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ORIGINAL ARTICLE

The Frequency of Fcγ Receptor IIa Gene Polymorphism in Children with Chronic Immune Thrombocytopenic Purpura

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ABSTRACT

Background: In childhood, immune thrombocytopenia (ITP), anti-platelet autoantibodies mediate platelet clearance through Fc-γ receptor (FcγR)–bearing phagocytes. The current study aimed to assess the frequency of FcγRIIa gene polymorphism in Egyptian children with chronic ITP and its relation to the disease process and chronicity compared to controls.

Methods: The current case-control retrospective study was conducted in the Hematology unit of the Pediatric Department, at Zagazig University Children's Hospital, on 40 chronic ITP pediatric patients and 40 matched age and sex-healthy controls, during the period from January 2018 until January 2019. All patients were subjected to detailed full history taking, complete clinical examinations, and routine laboratory investigations for ITP. Genotyping for FcγRIIa was performed by using PCR-RFLP methods.

Results: The results revealed that mutant heterozygous (HR) were found to be significantly higher among ITP patients (57.5%) compared to the control group (10%) P<0.001. The frequency of the FcγRIIa-131 (R) allele in children with ITP was significantly higher compared with control children (48.7% versus 25%; P = 0.001).

Conclusions: The significant association between FcγRIIa-131 (HR) mutant heterozygous gene polymorphism and chronic immune thrombocytopenic purpura may indicate its possible role in disease susceptibility and the development of chronicity.

Keywords: Immune thrombocytopenia; FcγRIIa polymorphisms; PCR-RFLP.

INTRODUCTION

Immune thrombocytopenia (ITP) is defined as a persistent or transient reduction of platelets' number below 100 × 10^9/liter, due to platelets destruction by auto-antibodies or impaired production. According to duration, ITP is classified into acute ITP (disease duration < 3 months), persistent ITP (disease lasting between 3 month and 12 months duration), and chronic ITP (disease duration >1 year) [1].

FcγRIIa is one of the FcRs family located on chromosome 1. Polymorphic variants of FcγRIIa have a high affinity for distinct immune-globulin G, resulting in highly variable destruction of immune complexes [2].

It contains functional single-nucleotide polymorphism (SNP), which alters the binding affinities of these receptors for the γ chain of the Fc fragment of immunoglobulin G. FcγRIIa polymorphism is one of the most important genetic polymorphisms that is closely associated with ITP [3]. Platelets with associated IgG (immune-globulins against platelet membrane glycoproteins GPIIb/IIIa and GPIb/IX) are targeted for destruction by the binding of the Fc portion of IgG to platelet-specific membrane leading to its phagocytosis by reticuloendothelial cells, mainly macrophages, which express receptors of the Fc portion (FcRs) [4].

This study aimed to assess the association of FcγRIIa gene polymorphism in pediatric patients with chronic ITP and its relation to disease process and chronicity compared to healthy controls.

METHODS

A retrospective case-control study was conducted on 40 children diagnosed with chronic ITP and 40 age and sex-matched healthy subjects as a control recruited from the outpatient hematology clinic, Zagazig University Children's Hospital during a period from January 2018 until January 2019.

Inclusion criteria: patients with chronic ITP (disease duration more than 1 year from the start)

of both sex, age> 1 year-18 years, and those who approved to share in the study were included in this work.

Exclusion criteria: those with acute ITP (disease duration less than 3 months since diagnosis), persistent ITP (disease duration from 3 to 12 months since diagnosis), secondary ITP, age below 1 year, and those who refused to share in the study were excluded from this work.

Patients were subjected to full history taking, along with complete physical examination including "site and shape of bleeding; petechiae, bruises, ecchymosis, epistaxis, etc." and routine laboratory investigations including complete blood count with manual platelet count, and C3, ANA, anti-DNase to exclude secondary causes, along with bone marrow examination.

Genotyping of FcγRIIa gene: both cases and controls were subjected to FcγRIIa genotyping performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

DNA extraction and storage: blood samples of 5 ml of peripheral venous blood, were obtained from each participant under complete aseptic condition and were collected in sterile K ethylene di amine tetra acetic acid (EDTA K2 tube) and stored at -20 °C. All the reagents were highly purified analytical PCR materials. All the tip pipettes, and tubes used for DNA extraction were RNAse, DNAse-free tubes to bypass contamination obtained from Thermo Fisher Scientific (Ballocs. UAB).

