Original Article

The Correlation between Overexpression of GATA-1 Variants Valine 205 Methionine and R216Q with Occurrence, Prognosis, and Therapeutic Approaches for Immune Thrombocytopenic Purpura

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Abstract

Objective: Immune thrombocytopenic purpura (ITP) is an autoimmune disorder that is characterized by symptoms of hemorrhage and thrombocytopenia due to the production of autoantibody against platelet glycoproteins. The occurrence of gene defects caused by mutation has been recently identified as a main factor in the onset of megakaryocytic disease. **Materials and Methods:** To conduct this study, 140 patients and controls with no history of platelet disorders were selected. After the collection of samples, the prevalence of GATA-1 gene mutations was evaluated using polymerase chain reaction technique, which was confirmed by sequencing. **Result:** The results revealed no significant information on the occurrence of valine 205 methionine (V205M) and R216Q variants in ITP patients. However, data analysis indicated clinically significant differences between control group and ITP patients in some hematological parameters such as red blood cell, hemoglobin, hematocrit, and lymphocyte count. **Conclusion:** V205M and R216Q mutations were not detected in ITP patients.

Keywords: *GATA-1 mutation, immune thrombocytopenic purpura, platelet disorder, R216Q variant, V205M variant*

Introduction

Immune thrombocytopenic purpura (ITP) is an autoimmune disease associated with platelet destruction, which leads to bleeding disorders in patients.[1] Autoantibodies against platelet glycoproteins, including glycoprotein IIb/IIIa (GPIIb/IIIa) and GPIb/IX, are a main reason for platelet destruction.[2] The autoantibodies eliminate platelets in the reticuloendothelial system due to the mentioned mechanism, leading to occurrence of thrombocytopenia.[3] However, binding of autoantibodies to GPIIb/IIIa on megakaryocyte membrane causing megakaryocytic maturation disorder could be another reason for thrombocytopenia in ITP.[4,5] According to the new classification, ITP is categorized into three subgroups. Newly diagnosed ITP occurs within 3 months of diagnosis, persistent ITP is present 3–12 months after diagnosis, and Chronic ITP lasts more than 12 months after diagnosis.[6] In addition, ITP could occur after bacterial or viral infections, which is known as acute ITP.[7,8] The pathophysiology of ITP is heterogeneous, and genetic disorders such as mutations, the expression of several gene variants, and polymorphisms in vital factors of platelet production and function may play an essential role in the disease.[9,10] Different types of genes are involved in platelet production and function by controlling and regulating the expression of platelet receptors, including growth factors, cytokines, signaling molecules, and transcription factors.[11] Therefore, any mutation leading to disruption in the function or expression of these genes can lead to impaired platelet production and function,[12,13] which predisposes to platelet disorders.[14,15] GATA-1 is a crucial factor in platelet production, maturation, differentiation, and function by binding to its cofactor, namely friend of GATA-1 (FOG-1) and exerting its effect as GATA-1:FOG-1 complex.[16,17] Several variants are expressed due to GATA-1 mutations, each of which can induce a different platelet disorder.[18] Valine 205 Methionine (V205M) and Arginine 216 Glutamine (R216Q) are two common variants of GATA-1. The expression of V205M and R216Q disrupts

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GATA-1 binding to FOG-1 and to DNA, respectively, both of which lead to macrothrombocytopenia.[19,20] On the other hand, studies have shown a direct relationship between GATA-1 expression with increasing production of Interferon-γ (INF-γ). According to investigations, upstream INF response region (UIRR) augments INF-γ production in cells with high expression levels of GATA-1.[21] Moreover, defects of inflammatory cytokines (e.g. INF-γ) could result in disruption of immune system and cause-related diseases, including ITP. This research seeks to address the following questions:

1. Is there any significant relationship between GATA-1 mutations and the occurrence of ITP?
2. Can GATA-1 variants be considered as a significant factor in ITP diagnosis, prognosis, and follow-up?
3. Is there any chance for GATA-1 to be a significant factor in improving treatment approaches in ITP patients?

