Research Article

Association Of Toll-Like Receptor 4 (TLR4) Expression With Psoriasis Pathogenesis In Patients From Basrah, Iraq

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Abstract

Psoriasis is a long-term inflammatory skin disease that occurs when a person's genes are affected by some environmental triggers. The triggers are associated with immune dysregulation by complex inflammatory mechanisms, causing the typical pathological changes in the skin. This study aimed to investigate the role of Toll-like receptor 4 (TLR4) in psoriasis progression by measuring its serum concentration using ELISA and assessing its tissue expression in skin biopsies via immunohistochemistry (IHC). We collected 35 venous blood samples from psoriatic patients and 22 from healthy controls, along with 5 skin biopsies from patients and one from a control, Statistical analysis was performed using independent t-tests, and $p < 0.05$ was considered significant. The results showed a statistically significant increase in TLR4 expression in psoriatic patients compared to healthy individuals. "These findings suggest that TLR4 plays a pivotal role in sustaining inflammatory pathways and could serve as a potential therapeutic target in psoriasis."

Keywords: Toll-like receptor 4 (TLR4); Psoriasis; Psoriasis pathogenesis; TLR4 expression; Innate immunity; Inflammatory response; Cytokines; Immune dysregulation; Basrah; Iraq.

Introduction

Psoriasis is a chronic inflammatory skin disorder that occurs in genetically susceptible individuals upon exposure to environmental triggers. These factors lead to a dysregulated immune response involving multiple inflammatory pathways, resulting in structural and functional changes in the skin and the development of psoriatic lesions [1]. Histopathological hallmarks include acanthosis (epidermal thickening), hyperkeratosis (thickened stratum corneum), and parakeratosis (nuclei retention in the stratum corneum) [2]. Research over the past fifty years has increasingly highlighted the central role of both innate and adaptive immunity in psoriasis pathogenesis. Psoriatic lesions contain increased numbers of immune cells, including natural killer (NK) cells, neutrophils, mast cells, T lymphocytes, and dendritic cells. These cells secrete many immunological mediators, including tumor necrosis factor-alpha (TNF-α) [3, 4]. Some of the most important immune factors involved are the Toll-like receptors (TLRs), especially TLR4. These receptors are found in immune cells, and are essential for recognizing self and non-self-molecules to trigger innate and adaptive immune responses [7]. TLR4 plays a crucial role in the initiation and shaping of psoriatic plaque formation and promotes the release of pro-inflammatory cytokines, such as TNF-α and IL-6, which further amplify the inflammatory process [8, 9]. Although a global association has been established between TLR4 and psoriasis, no study in Iraq has studied these two levels simultaneously, especially in Basrah, and thus this study filled a research gap in the region. This study aimed to: Measure serum levels of, TLR-4 in psoriatic patients and healthy controls using ELISA. Evaluate the tissue distribution of TLR4 in patient and control skin samples using immunohistochemistry.

Materials and Methods

Sample Collection

Blood Samples:

The study included 35 venous blood samples from psoriasis patients (male and female, aged 8–55) from Basrah Governorate, Iraq. Patients who had received treatment in the past three months, or those with cardiovascular disease, diabetes, autoimmune disorders, or pregnancy were excluded. Twenty-two blood samples were also collected from healthy individuals as a control group. All samples were obtained after clinical examination by a

dermatologist at Basrah Teaching Hospital. Serum was separated by centrifugation and stored at -20°C until ELISA analysis. Ethical approval was obtained from the University of Basrah and the Basrah Health Department ethics committee No. (610) issued in 8/9/2024

Tissue Samples:

Five psoriatic plaque samples were collected from patients aged 20–35, along with one control sample. Patients with autoimmune diseases, cancer, or immunodeficiency were excluded from the biopsy group.

Measurement of Immune Factors

TLR4 serum levels were quantified using a commercial ELISA kit (Bioassay Technology Laboratory, China) according to the manufacturer's instructions.

