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Original article

Detection of Toll like Receptor 2 Single Nucleotide Gene Polymorphism in Psoriasis

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Abstract

Background: Psoriasis is a complicated inflammatory condition of chronic nature with several etiologies. There is evidence that environmental, genetic, and immunologic factors are involved in its pathogenesis. Toll like receptor (TLR) has been identified among human beings. One of most important toll-like receptors is toll like receptor 2 which is involved in variety of autoimmune disorders. **Aim**: To determine the role of toll like receptor 2 single nucleotide gene polymorphism in psoriasis patients compared to healthy controls. **Patients and methods:** Blood samples from 30 patients with fully developed psoriasis were collected as well as from 30 healthy controls and those blood samples were analyzed using real time PCR. Each patient's PASI (Psoriasis Areas and Severity Index) score was determined. **Results:** The detection of single nucleotide gene polymorphism was significantly higher in patients compared to controls with no correlation to age, sex or disease severity

(*P-value* > 0.05). **Conclusion**: Toll like receptor 2 single nucleotide gene polymorphism could be involved in the pathogenesis of psoriasis.

1. Introduction:

Psoriasis is a chronic inflammatory skin disease characterized by scaly indurated erythema. It has been recognized not only as a skin disease but also as a systemic disease. [1] The incidence of occurrence of psoriasis in developed countries is 1-4%. [2] The etiology of psoriasis is thought to originate from interaction of genetic, environmental, infectious and lifestyle factors. [3] Psoriasis is associated with several comorbidities, including cardiovascular disease, lymphoma, and depression. [4] The Psoriasis Area Severity Index (PASI) is the most widely used scale for assessing the severity of psoriasis and for therapeutic decision making. On the basis of the PASI score, patients have been stratified into 2 groups: mild disease and moderate-to-severe disease. [5] Toll-like receptors (TLRs) play an essential role in the pathogenesis of autoimmune diseases. TLRs belong to the family of pattern recognition receptors (PRRs) that recognize a wide range of pathogen-associated molecular patterns (PAMPs). [6] Among TLRs, TLR2, which has been documented to be expressed by several cell types, for instance, macrophages, can recognize various PAMPs including the membrane components of Gram-negative/positive bacterial cell wall, parasites, and so on. The mutation in TLR2 is believed to be associated with increased susceptibility to infectious diseases. [7] TLR2 contains several non-synonymous single-nucleotide polymorphisms that may impact the activation of its signaling cascade and alter the responsiveness to, or the course of, various infectious diseases, including those caused by pathogenic spirochetes. [8] The aim of the present study was to detect the association between toll like receptor 2 single nucleotide gene polymorphism and psoriasis.

2. Subjects And Methods:

2.1. Study design, duration, and settings

A case control study was conducted in the period between July 2022 and May 2023. All study participants were enrolled from the outpatient dermatology clinic, Faculty of Medicine, Beni-Suef University hospital.

2.2. Study subjects

The study included 60 participants divided into two groups, the case group included 30

cases diagnosed with psoriasis, and the control group included 30 healthy persons with matched age and gender.

2.2.1. Inclusion criteria: All psoriatic patients regardless of their age and sex, Apparently healthy controls with age and sex matched to the enrolled patients.

2.2.2. Exclusion criteria: Patients with other skin diseases, cutaneous tumors or autoimmune diseases.

2.3. Sample size

Based on the results of previous studies, sample size was determined using G power version 3.1.9.4. A total of 23 cases and 23 controls are required, however the sample size was increased to 30 cases and 30 controls to enhance the study power.

2.4. Ethical considerations

Approval for this study from the Research Ethical Committee at Faculty of Medicine Beni-Suef University (FM-REC) was taken before the beginning of the study, approval number (FMBSUREC/11092022/MOHAMMED).

Informed written consent was obtained from all participants before inclusion in this study. Confidentiality and personal privacy were respected in all levels of the study. Data wasn't used for any other purpose.

