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Serum Toll-Like Receptor 2 Concentration in Pediatric Atopic Dermatitis: A Case-Control Study

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Abstract

Background and Aims: Atopic dermatitis (AD) is a chronic, relapsing inflammatory skin disease. There is increased evidence that Toll-like receptors 2 (TLR2), which are a part of innate immunity, play a role in the pathophysiological changes in AD. The study aims to estimate the serum TLR2 concentration among children with AD compared to healthy controls, and to explore the relationship between serum TLR2 concentration and AD severity and total Immunoglobin E (IgE) level.

Patients and Methods: This case-control study enrolled 156 participants, with 76 atopic dermatitis (AD) cases and 80 controls. It was conducted at the outpatient clinics of Al-Fayhaa Teaching Hospital between December 2020 and March 2021. The study involved interviewing the parents of the participants, detailed clinical examination with assessment of disease severity, and laboratory tests including total IgE and serum TLR2 concentration.

Results: Serum TLR-2 concentrations were lower in AD cases (median= 761.9 pg/ml) than in controls (median=1528.5 pg/ml; P=0.00002). Significantly higher TLR2 levels were seen in patients with elevated IgE (P=0.013) and severe form of AD (P=0.005).

Conclusions: Although lower TLR2 concentrations could indicate their role in AD pathogenesis, higher TLR-2 levels were seen in more severe cases or patients with elevated IgE. This suggests a complex mechanism of TLR2 in the immune dysregulation that occurs in AD. This might refer to the involvement of TLR2 in both the onset and exacerbation of AD, particularly in severe forms.

Keywords: Atopic Eczema, Atopic dermatitis, Toll-like receptors

1. INTRODUCTION

Atopic dermatitis (AD), also known as "atopic eczema", is a chronic, relapsing inflammatory skin disorder and is considered a significant health problem, especially in children. It is a highly prevalent disease and has an impact on the quality of life [1,2].

Innate immunity is a major contributor to the pathogenesis of AD. The skin barrier serves to prevent the invasion of pathogens and allergens. Tight junctions (TJ) are transmembrane proteins that control skin permeability. These TJ are increased by Toll-like receptors 2 (TLR2) activation, which are part of innate immunity [3]. Patients with AD subjects have under-expression of both tight junction protein "claudin" and "antimicrobial peptides"; thus, TLR-2 might play a role in maintaining the integrity of the skin barrier [4].

TLRs are one type of pattern recognition receptors (PRRs) that belong to innate immunity. They are either intracellular or found on the cell surface. Their major function is to trigger inflammation and to develop antibacterial defense through the activation of type 17 helper T-cells (Th-17) and Th-1. These receptors can identify pathogen-associated molecular patterns of many Gram-positive and Gram-negative bacteria, fungi, and viruses. The Toll-like receptors 4 (TLR4) and TLR2 are intracellular. Impairment in their signaling, particularly hypo-responsiveness or reduced signaling, may stimulate Th2 response and subsequent development of AD [5].

Understanding the role of TLR2 in the skin and how they contribute to pathophysiological changes in AD can help in developing new therapeutic guidelines aiming to restore the skin barrier function and reverse the immune dysfunction that occurs in AD [6].

Objectives:

1. To estimate the serum TLR2 concentration among children with AD and compare it with that of healthy controls.
2. To explore the relationship between serum TLR2 concentration and the severity of the clinical features in children with AD, and the relationship between serum TLR2 concentration and total IgE level.

2. MATERIALS AND METHODS

A case-control study was conducted at the outpatient clinics of Al-Fayhaa Teaching Hospital between December 2020 and March 2021. Cases were recruited from the Dermatology outpatient clinic and were diagnosed by the attending specialist dermatologist using Hanifin and Rajka diagnostic criteria [7]. Age and sex-matched controls were selected from the other outpatient department, and they were free from any dermatological or infectious disease.

Followed by measurement of height and weight to calculate the body mass index (BMI). Among children under 2 years old, length was measured while lying down, and for those 2 years and older, height was measured while standing. BMI was divided into four categories: underweight, normal, overweight, and obese using "The WHO charts of BMI for age Percentiles" [8]. We used the Objective SCORAD tool to determine the severity of AD among cases. This tool involves assessment of the extent and intensity of the lesions, with a score less than 15 considered mild, 15 to 40 as moderate, and above 40 as severe AD [9].