Determination of FcγRIIa gene polymorphisms was done by using the polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) method. Genomic DNA of pediatric patients and controls was extracted from whole blood and processed by an established protocol for DNA extraction from blood samples by using a DNA extraction kit. (Thermo Scientific™ GeneJET™ DNA PURIF KIT, Ballics, UAB, catalog number K0721). (Thermo Scientific™ biotechnology, Seongnam-Si, Korea)

The extracted DNA was amplified by the polymerase chain reaction (PCR) using the recombinant 2X Taq PCR master mix (Dream taq™ green pc, code number k1081, LOT: 00643300)

The restriction enzyme BSH 12361 restriction endonuclease (Thermo fissure – Lithuania).

PCR analysis of FcγRIIa polymorphisms and its primer were performed by PCR-restriction fragment length polymorphism technique. as previously described in Koen et al. [5] (Table 1).

The PCR product, which is 337 bp, will be cleaved into two products 316, and 21 bp.

The FcγRIIa (H) allele was visualized as a 337 bp fragment, whereas the R alleles produced two fragments of 316 and 21 bp. The 21 bp product of the (R) allele is not seen on the gel and only the 316 bp products are detected. Therefore, in individuals lacking this polymorphism, the cleavage will not occur and a 337 bp band is detected.

If only one band 337(bp) is detected it is considered a normal FcγRIIa gene (wild type) (HH genotype).

If only one band (316 bp) is detected it is considered a homozygous mutation of the gene (RR genotype).

If the two bands (337 bp) and (316 bp) are present it is considered a heterozygous mutation of the gene (HR heterotype).

Ethical clearance: written informed consent was obtained from all participants, and the study was approved by the research ethics committee of the Faculty of Medicine, Zagazig University. The study was done according to the code of ethics of the world medical association (Declaration of Helsinki) for studies involving humans. Approval was taken from the institutional review board, Faculty of Medicine, Zagazig University.

STATISTICAL ANALYSIS

All data were collected, tabulated, and statistically analyzed using SPSS version 19. Continuous Quantitative variables were expressed as the mean ±SD and median (range), and categorical qualitative variables were expressed as absolute frequencies (number) and relative frequencies (percentage). The Mann-Whitney test was used to compare two groups of non-normally distributed data. Independent student's t-test was used to compare two groups of normally distributed data. Categorical data were compared using the Chi-square and single Chi-square goodness of fit tests. The odds ratio was used for risk quantification. The tests were two-sided with a p-value < 0.05 considered statistically significant (S), a p-value < 0.001 considered highly statistically significant (HS), and a p-value ≥ 0.05 considered statistically insignificant (NS).

RESULTS

There was no significant difference between the studied groups regarding demographic data as presented in Table 2. Regarding the clinical presentation of the studied group, Table 3 shows that ecchymosis and purpura were the commonest presenting symptoms. Also, Tables 4 and 5 illustrate the different treatment lines and treatment outcomes among the studied group.

Regarding laboratory data among the studied groups, there was a non-statistically significant difference between the studied groups regarding hemoglobin; however, the initial platelets counts were found to be significantly lower in ITP patients when compared to the control group (17.5 versus 316.5 respectively) as presented in Table 6.

Furthermore, it was concluded that the frequency of FcγRIIa (HR genotypes was significantly higher among ITP patients (57.5%) compared to the control group (10%), *P<0.001*

Also, the frequency of (The R allele) was significantly higher among ITP (48.7%) patients when compared to the control group (25%), with *P<0.001* (Table 7).

Lastly, Table 8 shows the relation between genotype polymorphism of the FcγRIIa and clinical data among the studied group.

Table 1: Primer and restriction enzymes for PCR-RFLP. primers from (Thermo Fissure– Lithuania).

Gene	SNP	Primers	Restriction enzyme
FcγRIIa	131 H/R	5′GGAAAATCCCAGAAATTCTC GC3′	BstUI
	rs1801274	5′CAACAGCCTGACTACCTATTA CGCGGG3′	

Table 2: Comparison of demographic data among the studied groups.

Variable	ITP group (N=40)		Control group (N=40)		Test	P value
Age: (years) **Mean ± SD** **Range**	7.98 ± 2.3 3 - 18		6.80 ± 1.4 2 - 12		2.772	0.007 (NS)
	N	%	N	%	χ2	P
Sex: **Female** **Male**	16 24	40 60	24 16	60 40	3.200	0.074 (NS)

ITP= Immune thrombocytopenic purpura; N=Number; S=significant difference (p<0.05); NS= non-significant difference (p>0.05); #= Mann-Whitney test.