Immunohistochemistry (IHC)

An anti-TLR4 primary antibody (Abcam, Cat. No. ab22048) was used. Tissue samples were fixed in 10% formalin, embedded in paraffin, and sectioned at 4–5 µm. After deparaffinization and rehydration, antigen retrieval was performed. Sections were incubated overnight with the primary antibody at 4°C, followed by a horseradish peroxidase (HRP)-conjugated secondary antibody. Staining was visualized with 3,3'-Diaminobenzidine (DAB), and slides were counterstained with hematoxylin, dehydrated, and mounted. Images were captured using a light microscope [10]

Statistical analysis

Statistical analysis was conducted using SPSS version 26.0. Data were expressed as mean ± SD. Independent t-test and one-way ANOVA were used to compare groups.

Results

TLR4 Serum Levels

TLR4 levels were significantly elevated in psoriasis patients across all age groups compared to controls, with a significantly different $p < 0.05$. This study, as shown in Table 1, revealed that in patients under 18, moderate cases had higher TLR4 levels (5.031 ± 3.560) compared to severe cases. while in the 19–28 age group, severe cases had higher levels (5.772 ± 4.856) than moderate cases. But in the 29–38 group, both mild and severe cases showed elevated TLR4. Also, in Figure 1, this study shows that female patients had higher TLR4 levels (4.238 ± 3.467) than males (3.108 ± 0.812).

Table 1. TLR4 concentration in patient and control samples

Age Group	Condition	Number	Mean ± SD
< 18	Moderate	6	5.031 ± 3.560
	Severe	2	3.080 ± 0.933
19–28	Moderate	7	2.787 ± 0.701
	Severe	5	5.772 ± 4.856
29–38	Moderate	3	2.776 ± 0.720
	Severe	2	3.125 ± 0.869
39–48	Moderate	2	2.800 ± 0.014
	Severe	6	2.921 ± 0.567
49–55	Moderate	2	2.601 ± 1.021

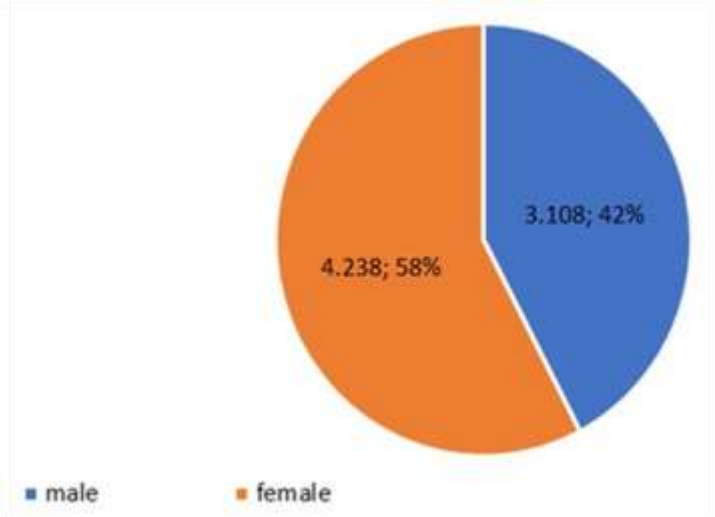

Fig.1. Describes TLR4 levels by gender

Immunohistochemistry study

IHC analysis of psoriatic plaques showed positive TLR4 expression, with brown cytoplasmic staining in approximately 60%, 60%, and 50% of cells in Figures 3, 4, and 5, respectively. In contrast, control tissue showed no TLR4 staining (Figure 2).

The immune response stimulated in the skin of psoriasis patients showed distinct staining in both the cytoplasm and nucleus of the epidermal layer. The staining was of moderate intensity and exhibited a heterogeneous distribution across the epidermis. The positively stained epidermal cells demonstrated an expression rate of up to 50% (Figure 3).

Furthermore, the current study results, as illustrated in Figures 4 and 5, revealed more intense staining within the epidermis, progressing toward the superficial layers and reaching the basal layer. A marked expression of TLR4 receptors was observed in the psoriatic skin regions, with widespread, more uniform, and diffuse staining. The percentage of TLR4-expressing cells reached approximately 60%.

