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Impact of ABCB1 gene polymorphisms on therapeutic efficacy of oral prednisolone in Egyptian ITP patients

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Abstract— **Background:**Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by heterogeneous bleeding manifestations as well as marked-inter individual variation in response to glucocorticoid (GCs); the 1st line ITP treatment. This may be due to Pgp protein; the product of ATP binding cassette B1(ABCB1) gene. It is involved in effluxing drugs as steroids outside the intracellular space thus decreasing its intracellular concentration. **Methods:** The study investigated the relation between single nucleotide polymorphism (SNPs) in ABCB1gene and the variable response to oral prednisolone treatment in 100 Egyptian ITP patients. Clinical response was measured during 1st 28 days and compared with C3435T and G2677T SNPs determined by PCR REPLF technique.**Results:** No significant difference in C3435T between ITP patients and control. Carriers of mutant allele of G2677T were significantly higher in ITP patients compared with the control group *(p value :02)* suggesting possible role in disease predisposition.

Conclusion— There was no significant relation between these two SNPs and age of onset of the disease , initial platelet count, response to steroid therapy.

Keywords— ITP, ABCB1, Glucocorticoids, polymorphisms

Introduction

Immune thrombocytopenia (ITP) is an autoimmune disorder caused by autoantibody and cell-mediated increased platelet destruction and/or decreased platelet production ***(1)***. It is manifested clinically by heterogeneous bleeding manifestations ranging from petechiae, ecchymoses, mucosal bleeding as vaginal, GIT bleeding, etc to serious rare intracranial Hge***(2).*** There is a great possibility that certain genetic factors have a pivotal role in the pathogenesis of ITP as disease susceptibility, and variable inter individual response to GCs therapy (3 ***)*** . Glucocorticoids remain as the mainstay and first-line therapy used worldwide in the treatment of ITP ***(4).*** Eighty to ninety percent of patients have an adequate response to GCs ,however, still minority of patients remain unresponsive. While treating patients with GCs some patients necessitate keeping them on high doses, repeated courses of GCs to keep a safe platelet count without bleeding, those patients defined as steroid dependent, and this making them more liable for steroid

toxicity ***(5)***. This inter-individual variation to steroid therapy may be due to group of transport proteins one of them is Pgp –efflux protein encoded by ABCB1 gene. Functional P-gp is highly expressed in several hematological cells as natural killer (NK) cells, CD4and CD8lymphocytes these cells have an important role in the pathogenesis of ITP (6 ***).***This protein is involved in pharmacokinetics; absorption, distribution, and disposition of drugs by pumping effluxing them from the intracellular space into the intestinal lumen decreasing drug concentration intracellular (7***)***.Glucocorticoids as substrates of Pgp.ABCB-1 expression in lymphocytes has been reported to be negatively correlated with the response to prednisone. also certain single-nucleotide polymorphisms (SNPs) in the ABCB-1 gene are associated with altered drug disposition(8***)***.more than 50 single nucleotide polymorphism (SNPs) were identified for ABCB1 three of them (C1236T, C3435T and G2677T/A) were frequently studied . Frequency of these polymorphisms and its effect vary widely among different ethnic populations, indicating that ethnicity has a major impact on genetic distribution and effect (9). The aim of this study was to clarify the relation of SNP C3435T and G2677T/A in Egyptian patients with ITP and predisposition to disease , response to oral GCs, and disease course.

