Association of Interleukin-1B and Interleukin-1 Receptor Antagonist Gene Polymorphisms with Prognosis and Development of Immune Thrombocytopenia in Children

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Abstract

Background: Immune thrombocytopenia (ITP) is one of the most prevalent childhood autoimmune diseases and characterized by immune-mediated destruction and reduced generation of normal platelets and is linked to a variety of bleeding symptoms. In order to understand the pathophysiology and prognosis of ITP, it is crucial to research the polymorphisms in several interleukin-1B and interleukin-1 receptor antagonist cytokine genes.

Aim: to identify the correlation between IL-1R and IL-1B receptor antagonist gene polymorphism and development of ITP and its impact on its prognosis and severity.

Methods: This study was carried out in The Clinical Pathology Department and Pediatric Department, Faculty of Medicine Zagazig University. This case control study was conducted on 25 pediatric patients suffering from immune thrombocytopenic purpura (ITP) newly diagnosed with follow up of treatment as a patients group and 25 apparently healthy pediatric individuals as a control group. Both study groups were subjected to Interleukin1beta (IL-1B-511, IL-1B-31) and Interleukin1receptor antagonist (IL1RA) polymorphism using restriction fragment length polymorphism RFLP(PCR)

Results: There is statistically significant relation between ITP and IL-1B-31, IL-1Ra, IL-1B-511 alleles or genotypes distributions. Mutant TT genotype of IL-1B-31significantly elevates risk of ITP by 18.62 folds, IL-1B-511 Mutant CC genotype significantly elevates risk of ITP by 3.5 folds and I/II hetero genotype of IL-1Ra significantly increases risk of ITP by 5.76 folds. Mutant TT genotype of IL-1B-31 significantly increases risk of non-severe ITP versus control group by 25.1 folds and T allele significantly increases risk of severe ITP versus control group by 5.75 folds, IL-1B-511 mutant CC genotype significantly elevates risk of non- severe and sever ITP versus control group by 12.6 folds and C allele significantly increases risk of severe ITP versus control group by 5.7 folds. and I/II hetero genotype of IL-1Ra significantly elevates risk of non-severe ITP versus control group by 9.4 folds. There was statistically significant relation between response to treatment and IL-1B-31 genotypes and alleles among ITP cases as wild CC genotype and C allele significantly decreases risk of non-response to medication. There was no statistically significant relation between response to medication and IL-1B-511 and IL-1Ra.

Conclusion: IL-1B and IL-1RA polymorphisms may play an important role as a genetic risk factor for the development and progression of childhood ITP and IL-1B-31 and IL-1B-511 polymorphisms could be used as a genetic predictor to determine the degree of severity of ITP. IL-1B-31 polymorphism could be used as genetic indicator to detect the response to treatment in ITP pediatric patients.

Keywords: Interleukin-1B, Interleukin-1 Receptor Antagonist, Gene Polymorphisms, Immune Thrombocytopenia

1. Introduction

Immune thrombocytopenia purpura (ITP) is an immune-mediated disorder induced via autoantibodies towards glycoproteins (GP) Ib/IX and GPIIb/IIIa present in platelet membranes

(1).

Clinically, ITP can be divided into three categories: newly diagnosed (for all patients at diagnosis), persistent (for instances with ITP permanent between 3 and 12 months from diagnosis, and cases in which spontaneous remission is not accomplished after the medication is stopped in between 3 and 12 months from diagnosis), and chronic ITP (long-lasting for more than 12 months) (**2).**

Although the ITP's cause is uncertain, genetic as well as environmental variables seem to contribute to the disease's progression. It has been suggested that autoantibody synthesis by B cells, several cellular immune dysfunctions, and cytokine dysregulation act a significant role in the ITP**'s** pathogenesis **(3).**

ITP has been linked to inflammatory cytokine gene polymorphisms and to elevated Th1/Th2 ratio. T cell activation and cytokine imbalances were discovered to have a significant function in the autoimmune disorders' pathogenesis, notably ITP. Cytokines are important participants in the equilibrium of Th1 and Th2 cells. Glucocorticoid therapy assists in normalize Th1 and Th2 levels in ITP cases because an imbalance in Th1/Th2 causes autoreactive B cell growth in ITP **(4).**

Stimulation of CD4+ T cells results in a proinflammatory reaction with the generation of autoreactive antibodies and interferon-γ and interleukin-2 (IL-2) elevated expression **(5).**

Polymorphisms in inflammatory cytokine genes, Fcγ receptors, human leukocyte antigen, and tumor necrosis factor are among the genetic variables linked to ITP **(6).**

FurthermoreIL-10, IL-1Ra, and IL-4 polymorphisms results in childhood chronic ITP, while an IL-1β exon 5 polymorphism is correlated with childhood ITP **(1).**

