Impact of serum immunoglobulins level and IL-18 promoter gene polymorphism among Egyptian patients with idiopathic thrombocytopenic purpura

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ABSTRACT

Objectives: Based on the concept of immune dysregulation in immune thrombocytopenic purpura (ITP) and that Interleukin-18 (IL-18) is an inflammatory cytokine that plays an important role in autoimmune disease by inducing interferon-γ secretion; this study aimed to assess a possible association between the IL-18 promoter polymorphisms (−607 C/A site) and genetic susceptibility to ITP and the impact of the immunoglobulins (Igs) concentrations level on disease severity and response to therapy.

Methods: A cross-section study was done on 105 patients’ age range from 10 to 28 years, with newly diagnosed ITP at the Oncology Center Mansoura University over the past 2 years and 100 healthy subjects as a control group. For all patients and controls, the IL-18 promoter polymorphism (−607 C/A site) as well as serum Ig (IgG, IgM, IgA) concentration was determined.

Results: The IL-18 promoter polymorphism (−607 C/A site) was not significantly different between ITP patients and normal controls. The number of patients respond to standard line of therapy was significantly higher in those with low IgA levels as compared to those with high IgA levels ($P = 0.02$). On the other hand, the number of patients respond to standard therapy was significantly higher in those patients with high IgM levels as compared to those with low IgM levels ($54.7\% \text{ vs. } 36.5\%$) ($P < 0.05$). The number of patients with bleeding manifestation was significantly higher among those with high IgA as compared to those with low IgA ($43 \text{ of } 79, 54.4\% \text{ vs. } 36 \text{ of } 79, 45.6\%; P = 0.04$). A change in IgG levels was not associated with response to treatment, bleeding tendency, or platelet counts.

Conclusion: There is no association between IL-18 promoter polymorphisms (−607 C/A site) and genetic susceptibility to ITP. High IgA and low IgM levels are a bad index for treatment response to standard therapy.

KEYWORDS

ITP; IL-18; immunoglobulin

Introduction

Immune thrombocytopenic purpura (ITP) is a common autoimmune disorder that is characterized by a low platelet count. It is mediated by platelet autoantibodies that accelerate platelet destruction and inhibit their production in the absence of any apparent initiating and/or underlying cause of the thrombocytopenia.1,2 The autoantibodies developed, in ITP patients, predominantly directed against platelet surface glycoprotein (gp) gpIIb–IIIa and gpIIb–IX. Most of the antibodies are of the IgG subtype and to less extent IgM and IgA. The interaction of these autoantibodies to platelet receptors helps in activation of the macrophages and complement-induced lysis which leads to platelets destruction. The macrophages express the platelet antigens on its surface and secrete cytokines after its destruction, which leads to its expression to CD4 T-cell and stimulation of cellular immune response. The activated CD4 T-cell recognizes other platelet gp as gpIIb–IIIa, which leads to acceleration and aggravation of the autoimmune response.3 In patients with active ITP, it was observed that there is an increase in the Th1–Th2 ratio together with impaired regulatory compartment, including T regulatory and B regulatory cells,4 and increased levels of both IFN-γ and IL-185; suggesting generalized immune dysregulation.6 Interleukin-18 (IL-18), which was initially described as an IFN-γ inducing factor in T-cells and natural killer cells, is a pleiotropic cytokine that plays an important role in the regulation of innate and acquired immune responses and affects many autoimmune diseases by controlling T helper cells.7,8 Previous study among Egyptian ITP patients reported that there is inverse correlation between serum and mRNA IL-18 levels and platelet counts.9 Single-nucleotide polymorphism (SNP) is one of the most common forms of genetic variations that affect the human genome and mediate the individual
susceptibility to some diseases. IL-18 polymorphism is one of the most important genetic polymorphisms that closely associated with ITP. The IL-18 607 A/C polymorphism is associated with the etiology and the pathogenesis of many autoimmune diseases such as systemic lupus erythematosus. To our knowledge, few investigations have been undertaken on the association between IL-18 gene polymorphisms and ITP.

