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Microbiological, Histopathological and Epidemiological Assessment of patients with *Staphylococcus aureus* in Babylon Province: A Cross-Sectional Study

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Abstract

Atopic dermatitis (AD) is a chronic skin disease with a wide prevalence, with a rate of 20% in children and 6.3% among adults. (AD) is characterized by dry skin, irritation, and constant, annoying itching, the main cause of which is an imbalance in the immune response and increased secretion of helper T cells of the first type Th1 and the second type Th2. *Staphylococcus aureus* bacteria are associated with the disease. This study aimed to analyze the microbial content and histological changes associated with atopic dermatitis. The study included recruiting 180 patients who were clinically diagnosed with (AD). Swabs were inoculated from active skin lesions to culture the bacteria and identify the most abundant species. Histological analysis was measured and histological changes were identified. Then, patient data were collected to determine the prevalence of the disease in Babylon Governorate. Statistical analyses were conducted using chi-square and P-value percentages Results: *Staphylococcus aureus* was the most prevalent (60.25%) at the significance level (P=0.0001) and the prevalence of the disease in females was 52.49% compared to males 47.51% at the significance level (P=0.0001). Histological changes associated with the disease have been identified. *Staphylococcus aureus* is abundantly associated with patients, confirming the importance of the microbial role in the mechanism and severity of the disease.

Keywords: Atopic dermatitis; *Staphylococcus aureus*; Histopathological; Skin microbiome; Epidemiology

التقييم الميكروبي والنسيجي والوبائي للمصابين بالتهاب الجلد التأتبي وعلاقته بالمكورات العنقودية الذهبية في محافظة بابل: دراسة مقطعية

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الخلاصة

التهاب الجلد التأتبي (AD) هو مرض جلدي مزمن ينتشر على نطاق واسع، بمعدل 20% عند الأطفال و6.3% بين البالغين، يتميز (AD) بجفاف الجلد وتهيج وحكة مستمرة ومزعجة، السبب الرئيسي لذلك هو اختلال التوازن في الاستجابة المناعية وزيادة إفراز الخلايا التائية المساعدة من النوع الأول Th1 والنوع الثاني Th2. ترتبط بكتيريا المكورات العنقودية الذهبية بهذا المرض. هدفت هذه الدراسة إلى تحليل المحتوى الميكروبي والتغيرات النسيجية المرتبطة بالتهاب الجلد التأتبي. وشملت الدراسة 180 مريضا تم تشخيص إصابتهم سريريا بمرض (AD). تم تلقيح مسحات من الآفات الجلدية النشطة لزراعة البكتيريا وتحديد الأنواع الأكثر وفرة. من ثم قياس التحليل النسيجي وتحديد التغيرات النسيجية. جمعت بيانات المرضى لتحديد مدى انتشار المرض في محافظة بابل. تم إجراء التحليلات الإحصائية باستخدام مربع كاي ونسب القيمة P النتائج: كانت المكورات العنقودية الذهبية هي الأكثر انتشارًا (60.25٪) على مستوى الدلالة (P = 0.0001) وكان معدل انتشار المرض لدى الإناث 52.49٪ مقارنة بالذكور 47.51٪ على مستوى الدلالة (P = 0.0001). وقد تم تحديد التغيرات النسيجية المرتبطة بالمرض. ترتبط المكورات العنقودية الذهبية بكثرة بالمرضى، مما يؤكد أهمية الدور الميكروبي في آلية المرض وشدته.

1. Introduction

Skin diseases are explicitly referenced in historical Greek mythology, and certain regions were believed to have regenerative powers for skin diseases one such place turned out to be the Peloponnese, which is defined as healing place in mythology. According to legend the Anigrids nymphs lived with in the historic site of Trifilia with the river Anigros, and people with pores and skin diseases could visit them to seek healing by living in a sacred water cave dedicated to them. In their system of understanding, skin diseases were represented an early attempt to provide an explanation for the causes of skin diseases[1].Atopic dermatitis (AD) is a chronic and complex pore and skin disease, where factors percentage the same mechanism of the disease as well as hypersensitivity especially excessive immune globulin E as well as changes in immune and cellular responses in addition to hypersensitivity especially high immunoglobulin E[2].