In ITP, IL-1 family members are associated with the megakaryocytopoiesis' activation, regulation of platelet generation, and autoantibodies' production. The IL-1family contains three associated genes, IL-1B, IL-1A, and IL-1Ra, and every act a various functional role in autoimmune disorders and ITP **(7).**

IL-1Ra and IL-1B are located on chromosome 2q14 within a 360-kb region. IL-1 beta (IL-1β) is a proinflammatory cytokine secreted via macrophages in systemic inflammatory responses and controls inflammatory reaction and immune reaction by accelerating cytokines involving IL-6 and IL-12 **(1).**

IL-1B has two diallelic polymorphisms in the promoter region at locations -511 and -31, and one in the fifth exon at position +3954. The promoter polymorphism 31 T > C (rs1143627) influences IL-1B expression. Intron 2 contains a penta-allelic polymorphism region with varied numbers of an 86-base pair (bp) tandem repeat sequence. As The diameters of the IL-1Ra PCR products were determined related to the 100-bp DNA ladder. The following alleles had various product sizes: I-IL-1Ra 1, 410 bp; II-L-1Ra 2, 240 bp; III-IL-1Ra 3, 500 bp; IV-IL-1Ra 4, 325 bp and V- IL-1Ra5, 595 bp. **(1).**

Many studies evaluated the correlation between numerous interleukins involving IL1B and IL-Ra polymorphism with ITP **(8).**

Prior works have recorded that IL1Ra∗1 (412 bp) variant was the most popular, accompanied via IL1Ra∗2 (240 bp) and other variants. Because the major role of the IL-1Ra gene is to create IL-1 receptor antagonist protein via various cells such as epithelial cells, immune cells, and adipocytes, and -proinflammatory regulation of IL-1 may result in IL-1-related immune and inflammatory responses. Thus, gene's inactivation via trinucleotide repeat mutation [e.g., 548 bp (IL-1Ra3, five repetitions) and 595 bp (IL-1Ra5, six repeats)] is accountable for IL-1

hyperactivity (and β-proinflammatory) as well as the onset and persistence of autoimmune disorders **(9).**

We aimed at this work to identify the correlation between IL-1R and IL-1B receptor antagonist gene polymorphism and development of ITP and its impact on its prognosis and severity

2. Subjects and Methods

2.1. Study Population

This study was carried out in The Clinical Pathology Department and Pediatric Department, Faculty of Medicine Zagazig University.

Ethical consideration: This study was approved via the International Review Board (IRB) of the Faculty of Medicine-Zagazig University (Approval no. 4573/6-5-2018). The collection of samples began in November 2020 and completed in November 2021.Using a specialized form, all participants and their parents provided informed written consent for the utilization their samples and clinical data in this study and to publish the findings in line with the Helsinki Declaration.

The sample size was computed utilizing EPI INFO 7 (Atlanta, GA, USA), Assuming that IL-1Ra(heterozygous) in children with ITP is 75% and in normal children 32%. The sample was calculated to be 50 children (25 patients, 25 control) at confidence level 95% and power of the test 80%. **(6)**

This case control study was conducted on 25 pediatric patients suffering from immune thrombocytopenic purpura (ITP) newly diagnosed with follow up of treatment as a patients group and 25 apparently healthy pediatric individuals as a control group

Inclusion criteria:

All pediatric newly diagnosed patients with ITP.

Exclusion criteria:

1- Patient refusal.

2- Patient with other type of diseases.

3- ITP associated with secondary causes

4- ITP cases on therapy

Both study groups were exposed to Full history acquiring, clinical data acquisition and Clinical examination. laboratory examinations (complete blood count [CBC] by "Sysmex Xn300" (Sysmex Corporation, Kobe, Japan), Kidney and liver functions tests utilizing automated analyzer "Roche Cobas 8000-c702" (Roche Diagnostics, Mannheim, Germany) and Bone marrow aspiration and examination if required.

2.2 The definition of severity of ITP and response of ITP to treatment via the international working group (IWG) is as follows: Evaluation of severity as severe ITP by determining whether an ITP patient had severe bleeding that needs to be treated once diagnosed or greater therapeutic intervention as the disease progresses.