Clinical significance

Any mutation impairing the expression and regulation of megakaryopoiesis-related genes (such as GATA-1) may lead to megakaryocytic disorders. Alternatively, as mentioned, a direct relationship has been detected between GATA-1 expression and the increase in INF-γ level. Considering the ITP occurrence due to the dysfunction of inflammatory cytokines, it has been hypothesized that GATA-1 mutations (such as V205M and R216Q) leading to GATA-1 impairment may have a role in ITP. The results of this study could introduce a new significant diagnostic and prognostic factor in ITP, although further studies are required in this respect.

Materials and Methods

Patients and controls

In this research, we evaluated ITP patients referred to Ahvaz Baghaei 2 Hospital between 2016 and 2018. The diagnosis of ITP was based on the American Society of Hematology criteria (13H). In addition, those with similar conditions in terms of thrombocytopenia, such as patients with HIV infection, myeloproliferative disorders, and SLE, were excluded from this study. All clinical symptoms of patients were reviewed upon admission based on the data recorded in their files [Table 1]. In this research, 70 patients with ITP (acute = 54 and chronic = 16) and 70 healthy individuals with no history of ITP or other hematological disorders were recruited [Figure 1]. This study was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (IR. AJUMS.

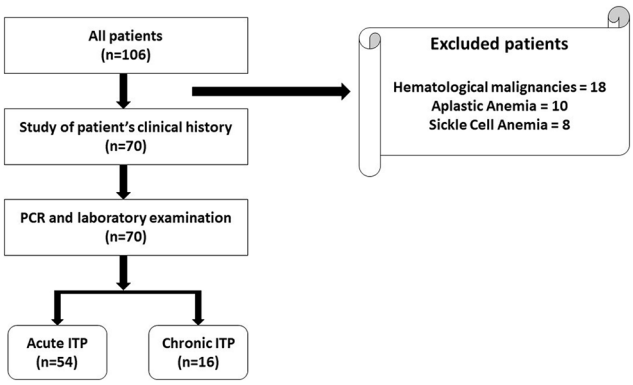

Figure 1: Flow diagram showing selection and exclusion of patients, polymerase chain reaction, and laboratory results. Immune thrombocytopenic purpura indicates immune thrombocytopenia

Table 1: Demographic and clinical characteristics of immune thrombocytopenic purpura patients and controls

Characteristics (variables)	ITP patients (*n*=70)	Non-ITP patients (*n*=70)	Total (*n*=140)	*P*<0.05	OR
Age (years), mean *n* (%)					
1-18	54 (77.1)	56 (81.2)	110 (78.6)	0.532	0.770
19-60	16 (22.9)	13 (18.8)	29 (20.7)		
Sex, *n* (%)					
Male	25 (35.7)	41 (58.6)	66 (47.1)	0.007	2.545
Female	45 (64.3)	29 (41.4)	74 (52.9)		
Platelet (10^3/µl), mean±SD	28.38±17.67	277.64±63.65	-	<0.001	-
PDW, mean±SD	16.61±13.32	15.03±4.22	-	<0.001	-
MPV (fl), mean±SD	13.32±1.89	11.84±2.32	-	<0.001	-
RBC (10^6/µ), mean±SD	4.26±0.68	4.84±0.69	-	<0.001	-
Hb (g/dl), mean±SD	11.26±1.83	12.87±1.9	-	<0.001	-
HCT (%), mean±SD	34.01±5.65	38.12±4.16	-	<0.001	-
MCV (fl), mean±SD	79.89±6.62	38.12±4.16	-	0.608	-
MCH (pg), mean±SD	27.76±7.53	26.39±2.80	-	0.728	-
MCHC (g/dl), mean±SD	32.89±2.05	33.29±1.21	-	0.601	-
WBC (10^3/µ), mean±SD	9.380±3.64	9.34±3.6	-	0.922	-
Lymphocyte (%), mean±SD	31.76±17.57	4.85±3.13	-	<0.001	-

