comparable to that produced by the prick of a histamine solution. The reaction could not be attributed to the mechanical injury inflicted by the prick; since prick with normal saline did not produce any oedematous or erythematous reaction. Also, histamine was used to ensure that they had not taken any antihistamine medications which could affect the result.

Result showed that 7/10 and 8/10 of scabietic volunteers had positive result when pricked with 1.2 μg/ml and 2.4 μg/ml of sarcoptic proteins extract, respectively, producing cutaneous anaphylaxis or immediate hypersensitivity. The reaction is deemed to be the type-1 or immediate hypersensitivity because it developed rapidly (within 20 minutes);
Table 1: Skin test to scabies mite and HDM extracts in scabietic patients and control group.

<table>
<thead>
<tr>
<th>History of Scabies</th>
<th>Prick Test</th>
<th>SS(μg/ml)</th>
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Figure 2: Positive prick test to scabies mite and house dust mite (HDM) extracts. His: histamine; N: normal saline; SS1: Sarcoptes scabiei mite extract (1.2 μg/ml); SS2: Sarcoptes scabiei mite extract (2.4 μg/ml).

Wheat and flare were specific for type-1 hypersensitivity, since all individuals were either active scabietics or had scabies infestation less than a year until that time.

The same result was approved when sensitized animals were intradermal injected with the mite proteins; immediate hypersensitivity or wheat was produced (Tarigan, 2006). The data of the current work disagreed with the result study done by (Falk and Bolle, 1980) which showed that patients with scabies who were prick tested by the scabies mite extract were negative in each case, nevertheless, 7/12 cases had a positive intracutaneous response of the immediate type to the scabies mite extract. The reason may be due to sufficient protein concentrations of mite were used in present study for prick test (1:1000 and 1:2000) if compared with 1:2500 that done by (Falk and Bolle, 1980).

Heating the sarcoptic proteins solution at 60°C for 60 minutes did not lose their allergenicity, this means that all or at least some of the mite allergens present in the mite extract are heat stable.

In a previous study, the sarcoptic allergens responsible for the hypersensitivity reaction in sensitized animals are 30-40 kDa and <10 kDa proteins, therefore we suspect that the sarcoptic heat stable allergens are in these range, as showed in a previous work (Tarigan, 2006).

The mite proteins were powerful allergens; since solution containing 1.2 μg/ml of proteins (which were previously treated with heat) produced a remarkable hypersensitive reaction when pricked.

On the other hand, 40% of scabietic patients had a positivity against HDM extract when pricked, whereas none of controls had a positive result. Other study showed in scabietic patients, the persons with a positive intracutaneous response to the scabies mite extract also had positive intracutaneous responses to DP, and five of these also had a positive prick test to HDM (Falk and Bolle, 1980). Another study demonstrated that extracts of HDM antigens exhibited significant greater positive results in scabietics than non-scabietic control subjects once skin prick tested (Mostafa et al., 1998).
Results of (Harmanyeri et al., 2002) proved that skin prick test was positive against HDM in 32% of scabies patients. Whereas (Taşkapan and Harmanyeri, 2005) revealed that 88% of patients with scabies had immediate-type reactivity (prick test and/or intradermal test positivity) in contradiction of HDM.

CONCLUSIONS

The current study concluded that the sarcoptic allergens that responsible for the hypersensitivity reaction are heat stable. There is a cross reactivity between Sarcoptes scabiei and HDM antigens. According the findings of this study, we recommend purification of sarcoptic mite proteins using gel filtration, ultrafiltration and chromatography to obtain purified proteins as a first step for antigen preparation that can be used in allergy tests.

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