Response of ITP to therapy is defined as a. Complete response (CR) is defined as no bleeding and two platelet counts of 100,000 or greater taken seven days apart. When the platelet count falls to less than 100,000 twice, one day apart, there is a loss of CR.

b. Response (R) is described as a platelet count of 30,000 or higher, platelet counts that are at least two times the baseline level, separated by seven days, and no bleeding.
c. No response (NR) is platelet count fewer than 30,000 or less than two-fold elevation from base line recorded twice one day apart and exitance of bleeding. **(10)**

2.3 Sampling

Each patient gave a 5 mL sample of venous blood under complete aseptic circumstances. The samples were divided into three groups: 2 mL on sterile plain vacutainer tubes for kidney and liver functions assays, 1.0 mL on sterile EDTA vacutainer tubes for full CBC, and 2.0 mL on sterile EDTA vacutainer tubes for IL-1B and IL-1Ra gene polymorphism by PCR REFLP. (Restriction Fragment Length Polymorphism).

2.4 DNA extraction

DNA extraction from blood using a genomic DNA extraction methodology and a genomic DNA purification kit from Thermo Scientific. (ThermoFisher Scientific Inc., Waltham, MA, USA). Additionally, a Qubit Fluorometer was used to measure the amount of genomic DNA present in the samples. (ThermoFisher Scientific Inc., Waltham, MA, USA).

2.5 Amplification and Detection of IL-1Ra and IL-1B gene polymorphism gene polymorphisms via restriction fragment length polymorphism (RFLP) (Thermo Scientific, USA)

Principle:

Following conventional PCR amplification, restriction enzymes that can recognize and cut DNA at a specific sequence are used to fragment the DNA sample. Agarose gel electrophoresis is used to split the generated restriction fragments into several length categories.

Reagents and procedures:

The IL-1B and IL-1Ra gene polymorphism *by PCR* REFLP (restriction fragment length polymorophism) Primer sequences of IL-1B-31 C＞T rs1143627 and IL-1B-511 T＞C rs16944 were as follows:

forward-5′-AGCTTCCACCAATACTCTTTTCCCCTTTCC-3′, reverse 5′TACACACAAAGAGGCAGAGAGACAGA-3′and forward 5′-TGG CATTGATCTGGTTCATC-3′ and reverse 5′-GTTTAGGAATCTTCCCA CTT-3′.

The PCR was carried out in a final volume of 20 µl comprising 10 µl of 2x *i-Taq*^{TM} PCR Master Mix (Thermo Scientific,*USA),*1 ul of each primer (Thermo Scientific, *USA)*, 6 µl of genomic DNA and 2ul of deionized water.

PCR Protocol:

DNA thermal cycling was used to perform the amplification. (Applied Biosystem, Singapore) Denaturation was place at 95 °C for 5 min, then 35 cycles were performed at 95 °C for 30 sec, 60 °C for 45 sec, and 72 °C for 40 sec. Terminal elongation took place at 72 °C for 7 min.

Restriction Digest Reaction:

For IL-1B-31, Alu1 restriction endonuclease from Thermo Scientific in the United States was used to digest the PCR-amplified products. 10 ul of PCR amplicon, 1 ul of the enzyme Alu1, 2 ul of 10X buffer, and 7 ul of nuclease-free water in a full volume 20 µl were used for the digestion. The specimens of the digested PCR amplicon were incubated overnight at 37°C. After loading the specimens onto the agarose gel, electrophoresis was carried out. The C-to-T mutation that results in the variant allele of IL-1B-31 results in homozygous mutant people (TT) showing two fragments of 174 and 99 base pairs, heterozygotes (CT) showing three fragments of 273,174, and 99 base pairs, and wild-type people (CC) showing just one band of 273 base pairs. (Figure 1)

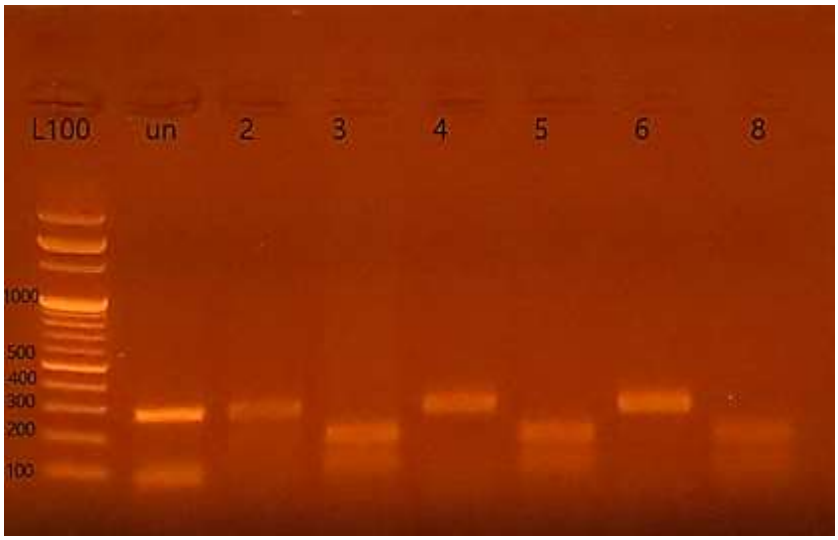

Figure (1): genotypes of IL-1B-31 polymorphisms

The IL-1B-511 PCR amplified products were digested utilizing AVA1 (Thermo Scientific. USA) restriction endonuclease. The digestion was conducted in a full volume 20 µl comprising 10 ul of PCR amplicon, 1 ul of enzyme AVA1, 2 ul of 10X buffer and 7 ul of nuclease- free water.