The disease (AD) is characterized by the onset of chronic and continuous scaly eczema lesions on the skin followed by itching and pain in the affected person there are many reasons for this however the main cause is an imbalance with type 601 Th1 and Th2 immune helper T cells[3].The disease is spreading worldwide affecting both young and old it is characterized by dry skin ,persistent irritation itching redness in the area, and infiltration of immune cells into the skin. The disease is considered a non-fatal disease[4].A benchmark study of the prevalence of atopic dermatitis in children confirmed that this changed to 20% in high -cost nations[5].Another study showed that its rate in children and adolescents was 11.1% while in adults was 6.3% of infected cases[6].the occurrence of an imbalance in the skin microbiome environment the high settlement or opportunistic Gram-positive *Staphylococcus aureus* bacteria and the loss of normal skin microbes leading to an imbalance in skin function is a distinct feature that accompanies (AD) leading to an exacerbation of the disease[7][8].

Staphylococcus aureus is harmful for people with (AD) [9].These bacteria produce collagen adhesion proteins that make it better to bind and harbor host invasion[10]. It is (AD) a disease whose symptoms are similar to other diseases, but histological features such as spongy dermis (Spongiosis) infiltration of lymphatic immune cells around the blood vessels, and other distinctive features and swelling of the keratinous layer[11].

The study aimed (1) Knowing the most abundant species among infected patients (AD) in Babylon Governorate (2) Knowing the prevalence of the disease and age groups, and studying the accompanying histological changes**.**

2. Materials and methods

2.1Ethical approval

Ethical approval was obtained from Imam Sadiq Teaching Hospital in Babylon Governorate, in accordance with the ethical principles of research on the clinical aspect of patients, and informed written ethical approval was obtained from all patients and parents of young people who agreed to enter the experiment according to a book issued by the hospital administration.

2.2 Inclusion Criteria

- Age ≥ 5 years

- Clinical diagnosis of atopic dermatitis
- No systemic or topical treatment within the previous 4 week

2.3 Exclusion Criteria

Samples affected by concomitant skin diseases or those receiving Corticosteroids therapies or other concomitant chronic diseases were excluded.

2.4 Samples collection

Samples (180) of tissue swabs and biopsies were collected from patients with atopic dermatitis who visited the dermatology consulting clinics at Imam Al-Sadiq Hospital in Babylon Governorate, aged (70-5) years and of both sexes, and were diagnosed by dermatology consulting doctors. The sterile swabs were partially moistened with a 2.5% saline solution by rotating the swab 5 times in the affected skin area after the information was recorded The swabs were then transported with their carrier medium Transport Media in a special box to transport and preserve the samples to the Microbiology Laboratory in order to implant them on public media in the medium of Blood Agar and the differential medium of MacConkey Agar and incubated at a temperature of 37 m for 24 hours for the purpose of growing and isolating bacteria.

2.5 Epidemiological Spread of disease

A data collection process was conducted from five hospitals in Babylon Governorate that contain dermatology consultations for the period from 1/6/2025 to 31/12/2025 relying on data recorded in the following hospitals (Imam Al-Sadiq Hospital, Al-Hillah Teaching Hospital, Marjan Teaching Hospital, Al-Musayyib General Hospital, Al-Hashimiya General Hospital), including most of Babylon Governorate, north and south of Babylon and the governorate center, where data was taken from the aforementioned hospitals, including (age, gender, residence, sex, Diagnosis and date on which the patient is seen).

2.6 Bacterial Culture and Identification

Samples taken from infected individuals were inoculated onto blood agar dishes and MacConkey Agar and incubated at 37°C for 24 hours. The samples were initially examined phenotypically based on the specific culture characteristics of each bacterial species in terms of hemolysis as well as the edges of the developing colonies. They were stained with Gram stain to distinguish between negative and positive bacteria[12]The catalase test was performed to confirm whether the bacteria were producing the catalase enzyme, causing a gas bubble product reaction[13].The single selected colony was transferred to the Mannitol Salt Agar[14]. and tube coagulase test was performed for coagulase to confirm the isolation of *Staphylococcus aureus* or other coagulase-negative species[15].