Following examination, five milliliters of venous blood were collected from the participants into a serum gel tube, which was then centrifuged and separated into two portions, one for total IgE testing and the

other for TLR2 Enzyme-linked Immunosorbent Assay (ELISA) testing. These portions were kept frozen for one month at -20°C.

The total IgE test was conducted using the ALFA (Allergy Lateral Flow Assay). The TLR2 ELISA kit from (Mybiosource (USA, Catalog No: MBS268902) was used for the quantitative determination of TLR2 serum concentration using the method recommended by the kit's manufacturer.

Official and Ethical endorsement

All procedures followed were following the Helsinki Declaration of 1975, revised in 2000. Ethical approval was granted from the Ministry of Higher Education, University of Basrah, College of Medicine, Research Ethics Committee (on 04/11/2020- No. 3S-343) and the Ministry of Health and Environment, Basrah Health Directorate (on 25/11/2020- No. 653). The parents of enrolled patients were informed about the research subject, and consent was obtained from them.

Statistical Analysis

The data were coded and analyzed using the Statistical Package for the Social Sciences (SPSS) version 26. Numeric variables were described as mean and standard deviation. Non-normally distributed data were expressed as median with minimum and maximum values. An independent two-sample t-test and a Mann-Whitney U test were used to assess the significance of differences between groups. The normality of distribution was tested using the Shapiro-Wilk test. Categorical data were formulated as frequencies and percentages. The chi-square test and Fisher's exact test (where appropriate) were used to test the significance of the association between categorical variables. A P-value of < 0.05 was the criterion of statistical significance.

3. RESULTS

A total of 156 participants, consisting of 76 AD cases and 80 controls, were recruited. The mean age of cases was 7.4 ± 4.4 years, while the control group had a mean age of 7.1 ± 4.6. Males represented 44 (57.9%) and (67.5%) of the cases and controls, respectively. The majority of participants in both groups had a normal BMI, with 63 (82.9%) AD cases and 76 (95.0%) controls falling in this category. However, the proportion of AD cases who were overweight or obese was higher than that of controls(P=0.015). The mean SCORAD score for the cases is 28.99 ± 5.63. Moderate disease was seen in 89.5% of the patients, as presented in **Table 1.**

Table 1: Baseline characteristics of the study groups.

	AD – group*	**Control-group**	**P-value**
Age (mean ± SD)			
Gender			
Male	44 (57.9%)	54 (67.5%)	0.215
Female	32 (42.1%)	26 (32.5%)	
BMI Interpretation*			
Normal	63 (82.9%)	76 (95.0%)	**0.015****
Overweight	5 (6.6%)	2 (2.5%)	
Obese	8 (10.5%)	2 (2.5%)	

	AD – group*	Control-group	P-value
Total IgE			
High	31 (40.8%)	13 (16.2%)	**0.001**
Normal	45 (59.2%)	67 (83.8%)	
Severity			
Mild	2 (2.6%)	-	
Moderate	68 (89.5%)	-	
Severe	6 (7.9%)	-	
* AD: Atopic Dermatitis; BMI : Body Mass Index. **Fisher's Exact test was used to compare normal vs. overweight and obese.			

Cases with AD had a significantly lower mean rank of serum concentration of TLR2 compared to the controls (P=0.00002). Among cases, the median level of TLR2 serum concentration was 761.9 pg/ml, which ranged from 326 to 2943 pg/ml. Whereas among controls, the median level of TLR2 serum concentration was 1528.5 pg/ml, ranging from 475 to 9164 pg/ml, as demonstrated in **Table 2**

Table 2: Serum concentration of TLR2 among the two study groups.

Study group	Serum concentration of TLR2 (pg/ml)		
	No.	Mean Rank	Median
AD group	76	62.7	761.9
Control group	80	93.5	1528.5
Total	156		
Mann-Whitney U = 1838.5	**P-value= 0.00002**		

Patients with AD were further investigated for the relationship between serum concentration of TLR2 and total IgE level. Significantly higher TLR2 concentrations were seen among patients with elevated total IgE levels (P=0.013), as presented in **Table 3.**

Table 3: Comparison of serum concentration of TLR2 according to IgE levels .