Patients and method

The study was reviewed and approved by the local ethics committee and was conducted in accordance with the Helsinki Declaration. Informed consent was obtained from all participants.This was an observational study conducted at hematology clinic, inpatient department of EL- Minia and El Kasr El Einy university hospital at the period Dec 2015 to Dec 2017. The ***study involved 2 groups ,group 1 which involved*** 100 ITP patients and **group 2** which involved 100 age and sex matched healthy volunteers. written informed consent was taken from both groups.Patients were diagnosed as ITP according to The American Society of Hematology (ASH) 2011 (10) as follow**:** Isolated thrombocytopenia (platelet count <100 x 10^9/L.),normal red cell indices, white blood cell count, and platelet morphology and no other causes of thrombocytopenia . the study exclude patients younger than 18 years old, Pregnant females ,Patient with thrombocytopenia due to other cause (HCV, HIV, chronic liver disease, others autoimmune disease, SLE, anti-phospholipid syndrome, drug induced thrombocytopenia as quinine and heparin induced thrombocytopenia and Lymphoproliferative disease)

All groups s were exposed to t**horough history taking and Complete physical examination, the Laboratory work up as follow:**

1- Complete blood count
2- Work up to exclude secondary causes of thromboytopenia
   a. Peripheral blood smear: to evaluate the size and morphology of platelets, RBCs, and WBCs
   b. ANA, anti-ds DNA titer
   c. Anti-phospholipid Ab. , Anti-cardiolipinAb (IgG, IgM)
   d. HCV Abs., HIV Abs by ELISA
   e. H pylori Ag in stool

D- Imaging:

1- Abdominal US: for assessment of liver, spleen, and abdominal LNs.

E- Treatment plane: All patients have received glucocorticoids 1mg/ kg per day .After the 28-day of continuous GCs treatment , Patients were assessed for response. According to GCs response , patients were grouped into 3 groups ***(5):***

1- The GCs responsive: A platelet count more than 30 000/dL or greater than 2-fold increase in platelet count from baseline measured on 2 occasions 7 days apart and the absence of bleeding. The quality of response is further divided into

- **Partial response**: platelet count > 30000 /L and at least 2-fold increase the baseline with cessation bleeding
- **Complete response**: A platelet count >100 000/L measured on 2 occasions 7 days apart and the absence of bleeding

2- ***The GC non responive group:*** platelet count less than 30000 /L or a less than 2-fold increase in platelet count from baseline or the presence of bleeding. Platelet count must be measured on 2 occasions more than a day apart

3- ***The GC dependent group;*** The need for ongoing or repeated doses administration of corticosteroids for at least 2 months to maintain a platelet count at or above 30 $*10^9$/L and/or to avoid bleeding .

F- Molecular studies

ABCB1 gene genotyping was done to all individuals included in this study for detection of 2 SNPS (C3435T and G2677T/A) using polymerase chain reaction restriction fragment length polymorphism (PCR-RELF) Technique .

***For* (G2677T) SNP:**

It determines the presence of the T allele at position 2677**:**

- Upstream primer: 5~-TGCAGGCTATAGGTTCCA GG-3~
- Downstream primer: 5~-TTTAGTTTGACTCACCTTCCCG-3~

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For (C3435T) SNP:

It determine the presence of the T allele at position 3435

- Upstream primer: 5~-GATCTGTGAACTCTTGTTTTC-3~
- Downstream primer: 5~-CTTGTTTTCAGCTGCTTGATGGCAA-3~

Statistical analysis

Statistical analysis was carried out using the SPSS program" Quantitative variables are presented as the mean ± standard deviation. Allelic and genotypic frequencies were estimated by genotype count. All statistical tests were two-tailed, and $p<0.05$ was chosen as the level of significance. The $\chi 2$ test was employed to perform univariate analysis of the association of each SNP with disease and categorical clinical features. The Kruskall- Wallis test was used to investigate the relationship between genotypes and quantitative clinical parameters .The association between genotypes and clinical characteristics was analyzed by logistic regression and expressed as odds ratio (OR) with 95% confidence interval (95% CI)

Results—

Invstigations to exclude 2ry thrombocytopenia	**Negative**
HCV ab , HIVab :	
ANA,	**Negative**
AnticardiolipinIgG , IgM	**Negative**
H Pylori Ag in stool	**Negative**
Bone marrow aspirate	**Hypercellular, giant megakaryocytes , no infiltration**
Abdominal US	**Normal liver, no splenomegaly**

