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ORIGINAL ARTICLE

Interleukin 10 Gene Polymorphism in Pediatric Immune Thrombocytopenia

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ABSTRACT

Background: We aimed to study interleukin 10 gene polymorphism and evaluate its prognostic significance in children with primary immune thrombocytopenia. **Methods:** The study was conducted at Pediatric Hematology Outpatient Clinic on 63 immune thrombocytopenic purpura (ITP) patients who were divided into: 21 patients with newly diagnosed ITP, 21 patients with persistent ITP, and 21 patients with chronic ITP. As a control group, 21 children of the same age and gender who appeared to be in good health were included. The polymerase chain reaction-restriction fragment length polymorphism assay (PCR-RFLP) was used to examine interleukin 10 (IL10) (-627) gene variants in both patients and controls. **Results:** There was highly significant difference between patients and controls as regards frequency of A and C alleles of IL10 (627) where A allele was more prevalent in patients than controls. Among patients, A allele was more prevalent in chronic ITP patients compared to newly diagnosed patients or those with persistent ITP. The AA genotype was more prevalent in patients than controls. Among patients, the AA genotype was more prevalent in those with chronic ITP than newly diagnosed patients or those with persistent ITP. **Conclusions:** We concluded that IL-10 gene polymorphism may contribute to susceptibility and chronicity of ITP in children as AA genotype was higher in patients than controls and in chronic patients compared to those with newly diagnosed ITP.

Keywords: Interleukin; ITP; Children; Gene polymorphism

INTRODUCTION

Autoimmune disease is characterized by a breakdown in self-tolerance, or the immune system's capacity to properly discriminate between self and non-self and refrain from attacking self [1]. The hallmark of childhood immune thrombocytopenia is characterized by isolated thrombocytopenia (platelet count <100,000/micro L with normal hemoglobin and white blood cell count). Most of the time, the etiology of ITP is still unknown, however other immunologic or environmental triggers, such as viruses, might cause it [1,2]. Previously, ITP was referred to as immunological thrombocytopenic purpura or idiopathic thrombocytopenic purpura. The name "immune thrombocytopenia" now in use maintains the commonly known abbreviation "ITP," but it now acknowledges that the disease is mediated by the immune system and that patients may have little to no bleeding or purpura [2]. ITP is caused by an aberrant autoantibody binding to circulating platelet membranes, often immunoglobulin G (IgG) with specificity for one or more platelet membrane glycoproteins [3].

Cytokine IL-10 inhibits immunological responses and has anti-inflammatory properties. Th2 cells, mast cells, and macrophages all release IL-10. In addition, cytotoxic T cells secrete IL-10 to suppress the activation of natural killer cells triggered by viral infection. IL-10 prevents the production of certain cytokines that are implicated in inflammation, such as TNF-α, IL-2, granulocyte-macrophage colony-stimulating factor, and interferon γ (IFN-γ). It may cause some T cells, B cells, and mast cells to become more active [4].

The different amounts of cytokine production are explained by the polymorphism of the cytokine genes, which are linked to the control of the inflammatory process mediated by the immune system. Because different cytokine gene alleles have been linked to a range of immune-inflammatory disorders, cytokine gene

polymorphisms have garnered attention recently [3].

Our goal was to investigate the predictive impact of the Interleukin 10 gene polymorphism in children with primary immunological thrombocytopenia.

METHODS

This case-control study was carried out at Zagazig University Hospitals in the Pediatric and Biochemistry Departments after receiving approval from the local institutional review board (IRB number 5167) and informed written consent from the parents of all patients prior to the commencement of the study. The Helsinki Declaration, issued by the World Medical Association to ensure the protection of individuals participating in medical research, was strictly adhered to during this study. Every research subject and/or their caregiver provided informed permission.

The different amounts of cytokine production are explained by the polymorphism of the cytokine genes, which are linked to control of the inflammatory process mediated by the immune system. Recently, cytokine gene polymorphisms have gained attention because several cytokine gene alleles have been connected to a variety of immune-inflammatory diseases [3].