2.5. Data collection methods and tools

Patients as well as healthy controls were subjected to the following:

1- **Complete history taking:**

- ✓ Age, sex, marital status, residence, special habits of medical importance and history of previous gestations.
- ✓ Onset, course, and duration of the disease (in the patients' group).
- ✓ Consanguinity and similar conditions within family members.

2- **Complete general and dermatological examination.**

3- **Psoriatic patients were categorized based on severity via PASI score**:

To form the score, the 3 characters of psoriatic plaques (redness, scaling and thickness) are given a number from (0 - 4), 4 being the worst score. The degree of affection of every area of the body is scored from (0-6).

Adding up the scores gives a range from (0 - 72), if the score <10, it indicates mild disease, while >10 indicates moderate to severe affection.

4- Sample collection and storage:

Under all aseptic precautions, blood samples (3 ml blood) were drawn from peripheral veins. Samples were then subjected for centrifugation, serum was separated and kept frozen at -20°C till analysis of toll like receptor 2 single nucleotide gene polymorphism by real time PCR according to the manufacture and kits instructions. Quantitative PCR (Q-PCR) was carried out by ABsolute™ QPCR SYBR® Green Mix kit (Thermo fisher scientific) using same primers of real time PCR experiments, designed by others and rechecked with Prime3plus software, to human specific toll-like receptors (TLR) 2.

PCR amplification

PCR for TLR-2 was 5 µl of template DNA, 1 µl of gene primer (ATCCTCCAATCAGGCTTCTCT) Amplicon Size (bp) 163. Primers are provided as a 40 µl solution containing both primers at a final concentration of 50 µM in 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA. Dilute with water as needed prior to use. This amount is sufficient for 1000 x 20µl PCR reactions assuming a final primer concentration of 0.1 µM. Add cDNA template. Recommended reaction conditions: primer concentration - 0.1 µM, MgCl2 concentration approx. 4 mM, SYBR Green Master Mix. Program: 95°C - 58°C - 72°C (45 cycles). A thermal cycler was used for the amplification processes (CATC; Rapidcycler, BioGene Ltd., Kimbolton, Cambridgeshire). The thermal cycling profile consisted of a 2-minute initial denaturation at 94 °C, 30 amplification cycles of 1-minute denaturation at 94 °C, 1-minute primer annealing, and 2-minute extension at 72 °C, and a 4-minute final extension at 72 °C. The TLR-2 amplicons generated fragments with a mean size of 209 bp. The sizes of the amplified products were determined using a DNA ladder of known length (100 bp). [9]

3. Results:

The current study included 30 psoriasis patients from both sexes and 30 healthy controls were collected, they were age and sex matched to the psoriasis cases. The psoriasis patients were females and males patients (53.3%), their age ranged from 6 to 60 years, the average age was 29.13±13.7. The largest percentage of the studied patients had gradual onset (90.0%) and progressive course of the disease (70.0%). Moreover, the average duration of the disease was .41±5.14 and ranged from 0.25 to 23 months. Positive family history was reported by (30.0%) of patients and previous treatment was reported by (50.0%).

Table (1) demonstrated that the mean PASI score for the studied patients was17.76±14.49. Half of the studied patients scored > 10 points in PASI scoring system and was classified as severe chronic plaque psoriasis patients (50.0%).

Table (1): Distribution of the studied patients as regards PASI (Psoriasis area severity index) scoring system.

	Mean	**SD**	**Minimum**	**Maximum**
PASI score	**17.76**	**14.49**	2.70	48.40
Grading	**Frequency (n=30)**		**Percentage (%)**	
Mild chronic plaque psoriasis (<°)	5		16.7	
Moderate chronic plaque psoriasis (5-10)	10		33.3	
Severe chronic plaque psoriasis (> 10)	**15**		**50.0**	

There was a significant moderate positive correlation between disease duration and PASI score for the studied patients (p=0.04, r=0.384), ***(Table 2 and figure 1).*** The toll like receptor 2 (TLR2) single nucleotide gene was significantly detected among (83.3%) of patients and (6.7%) of controls at (*p value= 0.001*), ***(Figure 2).***

Table (2): Correlation between age and disease duration for the studied patients and their PASI scoring.