2.7 Histopathological Examination

Samples taken from patients were fixed in a 10% formalin solution and after about 48 hours were removed from the formalin and washed several times with running water. This process was done by drawing water from the tissue by passing it through a series of

ascending ethyl alcohol concentrations starting (70-100%) for two hours at each concentration and then diluting it in xylene twice for 30 minutes at each pass. It was placed in wax melted at 58°C for two hours at a time, They were then buried in melted wax at 58°C until the samples froze, The patterns were cut and placed in a heater to stretch the and held on glass slides, I dyed the models after putting them in the oven to remove the wax from them, then I went through xylene for 10 minutes, then with a series of descending alcohols from 100-70 for two minutes in each stage, then I put them in haematoxylin dye for 5-10, washed them, and put them in eosin for 4 minutes, then rinsed them with alcohol and fixed the models with DPX[16].

2.8 Statistical Analysis

The Statistical Packages of Social Sciences-SPSS (2019) statistical program was used to analyze the data to study the effect of different factors on the studied traits. The significant differences between the means were compared using the T-test and the least significant difference test (Least Significant Difference -LSD) for more than two means. The chi-square test (Chi-Square: $\chi 2$) was also used to compare the significant differences between the sample distribution ratios according to the different factors as well as estimating the correlation coefficient between some variables in the studied sample.

3.Results

3.1Epidemiology Characteristics

Table 1 shows the prevalence data for the disease in Babylon Governorate over 6 months, as a percentage. Statistical analysis revealed a significant difference in the distribution between the sexes of those infected at *12.72. The rate of mites in males was lower at 47.51% compared to females at 52.49%

Table 1- Percentage distribution of studied epidemiology by age group

Gender	Number	Percentage %
Males	2429	47.51%
Females	2684	52.49%
Total	5113	100 %
Chi-square(χ^2)	---	*12.72
(P-value)		(0.001)

The data is displayed as a number for the male and female categories as a chi-square at Significant (P-value) (0.001).

Table 2 shows the disease data as a percentage. Statistical analysis revealed a significant difference in distribution between age groups. The age group 20≥ appeared at 68.39%, followed by the group 5-19 at 25.87%, then the group 0-4 at 5.74%. It had a high statistical significance (P = 0.0001)

Table 2- Number and percentage of epidemiological distribution of the studied sample by age group

Age group	Number	Percentage %
0 – 4	293	5.74%
5 – 19	1323	25.87%
20 ≥	3497	68.39%
Total	5113	100%
Chi-square(χ^2)	---	**3140.3
(P-value)		(0.0001)

The data is displayed as a number for the age group categories as a chi-square at highly significant (P-value) (0.0001).