ITP: Immune thrombocytopenic purpura; WBC: White blood cell; RBC: Red blood cell; Hb: Hemoglobin; HCT: Hematocrit; MCV: Mean corpuscular volume; MHC: Mean corpuscular hemoglobin; MCHC: Mean corpuscular Hb concentration; PDW: Platelet distribution width; MPV: Mean platelet volume, SD: Standard deviation, OR: Odds ratio

REC.1397.675), and informed consent was obtained from each patient participating in the investigation.

DNA extraction

About 2 mL peripheral blood was drawn from all the participants in an ethylenediaminetetraacetic acid (EDTA) anticoagulated tube. DNA was extracted from blood samples using a Roche kit (Germany) based on the manufacturer's protocol. All the extracted DNAs were stored at −20°C after measuring and analyzing the quantity and quality of DNA samples using NanoDrop One Microvolume ultraviolet-visible spectrophotometer (Thermo Fisher, USA) with a concentration of 100–200 ng/μl and 1.8–2.0 ratio in 260/280 nm.

Polymerase chain reaction

GATA1 variants V205M and R216Q were analyzed using PCR. The genomic loci of the two variants are close to each other, so we designed a single pair of primers (forward and reverse) to cover both of them, the sequences of which are shown in Table 2. For each sample, a separate microtube was used. The total volume of reaction solution was 50 μL, including 28 μL distilled water, 20 μL × 2 PCR master mix, 1 μL DNA, and 0.5 μL of each forward and reverse primers. The conditions for performing 35 PCR cycles were as follows: 95°C for 5 min, 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min with final extension for 3 min at 72°C.

Afterward, gel electrophoresis was performed by 2% agarose gel in ×1 tris-borate-EDTA buffer (PAYA PAZHOOHESH, Iran) to check for the presence or absence of PCR production. For each sample, a 506-bp single band associated with both V205M and R216Q variants was indicated on the gel [Figure 2]. It is worth noting that we sequenced all the samples to detect the mentioned variants separately.

DNA sequencing

Sanger sequencing was used for confirmation of PCR amplicons, and all the samples were sequenced using the following protocol: 5 μL forward primer, 5 μL reverse primer, 0.7 μL Big dye, 4 μL buffer, 3 μL H_2O, and 5 μL formamide. Final reaction was sequenced by ABI-3130XL [USA, Figure 3], and DNA analysis was performed using ABI PRISM 3130 × l DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

We used mean, standard deviation, median, and mid-range quartiles in quantitative variables to describe the data, while frequency and percentage were used for qualitative variables. For single-variable analysis of data, we used *t*-test (in case of normal data) and Mann–Whitney test (in case of nonnormal data). The normality of data was checked using the Kolmogorov–Smirnov test and linear regression was employed for multivariable data analysis.

Table 2: Primers used for polymerase chain reaction of GATA-1 variants

Technique	Primers	Sequence
PCR	Forward	5'-CTGCCCTACTTCAAGCTACCGC-3'
	Reverse	5'-ACTGATATTGACAGGCTAGTTG-3'