Immunoglobulins (Igs) are synthesized by B cells and act as antigen binding in the humoral immune response. Structurally, the Igs consist of two light chains and two heavy chains. The Ig isotype identified according to the type of heavy chain produced, of which five types are identified (IgA, IgD, IgG, IgE, and IgM), each of them own specific composition and functions related to the immune response. IgG is the most abundant Ig in the body, IgM is the initial Ig expressed in response to an immune process and both of them have a role in neutralizing toxins and other immunogens. On the other hand, IgE specifically is closely associated with hypersensitivity and allergic responses.

This study aimed to assess (1) the immune system deregulation in patients diagnosed as ITP and correlates the relation of these Igs levels to the initial platelet counts, severity of the disease, treatment response, and clinical outcome and (2) to test the impact of polymorphic sites in the promoter regions of IL-18 at the 607 position on the genetic susceptibility for ITP.

Material and method

A cross-section study was carried out on 105 patients in the age range of 10–28 years, with newly diagnosed ITP at the Oncology Center Mansoura University; ethnic background and sex-matched healthy controls (n = 100) were included in this study. The diagnosis of ITP was based on the American Society of Hematology (ASH) guideline for immune thrombocytopenia. Patients with other hematologic or immunologic disorders were excluded from the study. Standard treatment was including steroids, intravenous Ig or intravenous anti-D. The treatment response was assessed according to the ASH guidelines for immune thrombocytopenia. Subjects who had not responded to any or all of these agents were defined as non-responder to standard treatment. Informed consent was obtained from all patients. For all patients and controls, serum Igs (IgA, IgM, and IgG) concentration levels were measured by a commercially quantitative turbidimetric technique (Spinreact S.A.U., Girona, Spain); the principle of the test is to form insoluble complexes when anti-human Ig antibodies are mixed with samples containing Ig. The scattering light of the immunocomplexes depends on the Ig concentrations in the patient samples and can be quantified by comparison from calibrators of known Ig concentrations. The Igs levels were analyzed in relation to the platelet counts, differences in response to standard treatment, and bleeding history.

Venous blood samples were collected from newly diagnosed patients with ITP in two tubes; one was standard sterile tube containing ethylenediaminetetraacetic acid for complete blood count; peripheral smear; DNA extraction for IL-18 promoter polymorphism analysis and the second one blank tube for serum samples, which centrifuged at 3000 rpm (1600×g) for 10 minutes and the serum stored at −70°C until IgG, IgM, and IgA assay.

Subjects were categorized according to the median levels of IgA, IgM, and IgG, and then analyzed in relation to the treatment response, the incidence of bleeding events, and platelets count.

The IL-18 promoter gene polymorphism was amplified by polymerase chain reaction (PCR) from extracted DNA using the primer pair; forward primer (5′-CTTTGCTATCATCAGGAA-3′), reverse primer (5′-TAACCTCATTGGACTCC-3′). Cycling conditions were initial denaturation at 95°C for 4 minutes, 36 cycles at 95°C for 30 seconds, 64°C for 60 seconds, and 72°C for 30 seconds, and final extension at 72°C for 10 minutes. The PCR products were digested by MseI for A–C transition. The IL-18 CC genotype shows three DNA bands at the positions (199, 73, and 29 bp), the AA genotype shows three bands (101/98, 73, and 29 bp), and the AC genotype shows four bands (199, 101/98, 73, and 29 bp).

Statistical analysis

The statistical analysis of data was done by using excel program and SPSS version 16 (statistical package for social science). Qualitative data were described in the form of numbers and percentages. Quantitative data were described in the form of median and ranges. Statistical analysis was done by comparison between groups using the chi-square test regarding qualitative data, while quantitative nonparametric data comparison was performed using one-way ANOVA and the paired sample t-test. The probability of being by chance (P value) was calculated for all parameters (P is significant if ≤0.05 at confidence interval 95%).