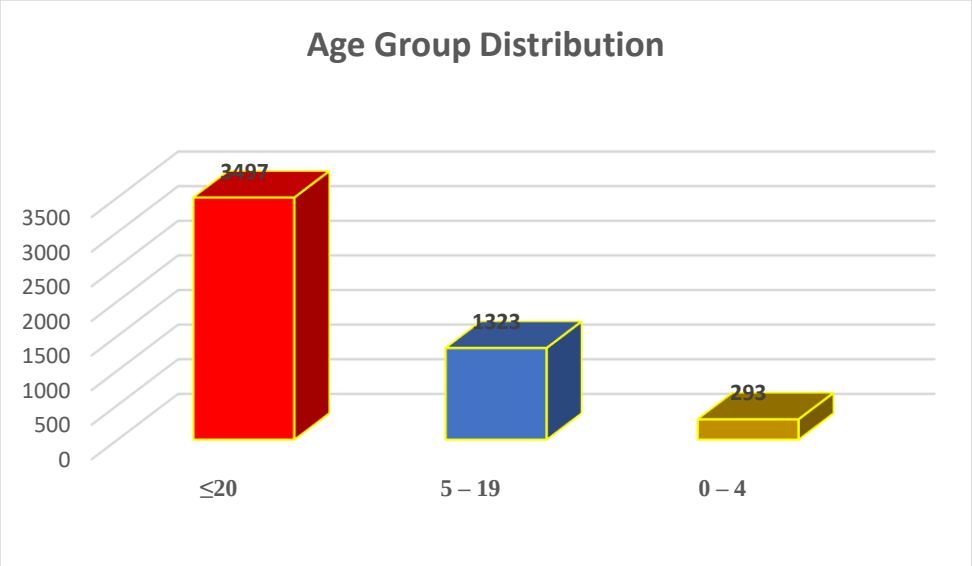

Figure -1 Shows the age group distribution in the study

3.2 Microbiological Characteristics

Table 3 displays data on positive-growth and negative-growth bacterial growth of infected people and the isolation rate. The analysis revealed a high significant difference in growth in samples, 156 samples, there is growth at a rate of 86.67, compared to no growth at a number of 24 at a rate of 13.33.

Table 3- Percentages of samples with and without growth

Bacterial growth	Number	Percentage %
Growth	156	86.67
No Growth	24	13.33
Total	180	100%
Chi-square(χ^2)	---	**96.80
(P-value)		(0.0001)

** (P≤0.01).

Data are presented as a percentage number of variables according to growth or non-growth as a highly significant chi-square (P≤0.01).

Table 4 *Staphylococcus aureus* is the most abundant isolate in patients, accounting for 60.25% of isolates, followed by *Staphylococcus epidermidi*s 35.26% and *Streptococcus pyogenes* 4.49%, with *Staphylococcus aureus* showing a statistically significant difference (0.0001).

Table 4- Number and percentage of epidemiological distribution of the studied sample according to the type of bacteria isolated

Type of bacteria isolated	Number	Percentage %
Staphylococcus aureus	94	60.25
Staphylococcus epidermidis	55	35.26
Streptococcus pyogenes	7	4.49
Total	156	100
Chi-square(χ^2)		**73.792
(P-value)		(0.0001)

** (P≤0.01)

The data is displayed as a number for the Type of bacteria isolated categories as a chi-square at Highly Significant (P-value) (0.0001).