As per our local guidelines, every patient underwent routine laboratory testing for the purpose of diagnosis and ITP follow-up. The 2011 ASH clinical practice guidelines on the evaluation and management of immune thrombocytopenia (ITP) served as the basis for the diagnosis of ITP.

The IL10 (-627) gene variations in patients and controls were investigated using the polymerase chain reaction-restriction fragment length polymorphism assay (PCR-RFLP).

Sample Collection: In order to perform PCR-RFLP genotyping for the IL10 gene polymorphism, five milliliters (mL) were extracted from each patient and control group and placed in a standard, sterile vacutainer.

***DNA extraction*:** Every patient had routine laboratory testing for the purpose of diagnosis and ITP follow-up, in accordance with our local guidelines. The diagnosis of immune thrombocytopenia (ITP) was based on the 2011 ASH clinical practice recommendations on the evaluation and management of ITP.

***Reaction mixture*:** 12.5 µL of PCR master mix (0.1 units/µL Taq DNA Polymerase, 32 mM (NH4)2 SO4, 130 mM Tris HCl, 5.5 mM MgCl2, and 0.4 mM of each dNTP), 5 µL genomic DNA, 1 µL of each primer, and 6.5 µL distilled water made up the 25 µl reaction mixture. To achieve a concentration of 25 pmol, primers were produced.

Primers: 5′-CCTAGGTCACAGTGACGTGG-3′ is the forward primer, and 5′-GGTGAGCACTACCTGACTAGC-3′ is the reverse.

Steps:There was an initial heat activation stage at 94°C for three minutes after 35 cycles of denaturation at 94°C for one minute, annealing at 50°C for one minute, extension at 70°C for one minute, and final extension at 70°C for three minutes. Next, utilizing gel electrophoresis on a 4% agarose gel (electro-4, Thermal Hybaid, Promega, USA), the amplification products were separated in parallel and the presence of DNA bands was assessed using ultraviolet trans-illuminator set at a wavelength of 312 nm. There was an amplified IL-10 (-627) polymorphism using 412-bp segments. The amplified PCR products (412 bp) were digested using Fermentas AM, Egypt's buffer, which contained 1 µL (10 units) of the common restriction enzyme RsaI, at 37°C for an entire night. One band at 412 bp emerged in people without this polymorphism and was given the name C/C (genotype, or wild type). Heterozygous for this polymorphism, the initial 176-bp, 236-bp, and cleaved 1 412-bp bands were demonstrated these individuals were assigned to the heterozygous C/A genotype. It was identified as A/A (homozygous A allele) if two bands, measuring 176 bp and 236 bp, appeared.

STATISTICAL ANALYSIS

2015 IBM Corp was used for data collection, tabulation, and statistical analysis. Armonk, NY-based IBM Corp, Version 23 of IBM SPSS Statistics for Windows was used. The mean± SD and median (range) were used to convey quantitative data, while number and percentage were used to express qualitative data. A normally distributed variable was compared between two groups using the independent t test. When comparing more than two groups of normally distributed data, an ANOVA (F test) was employed. The percentage of categorical variables was compared using the Chi-square test. There were two sides to every test. A p-value was considered statistically significant if it was less than 0.05, and statistically irrelevant if it was greater than 0.05.

RESULTS

Patients with chronic ITP (mean age 11.2±3.0) were significantly older than patients with newly diagnosed ITP (mean age 5.9±1.6), persistent ITP (mean age 7.5±1.6) and controls (mean age 7.3±2.7). Both the patients and the controls were watched with regard to sex. In terms of diagnostic age, there was no statistically significant variation among the patients. The most frequent presentation in our patients was purpura, which

was followed by external bleeding and ecchymosis. When it came to the initial clinical presentation, there was no statistically significant difference between the groups (Table 1). Regarding the frequency of the A and C alleles of IL10 (627), the patients' and controls' differences were quite significant with the A allele being more common in the patients group. Patients with persistent ITP or those who had newly diagnosed had a different allele prevalence than those with chronic ITP (Table 2).

