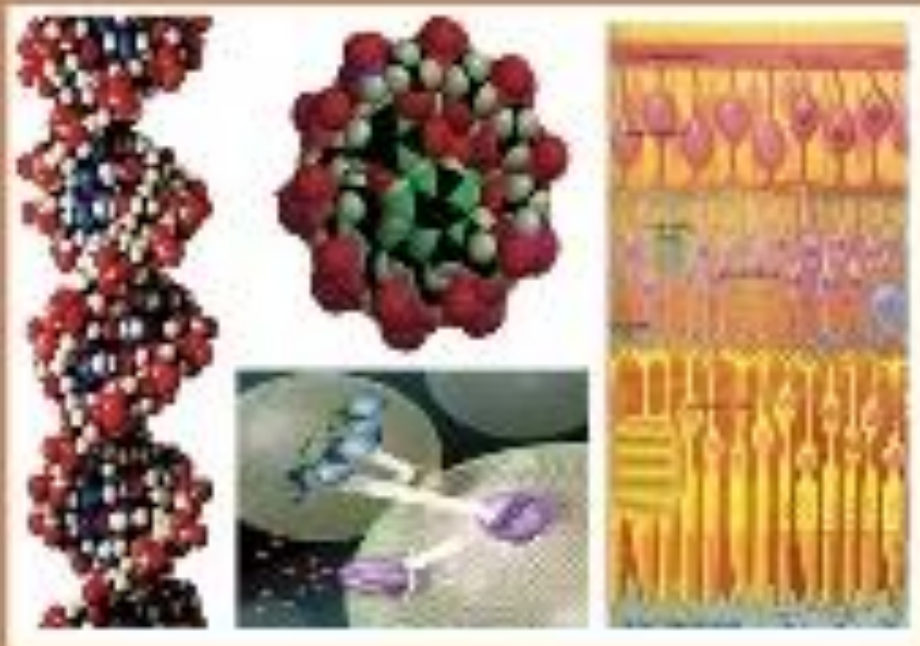




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Isolation and Diagnosis of Some Isolated Bacterial Types of Psoriasis Patients and Measurement of Some Immunological Parameters Such as IL-17 And Complements C3 and C4

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ABSTRACT

80 samples were collected from patients at Samarra General Hospital / Salah Al-Din General Hospital, 60 samples from patients with skin psoriasis and 20 samples from healthy people in the period from September 2022 to January 2023 and for the ages of 2-80.

Some bacterial species associated with psoriasis patients have been isolated, the most isolated bacteria were Staphylococcus aureus, 33.3% of the 60 bacterial isolates were isolated, it isolated 18 isolates of Staph epidermidis with 30% of the total isolates and Staphylococcus hominas isolated by 15%, gram-negative isolates included 13.3% of P. aeruginosa and five isolates of E. coli (8.3%), some immune markers were compared between a group of psoriasis patients and a healthy (control) group, the estimated C3 supplement level for patients is 265.1 ± 215.77 , while the rate for healthy people is 652.18 ± 102.3 , it is noted that this indicator is higher in patients with psoriasis compared to healthy people, it also indicates that the estimated C4 supplement level for patients is 392.92 ± 63.7 , while the rate for healthy people is 167.94 ± 38.7 . It is noted that this indicator is higher in patients with psoriasis compared to healthy people, the IL-17 level of interleukin was 765.29 ± 85.4 , while the rate for healthy people is 212.37 ± 39.6 . It is noted that this indicator is higher in patients with psoriasis compared to healthy people, the results also showed significant differences between the levels of immune indicators between patients and healthy people at a probability level (p value = 0.05).

INTRODUCTION

Psoriasis is associated with a number of comorbidities and significantly affects patients' health-related quality of life (Raval *et al.*, 2011, Bander *et al.*, 2015). Psoriasis can affect the scalp, face, soles, feet, nails and genitals. (Augustin *et al.*, 2018) Psoriasis affects both males and females, with early onset in females and those with a family history, the age of onset is seen in the ages of 30-39 and 60-69 years in men, and before 10 years in women (Parisi *et al.*, 2020). This rapid transition occurs due to the fact that the immune system accidentally attacks some cells instead of attacking bacteria, so the body begins to quickly produce new cells to compensate for those missing cells, autoimmune diseases impose a large burden of disease and death on the world's population. (Davidson and Diamond, 2020) has been confirmed to significantly affect chronic inflammation (Wu *et al.*, 2015).

