CORRELATION BETWEEN SKIN MICROBIAL INFECTION AND IgE, LPS IN SERUM WITH ATOPIC DERMATITIS

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ABSTRACT
This study was aimed to find the most prevalent microbial isolates, as well as measurement of total serum IgE, LPS levels and their correlation with Atopic dermatitis occurrences. A 88 patients were collected randomly (47 females and 41 males), through a period Sep. 2019 to Feb. 2020 from Al Zafaraniyah General Hospital. Patients ages ranged between 10 months to 30 years. In addition, the control samples are collected randomly from 20 apparently healthy people. Microbial isolation results showed that the most prevalence microbial isolates in atopic skin patients were; *Staphylococcus* spp. 81 (30.10%), fungi 65 (24.20%), *Bacillus* spp. 53 (19.70%), *Enterobacteriaceae* spp. 32 (11.90%), *Acinetobacter* spp. 16 (5.90%), *Corynobacterium* spp. 10 (3.70%), *Streptococcus* spp. 8 (3.00%) and *Pseudomonas* spp. 4 (1.50%) compared with control group. Antimicrobial susceptibility test was carried out for nine antimicrobial agents. The results showed that, most bacterial isolates become a multidrug resistant especially *Staphylococcus* spp. and *Enterobacteriaceae* spp. whilst two other tests in a concern with the AD patients which are IgE and LPS, both parameters results show that, the age from 0 - 2 years, is recorded higher serum level than other age categories.

Key words: atopic dermatitis, IgE, LPS, antimicrobial susceptibility, patients, disease

المستقبل

هدفت هذه الدراسة إلى إيجاد أكثر العزلات الميكروبية انتشارًا، بالإضافة إلى قياس مستويات *IgE*، *LPS* في مصل الدم بالتهاب الجلد التحسسي، بالتعاون مع مروج عبد الستار العبيدي، باحث، استاذ قسم التقنيات الاحيائية - كلية العلوم - جامعة بغداد.

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INTRODUCTION
Atopic dermatitis (AD) is a persistent inflammatory skin relapsing condition marked by dry skin, irritation and severe itching that may be exacerbated by several causes, such as allergens, diseases, changes in season and environment or psychological tension. Nearly half of the cases are diagnosed before the age of 1 year and more than one-third of the patients are recurrent during adulthood. (23). Scholz and Kilian (26) documented that within the skin areas, the diversity and density of the organs and follicles of hair may vary significantly and thus, result in a complex chemical and physical and site of geographically distinguished niches for the progress of bacteria. For example, Cutibacterium (earlier Propionibacterium) in addition to the species of Staphylococcus control the sebaceous areas (as in the face or the torso) while, the Corynebacterium, Staphylococcus in addition to the beta-Proteo bacteria are mostly determined to be existing in the areas of high moisture, that may include the arm pits, the elbows and the knee creases. The activity of skin normal flora to assemble begins just after birth and later, keeps to progress primarily in accordance to the body site over a period of several weeks, (6,21). In adulthood, regardless of how the skin is in contact to the environmental conditions, the composition of the microorganisms keep on continuously and unexpectedly to be at stable conditions with time, (20). This leads to the belief that the stabilization mechanism will support the relationships between the commensal microbes, not only that, but also between the microbes and their host, in a reciprocal way. Adam (1) reported the dominating effects of local antimicrobials and antiseptics of the clusters of skin bacterial societies in addition to their effects in the resisting behavior of S. aureus colonization. In normal people, it is known that the existence of IgE is normally limited, however, as the person becomes an AD patient, the IgE increases. This tendency covers almost 70-90% of AD (4), while the remaining 10-30% of the AD patients show no increase in their total IgE levels. This means that, though total concentrations of total serum IgE could be at low or medium levels, it is still possible to encounter significant raise of certain or specific IgE to different allergens, (3,19). Lipopolysaccharide (LPS) is an essential cell wall that is responsible for protecting the body against extreme environmental pressures, and is crucial for the integrity and viability of bacterial cells. Among most Gram-negative bacteria, they are constituent of and highly preserved, this function is linked to Lipopolysaccharide's ability to induce inflammatory response, (10). The major aim of this study was to determine the most prevalent microbial isolates, as well as measurement of total serum IgE, LPS levels to find the correlation between these parameters and AD occurrences.

