

Research Article

Impact of IL-1Ra Gene Polymorphism on the Etiology and Fate of Disease in Children with Immune Thrombocytopenic Purpura

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Background. Immune thrombocytopenic purpura (ITP) is considered to be one of the common childhood autoimmune diseases, and the current study was initiated to study the effect of various factors, particularly interleukin 1 receptor antagonist (IL-1Ra) gene polymorphism, on the course of the disease. **Methods.** The current case-control study involved 60 newly diagnosed children presented with ITP (also included 60 age- and sex-matched healthy children). All enrolled individuals had complete blood count and molecular study to determine the polymorphic state of IL-1Ra gene using conventional polymerase chain reaction. **Results.** Sixty patients with ages 1-14 years and having a male/female ratio of 1 : 1.61 were enrolled in the current study. Forty-five children (75%) recovered within the first year, and 15 (25%) children developed chronic ITP. IL-1Ra*2 variant was found to be significantly associated with control groups ($P = 0.011$), while IL-1Ra*3 was significantly associated with patients ($P = -0.0163$). Other factors having significant association with the remission rate include a previous history of immunization ($P < 0.0001$) and the symptoms at presentation ($P = 0.0009$). **Conclusions.** The current study revealed a significant correlation of IL-1Ra gene polymorphism to the etiology and the course of the disease.

1. Introduction

Immune thrombocytopenia (ITP) is a commonly occurring blood disorder and is one of the commonest causes of childhood thrombocytopenia. This autoimmune disease occurs due to destruction of platelets following binding of autoantibodies to the glycoproteins located on the platelet membrane (GPIIb/IIIa and GPIb/IX) [1].

As per the guidelines from the International Working Group, clinical classification of ITP includes three types: newly diagnosed, persistent (ITP cases that lasted for 3–12 months after initial diagnosis and did not resolve spontaneously following stoppage of treatment for 3–12 months), and chronic (ITP cases that lasted beyond 12 months) [2, 3].

ITP can also be classified as primary (without any associated disease) and secondary types (with associated disease). Globally, the childhood ITP incidence is 2–7 per 100,000 per year, which is much lower compared to the incidence of adult ITP (pediatric ITP rarely progresses to chronic level) [4–6].

The etiology of ITP is not yet completely understood. Studies have shown that both environmental and genetic factors play a significant role in ITP etiology. Microbial agents like cytomegalovirus, varicella-zoster virus, Epstein-Barr virus, human T-lymphocytic virus, etc. might be pivotal in the etiopathogenesis of ITP [3, 7, 8].

Another documented cause of ITP in children is MMR (mumps, measles, and rubella) vaccination. Although there is a documented cause-effect relationship between MMR vaccine and childhood ITP, the incidence is very low—occurring in 1–3 children per 100,000 doses of MMR vaccine [9]. Moreover, ITP incidence due to MMR vaccine is negligible compared to the incidence of diseases prevented by the vaccine [9]. Therefore, the risk-benefit ratio of MMR vaccination significantly outweighs that of vaccine-associated ITP.

In addition to microbial agents, the immune system of the affected person also plays an important role in ITP. B lymphocytes of the affected person produce antiplatelet antibodies, but T cells are a key factor in the etiopathogenesis of

ITP. Increased cytokine response due to cytokine network dysregulation is another documented factor associated with ITP pathogenesis [3, 9, 10].

Genetic polymorphism of the cytokine genes is known to be linked with ITP etiopathogenesis. In addition, the majority of the autoimmune diseases including ITP are linked with abnormal functioning of T cells and cytokines as they contribute significantly to disease pathogenesis.

Polymorphisms of genes encoding the cytokines like human leukocyte antigen, IL-4, tumor necrosis factors, IL-10, IL-1 family of cytokines, etc. are also involved in the pathogenesis of ITP. In the IL-1 family of cytokines, the IL-1A, IL-1Ra, and IL-1B also play key roles in ITP etiopathogenesis [10].

The standard treatment strategy for ITP management includes drugs like corticosteroids, intravenous immunoglobulin (IVIg), romiplostim, anti-D immunoglobulin, high-dose immunosuppressive drugs, and surgical interventions like splenectomy. However, approximately 20% of ITP cases are resistant to treatment [11]. In the current study, we assessed the importance of genetic polymorphism of IL-1Ra in the etiopathogenesis of ITP along with the prognosis of the disease.