PCR: Polymerase chain reaction

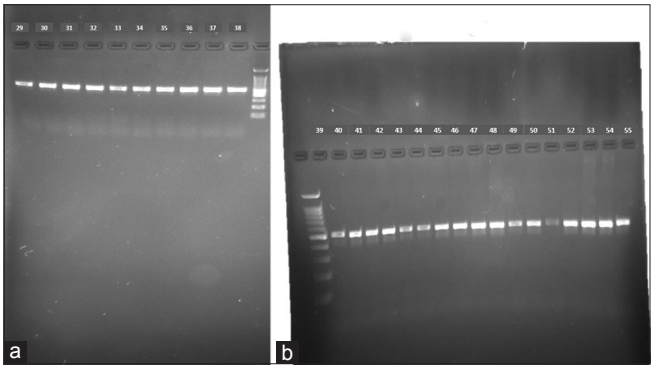

Figure 2: Evaluation of GATA-1 valine 205 methionine and R216Q variants by electrophoretic separation of polymerase chain reaction products on agarose gel. In this figure, we showed the results of 27 specimens, (a) number 29–38 and (b) number 39–55. A single pair of primers including a forward and a reverse was used for each sample. Also, the presence of these two variants of GATA-1 can be diagnosed according to the single band because of their closeness on the gene, and sequencing is required for detecting any of them separately

In cases in which the normality condition for response variable was not established, the logarithm of that variable was used. We performed all analyses using IBM SPSS Statistics for windows version 22.0. (Armonk, NY, IBM). $P < 0.05$ was considered to be statistically significant.

Results

First, we evaluated the presence of GATA-1 mutations (V205M and R216Q variants) in each of the control and ITP patients. According to the results, none of the cases had any of V205M and R216Q mutations in their GATA-1 gene; thus, our study shows no significant data about the occurrence of V205M and R216Q variants in ITP patients. Nevertheless, data analysis showed clinically significant differences between control group and ITP patients in a number of hematological parameters, which will be discussed below.

Comparison of platelet-related parameters between immune thrombocytopenic purpura and control groups

It is obvious that platelet count is decreased in ITP patients and is a significant diagnostic factor of the disease, which was also observed in this research ($P < 0.001$). As mentioned in Table 1, mean platelet volume (MPV) was decreased in ITP patients compared with control group, and there was a significant difference between the two groups ($P < 0.001$). Similar to MPV, there was a significant difference between ITP patients and healthy cases in mean platelet distribution width, which was increased in the former ($P < 0.001$).

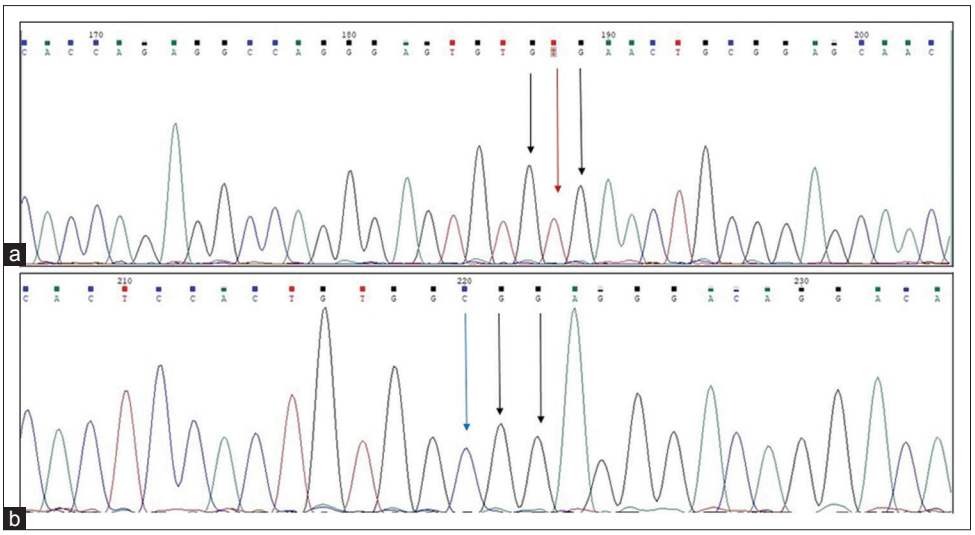

Figure 3: Evaluation of valine 205 methionine and R216Q mutations by direct sequencing technique in patients with immune thrombocytopenia Immune thrombocytopenic purpura. (a) Valine 205 methionine variant. (b) R216Q variant

Comparison of other hematological parameters between immune thrombocytopenic purpura and control group