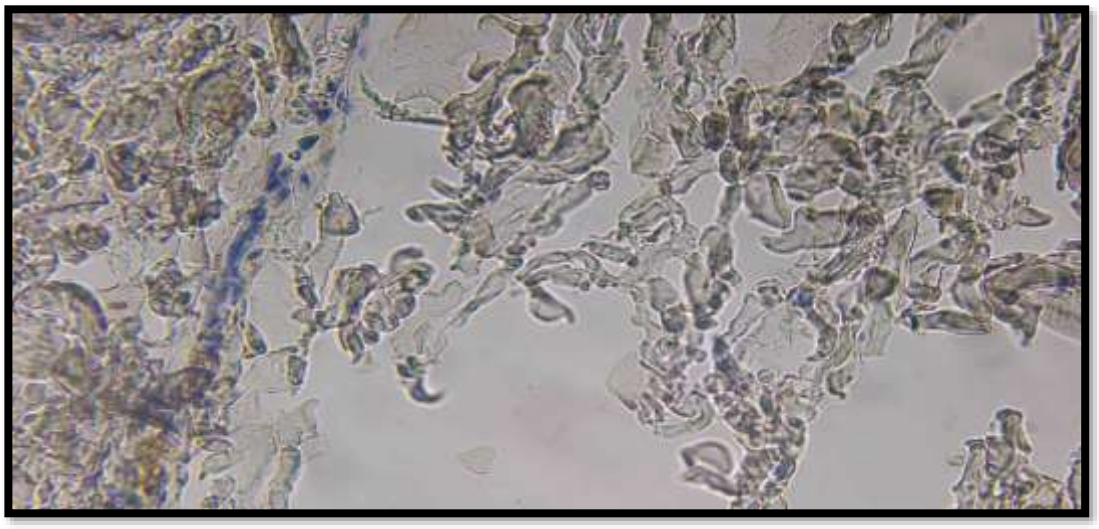

Fig. 2. Immunohistochemical section of the skin biobsy of control showed negative TLR4 staining (black arrows) with 0% staining contribution. IHC 40X.

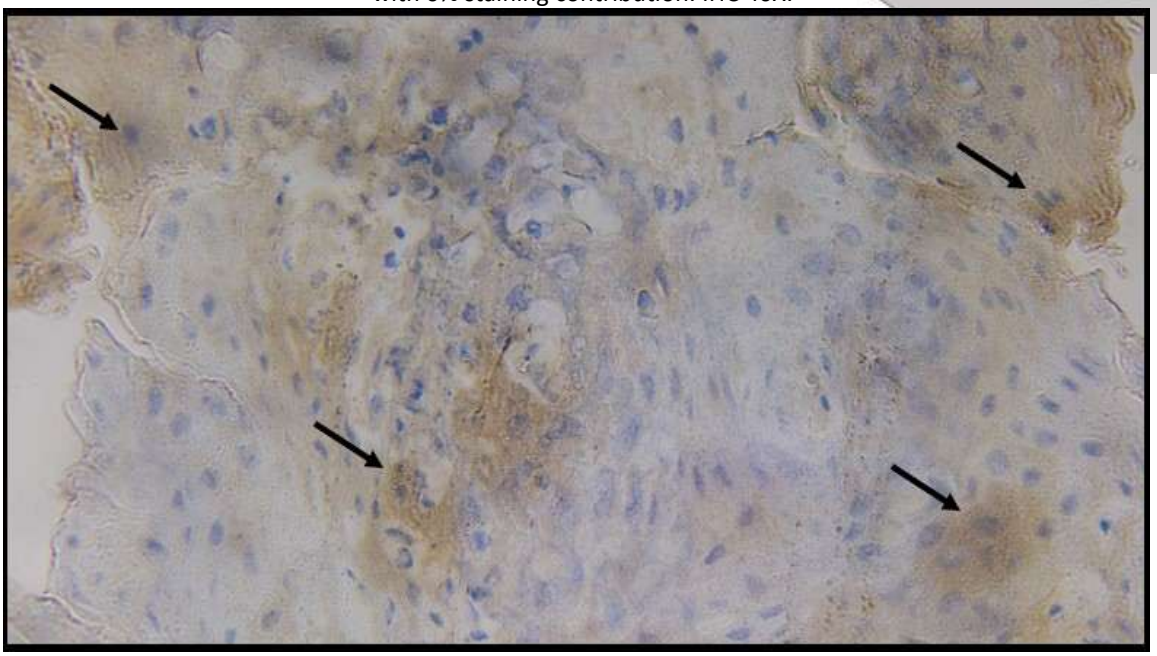

Fig. 3. Immunohistochemical section of the skin biobsy of psoriasis showed positive TLR4 staining (black arrows) in which the brownish IHC staining refered to positivity with 50% contribution. IHC 40X