Results

Descriptive data

One hundred and five newly diagnosed patients with ITP were included in this study, they were 80 females (76.2%) and 25 males (23.8%), with a median age of 22 years (range, 10–28 years). Bleeding manifestation was observed in 79 (75.2%) (Table 1).
Table 1 Demographic and clinical data of the 105 patients

| Variables                   | Study group (n = 105) |
|-----------------------------|-----------------------|
| Age (years)                 |                       |
| Median (range)              | 22 (10–28)            |
| Gender                      |                       |
| Males; n (%)                | 25 (23.8)             |
| Females; n (%)              | 80 (76.2)             |
| Total leucocytic count (×10^9/l); median (range) | 5 (3–9) |
| Hemoglobin concentration (g/dl); median (range) | 9.5 (6–13) |
| Platelet count (×10^9/l); median (range) | 22 (7–56) |
| ANA positive; n (%)         | 15 (14.3)             |
| Anti-DNA positive; n (%)    | 10 (9.5)              |
| LDH (IU/ml); median (range) | 350 (250–550)         |
| Fever/infection; n (%)      | 46 (88.5)             |
| Bleeding manifestation; n (%) | 79 (75.2)          |
| IgA (mg/dl); median (range) | 324 (146–501)         |
| IgM (mg/dl); median (range) | 82 (11–221)           |
| IgG (mg/dl); median (range) | 1592 (215–2248)       |

ANA, antinuclear antibodies; LDH, lactic dehydrogenase; Anti-DNA, anti-double-strand antibodies; IgA, immunoglobulin A; IgM, immunoglobulin M; IgG, immunoglobulin G.

Table 2 Variation in IgS median concentrations among the studied group

| Variation in IgS median concentration | Studied group n (%) |
|---------------------------------------|---------------------|
| IgA (mg/dl) ≤324 (median)             | 52 (49.5)           |
| >324 (median)                         | 53 (50.5)           |
| IgM (mg/dl) ≤82 (median)              | 53 (50.5)           |
| >82 (median)                          | 52 (49.5)           |
| IgG (mg/dl) ≤1592 (median)            | 52 (49.5)           |
| >1592 (median)                        | 53 (50.5)           |
| Total                                 | 105                 |

IgA, immunoglobulin A; IgM, immunoglobulin M; IgG, immunoglobulin G.

The median IgA level was 324 mg/dl (range, 146–501 mg/dl), a total of 53 subjects (50.5%) had equaled to or less than the median level and 52 subjects (49.5%) had elevated level than the median (normal range, 70–400 mg/dl). The median IgM concentration was 82 mg/dl (range, 11–211 mg/dl), 52 ITP patients (49.5%) had an elevated IgM than the median level and 53 subjects (50.5%) had concentration equal to or less than the median level (normal range, 40–230 mg/dl). The IgG serum concentration level was 1592 mg/dl (range, 215–2248 mg/dl). The ITP patients with high IgG concentration above median were 53 (50.5%) and 52 subjects (49.5%) had a low IgG below median (normal range, 700–1600 mg/dl) (Table 2).

**Relationships of IgA levels with response to treatment, bleeding manifestation, and initial platelet count (Table 3)**

Most patients with IgA level below or equal to median had good response to standard treatment (30 of 53, 56.6%; vs. 23 of 53, 43.4%, P = 0.02).

The number of patients with bleeding manifestation was significantly higher among those with high IgA as compared to those with low IgA (43 of 79, 54.4%; vs. 36 of 79, 45.6%; P = 0.04).

The platelet count was not statistically different between subjects with high IgA (> vs ≤324) as compared to those with low IgA (P = 0.58).

**IgM levels relationships with response to treatment, bleeding tendency, and platelet count (Table 4)**

The number of ITP patients responded to standard therapy was significantly higher in the subgroup with high IgM levels as compared to those with low IgM levels (54.7 vs. 36.5%) (P < 0.05). The incidence of bleeding manifestation was not significantly different in patients with high vs. those with low IgM levels (P > 0.05). On the other hand, the number of ITP patients with lower platelet count was significantly higher among patient groups with lower IgM as compared to those with high IgM (P = 0.03).

**Relationships of IgG levels with response to treatment, bleeding tendency, and platelet count**

The ITP patients with high IgG levels were not significantly different as compared to those with low IgG level regarding response to treatment, bleeding tendency, nor platelet count (P = 0.95, 0.77 and 0.8, respectively) (Table 5).