This investigation showed a significant increase in red blood cell (RBC) counts ($P < 0.001$), hemoglobin (Hb) ($P < 0.001$), and hematocrit ($P < 0.001$). However, the most striking result emerging from the data was that the mean lymphocytes counts were much higher in ITP patients than in healthy cases ($P < 0.001$). On the other hand, the data show no significant change in mean of some parameters as follows: mean corpuscular volume (MCV; $P = 0.608$), mean corpuscular Hb (MCH; $P = 0.728$), mean corpuscular Hb concentration (MCHC; $P = 0.601$), and WBC ($P = 0.922$) [Table 1].

Discussion

ITP is a multifactorial disease and environmental as well as genetic factors play a role in its incidence.[22] Dysregulation of megakaryocytic-related genes can disrupt normal megakaryopoiesis or even break the immune system tolerance, which results in immune deficiency.[23,24] Based on the investigations, GATA-1 is among the effective genes in megakaryopoiesis, which is obligatory for normal platelet function.[25] Therefore, any mutation and dysfunction in this transcription factor can cause different types of platelet disorders.[26] V205M and R216Q are two of the most important GATA-1 variants, which have been detected in a number of platelet disorders such as macrothrombocytopenia.[19,20] In this study, we examined the occurrence of GATA-1 V205M and R216Q mutations and their effect on ITP patients compared with control group. In 2006, White *et al*. examined R216Q variant of GATA-1 among gray platelet syndrome patients and a control group, and their results showed that the expression of the mentioned variant was significantly higher in patients than the control group.[27] Moreover, Hughan *et al*. in 2005 investigated GATA-1 mutation and R216Q expression in thrombocytopenic patients having platelet function disorders. They detected GATA-1 mutation and the expression of its variants (including R216Q) in a majority of patients and concluded that there was a significant correlation between this mutation and platelet dysfunction leading to platelet diseases.[20] On the other hand, Freson *et al*. performed a similar series of experiments in 2003 and showed that V205M, D218G, and D218Y mutations were associated with X-linked macrothrombocytopenia.[28] The mentioned investigations, as well as similar ones, confirm that GATA-1 mutation and expression of its variants, including V205M and R216Q, lead to platelet disorders, especially thrombocytopenia; nevertheless, no study has investigated the occurrence of GATA-1 mutations in ITP patients. According to researches, a close relationship has been identified between family members of GATA factor (including GATA-1) with IFN-γ response through UIRR so that the expression of GATA-1 increases IFN-γ levels in cells; in contrast, cells with no GATA-1 expression showing considerable decrease in IFN-γ levels have been found to be more susceptible to immune disorders such as ITP.[29-31] Therefore, for the 1st time, we have investigated the presence of GATA-1 V205M and R216Q mutations in ITP patients and compared them with control group, but none of the mutations was present in our study groups. If even a single mutation were detected in these patients, GATA-1 would be considered as a significant factor in ITP pathogenesis, prognosis, follow-up, and treatment. Anyway, in the present study, although there was no significant difference in a number of parameters such as MCV, MCH, MCHC, and WBC between ITP patient and the control group, analysis of some other hematological parameters, including RBC, Hb, and hematocrit (HCT) showed a significant increase in ITP patients relative to the control group. Moreover, a surprising finding was the significant

increase in lymphocyte counts of ITP patients compared with control group.

Conclusion

The main goal of the present study was to determine the expressions of V205M and R216Q GATA-1 mutations as significant pathogenic and prognostic factors of ITP patients. While this study did not confirm the occurrence of the two variants in ITP, it did show that there was a significant increase in means of some hematological parameters, including lymphocytes count, RBC count, Hb, and HCT, in ITP patients compared with the control group. As mentioned, the current investigation was the first study discussing GATA-1 mutation in ITP, so it has addressed many questions in need of further investigations in this field.

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Conflicts of interest

There are no conflicts of interest.

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