Table 3: Clinical data of the ITP studied group.

Variable	Studied group (N=40)	
Age of presentation (years) ***Mean ± SD*** ***Range***	5.51 ± 4.17 1 - 15	
	N	**%**
Initial clinical presentation: **Skin bleeding** **Ecchymosis** **Purpura** **Hematoma** **Muco-cutaneous bleeding** **Bleeding per gum** **Orifice's bleeding** **Bleeding per mouth** **Bleeding per nose** **Bleeding per vagina** **Bleeding with circumcision**	14 10 1 2 8 4 1	35 25 2.5 5 20 10 2.5
Associations: **HSM** **Splenomegaly** **Hepatomegaly** **Elevated liver enzymes** **H.pylori**	11 1 5 2 20	27.5 2.5 12.5 5 50

Table 4: Treatment received by the ITP studied group.

Variable	Studied group (N=40)	
	N	%
Received treatment:		
Corticosteroids	24	60
Combined (steroid and IVIG)	7	17.5
Thrombopoietin receptor agonist	6	15
IVIG	2	5
Splenectomy	1	2.5

Table 5: Outcome of the ITP studied group .

Variable	Studied group (N=40)	
	N	%
Outcome		
Complete response (platelets >100,000 mcl)	0	0
Partial response ((30 - 100,000/mcl)	32	80
No response (<30.000 mcl)	8	20

Table 6: Comparison of laboratory data among the studied groups.

Variable	ITP group (N=40)	Control group (N=40)	Test	P-value
Initial platelets:				
Mean ± SD	21.98 ± 15.7	331.4 ± 94.6	-7.704#	<0.001
Median	17.5	316.5		(HS)
Range	5 - 80	199 - 501		
Hemoglobin:				
Mean ± SD	10.6 ± 2.07	10.3 ± 1.05	0.733	0.466
Range	4.3 - 16	9 – 12.2		(NS)

HS= highly significant difference (p<0.001). NS= non-significant difference (p>0.05).

Table 7: Frequency distribution of genotype polymorphism among the studied groups.

Variable	ITP group (N=40)		Control group (N=40)		OR (95%CI)	P-value
	N	%	N	%		
Allele frequency:						
H allele	41	51.3	60	75	2.85 (1.461- 5.57)	0.001
R allele	39	48.7	20	25	0.36 (0.192-0.706)	(S)
FcγRIIa:						
HH genotype	9	22.5	28	70	Reference	<0.001
HR genotype	23	57.5	4	10	17.6 (4.87-65.6)	0.108
RR genotype	8	20	8	20	3.11 (0.905-10.6)	

HS=highly significant difference (p<0.001); S=significant difference (p<0.05).

Table 8: Relationship between genotype polymorphism (FcγRIIa) and clinical data among the studied groups.

Variable	HH (N=9)		HR (N=23)		RR (N=8)		Test	P-value
	No	%	No	%	No	%		
Initial clinical picture:								
Ecchymosis	4	44.4	7	30.4	3	37.5		
Purpura	2	22.2	6	26.1	2	25		
Hematoma	0	0	0	0	1	12.5		
Gum Bleeding	0	0	2	8.7	0	0		0.892

Variable	HH (N=9)		HR (N=23)		RR (N=8)		Test	P-value
	No	%	No	%	No	%		
Bleeding per nose	2	22.2	5	21.7	1	12.5	9.492	(NS)
Bleeding per vagina	1	11.1	2	8.7	0	0		
Bleeding with circumcision	0	0	1	4.3	0	0		
Outcome:								
No response: <30,000/ mcl	4	44.4	3	13	1	12.5	4.338	0.114 (NS)
Partial response: (30,000 - 100,000/mcl)	5	55.6	20	87	7	87.5		
Date of presentation:								
Mean ± SD	6.4 ± 4.70		5.7 ± 4.23		3.8 ±3.26		2.379	0.304 (NS)
Median	5.5		4.8		2.2			
Range	1.5-14		1 - 15		1 - 9			

NS: non-significant difference (p>0.05).

DISCUSSION

The sequencing of the human genome has allowed the identification of thousands of gene polymorphisms, most often single nucleotide polymorphisms (SNP) which may play an important role in the expression level and activity of the corresponding [5].

