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ORIGINAL ARTICLE

The Role of Toll-Like Receptor-2 in Acne Vulgaris of Iraqi Patients

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KEYWORDS

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ABSTRACT: The study was set up to investigate the role of Toll-like receptors-2 (TLR-2) in acne vulgaris. TLR-2 is a member of the innate immune system; it's a group of transmembrane receptors that recognize special microbial structures like pathogen-associated molecular patterns (PAMP) lead to the production of cytokine and inflammation. Ninety persons from both genders are included in the study, forty-five of them were patients and the same number was as control. Blood samples were taken from both groups and by using an ELISA kit, TLR-2 was estimated. The findings of the study referred that the adolescent, young adult and female gender was more effective in acne, and the male to female ratio was 1:1.25. Also, people with positive family history were more susceptible to acne. On the other hand, the results indicated there was a significant increase in sera levels of TLR-2 in patients with acne vulgaris compared to the controls group. Also, TLR-2 had a significant positive correlation with the severity of acne ($p>0.05$, $r=0.5$). We concluded that adolescent, young adult, female gender, and positive family history appeared to be the most possible factors associated with acne vulgaris. Also, It's illustrated that the high levels of TLR-2 in acne and the positive correlation of TLR-2 with acne severity, revealed that TLR-2 has a role in the bad prognosis of acne vulgaris.

INTRODUCTION

Toll-like receptors are essential elements of innate immunity and provide the first line of protection against infectious agents. TLRs cause signaling events in the intracellular that causes cellular expression and lead to the release of immune substance by sensing bacterial, viral, or fungal components [1]. TLR is one of the PRRs that recognize the PAMPs on antigen surface and plays a role in early immune [2], which helps stimulate adaptive immune responses [3].

Acne vulgaris is one of the widespread skin diseases, that affects the pilosebaceous follicle [4]. Several factors contribute to acne vulgaris development including increased sebum production, follicular hyperkeratinization, inflammatory mechanism, and hyper colonization of commensal bacteria Propionibacterium acnes that induce innate immunity and cause acne lesions [5]. Acne is a very common skin condition that affects 85% of adolescents and may persist into adulthood [6].

TLR-2 responses are important in various inflammatory disorders of immunity [7]. Numerous cells have TLRs on their surface, such as keratinocytes and sebocytes in the epidermis. TLRs are also found in many immune cells, such as macrophages, mast cells, dendritic cells, and monocyte [8, 9].

The aim of the study determines the levels of TLR-2 in acne vulgaris patients and compare them with healthy control to detect its role in acne prognosis.

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MATERIALS AND METHODS

The study included forty-five cases of acne patients from both genders who attended to AL-Shatrah general Hospital, Dermatology and Venereology Section, in the period between 1st November 2020 to 1st May 2021. The age range was (16-37 years). Exclusion criteria, Patients who have taken systemic or topical treatments for acne in the past month. Patients suffering from chronic systemic illness, females who are pregnant or breastfeeding. In addition, 45 non-acne were a control group of healthy subjects.

Estimation of acne severity

The clinical diagnosis of acne patients was confirmed by the dermatologist's physician. Patients were divided into three categories according to "The Agency for Healthcare Research and Quality". Acne vulgaris severity is classified as mild, moderate, and severe depending on account of the skin lesion in its different types, this is called "Combined Acne Severity Classification" [10].

Procedure

Three milliliters of venous blood samples were collected from all cases (patients and healthy controls) and put into the tube of a clot-activator to separate serum. The separated serum was centrifuged at 3000 rounds per minute (rpm) for 10 minutes and stored at freeze (-20°C). Serum levels of TLR-2 were determined by using ELISA Kit and according to the instructions of the provided company (Elabscience -USA).

Statistical analysis

Statistical analysis was done by using the SPSS program. It was determined at $P<0.05$ and the results were shown as mean ± SD. Student's t-test and chi-square were used. Pearson's correlation (r) was applied to determine the relationship.