The variant allele for IL-1B-511 is established by a T-to-C change, homozygous mutant individuals (CC) demonstrated 2 fragments of 190 and 115 bp; heterozygotes (CT) demonstrated 3 fragments of 305,190,115 bp; and wild-types (TT) demonstrated only 1 band of 305 bp. (Figure 2)

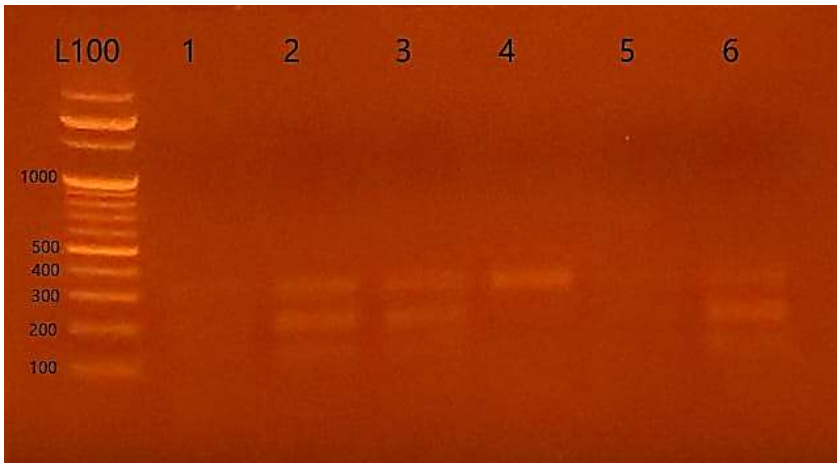

Figure (2): genotypes of IL-1B-511 polymorphisms

For IL-1Ra PCR amplicon sizes were recorded arranged to DNA ladder 100-bp. The product sizes of various alleles were as shown: II-L1Ra 2, 240bp; I-IL1Ra 1, 410 bp; III-IL1Ra 3, 500 bp; IV-IL1Ra 4, 325 bp; and V-IL1Ra5, 595 bp. Agarose gel electrophoresis was utilized to separate the digested products (Figure 3)

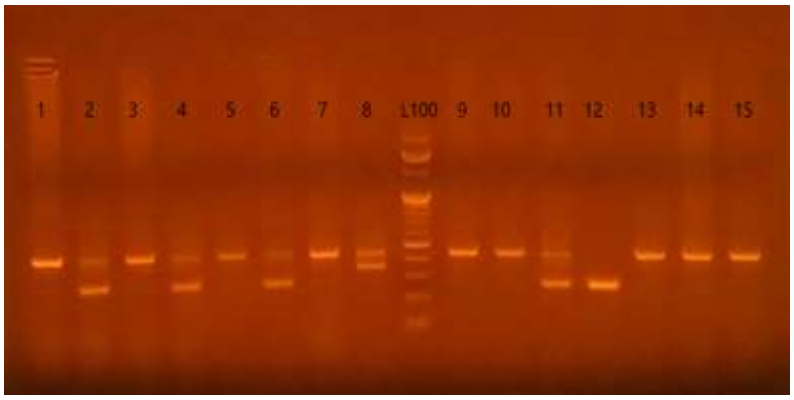

Figure (3): alleles of IL1Ra gene

2.6 Statistical Analysis

Data analysis was done utilizing version 26 of the statistical program Statistical Package for the Social Sciences. (IBM SPSS, Version 26.0. Armonk, NY, USA) The absolute frequencies of categorical variables were used to describe them, and the chi square assessment and, when suitable, the fisher exact assessment were utilized to contrast them. To determine the rough odds ratio and confidence interval, cross tabulation was utilized. Depending on the type of data, the standard deviations, means, or median and interquartile range of quantitative variables were utilized to characterize them. The Mann Whitney test (for data that were not regularly distributed) was utilized to contrast quantitative data between two groups. Using binary logistic regression, it was possible to pinpoint independent risk variables linked to certain health issues. P 0.05 was used as the statistical significance level. If $p \leq 0.001$, a greatly significant difference was evident.