**Table 3 The relation between IgA and response to standard treatment, bleeding tendency, and median platelet count**

| Immunoglobulin A (mg/dl) | IgA ≤ 324 | IgA < 324 | Total | P value |
|--------------------------|-----------|-----------|-------|---------|
| Response treatment       |           |           |       |         |
| Responder                | 30        | 18        | 48    | 0.02    |
| Non-responder            | 23        | 34        | 57    |         |
| Bleeding manifestations  |           |           |       |         |
| No                       | 17        | 9         | 26    | 0.04    |
| Yes                      | 36        | 43        | 79    |         |
| Platelet count ≤22,000   |           |           |       |         |
| 28                       | 30        | 58        |       | 0.85    |
| >22,000                  | 25        | 22        | 47    |         |
| Platelet count >22,000   |           |           |       |         |

**Table 4 The relation between IgM and response to standard treatment, bleeding tendency, and median platelet count**

| Immunoglobulin M (mg/dl) | IgM ≤ 82 | IgM < 82 | Total | P value |
|--------------------------|----------|----------|-------|---------|
| Response treatment       |           |          |       |         |
| Responder                | 19       | 29       | 48    | 0.05    |
| Non-responder            | 33       | 24       | 57    |         |
| Bleeding manifestations  |           |          |       |         |
| No                       | 9        | 17       | 26    | 0.068   |
| Yes                      | 43       | 36       | 79    |         |
| Platelet count ≤22,000   |           |          |       |         |
| 34                       | 24        | 58        |       | 0.03    |
| >22,000                  | 18        | 29        | 47    |         |

HEMATOLOGY 101
Table 5 The relation between IgG and response to standard treatment, bleeding tendency, and median platelet count

| Immunoglobulin G (mg/dl) | Response treatment | Bleeding manifestations | Platelet count |
|--------------------------|--------------------|------------------------|----------------|
|                          | IgG ≤ 1592         | IgG > 1592             | Total          | P value |
| Responder                | 34                 | 18                     | 48             | 0.95    |
| Non-responder            | 24                 | 29                     | 57             |         |
| No                       | 14                 | 38                     | 26             | 0.77    |
| Yes                      | 12                 | 41                     | 79             |         |
| ≤22,000/cmm              | 28                 | 24                     | 52             | 0.8     |
| >22,000/cmm              | 30                 | 23                     | 53             |         |

Table 6 IL-18 promoter polymorphism in ITP patients and controls

| Genotype frequency | ITP patients (n = 105) | Control group (n = 100) | P value |
|--------------------|------------------------|-------------------------|---------|
| −607 genotype      |                        |                         |         |
| CC                 | 41 (39.0%)             | 40 (40%)                | >0.05   |
| AC                 | 46 (43.8%)             | 46 (46%)                | >0.05   |
| AA                 | 18 (17.1%)             | 14 (14%)                | >0.05   |
| CA + AA            | 64 (61.0%)             | 60 (60%)                | >0.05   |

IL-18 genotyping by PCR–restriction fragment length polymorphism

There was no significant difference between the ITP patients and the control group as regard to the IL-18 promoter genotype polymorphism (−607 C/A site) (Table 6).

Discussion

Dysregulation in the immune system including derangement of T-cell function has been widely reported in ITP, with abnormal cytokine profiles correlated to loss of immune tolerance and defective B cell suppression.13,14 Autoreactive T-cells drive the generation of platelet-reactive autoantibodies by B cells as well as T-cytotoxic cell-mediated lysis of platelets.15 IL-18 appears to modulate inflammation at multiple checkpoints, acting not only on initiation and expansion of putative autoreactive Th1 responses but also via direct effects on multiple cellular targets, including macrophages, lymphocytes, and NK cells.16 Patients with active ITP displayed high plasma levels and IL-18 mRNA expression.15