		PASI score
Age	Pearson Correlation coefficient (r)	-0.083
	P value	*0.66*
Duration	Pearson Correlation coefficient (r)	0.384
	P value	*0.04**

Statistical analysis was done using Pearson correlation test.

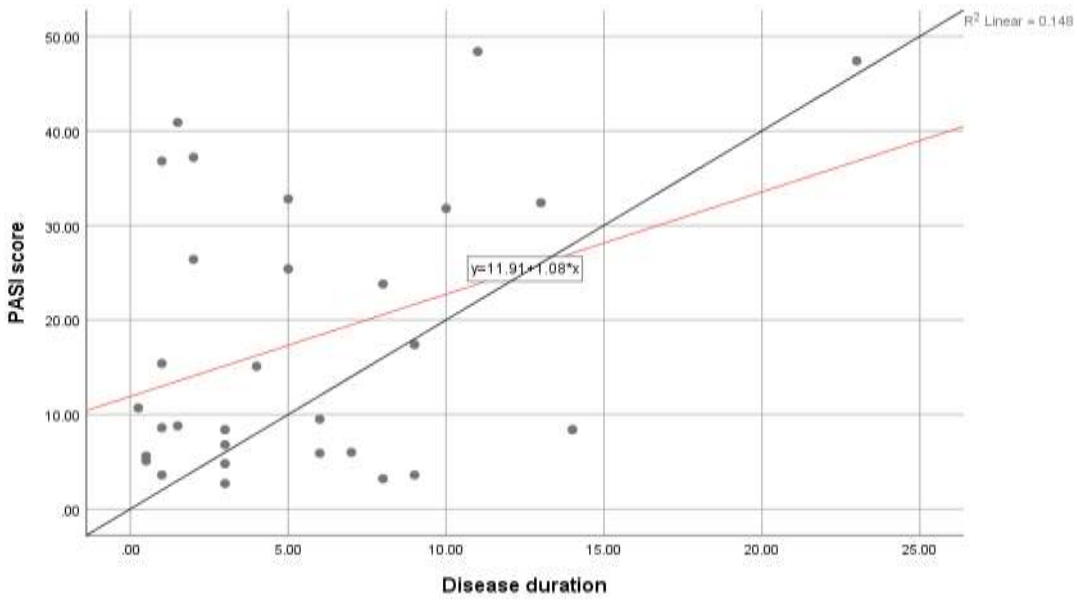

Figure (1): Correlation between disease duration of the studied patients and their PASI score