Total IgE	Serum concentration of TLR2 (pg/ml)		
	No.	Mean Rank	Median
Normal	31	33.3	537.5
High	45	46.1	973.7
Total	76		
Mann-Whitney U = 462.5	**P-value= 0.013**		

According to disease severity, patients were classified into a mild-moderate group and a severe group using the objective SCORAD. Patients with a severe form of AD had significantly higher concentrations of TLR2 compared to the mild-moderate disease group (P=0.005), as reported in **Table 4**.

Table 4: Comparison of serum concentration of TLR2 according to severity of AD.

Severity of AD	Serum concentration of TLR2 (pg/ml)		
	No.	Mean Rank	Median
Mild-Moderate	71	36.6	749.4
Severe	5	65.0	973.8
Total	76		
Mann-Whitney U = 45.0	**P-value= 0.005**		

4. DISCUSSION

Atopic dermatitis is one of the most common skin diseases, especially among children. In a recent study in Basrah, the prevalence of AD among children at outpatient dermatology clinics was 21.31%.[10] Clinical presentation varies with age and ranges from mild to severe disease. AD results in a significant impairment of the quality of life of patients and their families and represents an economic burden at the family and community levels [11].

Recent studies have shown that TLRs, particularly TLR2 and TLR4, play a significant role in the pathogenesis of AD. The dysregulation of TLRs in AD leads to an exaggerated immune response, leading to the release of pro-inflammatory cytokines, chemokines, and antimicrobial peptides, which contribute to AD development [5]. Understanding the role of TLRs in AD may pave the way for the development of new therapeutic strategies that target these receptors to alleviate the symptoms of AD [5,12].

In this study, patients with AD had significantly lower concentrations of TLR2 compared to controls. The exact role of TLR in the pathophysiology of AD is still unclear. Our results are parallel to the Lesiak et al. study, which found a significantly lower percentage of TLR2 expression in peripheral blood from AD patients using flow cytometry for its estimation [5]. Likewise, a histopathological study of skin biopsies taken from AD patients and controls showed a significantly lower expression of TLR2 in the uppermost layers of the skin of patients with AD [13].

Contrarily, a study by Tsybikov et al. in 2015 found that expression of TLR2 in patients with AD during flare-ups was significantly higher than in controls [14].

Other studies investigated TLR2 function by measuring the pro-inflammatory cytokine release rather than the actual concentration or expression of TLR2. A case-control study by Niebuhr et al.in Germany showed that peripheral blood macrophages had a significant impairment of TLR2 expression and function, estimated by measuring cytokines produced by TLR2 signaling by quantitative real-time polymerase chain reaction (PCR) [15]. A study by Hasannejad et al. found that cytokine production under the control of TLR2 was significantly reduced among patients with AD in comparison with controls [16]. Furthermore, a case-control study by Yu et al. found a diminished production of T-helper 1cell-mediated cytokines and an increase in T-helper 2 cytokines among AD patients, and this pattern was related to increased pathogenic colonization among them [17].

Other research failed to show either a positive or negative association between TLR2 and AD risk. A study by Terhorst et al. in 2006 concluded no difference in TLR2

function or expression among cases with AD and controls. This difference from the present study results was probably due to the variation in sample size (No.=13), and many types of atopy were included in their study [18].

In our study, patients with severe disease and patients with elevated total IgE levels had significantly higher TLR2 concentrations. Disease severity is correlated with total IgE level among cases of AD [19]. Our results are similar to Tsybikov et al. study in which a positive correlation was found between TLR2 expression and a higher SCORAD index. Over-stimulation of TLR2 as a response to *S. aureus* detection in severely inflamed skin can explain the high TLR2 concentration in severe AD [14]. It is noteworthy to note that the imbalance between mild-moderate case percentage (92.1%) and severe AD percentage (7.9%) in our study could potentially affect the interpretation of results regarding the relationship between TLR2 concentration and AD severity. The under-representation of severe cases reduces the statistical power, limits the generalizability, and suggests the need for research with more representation of this subgroup of patients.

Several studies have found an association between the genetic mutation of the TLR2 allele and the severity of the clinical picture of AD [12,20,21]. Among these, a case-control study by Salpietro et al. demonstrated that the TLR2 R753Q SNP, which refers to a specific single nucleotide polymorphism (SNP) in the TLR2 gene, was significantly more common in pediatric patients with severe clinical manifestations of AD [22].