There is a big vague about the factors that relate to disease inclination, severity, and treatment response. Immune dysregulation, as represented by alterations in the serum Ig levels, may increase disease severity as represented by failure to respond to treatment.17 Patient factors that should be considered in the decision to start treatment include presence of bleeding, patient age, platelet count, risk of traumatic injury, other causes of platelet dysfunction (aspirin, uremia), and liver disease.18 These alterations in Ig levels may represent an inflammatory or activated immune condition that makes the disease more difficult to control with specific treatments, resulting in isolated thrombocytopenia.17 Opsonization of Ab–platelet complexes by these antigen presenting cells facilitates intracellular processing of platelets and can lead to presentation by T-cells via MHC II as an array of ‘foreign’ platelet peptides. Presentation of platelet peptides by MHC II in a stimulatory context activates T-cells, leading to enhancement of the antiplatelet immune response and the possibility of epitope spread to additional platelet antigens.18

Due to the inadequate studies as regard to the immune system deregulation and their impact on ITP resistance to therapy in Egyptian patients, we intended in our study to measure the Igs levels in patients diagnosed as ITP and to assess the relationship of these levels to the platelet counts, bleeding manifestation, treatment response, and clinical outcome and to study the relation between IL-18 607 A/C polymorphism and the susceptibility to ITP.

In a cross-sectional analysis of cases with ITP, an elevation in IgA level than median was associated with resistant to the standard medical treatment and is also associated with high percentage of ITP patients with bleeding manifestation as compared to those with low IgA levels. However, initial platelet counts were not significantly different in the two groups, suggesting another mechanism for bleeding away from platelet count. George and Saucerman19 had approved that the total concentrations of IgG, IgA, and albumin were all higher than normal in platelets from patients with ITP. Antibodies mediate platelet activation through Fc-mediated GPIIb/IIIa crosslinking by IgG hexam erization as recently shown to occur on IgG-opsonized particles and cells.20

Beharka et al.21 and his colleagues in 2001 found that a tendency toward more resistant disease in ITP patients with an IgA greater than median was seen in the elderly patients which may be supported by the increase in serum IgA which was observed in all subjects known to have the autoimmune disease.

In addition to the underlying immune deficiencies which appeared, environmental and genetic factors may impact platelet turnover, tendency to bleed, and response to ITP therapy.22 As regard to IgM in our study, there was an association between low IgM levels and failure to respond to standard treatment and low platelet count. This could be attributed to the consumption of IgA in the opsonization and phagocytic process. On the other hand, there was no significant difference between the occurrence of bleeding tendency in ITP patients and the level of IgM. This finding was in agreement with Arnason et al.12 On the other hand, the changes in IgG level above or below median level were not associated with either response to neither treatment nor bleeding tendency or platelet count. This finding was in agreement with Arnason et al.12 who found that IgG levels do not
associate with major bleeding or treatment failure but associate with platelet count. In our study, there are a greater proportion of subjects with low IgM in the patients with high IgA level, as compared to subjects with elevated IgM but without statistical significance.

Cytokine-mediated immunity plays a crucial role in the pathogenesis of various autoimmune diseases. The IL-18 is among the cytokines responsible for immune-mediated pathologies and is probably one of the factors that contribute to pathogenesis of autoimmune disease. The results of Shan et al.\(^5\) showed that IL-18 and mRNA levels were significantly increased in patients with active ITP than in control subjects. Moreover, several studies have been reported that the SNPs in the promoter of the IL-18 gene at positions −607 C/A could induce higher IL-18 expression.\(^{23,24}\)

In our study, we found that there was no significant difference between the ITP patients and the control group as regard to the IL-18 genotype polymorphism. This in agreement with Zhao et al.\(^{10}\) and Li et al.\(^{26}\) who stated that there is no association between IL-18 promoter polymorphism and occurrence of primary thrombocytopenia.

In conclusion, the patients with elevation IgA level above median (324 mg/dl) were resistant to the standard medical treatment and also manifested with major bleeding. On the other hand, patients with low IgM levels below median (82 mg/dl) were associated with failure to respond to standard treatment and low platelet count. No significant differences in genotype and allele frequency were found between ITP patients and control group. We recommend initial measure of Ig level in patients with ITP to help us in the treatment protocol for each patient.

**Disclaimer statements**

**Contributors** Salah Aref: study idea and study plan; Mohamed Sabry: lab work; Sherin Abd El-Aziz: lab work; Tarek Abouzeid: clinical work; Mona Talaab: clinical work; Amr El-Sabbagh: manuscript writing.

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**Ethics approval** The study was approved by local ethical committee.

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