The AA genotype was more prevalent in patients than controls. Among patients, the AA genotype was more prevalent in those with chronic ITP (47.6%) than newly diagnosed patients (38.1%) or those with persistent ITP (33.3%) (Table 2). Apart from initial platelet count which was significantly lower in newly diagnosed patients with AA genotype compared to other genotypes, there was no significant difference among different genotypes as regards demographic and clinical characteristics as well as 1st line therapy (Table 3).

There was significant difference among patients with persistent ITP as regards IL-10 genotypes and gender, initial platelet count and 1st line therapy where AA genotype was associated with male gender, lower initial platelet count and steroids as 1st line therapy (Table 4).

There was no significant difference among patients with chronic ITP as regards IL-10 genotypes and any of studied demographic, clinical, laboratory or therapeutic characteristics (Table 5).

Table 1: Demographic characteristics of the studied groups

	Newly diagnosed ITP (N=21)		**Persistent ITP (N=21)**		**Chronic ITP (N=21)**		**Controls (N=21)**		**F**	**P**
Age(years)										
Mean ±SD **Range**	5.9±1.6 2.5-9.5		7.5±1.6 7-11		11.2±3.0 6.5-16		7.3±2.7 2.5-13		20.0	<0.00
Gender	N	%	N	%	N	%	N	%	X^2	P
Males **Females**	13 8	61.9 38.1	10 11	47.6 52.4	9 12	42.9 57.7	11 10	52.4 47.6	1.67	0.6
Age of diagnosis									F	P
Mean ±SD **Range**	5.9±1.6 2.5-9.5		6.9±1.5 3.5-10		6.7±1.5 5-10		- -		0.8	0.4

Table 2: Distribution of IL-10 (627) polymorphism among studied groups

	Newly diagnosed ITP (N=21)		**Persistent ITP (N=21)**		**Chronic ITP (N=21)**		**Controls (N=21)**		**X^2**	**P**
	N	%	N	%	N	%	N	%		
IL10(627) Genotypes										
CC **CA** **AA**	7 6 8	33.3 28.6 38.1	6 8 7	28.6 38.1 33.3	1 10 10	4.8 47.6 47.6	12 9 0	57.1 42.9 0.0	19.5	0.003
Alleles										
C **A**	20 22	47.6 52.4	20 22	47.6 52.4	12 30	28.6 71.4	33 9	78.6 21.4	21.6	<0.001

Table 3: Relationship between IL10(627) polymorphism and other parameters in patients with newly diagnosed ITP

	CC (N=7)		CA (N=6)		AA (N=8)		Test of Sig.	P
Age (years) : Mean ±SD	5.3±1.4		6.4±1.8		6.1±1.6		F 0.83	0.45
Gender:	N	%	N	%	N	%		
Males	4	57.1	5	83.3	4	50.0	X^2	0.42
Females	3	42.9	1	16.7	4	50.0	1.72	
Age at diagnosis (years) : Mean ±SD	5.3±1.4		6.4±1.8		6.1±1.6		F 0.83	0.45
Purpura	7	100	6	100	7	87.5	1.71	0.42
Ecchymosis	4	57.1	3	50	7	87.5	2.6	0.27
Wet bleeding	4	57.1	2	33.3	6	75	2.46	0.29
Initial platelet count Mean ±SD	15.3±5		23.5±11.9		7.75±8.2		5.7	0.01
Treatment:								
Conservative	1	14.3	2	33.3	1	12.5	1.12	0.57
Steroids	4	57.1	3	50	3	37.5	0.6	0.74
IVIG	1	14.3	0	0.0	1	12.5	0.9	0.63
Steroids + IVIG	1	14.3	1	14.3	3	37.5	1.35	0.5