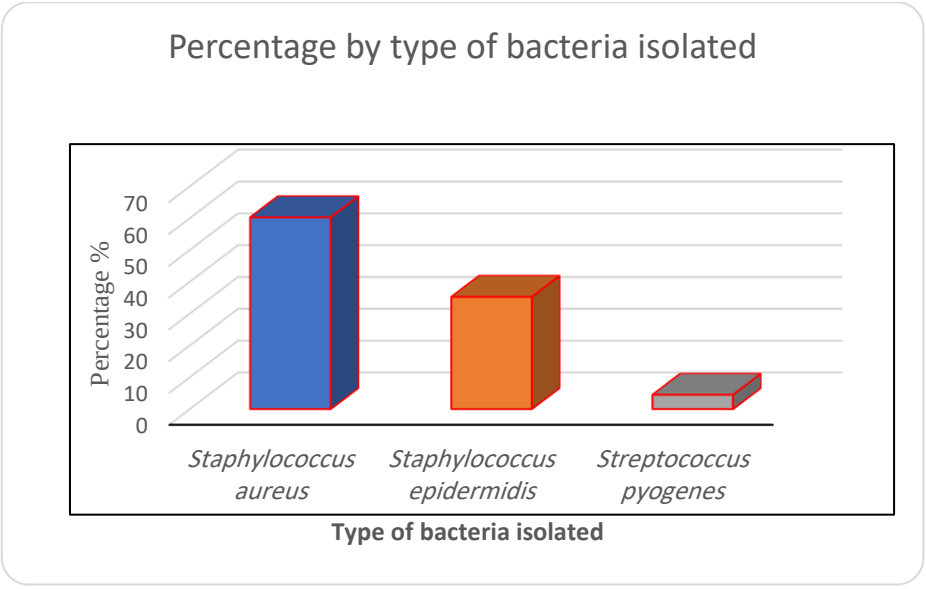

Figure -2 Shows the percentage of bacterial isolates in the study

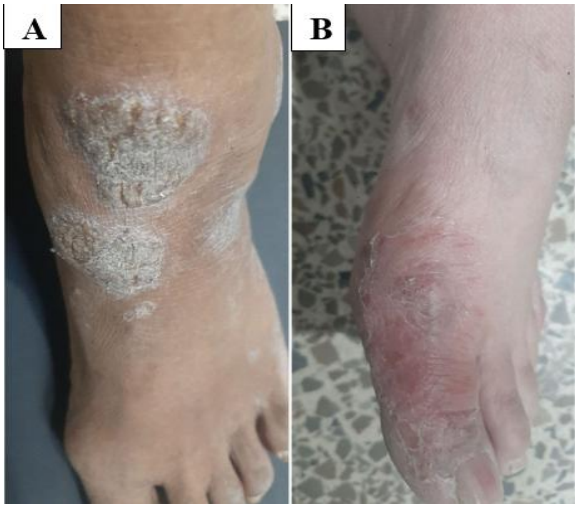

Figure -3 Clinical images of eczematous lesion areas, of adult female feet, A and B Shows the affected parts

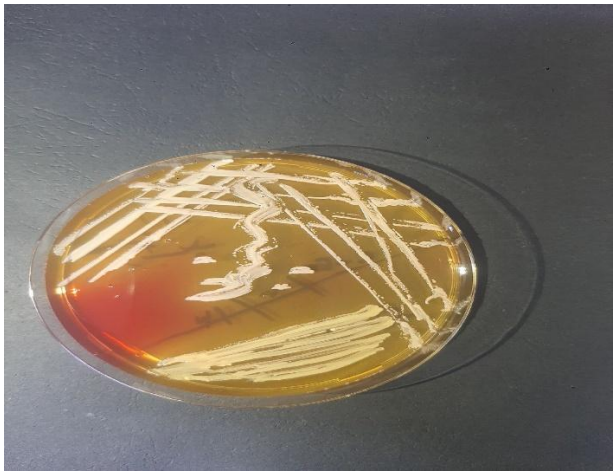

Figure -4 The Figure shows isolated colonies with a characteristic golden-yellow coloration, indicating mannitol fermentation on mannitol salt agar, which is a distinctive feature of *Staphylococcus aureus* isolates

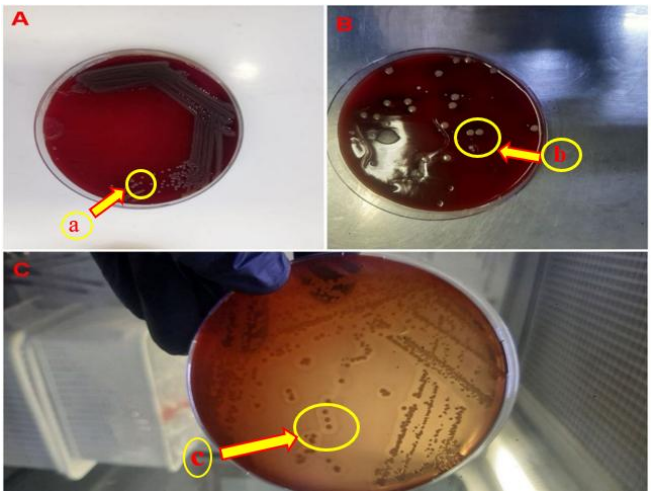

Figure -5 *Staphylococcus* cultured on human blood agar after 24 hours of incubation. The isolates exhibit morphologically similar colonies, characterized by a round shape, smooth surface, and creamy white colour. In addition, clear zones of haemolysis surrounding the bacterial colonies are observed, indicating beta- haemolysis activity.