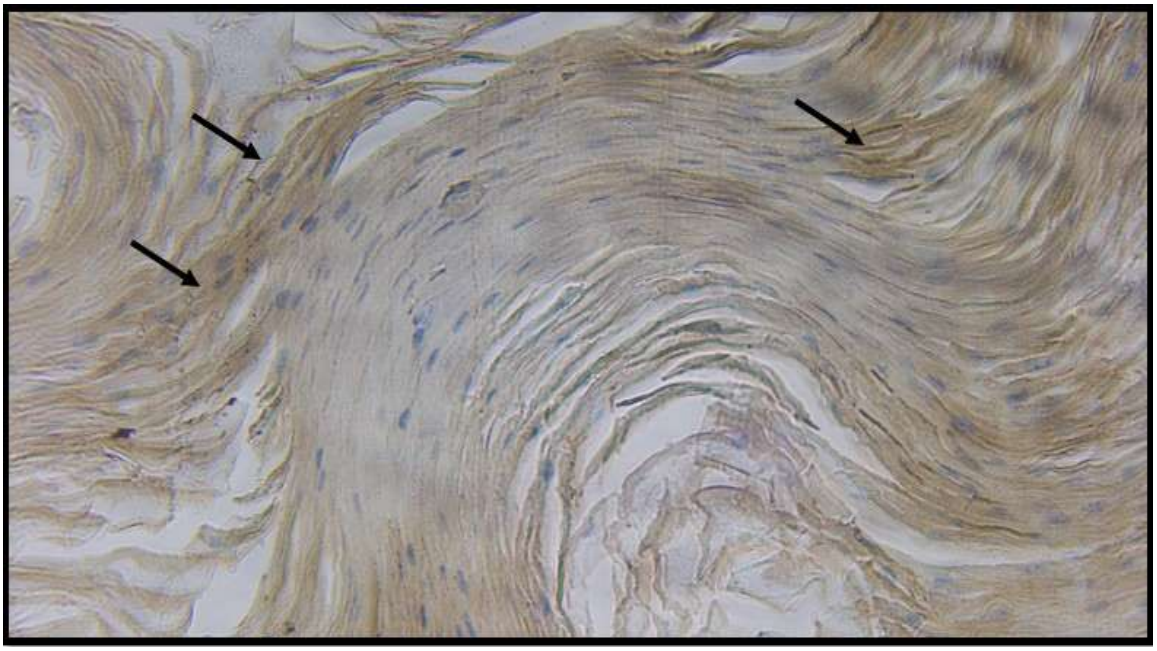

Fig. 4. Immunohistochemical section of the skin biobsy of psoriasis showed positive TLR4 staining (black arrows) in which the brownish IHC staining refered to positivity with 60% contribution. IHC 40X