3. Results

As per demographic data of the studied groups, the range age of patients group was (4-11) years and the range age of control group was (4-12) years and 68% of patients group was female and 32% was male compared to 52% and 48 % respectively in control group and there was no statistically substantial difference between the studied groups regarding age (p=0.519) or gender (p=0.077).

Clinical presentation, severity of ITP and treatment of studied patients are described in Table1, and shows that 52% of patients had ecchymosis, 28% had purpura and 8% had epistaxis. 9 patients (36%) had been classified as Severe ITP. 14 patients (56%) received first line treatment includes: steroids, IVIG and combination of them. 9 patients (36%) received Second line treatment includes: subcutaneous Thrombopoietin receptor agonist, oral TPO, Monoclonal CD20 Ab.

Eighteen patients of ITP were responders (72%) while seven patients (28%) were non responders.

Table (1): Clinical presentation, severity of ITP and treatment of studied patients.

	N=25	%
Skin		
Purpura	7	28%
Ecchymosis	13	52%
Mucus membrane		
Epistaxis	2	8%
Bleeding per gum	1	4%
Hematemesis	1	4%
Bleeding per rectum	1	4%
Severity:		
Severe	9	36%
Non-severe	16	64%
Treatment:		
First line	14	56%
Second line	9	36%
No treatment	2	8%
Response to treatment:		
Responders	18	72%
Non-responders	7	28%

As for IL-1B-31 genotypes and alleles distribution between the studied groups, there was statistically significant relation between ITP and IL-1B-31 genotypes and alleles. Mutant TT genotype of IL-1B-31 was higher in ITP patients (44%) contrasted to healthy control (4%) (p=0.002) and Mutant TT genotype significantly increases ITP's risk via 18.62 folds and T allele significantly increases risk of ITP by 9.8 folds. (Table 2)

Table (2): Comparison between the studied groups as per IL-1B-31 genotypes and alleles.

	Patients group	**Control group**	χ^2	**p**
	N=25 (%)	**N=25(%)**		
IL-1B-31: CC **(wild-type)** CT **(hetero)** TT **(mutant)**	 13 (52%) 1 (4%) 11 (44%)	 22 (88%) 2 (8%) 1 (4%)	9.714	0.002*
COR for TT (95% CI)	18.62(2.15 – 161.24)			
COR for TC (95% CI)	0.85(0.07 – 10.27)			
Alleles: C T	 27 (54%) 23 (46%)	 46 (92%) 4 (8%)	Fisher	<0.001**
COR for T (95% CI)	9.8(3.07 – 31.35)			

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

Regarding IL-1B-511 genotypes and alleles distribution between the studied groups, there was statistically significant relation between ITP and IL-1B-511 genotypes and alleles. Mutant CC genotype of IL-1B-511 was greater in ITP patiens (36%) contrasted to healthy control (4%) (p=0.015). Mutant CC genotype significantly increases risk of ITP by 3.5 folds and C allele significantly elevates risk of ITP by 5.52 folds. (Table 3)

Table (3): Comparison between the studied groups as per IL-1B-511 genotypes and alleles.

	Patients group	**Control group**	**χ^2**	**p**
	N=25 (%)	**N=25(%)**		
IL-1B-511: TT **(wild-type)** TC **(hetero)** CC **(mutant)**	 15 (60%) 1 (4%) 9 (36%)	 21 (84%) 3 (12%) 1 (4%)	5.194	0.015*
COR for CC (95% CI)	3.5(0.92 – 13.31)			
COR for TC (95% CI)	0.47(0.04 – 4.93)			
Alleles: T C	 31 (62%) 19 (38%)	 45 (90%) 5 (10%)	10.746	0.001**
COR for C	5.52 (1.86 – 16.34)			

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

There was statistically significant relation between ITP and IL-1Ra genotypes and alleles. I/II (hetero) genotype of IL-1Ra was higher in ITP patients (44%) contrasted to healthy control (12%) (p=0.025). I/II hetero genotype significantly elevates risk of ITP by 5.76 folds and II allele significantly elevates risk of ITP by 4.42 folds. (Table 4)

Table (4): Comparison between the studied groups as per IL-1Ra genotypes and alleles.

	Patients group	**Control group**	**χ^2**	**p**
	N=25 (%)	**N=25(%)**		
IL-IRa: **I/I (wild)** **I/II (hetero)**	 14 (56%) 11 (44%)	 22 (88%) 3 (12%)	Fisher	0.025*
COR for I/II (95% CI)	5.76 (1.36 – 24.36)*			
IL-IRa alleles **I** **II**	 39 (78%) 11 (22%)	 47 (94%) 3 (6%)	Fisher	0.041*
COR for II (95% CI)	4.42(1.15 – 16.97)*			

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

Multivariate analysis of factors correlated with ITP, IL-1B-31 CT, TT, IL-1B-511 CC, and IL-1Ra (I/II) independently increase risk of ITP by 1.414, 12.045, 5.881 and 3.682 folds consecutively. (Table 5)

Table (5): Multivariate analysis of factors correlated with ITP.