2. Material and Methods

The ethical committee at the University of Duhok/College of Medicine approved the study, and the researchers followed the Declaration of Helsinki for conducting medical research on human beings.

A total of 60 newly ITP-diagnosed children attending Heevi Teaching Hospital in Duhok/Iraq between November 1, 2017, and October 1, 2019, were enrolled in this study. These patients initially presented with isolated thrombocytopenia, but its secondary causes were excluded [11]. Also, another 60 age- and sex-matched children with no history of thrombocytopenia were enrolled as controls. After explaining the study procedure to the children's legally authorized representative (mostly parents), verbal informed consent was obtained from them. The complete history of the disease was obtained from parents of all enrolled children. Blood samples of all included children were collected in K2-EDTA tubes to examine complete blood count (using daily calibrated electronic hematology analyzer Swelabo-Sweden blood counter) and blood morphology. The remaining samples were frozen for DNA extraction using a modified salting-out extraction method adopted by Iranpur and Esmailzadeh that yields high quantity with high purity DNA [12, 13].

After extraction, amplification was done for IL-1Ra gene polymorphism using a set of primers, IL-1Ra-F: 5'-CTCAGC AACACTCCTAT-3', and IL-1Ra-R: 5'-TCCTGGTCTGC AGGTAA-3' [14]. Initially, a master mix was prepared using 2X Hot Start Master Mix from GeNet Bio (South Korea) and then amplified using a PCR thermal cycler (MultiGene™ OptiMax Thermal Cycler) from Labnet International Inc. (USA). PCR conditions were as follows: 94°C for 3 min, 30 cycles at 94°C for 30 s, 60°C for 30 s, and 72°C for 45 s, and

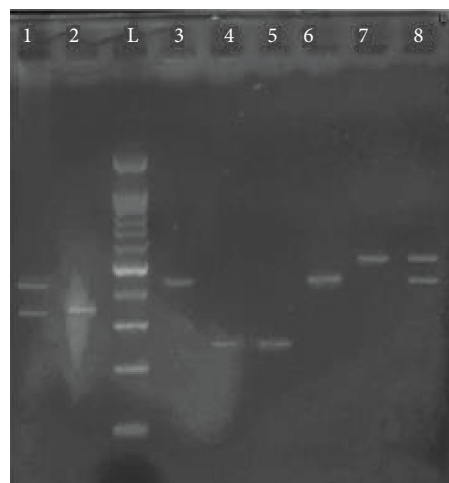


FIGURE 1: Various patterns of IL1Ra gene polymorphism seen. 1 represents heterozygous 325 & 412 bp IL-1Ra*4&1 variants; 2 represents homozygous 325 bp IL-1Ra*4 variant; L represents 100-1500 bp ladder; 3 and 6 represent homozygous 412 bp IL-1Ra*1 variant; 4 and 5 represent homozygous 240 bp IL-1Ra*2 variant; 7 represents homozygous 548 bp IL-1Ra*3 variant; and 8 represents heterozygous 412 and 548 bp IL-1Ra*1 and 3 variants.

final extension of 72°C for 5 min. The amplified products were then subjected to 2% agarose gel electrophoresis. Variable patterns are seen as shown in Figure 1. Resulting PCR products of 412 bp (IL-1Ra*1, four repeats); 240 bp (IL-Ra*2, two repeats); 548 bp (IL-1Ra*3, five repeats); 325 bp (IL-1Ra*4, three repeats); and 595 bp (IL-1Ra*5, six repeats) were obtained separately from different samples.

Data were analyzed with SPSS software (version 24). For continuous variables, Student's *t*-test was used, and for categorical variables, chi-square test or Fisher's exact test was used. *P* value < 0.05 was considered statistically significant.

3. Results

The main findings of the patients are listed in Table 1. It shows that 60 children with ITP and aged between 1 and 14 years (median 5.0 ± 3.47) were enrolled. The female to male ratio in the study population was 1 : 1.61.

A history of immunization within the last 45 days was documented for 9 patients (last immunization carried out within previous 14 days for 8 children and 3 weeks previously in one child) compared to 5 controls. Clinically small ecchymosis and petechiae were the most common presenting symptoms seen among 53 (88.3%) children. Only 7 (11.7%) children were presented with more severe symptoms including epistaxis, gastrointestinal bleeding, and hematuria. During the course of the disease episodes of epistaxis (*n* = 4; 6.67%), gastrointestinal bleeding (*n* = 3; 5.0%), and hematuria (*n* = 3; 5.0%) were documented. Ten patients (16.67%) suffered from these extracutaneous symptoms during the whole course of the disease. Clinically, only 2 patients (3.33%) had splenomegaly, and 1 patient (1.67%) had hepatomegaly.