RESULTS

The current study involved 90 subjects, 45 of them were patients with acne vulgaris, and 45 of healthy control people. As present in Figure 1, the study determines three age groups; the majority age group was 16-25 years old with 77.78% of patients and 71.11% of healthy control, whereas the 26-35 years old found in 17.78% of patients and 26.67% of controls. Finally, more than 35 years old was found in 4.44% of patients and only 2.22% of the control group. Between the age groups, no significant differences ($p>0.05$) was detected.

The current study findings showed that 44.44% of the patient group was male, while the occurrence in females was 55.56%. Also, there were 48.89% of males and 51.11% of females in the control group. The result shows no significant differences, as clear in Figure 2, $p>0.05$.

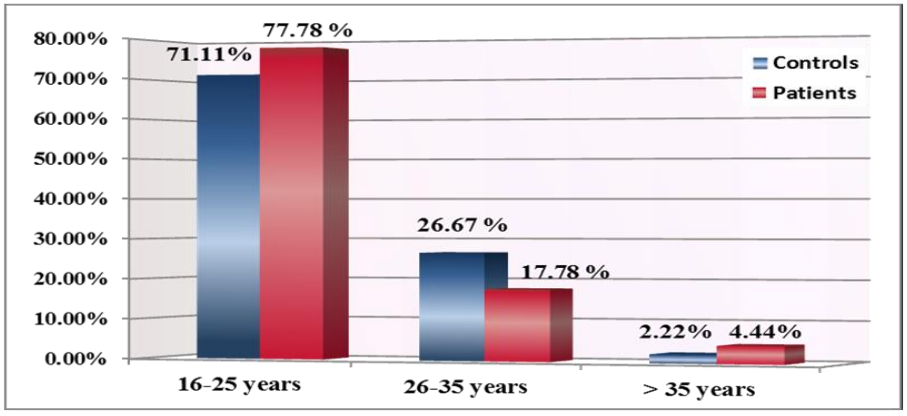

Figure 1. Distribution of Individuals According to Age (*p>0.05*).

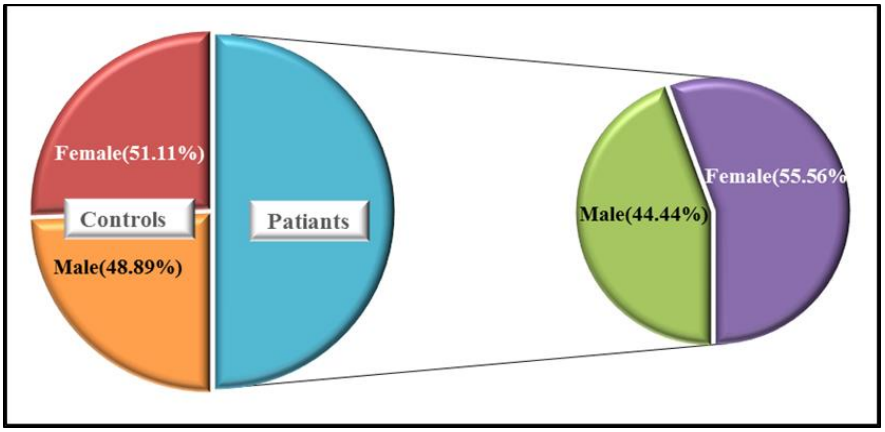

Figure 2. Distribution of Individuals According to gender (*p>0.05*).

This study indicated that a positive family history of acne appeared in 68.89% of the patient group and 24.44% of healthy controls, while negative family history appeared in 31.11% of patients and 75.56% of the control group. As listed in Table 1, a highly significant value was found between controls and patients (p<0.05).

Concerning the severity grades of acne, patients classified, a high percent of patients (37.78%) were in moderate acne, while 31.11% of patients in mild acne and the same percentage of patients were in severe acne with no significant value among different grades of acne (p>0.05). Figure 3 explains the distribution of acne patients according to severity grades.

Table 1. Distribution of subjects according to the family history of acne.