In ITP, FcγRIIa plays an important role in the phagocytosis of auto antibody-coated platelets [4]. This study was designed to evaluate the frequency of FcγRIIa gene polymorphism chronic immune thrombocytopenic purpura in children and their association with its clinical features.

We conducted a case-control study on 40 patients with chronic ITP; 24 males and 16 females and 40 age and sex-healthy-matched children as a control group. The mean age of our patient was 7.98 ± 2.3 years while it was 6.80 ± 1.4 in the control group with no statistically significant difference, P=0.007. Also, we did not find a statistically significant difference between cases and control regarding sex.

We found higher male predominance in our cases,P value =0.074. On the contrary Carcao et al. in their study on children with chronic ITP found higher female predominance (46 males and 52 females) [7].On the contrary, Papagiannia et al. in their study on chronic ITP patients reported younger age in their study than ours. The mean age of the studied group was 5.9±3.9 years, with higher female predominance (26 males and 27 females) in their study [8].

Also, Amorim et al. in their study on children with chronic ITP reported younger age of 5.39±4 than that reported in our study, with higher female predominance (15 males and 18 females) [9].

In our study, we found that the mean initial platelets count was 21.98 ± 15.7 x10^3/Ul in patients with chronic ITP versus 331.4 ± 94.6 x10^3/Ul in the control group with highly statically significant difference, P <0.001. Similarly, Zoheir et al. in their study on chronic ITP children found that the mean initial platelets count was 19.444.4±12.269.0 in the chronic ITP group versus 336.400.0±52.749.3 in controls, which is highly statistically significant, P <0.001 [10].

Opposite to our findings, Eyadaa et al. revealed that there was no statistical difference was observed between ITP patients harboring the normal or mutant alleles [6].

FC Gamma Receptor 2A (FcγRIIa) is the only FcγR expressed on platelets. Its polymorphism classified into (HH) homo-type (wild type) and mutant alleles (HR hetero-type, (RR) homo-type) with high affinity to human IgG [11].

In our study, we found that the FcγRIIa-131(H > R) polymorphism was more frequently detected in our chronic ITP patients compared to controls with a statistically significant difference of P=0.001.

On the contrary Amorim et al. reported that the genotype distributions and allelic frequency of the FcγRIIa-131 (R>H) gene were similar among chronic ITP patients and control, with no significant difference between them (p= 0.77) [9].

Our study showed that the (HR) genotype was found in 57.5% of ITP patients compared to the control group, which had 10% of the (HR) genotype with a highly statistically significant difference between genotypes among chronic ITP patients compared with controls, with P-value (<0.001). Also, Carcao et al. noted in their study conducted on 71 Canadian children with chronic

ITP that the genotypic frequency of FcγRIIa -131 (HR) was 43%. While the genotypic frequency among the controls was 53% (HR). They revealed that the frequency of the (HR) genotype of chronic ITP patients differed significantly from the control group (P-value=0.03). [7]

Close to our findings, Eyadaa et al. in their study conducted on 92 ITP Egyptian children, 80 of them were chronic, revealed that FcγRIIa (HR) genotype was found in 8.6% of ITP cases, whereas it was not detected in the controls [6].

In contrast to our results, Papagianni et al. in their study conducted on Greek ITP children found that there was no statistically significant difference between the two groups (patients and controls) regarding the FcγRIIa-131(HR) genotype, P=0.873 [8]. On the contrary, Pavkovic et al. in their study on adult ITP patients found no statically significant difference in genotype distribution and allele frequencies for FcγRIIa between cases and control [11]. This discrepancy between our data and all previously mentioned studies could be explained by the fact that these allelic variants have a random distribution with wide variation among ethnic groups and are also attributed to different sample sizes. Limitations: the small sample size and insufficient duration of the study could be one of the reasons of this discrepancy between our study and other previous studies and research among FcγRIIa genotyping.

CONCLUSIONS

We can conclude that a significant association of FcγRIIa-131 (HR) gene polymorphism with chronic immune thrombocytopenic purpura in children may indicate its possible role in disease susceptibility, genetic risk factor, pathogenesis, and development of chronicity

Recommendations: We recommend using a larger sample size in future studies for better results. Large-scale studies are still needed to support our results and to detect possible association of FcγRIIa gene polymorphism with the disease outcome.

Conflict of interest The authors report no conflicts of interest. The authors are responsible for the content and writing of the paper.

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