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The Effect of Toll-Like Receptor 2 Gene Polymorphism on Pediatric Pneumonia

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ABSTRACT

Background: To investigate the effect of Toll-like receptor 2 (TLR2) gene polymorphism on pediatric pneumonia.Methods: Overall, 80 patients with pediatric pneumonia (Observation group) treated in babil Children Hospital, Hillah from Sep 2019 to July 2020 were randomly enrolled. Meanwhile, 110 healthy children (Control group) in the same time period were randomly selected. PCR was applied to amplify the TLR2 (rs5743708) gene fragment. The TLR2 (rs5743708) polymorphism was detected, and the impacts of gene polymorphism difference on pediatric pneumonia were observed. Results: there was highly significant variation in genotype distribution ($p < 0.001$) in such a way that the heterozygous genotype R/Q and the homozygous genotype QQ were more frequent in patients group in comparison with control group in a significant manner ($p <0.05$). In terms of odds ratio (OR), the risk attributed to R/Q was 9.66 and that attributed to QQ was 3.02. On the other hand, the homozygous RR genotype was less frequent in patients group and can be regarded as a protective factor. Conclusion: TLR2 gene polymorphism might be related to the pediatric pneumonia and the population with Q allele at this locus may be more prone to pediatric pneumonia.

Keywords: TLR2; Gene polymorphism; Pediatric pneumonia; Community acquired pneumonia

Introduction

Pediatric pneumonia is an infectious disease attached great importance to by the pediatric department. Streptococcus pneumoniae, haemophilus influenzae and staphylococcus aureus are the main causes of bacterial pneumonia [1], and that respiratory syncytial virus, parainfluenza virus and influenza virus are the main causes of pediatric viral pneumonia [2]. Community acquired pneumonia (CAP) is the leading cause of children's mortality around the globe [3,4], with more than 150,000 children requiring hospitalization each year. It is the fifth largest epidemic disease in all pediatric hospitalizations in the United States which has a very high diagnosis value [5]. In healthy patients the innate immune system looks crucial for the early containment of microbial infections by triggering inflammation and coordinating the acquired immune response and the family of Toll-like receptors (TLRs) is a central component of this system and its description has permitted a better understanding of the molecular mechanisms concerning to antimicrobial and inflammatory responses, so the TLRs seem to play a key role in signaling molecules of pathogens and endogenous proteins related to immune activation given their ability to recognize evolutionary conserved pathogen-associated molecular patterns of microbial origin[6].Toll-like receptors (TLRs) play a central role in the initiation of cellular innate immune responses by virtue of their capacity to detect pathogens at either the cell surface or in lysosomes/ endosomes [7]. TLR2 has been implicated as the principal TLR in the

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recognition of S. pneumoniae [8], at least in part through an interaction with lipoteichoic acid, a constituent of the pneumococcal cell wall [9]. It has raised the question whether genetic factors can define the susceptibility to the disorder or the severity of symptoms. Although few studies have tried to answer the question [10], it seems to be too early to reach a conclusive results in this regard. Yet, previous studies have indicated that susceptibility to respiratory tract infections is determined by both genetic and acquired risk factors[11]. In studies on TLR2 on basis of single region and fragment, the etiology of diseases and all the clin-cal explanations except for inflammatory markers are not very clear. Therefore, in this study, the impact of TLR2 gene polymorphism on pediatric pneumonia was examined, hoping to find pediatric pneumonia screening indicators and to conduct better prevention and treatment of pediatric pneumonia.

Materials and Methods

Patients, subjects and study design

This is a case-control study. A total of 190 individual were involved in this study, with age ranging from (1 to 36) months. A 80 patients diagnosis with CAP divided into groups according to Age of disease. And 110 health individuals as control, who were observation in pediatrics hospitals in Babel governorate during the period from September 2019 to February 2020 by helping of clinicians in diagnosis of pneumonia patients. A questionnaire was taken from each patients and case sheets including, number, age, sex, feeding type, period of diseases and address.The sample are whole blood were collected from each participant, EDTA blood was used to extract DNA. DNA extraction was used to detect SNPs in TLR2 genes with the use of Restriction Fragment Length Polymorphism PCR (RFLP – PCR) and the data were statistically analyzed by SPSS software version 24.