MATERIALS AND METHODS
Patients and control
This study included total of 108 patients and control individuals are randomly selected from Al Zafaraniyah General Hospital, the diagnosis was performed by a dermatologist in the dermatologic clinic of the hospital. A 88 patients (47 females and 41 males). By using transport media, from each patient; 2 swabs were taken one from AD lesional skin and other from a non-lesional skin. Whereas 20 apparently health people(11 females and 9 males) are represented as a control, only one swab is taken from healthy skin for each subject. The specimens collection are carried out during Sep. 2019 to Feb. 2020.
For primary screening, collected swabs are cultured on different media (Mannitol salt agar, MacConky agar, Blood agar, Brain-Heart Infusion agar, Luria-Bertani agar and Sabouraud Dextrose agar)/ Himedia – India.(28) And then bacterial isolates were characterized by biochemical test such as (Indole, catalase, coagulase, Methyl Red, Oxidase, Urease, Voges–Proskauer), (23).

Antimicrobial susceptibility test
Kirby-Bauer disk diffusion method is used to detect antimicrobial susceptibility test (2,12) for nine antimicrobial discs including; Bacitracin (B), Novobiocin (NV), Caphalothin (CL), Azithromycin (AZM), Tobramycin (TOB), Levofloxacin (LEV), Coliston (CO), Sulfamethoxazole +Trimethoprim (TS) and Methicillin (ME)/ Mast group – England.
Blood sampling
Vein puncture blood specimens (3ml) were obtained from each patient and control subject. Blood was kept in a gel tube then centrifuged for 10 min at a 400 rpm, then the serum was collected and stored at (− 20°C), later. It’s used to analyzes LPS and total IgE. According to Ichiro (11) patients and control subjects are classify into 3 age categories; (0-2), (3-13) and (14-30) years.

Determination of IgE and LPS level
The tests are carried out through enzyme-linked immune sorbent assay (ELISA) on the basis of biotin double antibody sandwich technology to assay Human (IgE)/ Shanghai–China and lipopolysaccharides(LPS/LOS)/Shanghai–China. Dependent on company instructions, the procedures were done.

Statistical analysis
SPSS for Windows, version 22 Data processing was conducted using SPSS for Windows (SPSS Inc. Chicago, Illinois, United States). To decide if the parameters analyzed followed a Gaussian distribution. For continuous variables, data was expressed as mean ± standard deviation (SD). Differences between groups were tested by the student's t test, followed by the Levene’s variance equality test. The Games-Howell Post Hoc experiments were used with different comparisons. ANOVA tests were analysed according to Categorical variables were expressed as frequencies and proportions. Using the Chi-square test (χ²), proportions were compared. p value of less than 0.05 was consider to be statistically significant (8,9).

RESULTS AND DISCUSSION
AD patients have a particular ability to be compromised or colonized by a variety of microbial species. (5) epidermal barrier abnormalities play an important role in microbial colonization or infection in AD. In addition, the association with infectious complications of AD between innate and adaptive immune responses (13). Microbial investigation results showed that the most prevalence microbial isolates in Atopic skin patients were: *Staphylococcus* spp. was noticed to be 81 (30.10%), fungi 65 (24.20%), *Bacillus* spp. 53 (19.70%), *Enterobacteriaceae* spp. 32 (11.90%), *Acinetobacter* spp. 16 (5.90%), *Corynobacterium* spp. 10 (3.70%), *Streptococcus* spp. 8 (3.00%) and *Pseudomonas* spp. was 4(1.50%) compared with control group *Staphylococcus* spp. 15 (30.60%), fungi 15 (30.60%), *Bacillus* spp. 11 (22.40%), *Enterobacteriaceae* spp. 4 (8.20%), *Acinetobacter* spp. 2 (4.10%), *Corynobacterium* spp. 1 (2.00%), *Streptococcus* spp. 1 (2.00%), which recorded low percentages for all bacterial isolates than that recorded in patients group, as shown in figure (1 and 2 A,B).

![Figure 1](image-url)
Figure 1. Prevalence percentages of skin bacterial isolates in control group
These results compatible with that documented by Ong (22) who they found the correlation between S. aureus and AD, and speculated the explanation reason that most patients with AD are colonized by S. aureus on lesion skin. Also Nada et al., (18) showed that, S. aureus positive cases were correlated with 30% of the total cases of AD that were studied by them. On the another hand Slomski, (27) reported that the microbial agents such as S. aureus, candida and Trichophyton dermatophytes, all act in two different ways to stimulate the flares of AD. From the recent results it can be concluded that Staphylococcus spp. and fungi are the most prevalence microbial isolates that are found in AD patients. There was no significant difference are recorded between microbial diversity in lesional and non-lesional sites of skin in AD patients.  