	β	p	AOR	95% C.I.	
				Lower	Upper
IL-1B-31 (CC)		0.101			
IL-1B-31 (CT)	0.346	0.792	1.414	0.107	18.620
IL-1B-31 (TT)	2.489	0.032*	12.045	1.232	117.790
IL-1B-511 (TT)		0.341			
IL-1B-511 (CC)	1.772	0.146	5.881	0.541	63.991
IL-1B-511 (TC)	-0.059	0.962	0.943	0.081	10.929
IL-1Ra(I/II)	1.303	0.131	3.682	0.679	19.957

AOR adjusted odds ratio CI Confidence interval *p<0.05 is statistically significant **p≤0.001 is statistically highly significant

Regarding the relation between IL-1B-511, IL-1B-31, IL-1Ra polymorphism and severity of ITP is described in Table 6 as following, Among IL-1B-31, mutant TT genotype significantly increases risk of non-severe ITP compared to control group by 25.1 folds, and T allele significantly increases risk of severe ITP compared to control group by 5.75 folds and it significantly increases risk of non-severe ITP versus control group by 13.03 folds.

Among IL-1B-511, mutant CC genotype significantly increases risk of non- severe and severe ITP compared to control group by 12.6 folds, and C allele significantly increases risk of severe ITP compared to control group by 5.7 folds and it significantly increases risk of non-severe ITP compared to control group by 5.4 folds.

Among IL-1Ra, I/II hetero genotype significantly increases risk of non-severe ITP compared to control group by 9.4 folds and II genotype significantly increases risk non-severe ITP compared to control group by 6.1 folds but non significantly increases risk of severe ITP compared to control group.

Table (6): Relation between IL-1B-511, IL-1B-31, IL-1Ra polymorphism on severe, non-severe ITP and control subjects.

	Severe	Non-severe	control	Severe and control		Non-severe and control	
	N=9(%)	N=16(%)	N=25(%)	COR (95% CI)	p	COR (95% CI)	p
IL-1B-31:							
CC **(wild)**	6(66.7)	7(43.8)	22(88)	1(reference)		1(reference)	
CT **(hetero)**	0(0)	1(6.3)	2(8)	0	>0.999	1.57(0.12-20.1)	>0.999
TT **(mutant)**	3(33.3)	8(50)	1(4)	1.38(0.27–6.48)	0.693	25.1(2.7-236.7)	<0.001**
Alleles:							
C	12(66.7)	15(46.9)	46(92)				
T	6(33.3)	17(53.1)	4(8)	5.75(1.4–23.68)	0.017*	13.03(3.8-44.8)	<0.001**
IL-1B-511:							
TT	5(55.6)	10(62.5)	21(84)	1(reference)		1 (reference)	
TC	1(11.1)	0(0)	3(12)	1.4(0.12 – 16.5)	>0.999	0	0.539
CC	3(33.3)	6(37.5)	1(4)	12.6(1.1 – 148)	0.047*	12.6(1.3–119.2)	0.028*
Alleles:							
T	11(61.1)	20(62.5)	45(90)	1 (reference)		1(reference)	
C	7(38.9)	12(37.5)	5(10)	5.7(1.52 – 21.5)	0.006*	5.4(1.7 – 17.4)	0.005*
IL-1Ra:							
I/I (wild)	7(77.8)	7(43.8)	22(88)	1(reference)		1(reference)	
I/II (hetero)	2(22.2)	9(56.3)	3(12)	2.1(0.3 – 15.2)	0.591	9.4(2 – 44.83)	0.004*

IL-1Ra allele							
I	16(88.9)	23(71.9)	47(94)	1(reference)		1(reference)	
II	2(11.1)	9 (28.1)	3(6)	1.96(0.3 – 12.8)	0.602	6.1(1.5 – 24.8)	0.009*

COR crude odds ratio CI Confidence interval χ2Chi square test *p<0.05 is statistically significant **p≤0.001 is statistically highly significant

There is statistically significant relation between response to treatment and IL-1B-31 genotypes and alleles among ITP patients. Wild CC genotype and C allele significantly decreases risk of non-response to treatment, while there was no statistically significant relation between response to treatment and IL-1B-511 genotypes and alleles. Mutant CC and TC genotype and C allele non-significantly decreases risk of non-response to treatment. Also, there was no statistically significant relation between response to treatment and IL-1Ra genotypes and alleles. I/II hetero genotype non-significantly decreases risk of non-response. (Table 7)