Family history	Healthy		Patient		*P. Value*
	N	%	N	%	
Positive family history	11	24.44%	31	68.89%	*<0.05*
Negative family history	34	75.56%	14	31.11%	
Total	45	100%	45	100%	

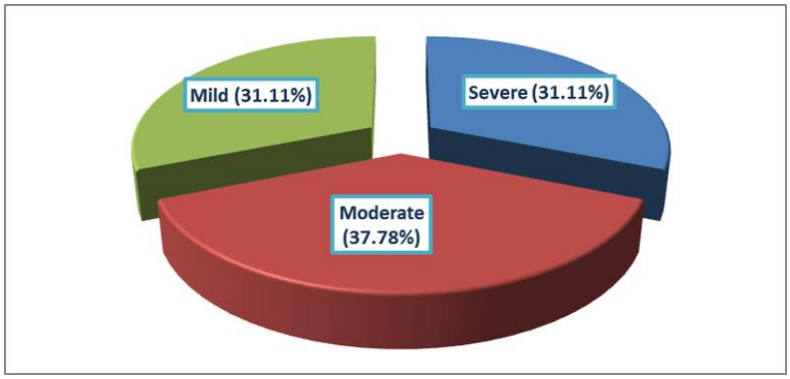

Figure 3. Distribution of Patients According to Severity Grades

The study showed a significant rise in TLR-2 level in the serum of patients compare to it is level in the control group (p<0.05). Also, the results indicated elevated levels of TLR-2 in male patients (1.83 ng ml^{-1}) compared with a male control (0.6 ng ml^{-1}), while TLR-2 level for female patients (1.20 ng ml^{-1}) compared with a female control (0.32 ng ml^{-1}), Table 2.

According to the relationship between TLR-2 sera levels of patients and acne severity, the results recorded that there was a positive correlation (r= 0.5) between the level of TLR-2 and Severity grades of acne with a significant value (p<0.05), Figure 4.

Table 2. Sera mean levels of TLR-2 in Acne Patients and Healthy group.

Parameter	Investigated groups	No.	Mean± SD	*P. Value*
TLR-2	Male patients	20	1.83 ± 0.45	*<0.05*
	Male control	22	0.60 ± 0.08	
	Female patients	25	1.20 ± 0.28	*<0.05*
	Female control	23	0.32 ± 0.05	

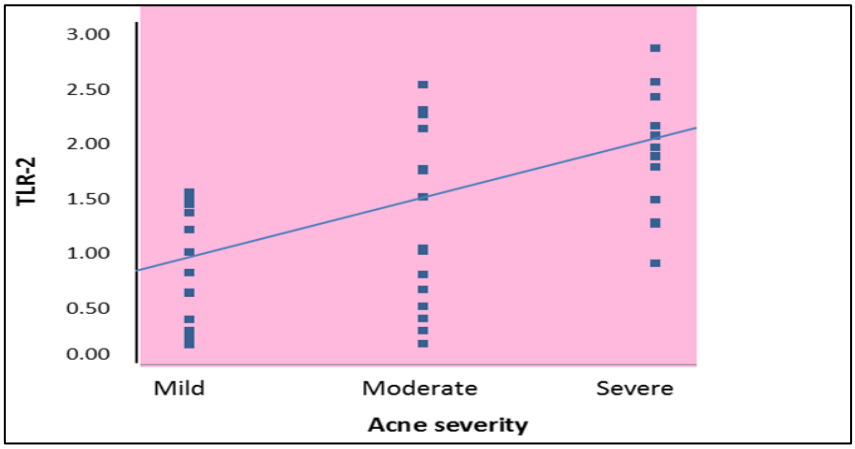

Figure 4. The positive correlation between TLR-2 sera levels and Severity Grades of Acne (r= 0.5, $p<0.05$).

DISCUSSION

This age of individuals ranges from 16-37 years old compatibles to a local study [11]. The average age was 23 years agreeing to some research by [12, 13]. Other studies found the average age was 24 years [14, 15] or 22 years [16]. This is a satisfactory result because adults have greater interest and awareness than adolescents. Moreover, during this period of life, a complete immune system receives additional support to protect itself from infection [17]. On the other hand, during puberty, internal factors like changes in sebum production are a result of hormonal change (especially androgen) or genetic factors. Also, external factors such as stress use of drugs, and utilized cosmetics lead to inflammation and acne prognosis [18].

The distribution results of samples according to sex found that the male to female ratio was 1:1.25. These results agree with some studies [11, 19]. Also, it is compatible relatively with some other published articles [16, 20, and 21].

