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ROLE OF INTERLEUKIN 8 -781C\T POLYMORPHISM FOR DIAGNOSIS AND PREDICTION OF PAEDIATRIC ASTHMA

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ABSTRACT

Background: Asthma is a chronic respiratory illness marked by inflammation and reversible obstruction of the airways, which may cause Wheezing, shortness of breath, dyspnea and cough.

Aim and objectives: Detect relationship between IL-8 -781C/T (rs2227306) polymorphism and pediatric asthma.

Subjects and Methods: This study was a case control study included 40 asthmatic pediatric patients and 40 healthy controls of matched age and sex in the period from June 2020 to December 2020 at Outpatient clinics, Pediatric Department at Zagazig University Hospitals.

Results: There is a statistically significant difference between the cases and control groups examined when it comes to level of IL-8 and Interleukin 8 -781C\T Polymorphism. CC and CT genotypes increased risk of asthma by 8.67 and 3.76 folds respectively. C allele increased that risk by 3.38 folds.

Conclusion: Our findings support IL-8's inflammatory function and suggest that it may be utilized as a criterion for the severity of asthma in children. More studies on the IL8 gene variants and their biological effects are also required.

Keywords: inflammatory, lung function, genotypes, Asthma, GINA, IL8 gene.

Introduction

Asthma is a chronic respiratory illness marked by inflammation and reversible obstruction of the airways, which may causeWheezing, shortness of breath, dyspnea and cough. Asthma affects about 350 million people globally, of all ages, and is responsible for roughly 350,000 fatalities each year [1]. Although asthma is a lifelong illness, it is the most prevalent chronic disorder among children and young people, with more severe symptoms [2]. Asthma is responsible for a large worldwide burden, which is primarily driven by direct economic expenditures to health-care systems [3]. There are significant variations in asthma prevalence across nations and people, ranging from 1.5 to 15.6 percent, as well as between ethnic groups within countries. These variations may be due to complicated interactions between environmental and genetic variables [4]. The CXC chemokine superfamily includes the IL-8 chemokine, which has two receptors: alpha (IL-8 RA, CXCR1) and beta (IL-8 RA, CXCR2) (IL-8 RB, CXCR2).IL-8 is a chemotactic cytokine that activates inflammatory cells by attracting neutrophils, mononuclear phagocytes, mast cells, and T lymphocytes.The inflammatory process, both acute and chronic, is initiated by IL-8. It has been linked to the development of bronchial asthma and other respiratory diseases [5]. On chromosome 4q13–q21, a gene with four exons, three introns, and a proximal promoter region encodes human IL-8. So far, fifteen IL8 polymorphisms have been identified, although only two have been linked to IL-8 mRNA levels [6]. A higher level of IL-8 has been linked to the polymorphism IL-8 - 781C/T (rs2227306) in the promoter region. The IL-8 - 781C/T (rs2227306) gene, which is located within the first intron, was found to help with gene transcription and regulation. In genetic association studies, these alterations have lately been related to susceptibility to inflammatory diseases including RSV bronchiolitis, ARDS, lung cancer and asthma [7]. In our study, we sought to investigate whether there was a relationship between the IL-8 - 781C/T (rs2227306) polymorphism and childhood asthma.

Patients and Methods

This study was a case control study included 40 asthmatic paediatric patients and 40 healthy controls of matched age and sex in the period from June 2020 to December 2020 at Outpatient clinics, Paediatric Department at Zagazig University Hospitals. We includeChildren aged between 5-15 years old*and* asthma was diagnosed according to GINA guidelines. **[8]**

Data collection: Sociodemographic data collected from the participants included the following domains: personal data (age and sex). Present history about the case and the degree of severity and whether controlled or not, full general examination and local chest examination was done, routine laboratory investigations (CBC), chest X ray, Spirometer and detection of Interleukin 8-781C/T (rs2227306) Polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) methods were used to identify the Interleukin 8 -781CT polymorphism.

Technique:

Blood sampling: A total of 2 ml of peripheral venous For DNA extraction, blood samples were obtained in EDTA-treated tubes. and then kept at 20 °C until further analysis.

Estimations of Human IL-8 Serum Levels: For the serum preparations**,** we collected 3 ml of venous blood in a tube with no anti-coagulant and then permitted to clot at room temperature. The serum was separated using centrifuge (13000g, for 15-minutes), then kept at – 20º C till usage. The determinations of the IL-8 serum levels have been done by means of a commercial IL-8 Human ELISA (enzyme linked immune-sorbent assess) Kit (R&D Systems, Minneapolis, MN, US). The IL-8 serum levels information was presented as mean ± (SD). A $P<0.05$ was considered significant.