Primers

The RFLP-PCR primer for detection of TLR2 (rs5743708) were designed by [12]. These primers were provided from Macrogen/Korea and restiriction enzyme SsiI (NEB/UK) (Table 1 and 2)

Polymerase Chain reaction

The PCR products were analyzed by agarose gel electrophoresis following steps:
1- A1.5% Agarose gel was prepared in using 0.5X TBE and dissolving in microwave for 5 minutes, and left to cool for 50°C.
2- Then 3µl ethidium bromide stain were added into agarose gel solution.
3- Agarose gel solution was poured in tray after fixed the comb in proper position and left to solidified for 15 minutes at room temperature, then the comb was removed gently from the tray.
4- The gel tray was fixed in electrophoresis chamber and filled by 0.5X TBE buffer.
5-A 10µl PCR product were loaded in to each well with added 5µl (DNA marker Ladder) in first well. Then electric current was performed at 100 volt and 80 AM for 1hour.
6- PCR products were visualized by using UV Trans illuminator

RFLP-PCR mix preparation

The RFLP-PCR master for detection of TLR2 (rs5743708) gene polymorphism was prepared by using *SsiI* restriction enzyme (New England Biolabs. UK) and this master mix done independent according to company instructions (Table 3). After that, this master mix placed in Exispin vortex centrifuge at 3000rpm for 2 minutes, then transferred into incubation at 37°C for

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overnight. After that, RFLP-PCR product was analysis by 3% agarose gel electrophoresis methods that mention in PCR product analysis. The (RR) wild type homozygote, was digested by restriction enzyme and 115 and invisible 58bp band, the (QQ) mutant type homozygote that showed undigested by restriction enzyme into 173bp, and the (R/Q) heterozygote, the product was digested by restriction enzyme into 173bp, 115bp, and invisible 58bp band.

Statistical analysis

data were statistically analyzed by SPSS software version 24, which were expressed as Kruskal Wallis test ,significant at $p > 0.05$

Table 1. The primers with their sequence and amplicon size**.**

Primers	**Sequence (5'-3')**		**Amplicon**
TLR2 (rs5743708)	Q	GAAGAGAACAATGATGCTGCCATTC	178bp
	R	CTAGGACTTTATCGCAGCTCTC	

Table 2. Company and country of origin of restriction enzymes were used in RFLP-PCR assay.

Gene	**Restriction enzyme**	**SNP**	**Company/Country**
TLR2 (rs5743708)	SsiI	R/Q	New England Biolabs. UK

Table 3. RFLP-PCR master mix

RFLP-PCR Master mix	**Volume**
PCR product	15µl
***SsiI* Restriction enzyme buffer 10X**	3 µl
***SsiI* (10 unit)**	1 µl
Free nuclease water	1µl
Total volume	20 µl

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Results

The results of TLR2 amplification and RFLP-PCR were illustrated in figures (1 and 2). The comparison of patients and control subjects frequency distribution according to TLR-2 (rs5743708) genotypes and alleles is shown in table 4.6. Regarding dominance mode of inheritance, there was highly significant variation in genotype distribution ($p< 0.001$) in such a way that the heterozygous genotype R/Q and the homozygous genotype QQ were more frequent in patients group in comparison with control group in a significant manner ($p<0.05$). In terms of odds ratio (OR), the risk attributed to R/Q was 9.66 and that attributed to QQ was 3.02. On the other hand, the homozygous RR genotype was less frequent in patients group and can be regarded as a protective factor. With respect to dominant mode of inheritance, genotype RR was a highly significant protective factor ($p< 0.001$) with an OR of 0.14. With respect to recessive mode of inheritance, genotype QQ showed no significant association with a disease sate ($p = 0.624$). In allele analysis, allele Q was a highly significant risk factor ($p < 0.001$) with an OR of 3.41.

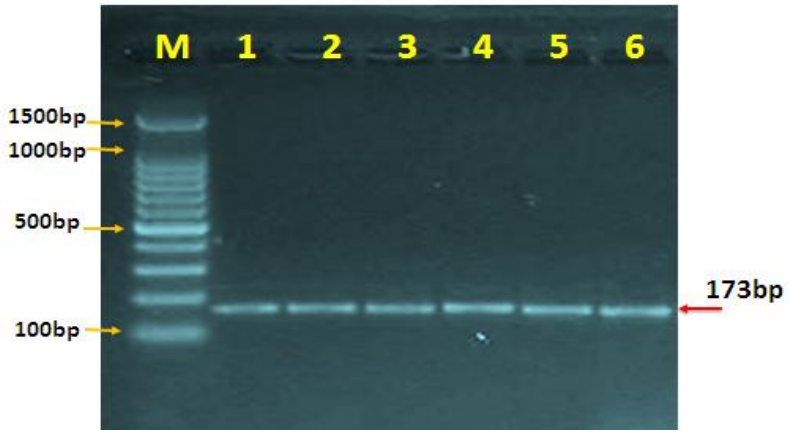

Figure 1. Agarose gel electrophoresisillustrate the PCR product analysis of TLR2at 1.5 % agarose. Where M: marker (1500-100bp), lane (1-6) some catalase gene PCR amplification at 173bp PCR product size.