**Antimicrobial susceptibility test:** One of the important objective of present work is to study the effects and consequences of using different antimicrobial drugs in elevation of resistance among bacterial species colonized AD patients and the control group subjects. Regarding to *Staphylococcus* spp.; the results of resistance against nine antimicrobial agents for the case of AD patients in this study are: Bacitracin 33(67.30%), Novobiocin 6(12.20%), Caphalothin 33(67.30%), Azithromycin 33(67.30%), Tobramycin 18(36.70%), Levofloxacin 6(12.20%), Colistin 26(53.10%), Sulfamethoxazole +Trimethoprim 8(16.30%), and Methicillin 24(49.00%). As shown in Figure 3, while in case of control group, the R values as presented in Figure 4 are: 9(75.00%), 10(83.30%), 0(0.00%), 11(91.70%), 10(83.30%), 6(50.00%), 4(33.30%), 8(66.70%), 3(25.00%), 5(41.70%) respectively.  

**Figure 2. Prevalence percentages of skin bacterial isolates in Atopic dermatitis group**  
A: for healthy skin and B: for Atopic dermatitis locus  

**Figure 3. Antimicrobials resistant percentages of Staphylococcus spp. isolated from AD patients**
Figure 4. Antimicrobials resistant percentages of *Staphylococcus* spp. isolated from control subjects

In addition the resistance of *Enterobacteriaceae* spp., the results of the resistance were noticed to be for each antimicrobials are: 31(88.60%), 6(17.10%), 28(80.00%), 20(57.10%), 9(25.70%), 7(20.00%), 20(57.10%), 12(34.30%), 16(45.70%) to Bacitracin (B), Novobiocin (NV), Caphalothin (CL), Azithromycin (AZM), Tobramycin (TOB), Levofloxacin (LEV), Colistin (CO), Sulfamethoxazole + Trimethoprim (TS) and Methicillin (ME) respectively figure 5 for the case of AD patients, as compared to the control group figure 6, which are 6(85.70%), 3(42.90%), 5(71.40%), 6(85.70%), 2(28.60%), 1(14.30%), 2(28.60%), 2(28.60%), 3(42.90%) respectively.

Figure 5. Antimicrobials resistant percentages of *Enterobacteriaceae* spp. isolated from AD patients

Figure 6. Antimicrobials resistant percentages of *Enterobacteriaceae* spp. isolated from control subjects
The third testing program is carried out on the resistance of species to Acinetobacter. Figure 7 is shown the test results for the resistance in case of AD patients. For each of the above specified antimicrobial, the resistance values (R) have the values: 5(45.50%), 2(18.20%), 4(36.40%), 6(54.50%), 1(9.10%), 1(9.10%), 3(27.30%), 5(45.50%) and 4(36.40%), for Bacitracin (B), Novobiocin (NV), Caphalothin (CL), Azithromycin (AZM), Tobramycin (TOB), Levofloxacin (LEV), Colistin (CO), Sulfamethoxazole plus Trimethoprim (TS) and Methicillin (ME) respectively. Furthermore, it was noticed that the resistance values (R) of the control group as given in figure 8, for each of the antimicrobials are: 9(75.00%), 0(0.0%), 11(91.70%), 10(83.30%), 6(50.00%), 4(33.30%), 8(66.70%), 3(25.00%) and 5(41.70%), respectively.

**Figure 7. Antimicrobials resistant percentages of Acinetobacter spp. isolated from AD patients**

**Figure 8. Antimicrobials resistant percentages of Acinetobacter spp. isolated from control subjects**

The fourth bacterial isolate concerned is Pseudomonas spp. These species show strong resistance to Bacitracin 1(50.00%), Caphalothin 1(50.00%), Azithromycin 1(50.00%), and Methicillin 1(50.00%), but of less sensitivity to Novobiocin (NV), Tobramycin (TOB) and Levofloxacin (LEV) figure 9.

**Figure 9. Antimicrobials resistant percentages of Pseudomonas spp. isolated from AD patients**
Finally, tests on resistance of *Streptococcus* spp. were carried out. Surprisingly, these species show strong resistance (100%) to Caphalothin, Sulfamethoxazole plus Trimethoprim and Colistin while the resistance to Tobramycin, Methicillin are both 2(66.70%) and Levofloxacin is 1(33.30%) but still sensitive to Bacitracin, Novobiocin and Azithromycin as given in figure 10.