Table (7): Relation between response to treatment and IL-1B-31, IL-1B-511 and IL-1Ra genotypes and alleles among ITP patients

	Non-responders	**Responders**	χ^2	**p**
	N=7 (%)	**N=18 (%)**		
IL-1B-31:				
CC **(wild-type)**	1 (14.3%)	12 (66.7%)	4.153	0.041*
CT **(hetero)**	1 (14.3%)	0 (0%)		
TT **(mutant)**	5 (71.4%%)	6 (33.3%)		
COR for CC (95% CI)	0.1(0.01 – 1.06)			
COR for TC (95% CI)	∞			
Alleles:				
C	3 (21.4%)	24 (66.7%)	Fisher	0.005*
T	11 (78.6%)	12 (33.3%)		
COR for C (95% CI)	0.14(0.03 – 0.58)			
IL-1B-511:				
TT **(wild-type)**	5 (71.4%)	10 (55.6%)	0.368	0.544
TC **(hetero)**	0 (0%)	1 (5.6%)		
CC **(mutant)**	2 (28.6%)	7 (38.9%)		
COR for CC (95% CI)	0.57(0.09 – 3.83)			
COR for TC (95% CI)	0			
Alleles:				
T	10 (71.4%)	21 (58.3%)	Fisher	0.521
C	4 (28.6%)	15 (41.7%)		
COR for C (95% CI)	0.56 (0.15 – 2.13)			
IL-1Ra:				
I/I (wild)	5 (71.4%)	9 (50%)	Fisher	0.407
I/II (hetero)	2 (28.6%)	9 (50%)		
COR for I/II (95% CI)	0.4 (0.06 – 2.63)			

IL-IR a alleles **I** **II**	12 (85.7%) 2 (14.3%)	27 (75%) 9 (25%)	Fisher	0.705
COR for II (95% CI)	2 (0.37 – 10.96)			

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

4. Discussion

Immune thrombocytopenia (ITP) is an autoimmune disease in which there are antibodies and cell-mediated inhibition of platelet formation and destruction, which raises the risk of bleeding**. (11)**

Childhood chronic ITP is caused by polymorphisms in inflammatory cytokine genes, such as IL-1B and IL-1Ra polymorphisms, as the IL-1 family has a distinct functional role in autoimmune disorders and ITP. **(6)**

Recent research has demonstrated that proinflammatory cytokine polymorphism play a significant role in the development probability and severity of ITP. **(12)** Therefore, it might be valuable to investigate the association between IL-1B and IL-1Ra receptor antagonist gene polymorphism and development of ITP and its impact on its prognosis and severity.

A larger percentage of ITP patients (44%) than healthy controls (4%) had the mutant TT genotype of IL-1B-31, according to the current study's analysis of the distribution of this polymorphism among ITP patients and healthy controls (p=0.002). Additionally, there is a statistically significant correlation between ITP and IL-1B-31 alleles and genotypes, with the mutant TT genotype significantly raising ITP risk by 18.62 times and the T allele considerably raising ITP risk by 9.8 fold.

In line with the current finding, **Yadav et al. 2017** demonstrated that the proportions of both heterozygous and homozygous variant IL-1B-31 genotypes were higher among patients (OR=2.33, P =0.034), and they had a significant correlation with ITP susceptibility, with a 1.52-fold extreme probability of susceptibility to ITP (OR=1.52, P =0.034). **(6)**

The T allele and C allele incidence between ITP patients and the control group (75.8% versus 83.3% and 24.2% versus 16.7%, respectively; p = 0.18), and **Karimov et al. 2021** findings that there was no statistically significant correlation between IL-1B gene polymorphism and the risk for the development of ITP, were in conflict with the current findings. These variations in the results could be attributed to variations in the population under study. **(12)**

Regarding the IL-1B-511 polymorphism's distribution among patients and healthy controls, ITP patients had a greater rate of the mutant CC genotype of IL-1B-511 (36%) than healthy controls (4%; p=0.015). Additionally, there is a statistically significant correlation between ITP and IL-1B-511 genotypes and alleles, with the mutant CC genotype considerably raising ITP risk by 3.5 times and the C allele significantly raising ITP risk by 5.52 folds.

In contrary to the current result **Yadav et al. 2017** demonstrated that IL-1B-511 was not correlated with ITP. **(6)**

The current findings also contradicted **Pavkovic et al. 2015** findings, which claimed that there were no appreciable variations in genotype distribution and IL-1B-511C/T allele frequencies between ITP patients and controls (p=0.845). These variations in outcomes might be related to variations in the adult population under study. **(13)**

Concerning the IL-1Ra Polymorphism's distribution among the cases and healthy controls, I/II (hetero) genotype of IL-1Ra was higher in ITP patients (44%) contrast to healthy control (12%) (p=0.025). There is

statistically significant relation between ITP and IL-1Ra genotypes, as I/II hetero genotype significantly increases risk of ITP by 5.76 folds and II allele significantly increases risk of ITP by 4.42 folds.