Table 4: Relationship between IL10 polymorphism and other parameters in patients with persistent ITP

	CC (N=6)		CA (N=8)		AA (N=7)		Test of Sig.	P
Age (years) : Mean ±SD	8.5±1.5		7.0±1.1		7.3±2.0		F 1.66	0.21
Gender:							X^2	
Males	2	33.3	2	25	6	85.7	6.2	0.04
Females	4	66.7	6	75	1	14.3		
Age at diagnosis (years) : Mean ±SD	7.7±1.3		6.4±1.0		6.7±2.0		F 1.2	0.32
Purpura	5	83.3	6	75	7	100	1.94	0.37
Ecchymosis	5	83.3	6	75	5	71.4	0.26	0.87
Wet bleeding	2	33.3	1	12.5	6	85.7	8.48	0.014
Initial platelet count Mean ±SD	15.7±6.6		24.2±5.3		11.8±7.9		F 6.8	0.006
Treatment:								
Steroids	2	33.3	1	12.5	6	85.7	8.48	0.014
IVIG	3	50.0	2	25.0	2	28.6	1.07	0.58
Steroids + IVIG	3	50.0	6	75.0	5	71.4	1.07	0.50

Table 5: Relationship between IL10 polymorphism and other parameters in patients with chronic ITP

	CC (N=6)		**CA (N=8)**		**AA (N=7)**		**Test of Sig.**	**P**
Age (years) : Mean ±SD	13.0±0.0		10.5±2.5		11.75±3.6		F 0.58	0.56
Gender:							X^2	
Males	1	100.0	4	40.0	4	40.0	1.4	0.49
Females	0	0.0	6	60.0	6	60.0		
Age at diagnosis (years) : Mean ±SD	5.5±0.0		6.95±1.1		6.6±1.9		F 0.46	0.63
Purpura	1	100.0	8	80.0	9	90.0	0.56	0.74
Eccymosis	0	0	8	80.0	9	90.0	4.79	0.09
Wet bleeding	0	0	3	30.0	6	60.0	2.63	0.26
Initial platelet count Mean ±SD	30.0±0.0		20.9±7.6		14.7±9.7		F 2.2	0.13
Treatment:							X^2	
Steroids	0	0.0	6	60.0	2	20.0	4.04	0.13
Steroids + IVIG	1	100.0	4	40.0	8	80.0	4.04	0.13
Rituximab	0	0.0	0	0.0	3	30.0	3.85	0.14

DISCUSSION

There are very few studies in Egypt which evaluated Interleukin 10 polymorphism in childhood ITP. In this study we aimed to study IL-10 gene polymorphism and evaluate its prognostic significance in children with ITP.

While the proportion of female patients with chronic ITP was higher than that of patients with persistent conditions and those who had just received a diagnosis did not vary statistically. Patients with chronic ITP in our study were significantly older than those with ITP that had just been diagnosed. persistent ITP, and controls (11.2 versus 5.9, 7.5, and 7.3 years respectively). Purpura was the most common presentation in our patients followed by ecchymosis and external bleeding (95.2% versus 66.7% and 57.1% respectively). There was no statistically significant variation between the groups in terms of the first clinical presentation. None of our patients had serious or potentially fatal bleeding.

Our results were in line with those documented in the literature, which states that children with ITP, regardless of gender preference, usually suffer from symptoms between the ages of 2 and 7. In a comprehensive meta-analysis, **Heitink-Pollé et al. [5]** discovered that a significant predictor of chronicity is older age at presentation (age ≥11 years; OR 2.47, 95% CI 1.94-3.15).

According to recent studies, there was a larger male to female ratio throughout infancy and a declining trend toward older age. ITP starts out suddenly. Almost all patients get bruises and petechiae, which are the most frequent initial clinical presentation [6]. In children with ITP, severe bleeding that could be fatal is uncommon (0.2–0.9%) [7].