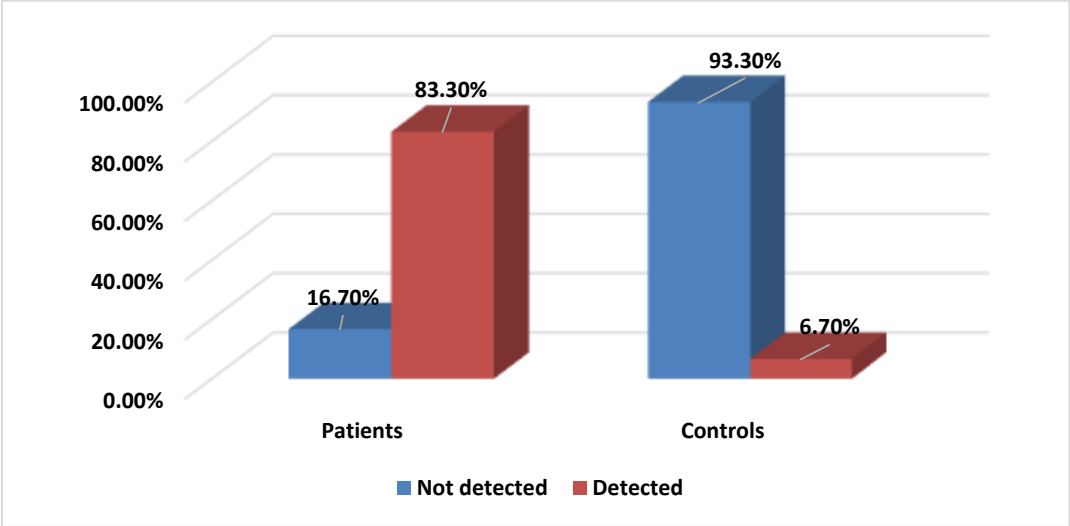

Figure (2): TLR2 gene detection among the studied participants

The prevalence of TLR2 among the studied female patients (51.5%) was non significantly higher than male patients (48.5%). And there was no statistically significant difference between them (*p=0.79*). Regarding age, the mean age for the studied participants with detected TLR2 gene (31.61±16.1) was non-significantly higher than participants with no detected gene (30.30±14.4) at *p=0.74*. Moreover, there was no statistically significant difference between the studied psoriasis patients as regards disease severity, ***(Table 3).***

Table (3): Association between demographic data and gene detection among the studied patients

Demographic data			TLR2 Gene		*P value*
			Detected	**Not detected**	
Sex	Males	N (%)	16 (48.5)	14 (51.9)	*0.79*
	Females	N (%)	17 (51.5)	13 (48.1)	
Age	Mean± SD		31.61±16.1	30.30±14.4	*0.74*
Disease severity	Mild	N (%)	2 (40.0)	3 (60.0)	*0.21*
	Moderate	N (%)	2 (20.0)	8 (80.0)	
	Severe	N (%)	1 (6.7)	14 (93.3)	

Statistical analysis was done using Qui square test and independent sample t-test.

4. Discussion:

Psoriasis is a common chronic, recurrent, immune mediated disease of the skin and joints. It has a strong genetic component but environmental factors such as infections can play an important role in the presentation of disease. There are several clinical cutaneous manifestations of psoriasis but most commonly the disease presents as chronic, symmetrical, erythematous, scaling papules and plaques. [10] TLR2 has been implicated in several auto-immune and inflammatory conditions, and its role in disease pathogenesis has been supported by numerous reports of TLR2 polymorphisms in humans linked to disease. [11] Toll-like receptor-2 acts on keratinocytes, recognition of pathogen-associated molecular patterns, cytokines, chemokines, inflammasomes, neuroendocrine regulatory mechanisms, diet and immune detection and other pro-inflammatory targets implicated in the activation of response. [12] The present study showed that the expression of toll like receptor 2 single nucleotide gene polymorphism was significantly higher in patients compared with the controls with no significant correlation to age, sex ***(Figure 1 & Table 2).*** This finding was in agreement with **Nakao et al (2020) who** showed that TLR-2 expression was increased in immature dendritic cells from patients with Psoriasis. Monocytes and mature dendritic cells did not show statistically significant differences. No difference was observed in the expression of TLR-4 in any cell type. The supernatant expression of cytokines showed a Th1 pattern, mostly with increased expression of TNF-alpha, IFN-gamma, and IL-2. Western