The ratio of males to females varies in different studies, but acne shows prevalence in female patients throughout all years of life [22]. This may be due to changes in hormones during the menstrual cycle, concerns about career and family, economic pressures, and poor sleep quality caused by a variety of conditions [23], environmental factors, and utilized cosmetics [24]. Acne affects over 85% of people in adolescents age, with females being influenced more commonly than males after adolescence [25]. In addition, females perform almost two-thirds of the dermatology visits for acne [26].

According to family history, we found that people with a positive family history of acne are more susceptible to acne than others. The result agrees with the study conducted in 2016 [27] and relatively with previous findings in 2010 [20] and in 2014 [28] which suggested that hereditary factors play a cardinal role in acne. The genetic study of acne in twins by Bataille et al. (2002) recorded that 81% of acne occurrences are attributed to genetic factors [29]. However, another date found positive family history of acne in 30% of acne patients [30].

Thus, available evidence determined that familial history was associated with an increased individual tendency to acne, appearance at a younger age, more widespread and

severe lesions, risk of recurrence, and lesions more difficult to treat [28, 31].

Family history had been identified as a significant risk factor, influencing the size, number, and stimulation of sebaceous glands, as well as hormonal control in the body [32].

Most patients were of moderate acne this finding agrees relatively with the study in 2019 [33]. The difference in results from one study to another is due to differences in some criteria that were used.

Also, the current study appeared highly significant increase in TLR-2 inpatients than control subjects, and this was agreed with Egypt's research in 2009 [34] and also agreed with other studies [35-38].

A previous study in 2018 investigated that P. acnes or peptidoglycan molecular stimulated inflammation in vitro and Vivo increase TLR-2 expression in sebocytes and keratinocytes. SOD3 suppressed TLR-2 expression in immune cells and also inhibited the expressions of NF-κB [39].

Blood monocytes from acne patients in contrast with healthy control expressed significantly higher levels of TLR-2 and following P. acnes stimulation, exhibited significantly greater TLR-2 expression. Treatment of acne patients with isotretinoin down-regulates monocyte TLR-2 expression and decreased cytokine response to bacteria after therapy [40].

Moreover, there were many drugs investigated for acne treatment. It was shown that acne therapy reduces the expression of TLR-2 when compared to before the treatment with plant and natural therapy [41, 42].

Toll-like receptors are the main PRR of the innate immunity that senses PAMPs [43]. It is found on the surface of different cells especially monocyte and keratinocyte found in the dermis and it is activated by P. acnes presence. This receptor recognizes the peptidoglycan found in the cell well of P. acnes [44]. This results in activation of the NF-κB pathway and the MAPK cascade. TLR-2 controls the production of pro-inflammatory cytokines, including IL-1α, TNF-α, IL-6, and IL-8 which lead to inflammation of the skin [45]. More immune cells will aggregate at the site of inflammation leading to more TLR-2 expression as acne severity increases [46].

The positive correlation between TLR-2 and severity with significant value may belong to the immune activation in response to TLR-2 interaction with bacteria P. acnes causing increased cytokine production and this rise in severity and inflammation. In particular, IL-8 is induced through the activation of TLR-2, leading to the recruitment of neutrophils to the pilosebaceous unit and a rise in TLR-2 expression at the site [9].

Previous mentions demonstrated that TLR-2 on macrophage increase expression around pilosebaceous with acne lesion development [47], while staining of acne lesions by Ozlu et al. (2016) found that TLR-2 level in papules and comedones was higher than severe pustules and nodules [37].

According to the current results, we concluded that the female gender appeared to be the most possible factor associated with acne vulgaris. Also, It's illustrated that the high levels of TLR-2 in acne patients and the positive correlation of TLR-2 with acne severity, revealed that TLR-2 has a role in the bad prognosis of acne vulgaris.

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Not applicable.

ETHICAL CONSIDERATION

To conduct the research ethical permission was obtained from the hospital and from all participants in this work patients and healthy. The patients Selection were accomplished with the assistance of Dermatologists in the hospital.

Conflict of interest

The authors declare no conflicts of interest.

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