![Figure 10. Antimicrobials resistant percentages of *Streptococcus* spp. isolated from AD patients](image)

In 2016, Iryohojin et al. found in their patent that, Bacillus bacteria are low pathogenic, have a strong inhibitory effect against pathogenic bacteria like dermal fungi and *Staphylococcus aureus*, also decompose and metabolize waste products released by the human body, and there is certain species of Bacillus that grow in the bathwater have therapeutic effects against AD. *Bacillus subtilis*, a Bacillus species, was used to produce the antibiotic bacitracin. Therefore, it can be suggesting that Bacillus spp. isolates from AD patients did not have an effect to counteract other pathogenic bacteria, may due to Bacillus isolates have no ability to produce substances that inhibit the growth of other pathogens, or that the microorganisms have become resistant to the Bacillus products, and this is what we find clearly through the resistance of the isolated bacterial species to Bacitracin. The results agreed in the present work as those results obtained by Kong et al., (14) who suggested that microbial community constructions at positions of disease predilection are different in AD patients as compared with the control samples. Microbial multiplicity during AD flares was directly related to the presence or absence of recent AD treatments, with recurrent treatment associated to greater bacterial diversity than no recent treatment. In general, the origin of the encountered resistance is attributed to creation of wide range of beta-lactamase enzymes and their modifications against aminoglycosides. This behavior will change the spots of quinolones and hence, resulting in various efflux mechanisms which makes the options for selecting proper empirical formula of antimicrobials a challenging task as noted by Maragakis et al, (16).

**Serum IgE level test**

Adaptive immune response dysregulation with high total and specific IgE levels has been associated with the severity of the disease and infectious complications, (25). The results are presented in Table 1 show the age groups under consideration. For the age patient group (0 to 2 years), the result of IgE is (320±0.11 IU/mL), for the age group (3 to 13 years) was (178±0.07 IU/mL) and for the age group (14 to 30 years) is (100±0.02 IU/mL). While the control group of the apparently healthy people, results for the same sequence of age groups are found to be (120 IU/mL), (180 ±0.05 IU/mL) and (170 ±0.05 IU/mL), respectively. Dehlink and his Colleagues (7) demonstrated that the level of serum Immunoglobulin E correlates well with cell-bound IgE. However, a high total IgE level alone is of inadequate value as a marker of allergy as it does not give any evidence to sensitizing allergens in a subject. Hence, consideration was paid more on serum Immunoglobulin E as a biomarker. As well as Aral et al., (4), concluded that some AD patients show no noticeable variation of the IgE level but, that does not mean there will be no definite increase in the IgE level of those patients.
Different small letters denote significant differences.

**Determination serum LPS level**
The beneficial physiology of LPS includes natural suppression of the responses of allergies (15). Detailed analysis in the present study highlighted that; Serum total LPS level results in table 2 show significant differences among different groups of age categories; (0.39±0.08) for the age group (0 to 2 years), (0.25±0.08) for the age group (3 to 13 years) and (0.11±0.04) for the age group (14 to 30 years) as compared to (0.12), (0.21±0.06) and (0.25±0.10) in case of healthy control groups respectively.

<table>
<thead>
<tr>
<th>Age categories</th>
<th>N</th>
<th>Control N</th>
<th>Patients P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2 y</td>
<td>1</td>
<td>120</td>
<td>5</td>
</tr>
<tr>
<td>3-13 y</td>
<td>7</td>
<td>180 ±0.05b</td>
<td>60</td>
</tr>
<tr>
<td>14-30 y</td>
<td>6</td>
<td>170 ±0.05d</td>
<td>10</td>
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<tr>
<td>P value</td>
<td>14</td>
<td>0.95</td>
<td>75</td>
</tr>
</tbody>
</table>

Different small letters denote significant differences at p≤ 0.01.

Detailed inspection of the test results show that patients with AD have lower values of gram-negative bacteria on their skins as compared to the healthy people (17). Myles with his collagous(2016), in his study, also indicated that the reason for such a property is attributed to the reason that AD patients, in general, have less gram-negative bacteria existing on their infected skin as compared to the healthy people therefore, the proposed transplant will increase those bacteria resulting in the suggested improvement of their symptoms.

**CONCLUSIONS**

In view of the results of the current study, the following conclusions can be presented: Staphylococcus spp., fungi and Bacillus spp. are more predominant microbial isolates found in lesional and non-lesional skin of AD patients. Most bacterial isolates are resistant to most empirical antibacterial agents. Serum immunoglobulin E level is not consider as a good indicator for AD diagnosis. Also, there is no correlation between serum LPS and AD.

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