blot analysis confirmed the increased expression of TLR-2. [13] **Donetti et al (2023)** showed Epidermal keratinocytes in normal skin expressed TLR1, TLR2 and TLR5. Cytoplasmic TLR1 and TLR2 were expressed throughout the epidermis, with higher staining of the latter on basal keratinocytes, while TLR5 expression was concentrated in the basal layer. In contrast, in lesional epidermis from patients with psoriasis, TLR2 was more highly expressed on the keratinocytes of the upper epidermis than on the basal layer, while TLR5 was down regulated in basal keratinocytes compared with corresponding non lesional psoriatic epidermis. [14] **Wang et al (2021)** propose to assess the modulation of TLR expression on psoriatic skin of patients treated with Adalimumab and systemic conventional therapies. They recruited fifteen patients: ten were treated with adalimumab and five with systemic conventional therapies; their clinical conditions were analyzed by PASI index and skin biopsies were evaluated for TLR1 and TLR2 expression by immunohistochemistry assays. Their data suggest adalimumab is not only able to improve the clinical condition of psoriatic patients, but also to modulate TLR1 and TLR2 expressions involved in psoriasis, as in healthy skin. Adalimumab is a most promising biological drug able to orchestrate immune and inflammatory responses in psoriatic lesions, recovering TLR expression on basal keratinocytes. [15] **Sabah-Özcan et al (2019)** demonstrated that the TLR2-rs4696480 genotype seemed to have a higher risk for psoriasis while the TLR2-rs11938228 polymorphism has not shown any significant association with the risk of psoriasis. There was no statistically significant difference between the mean age, gender, onset age, and PASI level and genotypes resulting in The SNP rs4696480 of TLR2 may have significant effects on the heritability of psoriasis in the Turkish population. [16] **Kim et al (2002)** investigated whether TLR2 mediates P. acnes-induced cytokine production in acne. Transfection of TLR2 into a nonresponsive cell line was sufficient for NF-kappa B activation in response to P. acnes. These data suggest that P. acnes trigger inflammatory cytokine responses in acne by activation of TLR2. As such, TLR2 may provide a novel target for treatment of this common skin disease. [17] There are other infectious dermatological disorders in which toll like receptor 2 gene polymorphism play an important role in its pathogenesis as proved by **Bakry et al (2013)** who aimed at exploring toll-like receptor 2 (TLR2) immunolocalization and its possible role in

the innate immune response against viral warts and molluscum contagiosum (MC). The study proved that upregulation of TLR2 is involved in the induction of defense mechanism against human papillomavirus and MC virus. [18] Toll like receptor 2 gene polymorphism is also included in therapeutic action of some drugs as proved by **Dispenza et al (2012)** who proved that isotretinoin induces remission of acne by normalizing the innate immune response to the commensal bacterium Propionibacterium acnes. Compared with normal subjects, peripheral blood monocytes from acne patients expressed significantly higher levels of Toll-like receptor 2 (TLR-2) and exhibited significantly greater induction of TLR-2 expression following P. acnes stimulation. Treatment of patients with isotretinoin significantly decreased monocyte TLR-2 expression and subsequent inflammatory cytokine response to P. acnes after 1 week of therapy. [19] On the other hand, in a study done by **Tagoe et al (2023)** assessed gene expression analysis of toll like receptor 2 in rat keratinocytes showing that A time-course of TLR2 activation in rat epidermal keratinocytes revealed that although there was rapid initial activation of innate immune pathways, this was rapidly superseded by widespread up-regulation of epidermal differentiation related proteins. [20] Taken together, these data define a dual role for Toll-like receptor 2 activation during epidermal barrier repair that may be a useful therapeutic modality in treating diseases of epidermal barrier dysfunction.

5. Conclusion and recommendations:

Our study revealed that the expression of toll like receptor 2 single nucleotide gene polymorphism is significantly higher in psoriatic patients in comparison with the controls. According to these findings, toll like receptor 2 gene polymorphism is suggested to play an essential role in the mechanisms that are responsible for development of the psoriasis.

Based on the results of our study and in conjunction with data from previous studies, we suggest:

- Further studies and large groups of subjects are essential to confirm the role of toll like receptor 2 single nucleotide gene polymorphism in the pathogenesis of psoriasis.

- Studies about toll like receptor 2 single nucleotide gene polymorphism in other proliferative skin diseases (squamous cell carcinoma, basal cell carcinoma) and

inflammatory skin diseases (atopic dermatitis, chronic eczema).

• Clinical trials on the efficacy of using toll like receptor 2 suppressors in the treatment of psoriasis.

Conflicts of Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Informed consent

A signed consent form was obtained from each study participant.

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