In addition, the discovery of the IL-17 pathway contributed to a clearer understanding of the underlying mechanism of inflammatory diseases, at present, treatments for inflammatory diseases have evolved from general immunosuppression to biology against the IL-17 signaling pathway, such as IL-17, IL-12/23, and IL-23 inhibitors (Nesmond *et al.*, 2019). The pathway of immune supplements plays an important role in the innate immune response and defense against bacterial infection, through opsonization and degradation of pathogens, complementary proteins facilitate the removal of immune complexes and damaged cells, recently, there is a new appreciation for the role of the supplement in neuroinflammation, aging and cancer, the complement system consists of about 50 membrane-bound soluble proteins, including the classic ingredients, C1-C9, although most soluble agents are produced by the liver, however, complementary proteins are also secreted by a variety of skin cells including mast cells, macrophages, keratinocytes and fibroblasts (Zhuang and Lyga, 2014). The supplement is traditionally considered part of the innate immune system, but its role in activating B and T cells also promotes adaptive immune responses, cytokines have an active role in pathogens of psoriasis. Pro-inflammatory cytokines are involved at every stage of psoriasis development. It contributes to the creation of a pathogenic vicious cycle, in which keratinocytes, endothelium, and immune cells release inflammatory mediators, it stimulate each other and maintains the chronic inflammatory process. Type I cytokines (TNF- α , IFN- γ , IL-2), and type 17 cytokines (IL-6, IL-17, IL-22 and IL-23) are most important in psoriasis (Coimbra *et al.*, 2010).

MATERIALS AND METHODS

The samples were transferred to the laboratory for cultivation on the primary

isolation cultured media, which are the center of Akar Al-Makunki and the center of Akar Al-Manitol, the dishes were incubated aerily at 37°C for 24 hours and bacterial isolates were diagnosed based on shapes, phenotypic examinations and biochemical tests, bacterial colonies developed on the culture media of the primary culture were diagnosed on the basis of the culture characteristics in terms of shape, size, color, texture, smell, and fermentation of lactose sugar in the middle of Akar Maknoki and mannitol sugar on the medium of salt mannitol, then it was subjected to a microscopic examination by taking a swab, fixing it with heat, staining it with Gram stain, and then examining it under the oil lens of the optical microscope, several biochemical tests have been performed such as catalase and oxidase test, IMVIC test set, urease test and plasma coagulation test as reported by approved diagnostic systems (Mahon *et al.*, 2014; Macfaddinn 2000) and fungal isolates were diagnosed as reported in Al-Attaqchi, 2017, Abdulbaqi *et al.*, 2018).

Immunological tests were conducted to obtain the serum of infected people and a control sample to detect the presence of C3, C4 and IL-17. Blood was collected through venous blood. 5 ml of blood was collected in anticoagulant-free plastic tubes to obtain the serum using a 3000 rpm centrifuge for 5 minutes. Serum samples were kept at -20°C until test time with Elisa Elabscience.

RESULTS AND DISCUSSION

Identification of Isolated Microbes:

Bacterial and fungal isolates isolated from patients with psoriasis were diagnosed based on their phenotypic characteristics on culture media, microscopic characteristics, Gram-stain coloration and biochemical tests. Diagnosis with API 20 E was confirmed in members of the Gram-negative enteric family, Gram-positive bacteria were also isolated using API Staph 20 E.

Table 1: Types of Microbial Isolated from the Skin of People with Psoriasis.

	Bacterial species	Number	Percentage
Bacteria	<i>Staphylococcus aureus</i>	20	33.30%
	<i>Staphylococcus epidermids</i>	18	30%
	<i>Staphylococcus hominas</i>	9	15%
	<i>Pseudomonas aeruginosa</i>	8	13.30%
	<i>E. coli</i>	5	8.30%
Total		60	100%