TABLE 1: Demographic and hematological data of all enrolled patients and controls.

Parameters	I TP children	Control children	P value
Age: range (years) (median \pm SD)	1–14 (5 \pm 3.47)	1–14 (5 \pm 3.21)	0.892
Sex			
Male: no. (%)	23 (38.3%)	25 (41.7%)	0.712
Female: no. (%)	37 (61.7%)	35 (58.3%)	
History of immunization no. (%)	9 (15.0%)	5 (8.33%)	0.259
Hb (g/L) (median \pm SD)	68–146 (115 \pm 14.1)	93–151 (117.5 \pm 16.5)	0.119
Mild Anemia (no. (%))	18 (30%)	17 (28.33%)	0.842
WBC ($\times 10^9$ /L) (median \pm SD)	4.3–18.2 (8.35 \pm 3.44)	4.2–16.9 (7.8 \pm 2.71)	0.715
Platelets ($\times 10^9$ /L) (median \pm SD)	2–98 (13 \pm 30.37)	167–399 (278 \pm 60.41)	<0.001

TABLE 2: Possible IL-1Ra genetic polymorphism variants seen among patients and controls.

IL-1Ra	Variants	Cases	Controls	Total
	1,1	41 (68.3%)	42 (70.0)	83 (69.2%)
	1,2	6 (10.0%)	14 (23.3%)	20 (16.7%)
	1,3	9 (15.0%)	2 (3.3%)	11 (9.2%)
	1,4	1 (1.7%)	0 (0%)	1 (0.85%)
	2,2	0 (0%)	2 (3.3%)	2 (1.7%)
	3,3	1 (1.7%)	0 (0%)	1 (0.85%)
	4,4	2 (3.3%)	0 (0%)	2 (1.7%)

TABLE 3: Fate of the disease and response to therapy.

No. of patient who achieved complete response within the first year	45 (75%)
Spontaneous remission	35 (58.33%)
Steroid	9
IV Ig	1
No. of patients who did not achieve complete response within the first year (chronic)	15 (25%)
Steroid	14
Steroid+IV Ig	1

Hematological data revealed that patients had significantly lower platelet count ($P < 0.001$) in comparison to control, with comparable hemoglobin ($P = 0.35$) and WBC counts ($P = 0.63$). In comparison to 17 controls, 18 patients suffered from mild nutritional anemia, including 1 patient with moderate anemia and 1 patient with severe anemia.

With respect to IL-1Ra gene polymorphism as shown in Table 2, IL-1Ra*1 (412 bp) was the most common variant among patients and controls. Alleles of IL-1Ra*2 (240 bp) variant were found to be significantly higher among controls ($P = 0.011$), while alleles of IL-1Ra*3 (548 bp) variant were found to be significantly higher among patients ($P = 0.0163$).

Table 3 reveals the fate of the disease and the response to therapy. As shown in the table, the majority of children (35 out of 60) with ITP recovered spontaneously without any medication and required only observation. Ten patients

required medication to achieve complete remission, and the remaining 15 patients did not achieve remission and became chronic patients.

Table 4 shows the impact of various factors on the fate of the disease. It indicates that those patients with a recent history of immunization achieved complete remission within the first 3 months. Older children aged more than 10 years achieved nonsignificant lower remission ($P = 0.245$). The same is applied to the age, gender, hemoglobin level, white blood count, platelet count, and symptoms of the enrolled individuals that do not have significant impact on the achievement of remission. Also, all patients with IL-1Ra*2 polymorphism variants achieved complete remission.

4. Discussion

Destruction (immune mediated) of platelets is the principal pathophysiological feature of ITP. Cell-mediated immunity and cytokine response are the two major players in the pathogenesis of the disease. Although the exact stimulus for cytokine dysregulation in ITP patients is yet to be established, it is known that, in ITP patients, contact of macrophages and dendritic cells (antigen presenting cells) with foreign microbial proteins (antigen) might trigger cytokine response. This may lead to increased synthesis of IL-1, IL-8, IL-6, and TNF- α , followed by a surge in chemokine production and then a fall in cytokine production to normal level [7, 8, 15, 16].