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 05, 2021

IL-8 Polymorphisms Genotyping: The whole DNA extraction kit was used to extract genomic DNA from entire blood (iNtRON Biotechnology, Korea). Polymerase chain reaction amplification and restriction fragment length polymorphism (RFLP-PCR) analyses were used to genotype the IL-8 SNPs. The 203-bp DNA fragment for the rs2227306 polymorphism was amplified using the primers [Forward: CTCTAACTCTTTATATAGGAATT; Reverse: GATTGATTTTATCAACAGGCA]. With 100 ng of template DNA in each tube, the PCR reactions were carried out in a total volume of 25 ul.They were amplified using the following cycle parameters: 20 pmol of each primer (Invitrogen, USA) and 12.5 ul of 2x Dream TaqTM Green PCR Master Mix (Thermo Scientific). Pre-denaturation at 94°C for 8 minutes, 35 cycles of denaturing at 94°C for 30 seconds, annealing at 54°C for 30 seconds, extension at 72°C for 30 seconds, and a final extension at 72°C for 10 minutes were the PCR amplification conditions for the rs2227306 region. 1 U of the restriction enzyme EcoRI was used to digest the PCR products (New England Biolabs, USA). The C allele of rs2227306 was found using 19- and 184-bp segments, whereas the T allele was discovered using a single 203-bp fragment. After separation on a 3 percent agarose gel stained with ethidium bromide, the digested fragments were seen using a UV illuminator.

Ethical considerations: The IRB "institutional review board" of Zagazig University's school of medicine accepted the research plan. All participants signed a written informed consent form (parents). To gather all data on the patient and the outcomes of investigations conducted during the study period, a file with the same number was created. They were told that any information collected would be kept strictly private, and that the study findings would be utilized only for research purposes. They have the option of declining without affecting their management strategy.

Statistical analysis: The data was coded, entered, and analyzed using Microsoft Excel software throughout the history, clinical examination, laboratory investigations, and outcome measures. The data was tabulated and analyzed using SPSS (statistical package for social science). Independent samples Student's t-test was used to compare between two groups of normally distributed variables while Mann-Whitney U Test is a statistical test for comparing two groups with quantitative variables that do not have a normal distribution. The Chi-square test (X2) or fisher were performed to compare and correlate two qualitative variables. The results were considered statistically significant and highly statistically significant when the significant probability (P value)was < 0.05* and <0.001** respectively.

Results

This study included 40 asthmatics with mean age 7.80 ± 1.786 and 40 control with mean age 8.4 ± 1.566.There was no statistically significant difference in age or gender between the groups examined. Also in our study we compared the pulmonary functions (FVC, FEV1, and FEV1/FVC) in the asthmatic and control groups (all were significantly lower in asthmatic group than control group).Table (1).

According to the asthma group we found the level of control, 15%, 42.5% and 42.5% were well controlled, partially controlled and poorly controlled respectively. According to asthma severity, 15%, 42.5% and 42.5% had intermittent, persistent moderate and persistent severe asthma respectively. According to type of asthma, 60% of patients had atopy, 40% had no atopy. Table (2)

According to IL-8 serum level, we compared the level of IL-8 in cases and control groups. There is a statistically significant difference between asthmatic [39.2 pg/ml (10.7 – 90.9)] and control groups [11.3 pg/ml (4.5 – 21.8)].Table (3)

Between the two investigated groups, there is a statistically significant difference regarding Interleukin 8 -781C\T (rs2227306) Polymorphism. CC and CT genotypes increased risk of asthma by 8.67 and 3.76 folds respectively. C allele increased that risk by 3.38 folds. Table (4)

We also analyzed the association of IL-8-781 C/T (rs2227306) Polymorphism with level of control. There is significant association between level ofcontrol and Interleukin-8-781 C/T (rs2227306) Polymorphism genotype or alleles. TT and CT genotypes decrease risk of poor control (COR 0 and 0.11 for TT and CT respectively) T alleles decrease risk of poor control (COR 0.11).Table (5)

There is significant association between asthma severity and Interleukin-8-781 C/T (rs2227306) Polymorphism genotype or alleles. TT and CT genotypes decrease risk of severe asthma (COR 0 and 0.11 for TT and CT respectively). T alleles decrease risk of severe asthma (COR 0.11).Table (6)

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 05, 2021

Table (1) Baseline data of the studied groups:

er	group %)	group %)		
r) D	786	66		
) D	8.998	2.35		*
%) D	12.38	2.289		*
VC D	7.045	409		*

χ^2Chi square test t independent sample t test Z Mann Whitney test
**p≤0.001 is statistically highly significant

Table (2) Disease-specific data of asthmatic patients:

er	group	
control: trolled controlled ontrolled		
: ent t moderate t severe		
asthma:		

Table (3) Comparison between the studied groups regarding IL-8:

er				
	group (range)	group (range)		
	7 – 90.9)	– 21.8)		*

**p≤0.001 is statistically highly significant Z Mann Whitney test

Table (4) Comparison between the studied groups regarding Interleukin 8 -781C\T Polymorphism:

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 05, 2021

	kin 8 -781C\T Polymorphism				
	group	**group**			**% CI)**
					7 – 36.36) * 7 – 16.15) nce)
					2 – 6.64) * nce)