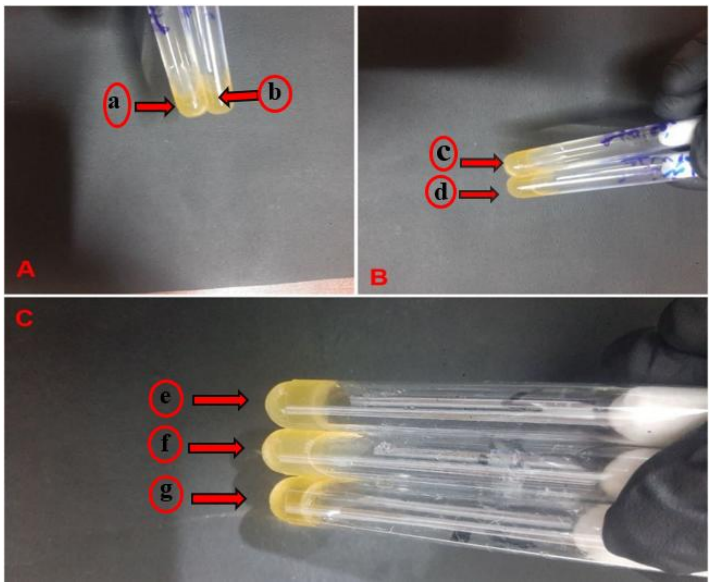

Figure -6 (A-C) demonstrates the biochemical coagulase tube test performed to determine whether the clinical isolates are coagulase-positive or coagulase-negative. Figures (a- d) represent a coagulase-positive clinical isolate as indicated by clot formation in the plasma. Figure (e- g) represents a coagulase-negative clinical isolate showing no clot formation after incubation.

3.3 Histopathological Findings

Histopathological analysis of skin biopsy samples stained with H&E highlights the main features of histological changes such as thickening of the stratum corneum, elongation of retinal protrusions, the appearance of spongy edema in the chronic case within the spinosum a edema , and perivascular lymphocyte infiltration in the papillary dermis.

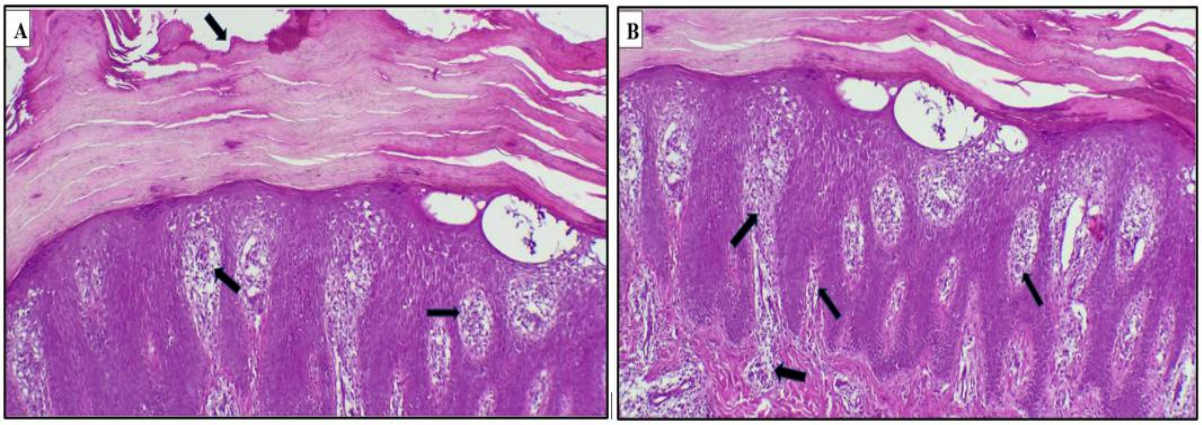

Figure -7 Histopathological features of atopic dermatitis in H&E-stained sections. (A) Hyperkeratosis and increased thickness of the stratum corneum, intracellular spongiform edema(arrow). (B) Lymphocyte infiltration around blood vessels Elongation of rete ridges into the dermis (arrow).