There are three structurally related polypeptide members in the family of IL-1 cytokine: IL-1a, IL-1b, and IL-1Ra (it characteristically attaches itself to the IL-1 receptors and suppresses biological functions of IL-1). Thus, maintaining the balance between IL-1 and IL-1Ra is of utmost importance for adequate host immunity [3, 16]. Biological functions of IL-1 include modulation of cellular proliferation and induction of production of different cytokines. In the case of ITP patients, IL-1 is possibly involved with upregulation of megakaryocytopoiesis, increased production of platelets, and production of auto antibodies [16].

The same gene can encode two different proteins of IL-1Ra; these two different proteins are produced by splicing of 2 different exons in an alternative fashion [3, 16]. Genes encoding the cytokines are polymorphic in nature and are responsible for the regulation of pathogenesis of several

TABLE 4: Effect of various factors on remission achievement.

Parameter		Cure	Chronic	
	<5.0	28	8	
Age (years)	5–10	13	4	<i>P</i> 0.245
	>10	4	3	
	Male	16	7	
Sex	Female	29	8	<i>P</i> 0.602
	Immunization	9	0	<i>P</i> < 0.001
Hb.		11.3 ± 1.71	11.5 ± 1.31	<i>P</i> 0.661
WBC		8.2 ± 3.47	8.35 ± 3.47	<i>P</i> 0.861
Platelets		23 ± 29.43	38 ± 30.77	<i>P</i> 0.384
Platelets	<50*10 ⁹ /L	25	12	<i>P</i> 0.091
	>50*10 ⁹ /L	20	3	
Symptoms	Mild	40	10	<i>P</i> 0.0009
	Severe	4	6	
	1	42	14	
IL-1Ra*	2	6	0	<i>P</i> < 0.001
	3	7	3	

autoimmune diseases. IL-1Ra is associated with several inflammatory diseases like ankylosing spondylitis, rheumatoid arthritis, alopecia areata, etc. [16–19].

In this study, it was found that IL-1Ra*2 was the most significant polymorphism associated with control group and IL-Ra*3 was the most significant polymorphism in patients. Similar to our study, several other studies have also found the association of genetic polymorphism of IL-1Ra with ITP.

Wu and his colleagues also published a study implicating the importance of genetic polymorphism of IL-1R in the etiopathogenesis of ITP [16]. In another study, Yadav and his colleagues evaluated the relation between several interleukins including IL-Ra with ITP [10]. They reported that the presence of allele-II of IL-1Ra led to 1.75-fold increase in the probability of ITP development. They also found that both hetero and homozygous types of genotypes of IL-1Ra are associated with ITP.

In our study, we found that a history of immunization and symptoms at presentation significantly influenced the remission.

Previous studies have documented that IL1Ra*1 (412 bp) variant was the most common, followed by IL1Ra*2 (240 bp) and other variants; this was in agreement with our results [21]. As the main function of IL-1Ra gene is to produce IL-1 receptor antagonist protein by different cells including epithelial cells, immune cells, and adipocytes, it is possible that α and β -proinflammatory modulation of IL-1 might lead to IL-1-related immune and inflammatory responses [21]. So inactivation of the gene through trinucleotide repeat mutation [e.g., 548 bp (IL-1Ra*3, five repeats) and 595 bp (IL-1Ra*5, six repeats)] is responsible for hyperactivity of IL-1 (α and β -proinflammatory) and induction and persistence of autoimmune diseases. In contrast, the current study showed hyperactivity of the gene [240 bp (IL-Ra*2, two repeats)] is

linked with a reduced chance of development of ITP and an improved recovery rate through counteracting the action of proinflammatory IL-1, mainly that of its β subunit.

5. Conclusions

In our study, the majority of childhood ITP cases recovered spontaneously and did not require any specific treatment. Our findings also indicate that different factors, such as previous history of immunization, severity of the disease, and IL-1Ra*2 (240 bp) variant, may be associated with increased recovery rate.

Abbreviations

ITP: Immune thrombocytopenic purpura
 IL-1Ra: Interleukin 1 receptor antagonist
 MMR: Mumps, measles, and rubella
 PCR: Polymerase chain reaction.

Data Availability

Data are available on request.

Ethical Approval

This study was approved by the ethical committee at the University of Duhok/College of Medicine.

Consent

Verbal informed consent was obtained after the study was explained to the children's legally authorized representative (mostly parents).

Conflicts of Interest

No conflicts of interests are declared by the authors.

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