Chi square test **p≤0.001 is statistically highly significant

Table (5) Relation between Interleukin 8 -781C\T and level of control:

	control				
	kin 8 -781C\T Polymorphism				
	oorly ontrolled	**and partially rolled**			**% CI)**
					nce) 2 – 0.6) *
					nce) 2 – 0.5) *

χ^2 Chi square test MC Monte Carlo test COR crude odds ratio CI Confidence interval p<0.05 is statistically significant
**p≤0.001 is statistically highly significant

Table (6) Relation between Interleukin 8 -781C\T Polymorphism and Asthma severity:

	severity				
	kin 8 -781C\T Polymorphism				
	nt severe	**tent and Persistent mod**			**% CI)**
					ce) 2 – 0.6) *
					nce) 2 – 0.5) *

χ^2 Chi square test MC Monte Carlo test COR crude odds ratio CI Confidence interval *p<0.05 is statistically significant
**p≤0.001 is statistically highly significant

Discussion

Asthma is a serious respiratory condition. It's one of the most prevalent lung diseases, with persistent airway inflammation as its hallmark **[9]**. The aim of our study was to find a relationship between IL-8 - 781C/T (rs2227306) polymorphism and pediatric asthma and its severity and its role in diagnosis and prediction of pediatric asthma.

Our study is a case control study included 40 asthmatic paediatric patients and 40 healthy controls of matched age and sex in the period from June 2020 to December 2020 at Outpatient clinics, Pediatric Department at Zagazig University Hospitals.

Many studies done before about IL 8 and reported its role in in the prognosis of asthma severity**. [10,11]**

In our study we found that the serum level of IL-8 in asthmatic group [39.2 pg/ml (10.7-90.9), p<0.001] is significantly higher than the control group's [11.3 pg/ml (4.5-21.8)].

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 05, 2021

Similar studies were done and investigated the link between IL-8 Gene Variants and Childhood Asthma Expression. The researchers found that the asthmatic group's IL-8 serum levels were significantly higher (48.82 ± 27.36 pg/ml) than the control group's (10.67 ± 4.62 pg/ml; P<0.0001) **[5]**. Similarly, Zhang & Bai **[10]** reported that the serum IL-8 level in asthmatic group (87.45 pg/mL; 5–7500) was significantly (P< 0.001) higher than that of the control group (10.9 pg/mL; 6.8–39.65). The increased release of IL-8 in the asthmatic group found in our research was consistent with a number of prior reports **.**Zhang et al., **[12]** and Sun et al., **[13].**

In our study according to asthmatic group, we analyzed the severity of this group, 15%, 42.5% and 42.5% were intermittent, persistent moderate and persistent severe asthma respectively. And also according to level of control of asthmatic group, 15%, 42.5% and 42.5% were well controlled, partially controlled and poorly controlled respectively. And according to type of asthma of asthmatic children, 60% of patients had atopy, 40% had no atopy. These findings were similar to what was mentioned by Charrad et al., **[5]**, where 68.82% of the studied asthmatic children were atopic, also 81.63% of them had mean IgE level >200 U/ml-1.

In the current study, as regarding comparison between the asthmatic and control groups regarding Interleukin 8 -781C\T (rs2227306) Polymorphism, there is a statistically significant difference between the groups examined. CC and CT genotypes increased risk of asthma by 8.67 and 3.76 folds respectively. C allele increased that risk by 3.38 folds.

In our study, there was significant association between level of control and Interleukin-8-781 C/T (rs2227306) Polymorphism genotype or alleles. TT and CT genotypes decrease risk of poor control (COR 0 and 0.11 for TT and CT respectively). T alleles decrease risk of poor control (COR 0.11). Also, there is significant association between asthma severity and Interleukin-8-781 C/T (rs2227306) Polymorphism genotype or alleles. TT and CT genotypes decrease risk of severe asthma (COR 0 and 0.11 for TT and CT respectively). T alleles decrease risk of severe asthma (COR 0.11).

Our results agreed with Charrad et al., **[5]**, where they looked at the link between two IL8 polymorphism (rs4073) (rs2227306) and asthma risk in Tunisian children. This study Charrad et al., **[5]**, reported that both (rs2227306) and (rs4073) had a strong association with increased risk of childhood asthma. Also, Heinzmann et al. **[14]** reported similar findings in the German asthmatic children. According to our results, the rs2227306 gene was significantly linked to an elevated risk of childhood asthma. These findings suggested that IL-8 genetic polymorphism expression may have a role in the development of pediatric asthma.

Conclusion

From all the mentioned data we can conclude that our findings support IL 8 -781C\T's inflammatory role and suggest that it has the potential to be utilized as a marker for the prediction of asthma severity in children. Significant association between level of control of asthma, asthma severity and Interleukin-8-781 C/T Polymorphism genotypes was reported with TT and CT genotypes and T allele decrease risk of poor control and asthma severity. Also according to serum IL 8, there is a statistically significant difference between the examined two groups .More studies on the IL8 gene variants and their biological roles are also required.

Conflicts of interest: None.

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ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 05, 2021

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