Alternatively, **Hamed et al. [8]** found that 84.4% of their ITP patients were female and 15.6% of their patients were male. Additionally, research on individuals with ITP by **Del Vecchio et al. [9]** and **Talaat et al. [10]** revealed that ITP in adulthood affects girls more than males. This may be related to the study population being different adults with ITP were the subject of earlier research, whereas only children with ITP were the focus of our investigation.

The initial mean platelet count of our newly diagnosed patients was 14.76 X103 /ul. Patients with newly diagnosed ITP exhibited significantly lower platelet counts (14.76, 17.7, and 18.4 X103 /ul, respectively) compared to those with persistent and chronic ITP. This should be considered in the context of the fact that in children with ITP, a greater platelet count at diagnosis is a risk factor for chronicity. **Grimaldi-Bensouda et al. [11]** observed that a higher baseline platelet count (Odds Ratio 1.03; 95%CI: 1.00, 1.06) was the only potential predictor of chronicity at 12 months, which provided good support for our findings. **Heitink-Pollé et al. [5]** discovered that patients with chronic ITP had a notably elevated platelet count at diagnosis, with a mean difference of 5.27 (95% CI 2.69-7.86).

In their extensive meta-analysis, **Heitink-Pollé et al. [5]** discovered that older age at presentation (age ≥11 years; OR 2.47, 95% CI 1.94-3.15) has an In our study, there was highly significant difference between patients and controls as regards frequency of A and C alleles of IL-10

(627) where A allele was more prevalent in patients than controls. Among patients, A allele was more prevalent among patients with chronic ITP as opposed to those who have just received a diagnosis or who have persistent ITP (71.4% versus 52.4% and 52.4% respectively). The AA genotype was more prevalent in patients than controls. Among patients, the AA genotype was more prevalent in those with chronic ITP than newly diagnosed patients or those with persistent ITP (47.6% versus 38.1% and 33.3% respectively).

Our results were in agreement with those reported by **Makhlouf and Abd Elhamid, [12]** where it was found that ITP patients had higher frequencies of IL10 A alleles than controls. The difference between ITP patients with newly diagnosed and chronic conditions was statistically significant in the distribution of IL10 gene polymorphisms, with the A allele being higher in the former group than in the latter. **Mouzaki et al. [13]** report that children with chronic ITP had elevated IL10 levels, which may indicate Th1 participation in the pathophysiology of ITP as seen by an increase in Th1 cytokines.

Conversely, **Wu et al. [14]** discovered that, in contrast to controls (15%), ITP patients with homotype A/A was statistically identical. This can be attributed to different ethnicities among different studies.

In our study, there was significant relationship between AA genotype and initial platelet count in patients with newly diagnosed and persistent ITP where platelet count was lower in these patients, also, there was significant relationship between AA genotype and male gender and those who received steroids as first line therapy in patients with persistent ITP.

Apart from this, there was no significant association between IL-10 gene polymorphism and any of studied demographic, clinical, laboratory or therapeutic characteristics. Our results are matched with **Makhlouf and Abd Elhamid, [12]** where acute patients with AA genotype had significantly lower initial platelet counts than other genotypes. Moreover, they found significant association between AA genotype and both lower hemoglobin levels and higher antiplatelet antibodies levels. On the other hand, **Makhlouf and Abd Elhamid, [12**] did not find any significant association between AA genotype and other studied demographic, clinical, laboratory or therapeutic characteristics.

***Limitations*:** One of the study's drawbacks was its small sample size; hence, bigger multicenter investigations are still required to corroborate these results. Another drawback was that we had to start with patients who had developed ITP from scratch, ascertain their genotype and baseline IL-10 level, and monitor any variations in their serum IL-10 levels throughout time. But many de novo ITP patients lost track of their progress, particularly after it had improved.

CONCLUSIONS

We concluded that IL-10 gene polymorphism may contribute to susceptibility and chronicity of ITP in children as AA genotype was higher in patients than controls and in chronic patients compared to those with newly diagnosed ITP.

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