As regard C3435T, table 1 shows the frequency distribution of wild CC genotypes as wild non mutant allele C was significantly higher compared to CT genotypes (CC versus CT) and T mutant allele(C versus T) (p value:***<0.001)*** in patient group as compared to control group. As regard G2677T, table 2 shows that T allele is significantly higher in patient group compared with control group than wild G allele but no significant difference at level of genotypes. There was also no statistically significant correlation between any of polymorphisms and age of onset of ITP or initial platelet count

Table 1: Distribution of C3435T polymorphism in studied population

	Control	ITP	P value (2)	OR / (95% CI)	P value (3)
C3435T					
CC	12(12.9%)	42(43.8%)		Ref.	
CT	70(75.3%)	42(43.8%)	***<0.001****	0.17 / (0.08-0.36)	***<0.001****
TT	11(11.8%)	12(12.5%)		0.31 / (0.11-0.88)	***0.028****
(1) **Wild**	82(88.2%)	84(87.5%)	*0.888*	Ref.	*0.888*
Mutant	11(11.8%)	12(12.5%)		1.1 / (0.4-2.5)	
C	94(50.5%)	126(65.6%)	***0.003****	Ref.	***0.003****
T	92(49.5%)	66(34.4%)		0.54 / (0.35-0.81)	

Table 2:Distribution of G2677T polymorphisms in studied population

	Response		P value (3)
	Complete	Partial	
G2677T 0/ **C3435T** 0	24(35.8%)	11(42.3%)	*0.574*
G2677T 0/ **C3435T** 1	8(11.9%)	1(3.8%)	
G2677T 1/ **C3435T** 0	32(47.8%)	14(53.8%)	
G2677T 1/ **C3435T** 1	3(4.5%)	0(0%)	

	Control	ITP	P value (2)	OR / (95% CI)	P value (3)
G2677T					
GG	23(23%)	16(16%)	*0.097*	Ref.	
GT	40(40%)	32(32%)		1.2 / (0.5-2.5)	*0.729*
TT	37(37%)	52(52%)		2 / (0.9-4.3)	*0.071*
(1) **Wild**	63(63%)	48(48%)	***0.033****	Ref.	
Mutant	37(37%)	52(52%)		1.8 / (1.04-3.2)	***0.034****
G	86(43%)	64(32%)	***0.023****	Ref.	
T	114(57%)	136(68%)		1.6 / (1.1-2.4)	***0.023****

There was no significant difference in genotype and allele frequency and distribution of any of both polymorphisms separately or combined as shown in table 4 found between early and late responders, partial and complete

Table 3: combined effect of both polymorphisms on time, quality of response to GC

	Time of response		P value (3)
	Early	Late	
G2677T 0/ **C3435T** 0	24(36.4%)	11(40.7%)	*0.685*
G2677T 0/ **C3435T** 1	8(12.1%)	1(3.7%)	
G2677T 1/ **C3435T** 0	32(48.5%)	14(51.9%)	
G2677T 1/ **C3435T** 1	2(3%)	1(3.7%)	

	Dependence		P value (3)
	Negative	Positive	

G2677T 0/ **C3435T** 0	11(34.4%)	25(39.7%)	
G2677T 0/ **C3435T** 1	5(15.6%)	4(6.3%)	*0.531*
G2677T 1/ **C3435T** 0	15(46.9%)	32(50.8%)	
G2677T 1/ **C3435T** 1	1(3.1%)	2(3.2%)	

0 wild , 1 heterozygous; mutant. ***(3) Chi square test or Fisher exact test if expected frequency <***:

Discussion

The study examined each of C3435T and G2677T polymorphisms of ABCB1 gene in Egyptian ITP patients and control healthy volunteers , its relation to response to steroid therapy. The study was done on 100 adult Egyptian ITP patients, 100 healthy controls. **At the baseline**, frequency distribution of each of the SNPs was examined and compared in ITP and control. The newly diagnosed ITP patients were given prednisolone orally 1 ml/kg (max. 60mg).out Of 100 ITP cases 97% were responders and 3 % were resistant. This result was similar to El-beblawy et al, 2015 as 91% were responsive and 9% were resistant ***(11)***. This was inconsistent with the result of Xuan et al., done on 471 ITP patients, as resistant cases was higher 24% and 76% were responsive and this may be explained by the difference in the number of patients in both study ***(12).*** After one month of oral steroid ttt , gradual withdawal over period of 6 months. 70% of total responders were steroid dependent (persistent group) and the other 30% were steroid non-dependent (remission group). Results differ from that found in El-beblawy et al 2015 as the steroid dependent cases were 19% and 81% were steroid non-dependent reflecting the difference in disease course between adults and pediatric (11)

Concerning C3435T distribution, there was significantly higher distribution of wild C allele compared to mutant T allele (C versus T) (P value; ***0.003)*** in patient as compared with control group. This result suggests no role of this polymorphism in predisposition to ITP. This is consistent with the study of ***El-beblawy et al, 2015 on*** Egyptian children with ITP which revealed that the frequency of the C3435T gene in all patients with ITP and control was not significant *(P value .090*) but El-beblawy et al ,2015 observe that children with wild CC genotype of this polymorphism significantly have the oldest age of ITP diagnosis *(P value:.02).*our results is also consistent with M. Xuan et al ,(12) on chines patients with ITP and Akin et al., 2011 in Turkey on pediatric ITP.**As regard other polymorphism G2677T**, the study revealed that mutant T allele was significantly higher when compared with wild G allele in patients and control group. This may give impression to possibility of relation to disease predisposition. This result is inconsistent with M. Xuan et al, (12) done on in China and found no statistical difference between ITP and normal persons . **Also, in the present study**There was no significant differences at age of diagnosis, initial platelet count between different genotypes or alleles of either C3435T or G2677T polymorphism**.** This differ than the study of El-beblawy et al, 2015 which reveled that there was a significant difference in age at diagnosis of C3435T gene with the CC genotype had the oldest age and lowest initial platelets count (P 0.029 and P 0.004)**.**

As regard C3435T, there was no statistical significant difference or special distribution in genotypes or alleles between groups of partial or complete response, early or late responders, dependent or non-dependent group.This suggests no role of this polymorphism in determining the disease course. this was consistant

with the results of Xuan et al, as patients received the same steroid oral regimen and they found no statistically significant difference in frequency or genotype distribution of C3435T polymorphism between the GCs-responsive and non-responsive group ***(12)***. Our results also were consistent with El-beblawy et al ,2015 but the patients received different ttt modalities in the form of high dose dexamesazone , high dose methylprednisolone, and IVIG, anti D Ig, The response was assessed as a whole as regarde time, best reached platelet count, and post-treatment platelet count. There was no significant difference in response to different modalities of treatment , time of treatment, and post-treatment platelet count in newly diagnosed patients with ITP between different genotypes and alleles of C34353T polymorphism (11). This can be attributed to the small number of patients receiving each ttt modality so it did not achieve significant statistical results.

Another study by Akin et al., 2011compared the C3435T polymorphism with the response to therapy in turkey children with ITP but patients were given high-dose methylprednisolone at a dose of 30 mg/kg/day for 3 days and 20 mg/kg/day for 4 days. They found no significant difference in C34353T genotype and allele distribution between responders and non- responders.***(13)***

As regard G2677T Polymorphism, we also found there was no statistical significant difference or special distribution in genotypes or alleles between groups of partial or complete response, early or late responders, dependent or non-dependent group. These results differ from Xuan et al., 2014,who found that homozygous mutant genotype had significantly better response to 28 days GCs therapy compared with wild genotype with a poor GCs response (12).

Conclusion

There was no significant differences at age of diagnosis, initial platelet count between different genotypes or alleles of either C3435T or G2677T polymorphism.

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