4. Discussion

The study explored the microbial, epidemiological and histological aspects of atopic dermatitis patients in an Iraqi group in a previously unstudied area, by analysing the microbial diversity of those infected and an epidemiological analysis of the disease prevalence data in addition to the histological analysis. The main results of the study were as follows: (I) *Staphylococcus aureus* is the most abundant isolate at 60.25% (II) The disease prevalence rate in the female group was higher at 52.49% Compared to males (III), the prevalence of the disease in the age group 20≥ was 68.39% higher than the rest of the groups.

The colonization rate (60.2) and the abundance of *Staphylococcus aureus* bacteria are of high moral significance (0.0001), which indicated in previous studies a morally significant result of the abundance of *Staphylococcus aureus* in atopic dermatitis and the lack of microbial diversity [17]. Our result is similar to that of Kadhum and Abood, which indicated that *Staphylococcus aureus* bacteria were the most abundant and significant isolates, with an isolation rate of 64% of isolates in Iraqi patients in Baghdad Governorate [18]. The difference in the percentage and significance among Mexican patients, where the isolation rate was (36.7%), was for those infected with (AD) [19]. The difference is due to the method of work, the group of infected people, the environment in which the infected people live, the areas where the sample was taken from the infected skin, and the type of the infected person's skin, whether it is oily or not [20].

The prevalence of the disease the female group was greater compared to males at (P = 0.001), which is consistent with scientific reviews that indicated that its percentage in females is higher compared to males for the reason that females after puberty have immune responses affected by sex hormones in patients, which enhances the secretion of oestrogen and progesterone from the activity of type II helper T cells Th2 Progesterone weakens the skin barrier [21]. Our results are consistent with an analytical study in English patients, as the study indicated a higher prevalence of the disease in females compared to males at (P = 0.001) in adulthood [22]. The results of our study did not agree with a study conducted in the Democratic Republic of the Congo, where its prevalence was higher among males compared to females at 54.4%. The reason for this difference is due to the sample size, the age groups studied, the geographical environment, and the ethnicity of the affected individuals [23].

The prevalence rate in the age group of 20≥ came from the rest of the groups at a rate of 68.39% at a high moral significance level P = 0.0001)) This is consistent with an analytical study conducted at the level of 20 countries around the world that found that there is a moral

increase in infection in adult groups, and the study confirmed that infection in adults is on the rise. Continuous [24]. The results of our study differ from a study conducted by Elsawi in the United States of America in a cross-sectional study, where their percentage was 11% of the total number of infected people. The difference is due to the size of the small sample that was studied, the age, the study methodology, and the division of age groups [25]. The expression of signs of recent differentiation increases significantly with the increasing age of the person infected with (AD), so the skin suffers from functional disorders in those infected due to structural and morphological changes such as skin thinning, so with age the human skin becomes aging accompanied by immune changes affected by the environment and microbial imbalance [26].

The study found an increase in the rate of bacterial growth in people infected with ((AD. If the study recorded a growth of 156 samples with growth compared to 24 samples with negative growth, this is consistent with previous studies that indicated that the rate of bacterial colonization in patients with atopic dermatitis is a higher abundance of colonization compared to healthy people [27].

The histological findings of the disease with keratinized layer hyperplasia, epidermal cell keratinization, spongy necrosis, and perivascular lymphocyte infiltration are consistent with conventional histological pathological signs of (AD) [27][28].

5. Study Limitations

Adopting traditional agriculture and not using PCR technology in analysing microbial diversity and not using tissue immunity in performing histological analysis**.**

6. Conclusion

The study aimed to identify the microbial, histological changes and epidemiological spread associated with atopic dermatitis. The study showed the association of *Staphylococcus aureus* with the disease and the imbalance of microbial skin diversity. The results may indicate the possibility of the contribution of bacterial colonization of *Staphylococcus aureus* to the severity of the disease and the immune-inflammatory state of the skin, which confirms the necessity of bacterial colonization interventions. We recommend studying the microbial axis using an analysis of the entire genomic content of those infected.

Conflict of Interest: The authors declare no conflicts of interest.

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