Previous studies have documented that the proportion of patients with Staph. Aureus on the skin lesion of psoriasis ranges from 0.03 to 0.64 in 15 recorded studies. In another study, it was found that Staph. Aureus is colonized in 60% of psoriasis lesions and 60% of bacterial isolates secrete toxins, those patients with toxin-producing isolates have a higher psoriatic region with an increased severity index score (Zhou and Yao, 2022). Researchers (Elfatoiki *et al.*, 2016) found 21% of *S. aureus* nasal staphylococcus in patients with psoriasis. Also, Boncompain *et al.* (2022) documented higher rates in psoriasis patients of 37.24%. Four previous studies have documented the prevalence of colonization of staphylococcus nasal 56, 44, 50 and 40% in psoriasis patients and 8, 28, 34 and 42.1% in healthy individuals, respectively (Graber *et al.*, 2011; Balci *et al.*, 2009; Nguyen *et al.*, 2008; Tomi *et al.*, 2005, Hussein *et al.*, 2017). According to data from the National Health and Nutrition Examination Survey (NHANES) within the United States, the prevalence was 30.4% between 2001 and 2004, the reason for the supremacy of Staph. aureus to possess many virulent factors, injuries occur through contact with the surface of the skin tissues of the host or through abrasions or wounds, secreting a number of enzymes, including the lipolysis enzyme Lipase and the enzyme Hyaluronidase, which helps it to spread and break down the base material of the connective tissue, causing imbalance and disorder in these tissues (2012., Janstova *et al.*). 18 isolates were isolated to Staph bacteria. epidermids and 30% of the total isolates, the reason for this is that it is opportunistic bacteria when the right conditions are available that cause infection and possesses

several virulent factors represented in their ability to produce biofilm and slime layer, in addition, it releases Toxins, exoenzymes and Efflux pumps (Namvar *et al.*, 2014, Dahham *et al.*, 2020). *Staphylococcus hominas* was also isolated by 15%, it is part of the natural microbiota in the human body, specifically the skin, human staphylococcus has been isolated as a presumptive pathogen in a small number of reported skin and soft tissue infections (SSTI), including infected abscesses and genital infections (Natsis & Cohen, 2018). Gram-negative isolates included 13.3% *P. aeruginosa* isolate and five *E. coli* (8.3%) isolates. *P. aeruginosa* is the most isolated Gram-negative bacteria, this is consistent with the findings of Yongsoon *et al.* (2018), which recorded the highest isolation rate at 76%, it also agreed with the study of researchers Hussein and Obaid (2017) conducted in Iraq and bacteria reached the highest isolation rates by 74.6%, the reason for the virulence of these bacteria, which are invasive pathogens that invade the skin and soft tissues and cause skin infections, may be attributed to the fact that they possess many different virulence factors that enable them to resist antibiotics such as the production of enzymes and toxins such as the metallo β -lactamases and broad-spectrum enzymes -Extended Spectrum β Shaikh *et al.*, 2015, Hussein *et al.* 2019).

The Level of Some Immune Markers in Psoriasis Patients Compared to Healthy People:

Through the Table 2, some immune markers were compared between a group of psoriasis patients and a healthy (control) group, the estimated C3 supplement level for patients is 265.1 ± 215.77 , while the rate for healthy people is 652.18 ± 102.3 , it is noted

that this indicator is higher in patients with psoriasis compared to healthy people, it also indicates that the estimated C4 supplement level for patients is 392.92 ± 63.7 , while the rate for healthy people is 167.94 ± 38.7 . It is noted that this indicator is higher in patients with psoriasis compared to healthy people.

The IL-17 level of interleukin was 765.29 ± 85.4 , while the rate for healthy people is 212.37 ± 39.6 . It is noted that this indicator is higher in patients with psoriasis compared to healthy people.

The results also showed significant differences between the levels of immune indicators between patients and healthy people at a probability level (p value = 0.05).

These findings are important because they reflect differences in the level of certain immune markers between psoriasis patients and healthy people, immune disorders are thought to play a role in the development of psoriasis, and these findings show differences in patients' immune system response compared to healthy people.

It is essential that doctors and researchers carry out more studies and analyses to understand in more detail how these differences in immunity affect the development and progression of psoriasis, these findings may contribute to therapeutic and research guidance to improve the care and treatment of people with psoriasis.

Table 2: Level of Some Immunological Indicators in Psoriasis Patients Compared to Healthy People.

Group	C3 (944-166)	C4 (75-217)	LL-17 (308-134)
Patients	265.1 ± 215.77 a	392.92 ± 63.7 a	765.29 ± 85.4 a
Healthy (control)	652.18 ± 102.3 b	167.94 ± 38.7 b	212.37 ± 39.6 b

Follador *et al.* (2010) report that cutaneous keratinocytes make and secrete C3. In addition, C3 derived from epidermal keratinocytes can also be attributed to the deposition of C3 on the lower membrane of epidermal cells in autoimmune or inflammatory disorders. Terui *et al.* (2000) reported that keratinocyte production from inflammatory mediators such as C3 was affected by fair leukocyte migration to the epidermis.