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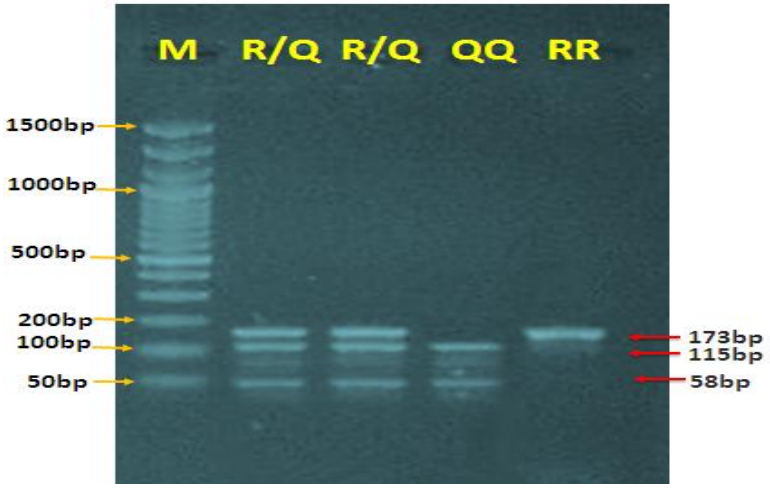

Figure 2. showed Agarose gel electrophoresis image that showed the RFLP-PCR product analysis of TLR2 (rs5743708) Gene polymorphism by using *SsiI* restriction enzyme in 2.5% agarose gel. Where M: marker (2000-50bp). Lane (RR) wild type homozygote, the product undigested by restriction enzyme and still 173bp bands. Lane (QQ) mutant type homozygote that showed digested by restriction enzyme into115bands, and 58bp bands. Lane (R/Q) heterozygote, the product digested by restriction enzyme into 173bp , 115bp and 58bp bands.

Table 4. The comparison of patients and control subjects frequency distribution according to TLR-2 (rs5743708) genotypes and alleles

TLR-2 (rs5743708)	**Genotype**	**Patients *n* = 80 *n* (%)**	**Control *n* = 110 *n* (%)**	***p* overall**	***p*2**	**OR**	**95 % CI**
Codominance	RR	25 (31.3 %)	84 (76.4 %)	<0.001 HS	Reference		
	R/Q	46 (57.5 %)	16 (14.5 %)		<0.001 HS	9.66	4.69 - 19.91
	QQ	9 (11.3 %)	10 (9.1 %)		0.026 S	3.02	1.11 - 8.26
Dominant	RR	25 (31.3 %)	84 (76.4 %)		<0.001 HS	0.14	0.07 - 0.27
	R/Q+QQ	55 (68.8 %)	26 (23.6 %)		Reference		
Recessive	RR+R/Q	71 (88.8 %)	100 (90.9 %)		Reference		

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	QQ	9 (11.3 %)	10 (9.1 %)		0.624 NS	1.27	0.49 - 3.28
Allele	R	96 (60.0 %)	184 (83.6 %)		Reference		
	Q	64 (40.0 %)	36 (16.4 %)		< 0.001 HS	3.41	2.11- 5.49

n: number of cases; **OR**: odds ratio**; CI**: confidence interval; **HS**: highly significant at $p \leq 0.01$; **S**: significant at $p \leq 0.05$; **NS**: not significant at $p > 0.05$

Discussion

In the current study, there was significant association between TLR-2 (rs5743708) gene polymorphism and respiratory tract infection in children; the heterozygous genotype R/Q and the homozygous genotype QQ were risk factors with 9.66 and 3.02 odds ratios, respectively, while the homozygous RR genotype was protective factor against respiratory tract infection and allele Q was a risk factor with 3.41 odds ratio. The significant association between lower respiratory tract infection and TLR-2 (rs5743708) gene polymorphism was documented by [13] and this finding is supportive to our finding. In another study, the susceptibility to respiratory tract infection in children in association with TLR-2 (rs5743708) gene polymorphism and they found that allele Q was a highly significant risk factor and this finding is similar to our finding.TLR-2 (rs5743708) gene polymorphism has been linked to a number of infections and this polymorphism (Q-allele) has been shown to be associated with changes in NFkB pathway, reduction in TLR-1 and 2 signaling and reduced cytokine production and thereby increasing susceptibility to infections including viral, bacterial and even fungal agents in various systems and organs of the body [14].

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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