CONCLUSIONS

TLR2 receptors have been shown to contribute to the pathogenesis of AD. Children with AD showed significantly lower serum TLR2 concentrations compared to controls. This refers to the impaired innate immune response. However, higher TLR2 levels were observed in patients with elevated IgE levels and severe AD. This suggests a complex mechanism of TLR2 in the immune dysregulation that occurs in AD and might refer to the involvement of TLR2 in both the onset and exacerbation of AD, particularly in severe forms. We recommend further studies that investigate the TLR2 expression among the Iraqi population with emphasis on those with severe disease and among patients with other forms of atopy.

Conflicts of interest: No conflicts of interest to be declared.

Data Availability: The datasets are available from the corresponding author on reasonable request.

Authors Contributions: Al-Rubaye A.: Conceptualized the study, designed the methodology, collected and analyzed the data, and drafted the manuscript. Performed the literature review and was responsible for interpreting the results. **Al-Yassen A.**: Supervised the study design and methodology, provided critical revisions to the manuscript, and contributed to the interpretation of the data. Guided the overall direction of the research, provided expert insights, and ensured the quality and accuracy of the final manuscript.

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تركيز المستقبل الشبيه بالتول رقم 2 في مصل الدم لدى الأطفال المصابين بالتهاب الجلد التأتبي: دراسة حالات و شواهد

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الملخص

الخلفية والأهداف: يُعَد التهاب الجلد التأتبي من أمراض الجلد الالتهابية ذات الطبيعة المزمنة. هناك أدلة متزايدة على أن المستقبلات الشبيهة بالتول والتي تعد جزءًا من المناعة الفطرية تلعب دورًا في التغيرات الفيزيولوجية المرضية في مرض التهاب الجلد التأتبي. تهدف هذه الدراسة إلى تقدير تركيز المستقبلات الشبيهة بالتول في مصل الدم بين الأطفال المصابين بمرض التهاب الجلد التأتبي ومقارنته بالأشخاص الأصحاء بالاضافة إلى استكشاف العلاقة بين تركيز المستقبلات الشبيهة بالتول في مصل الدم مع شدة المرض ومستوى الغلوبولين المناعي (IgE) الكُلّي.

منهجية الدراسة : شملت دراسة الحالات والشواهد هذه 156 مشاركًا متكوّن من 76 حالة التهاب الجلد التأتبي و80 من الاطفال الاصحاء كمجموعة مراقبة. أُجريت الدراسة في العيادات الاستشارية بمستشفى الفيحاء التعليمي بين ديسمبر 2020 ومارس 2021. وتضمنت الدراسة إجراء مقابلات مع أولياء أمور المشاركين، وإجراء فحص سريري تفصيلي مع تقييم شدة المرض، وفحوصات مختبرية بما في ذلك إجمالي تركيز IgE وتركيز المستقبلات الشبيهة بالتول في المصل.

النتائج: كانت تركيزات المستقبلات الشبيهة بالتول في المصل أقل لدى المصابين بالتهاب الجلد التأتبي (الوسيط = 761.9 بيكوغرام / مل) مقارنة بمجموعة الاطفال الاصحاء (الوسيط = 1528.5 بيكوغرام / مل) (P = 0.00002) . كما و تم التعرف على أن مستويات المستقبلات الشبيهة بالتول كانت أعلى بشكل معتد احصائياً لدى المرضى الذين يعانون من ارتفاع مستوى الغلوبولين المناعي (IgE) الكُلّي ولدى المرضى الذين يعانون من النمط الشديد من مرض التهاب الجلد التأتبي.

الاستنتاجات: قد تشير المستويات المنخفضة من المستقبل الشبيه بالتول رقم 2 (TLR2) إلى دورها في التسبب في التهاب الجلد التأتبي فضلا عن ذلك لوحظ وجود مستويات أعلى من TLR2 في الحالات الأكثر شدة أو لدى المرضى الذين لديهم ارتفاع في IgE. يشير ذلك إلى وجود آلية معقدة لدور TLR2 في اضطراب المناعة المصاحب لالتهاب الجلد التأتبي وقد يعكس هذا الدور مشاركة TLR2 في كل من بداية المرض وتفاقمه، لا سيما في الأشكال الشديدة منه.

الكلمات الدالة: الإكزيما التأتبية، التهاب الجلد التأتبي، المستقبلات الشبيهة بالتول.