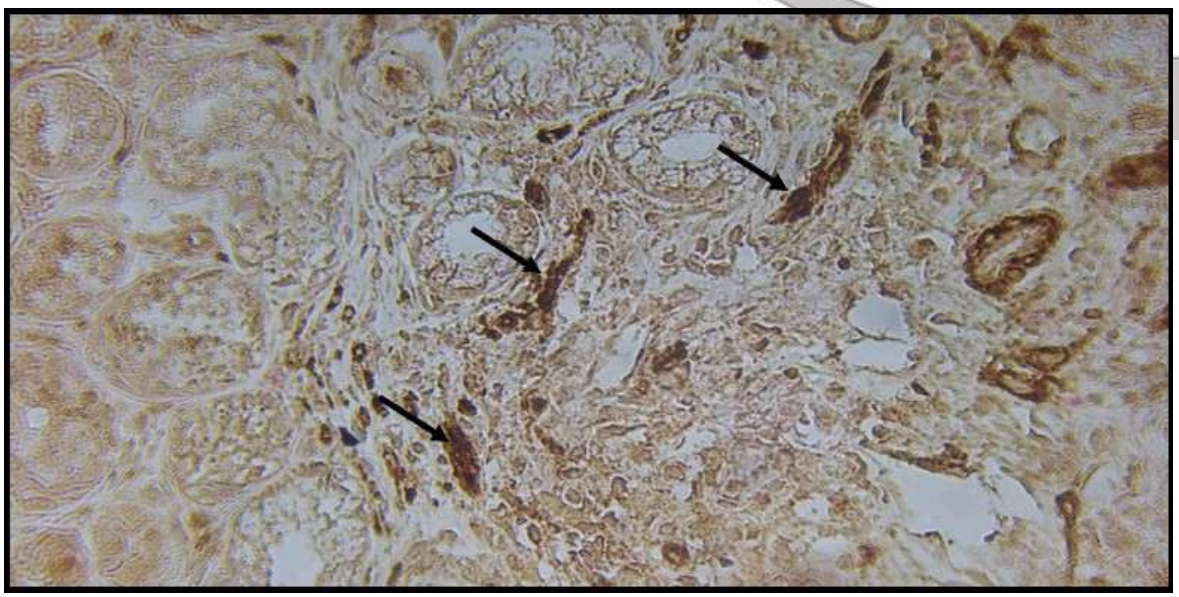

Fig. 5. Immunohistochemical section of the skin biobsy of psoriasis showed positive TLR4 staining (black arrows) in which the brownish IHC staining refered to positivity with 60% contribution. IHC 40X.

Discussion

Our results suggest a positive correlation between TLR4 concentration and psoriasis severity. TLR4 overexpression likely stimulates the production of pro-inflammatory cytokines such as IL-17, IL-23, and TNF-α, which are known to drive keratinocyte hyperproliferation and immune cell recruitment in psoriasis [11]. These results were consistent with previous studies, such as that conducted by Shehata et al. [12] which showed that psoriasis patients had elevated gene expression of TLR4 in peripheral blood mononuclear cells (PBMCs). This suggests that activation of TLR4 is linked to inflammatory signalling events like NF-κB, which aids in skin cell proliferation and lesion formation [13]. Also, a recent study showed that the expression of TLR4 is increased in the PBMCs of patients with psoriasis [14]. The levels of TLR4 were higher in our females which is similar with the results of Özkesici-Kurt et al. [15] indicating a potential effect of sex hormones on disease pathogenesis [16]. One more investigation of TLR4 found that the raised level of TLR4 is correlated with diabetic patients [17]. Moreover, in the study conducted by [18] the serum levels of TLR4 were seen to be elevated in the patients. Consistent with the suspected leukemogenicity of TLR4 in paediatric ALL.

Limitations and Future Directions

These results are somewhat limited due to the small number of samples, especially with tissue biopsies. The study was also a cross-sectional analysis which did not provide longitudinal follow-up data to associate with treatment response based on the level of TLR4 expression. Larger multicenter cohorts should be examined in future studies to validate TLR4 as a therapeutic biomarker and molecular mechanisms should be investigated using gene expression assays or protein signaling assays.

Clinical and Therapeutic Implications

So, as a close relationship between increased expression of TLR4 and severity of psoriasis, it is a potential novel immunotherapeutic target. Inhibition of TLR4 or NF-κB downstream pathways may be a possibility to control excessive inflammation in psoriatic skin. Additional translational studies are merited to assess the potential of TLR4 inhibitors (or TLR4 antagonists) to be used as adjunctive therapies.

Conclusion

The activation of pro-inflammatory pathways and TLR4 overexpression in serum and tissue support its important role in psoriasis pathogenesis. Overall, this study shows the critical role played by innate immune receptors in the maintenance of chronic skin inflammation, and sets a platform for future therapeutic interventions aimed at blocking TLR4 signaling.

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