The current study is consistent with previous studies by Kutukculer *et al.* (2005) and Chimenti *et al.* (2012) where serum levels of C3 and C4 were significantly elevated in psoriasis patients compared to health controls, which indicates that the complementary system is undoubtedly activated in psoriasis patients, high levels of C3 and C4 also indicated an increase in incorrect complementary system activity, this leads to significant tissue damage through increased deposition of human keratinocytes, which play a major role in causing disease.

In previous data regarding psoriasis patients related to C3, serum concentration C4 is a significant elevation in C3, besides, there is no significant increase in C4 in untreated psoriasis patients compared to the control group (healthy). Our results were consistent with that of Weigle (2000), who observed increased C3 levels and normal levels of C4 in psoriasis patients compared to the control group (healthy).

C3 derived from human keratinocytes in epidermal basement membrane deposits C3 contributes to autoimmune disorders or dermatitis such as psoriasis and plays a major role in causing the disease (Sequin *et al.*, 1993, El-Hilali *et al.*, 2016). The current results differed with that of Singh *et al.* (2009) who found that C4 levels had increased significantly.

Several studies have suggested that patients with an immune defect affecting the pathway of IL-17 signaling may suffer from chronic mucocutaneous candidiasis, it is a disorder characterized by persistent or

recurrent cases of nail or skin inflammation or diseases of the mucous or genital membrane (Liu *et al.*, 2011). The IL-17 pathway participates in the goal of inflammation that induces and maintains psoriatic disease.

The level of some immune indicators in patients with psoriasis by age groups:

The results presented in Table (3) show the level of some immune markers in psoriasis patients according to age groups. From the data provided, it can be seen that for the age group (1-10), patients are characterized by a relatively high level of complement C3, C4 and IL-17, with an average of 374.13 ± 29.73 , 843.40 ± 121.6 respectively, for ages 11-20, levels of C3, C4 and IL-17 were 420.68 ± 62.08 and 793.29 ± 168.4 respectively.

For the other age groups (21-30, 31-40, 41-50, 51-60, 61-70, 71-80) similar results showed a high level of C3, C4 and IL-17. Psoriasis affects both males and females, with early onset in females and those with a family history, its age of appearance shows a bimodal distribution with peaks ranging from 30 to 39 years and 60-69 years in men, and 10 years earlier in women.

In children and adolescents, a high

level of inflammation may be observed in the body in general, which is manifested by increased levels of creatinine serum and viron emitted, stimulation of the cellular immune system may appear with an increase in the number of specialized white blood cells such as leukocytes and monocytes, thus, there may be an accumulation of specialized immune cells in the affected skin, in adults, psoriasis patients in this age group may experience a high level of inflammation in the body, and this may have an impact on the levels of creatinine serum and ferron released, improved activity of immune cells that produce antibodies such as cellular plasma may be exhibited, leading to the accumulation of immune cells in psoriasis skin, however, it should be noted that the level of these immune markers may vary greatly from person to person, this may be influenced by the type and severity of psoriasis, as well as other individual factors such as genetics and environment. It is necessary for competent doctors to assess the patient's condition individually to determine the level of immune indicators and their effect on his state of health.

Table 3: Level of Some Immunological Indicators in Psoriasis Patients by Age Groups.

Age group	Number	C3 Normal range (944-166)	C4 Normal range (75-217)	LL-17 Normal range (308-134)
2-10	12	523.4 ± 238.77	374.13 ± 29.73	843.40 ± 121.6
11-20	17	514.4 ± 195.3	420.68 ± 62.08	793.29 ± 168.4
21-30	11	617.5 ± 221.53	396.46 ± 65.84	811.9 ± 134.4
31-40	18	491.8 ± 209.8	374.23 ± 35.39	780.31 ± 89.7
41-50	13	431.5 ± 210.27	369.07 ± 37.58	768.96 ± 93.4
51-60	12	440.3 ± 211.04	389.73 ± 40.87	744.2 ± 76.8
61-70	9	317.1 ± 225.96	475 ± 154.7	689.22 ± 97.8
71-80	8	0 ± 175.78	365 ± 0	599.47 ± 0

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