The existing findings were in line with **Pesmatzoglou et al.2012** who found a link between IL-1 Ra polymorphism and juvenile ITP. Compared to controls, children with ITP more frequently had the genotype I/II, and having allele II appears to raise the chance of developing ITP by 2.12 times. **(14)**

Yadav et al. 2017 findings showed that having the IL-1Ra allele-II was linked to a 1.75-fold higher chance of getting ITP supported the current findings. ITP was associated with both the homozygous and heterozygous variant genotypes of IL-1Ra allele-II. **(6)**

The existing outcomes were in line with those of **Mokhtar et al. 2016**, who found that mutant I/II IL-1Ra was more common in almost half of ITP cases than in the control group ($p = 0.039$). Additionally, ITP cases considerably higher than the controls in terms of the frequency of the IL-1Ra II allele ($p < 0.05$). **(15)**

The current findings, in contrast, did not support the findings of **Rasheed and Eissa, 2021**, who discovered that the IL-1Ra II variation had a significant relationship with control groups (P = 0:011) while the IL-1Ra III variant was strongly related with cases (P = −0:0163). **(9)**

Concerning the relation between IL-1B-511, IL-1B-31, and IL-1Ra genotypes and severity of ITP, the present study showed that T allele of IL-1B-31genotype significantly elevates risk of severe ITP compared to control group by 5.75 folds and mutant CC genotypes of IL-1B-511 significantly elevates risk of severe ITP compared to control group by 12.6 folds, and C allele significantly increases risk of severe ITP compared to control group by 5.7 folds. IL-1Ra, I/II hetero genotype significantly increases risk of non-severe ITP compared to control group by 9.4 folds and II genotype significantly increases risk non-severe ITP compared to control group by 6.1 folds but non significantly increases risk of severe ITP compared to control group

The current results were consistent with **Yadav et al. 2017** who found a substantial risk relationship between severe ITP and the variant allele of IL-B-31 (OR=1.64, P=0.039). Contrary to our findings, the IL-1B-511 genotype has no connection with either severe or non-severe ITP, and there is a substantial variation in the IL-1Ra genotypes' distribution between individuals with severe ITP and healthy controls. The homozygous variation showed a significant correlation (OR=5.83, P =0.014). Additionally, a substantial risk factor for severe ITP was demonstrated by the mutant IL-1Ra allele (II) (OR=1.9, P =0.0085). **(6)**

Concerning the relation between IL-1B-31, IL-1B-511 and IL-1Ra genotypes and response of treatment, there is statistically significant relation between response to medication and IL-1B-31 genotypes and alleles among ITP cases as wild CC genotype and C allele significantly decreases risk of non-response to treatment (p=0.005). While there was no statistically significant relation between response to treatment and IL-1B-511 and IL-1Ra.

These findings support **Yadav et al. 2017** who demonstrated that polymorphisms in IL-1B-511, and IL-1Ra exhibited no correlation with the response to steroid in ITP cases while in contrary to the present results they found also that IL-1B-31 genotypes Polymorphisms displayed no association with steroid response in ITP patients. **(6)**

5. Conclusion

IL-1B and IL-1RA polymorphisms may play an important role as a genetic risk factor for the development and progression of childhood ITP and IL-1B-31 and IL-1B-511 polymorphisms could be used as a genetic predictor to determine the degree of severity of ITP. IL-1B-31 polymorphism could be used as genetic indicator to detect the response to treatment in ITP pediatric patients.

6. The Limitations of the Study

The single-center sample gathering and small number of participants were the study's limitations; therefore, future research in multicenter settings with larger patient populations is advised to verify the present results.

Author Contributions: N.A.K. select the research idea, planned the practical experiments, and revised the findings; M.S.A., G.M.E and E.M.A. gathered, examined, and interpreted the patient data, including practical assessments; L.M.S. gathered and prepared the patients for the study. The manuscript's published form was accepted by all authors after they had read it.

Funding: no external funding for this study.

Institutional Review Board: Ethical approval for this study provided by The Zagazig University Institutional Review Board (ZU-IRB) (Approval no.4573/6-5-2018).

Informed Consent: All participants and their parents provided written informed consent before having their clinical data and specimens used in the study. This paper was then published in compliance with the Helsinki Declaration.

Data Availability: The relevant author may request access to the data at any time.

Conflicts of Interest: It is stated by the authors that they have no conflicts of interest.

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