Toll-Like Receptor 9 (TLR9) Gene C/T (rs352140) Polymorphisms in Adult Primary Immune Thrombocytopenia

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Abstract

Immune thrombocytopenia (ITP) is an autoimmune disease characterized by low platelet count and increased bleeding risk. The initial event(s) leading to antiplatelet autoimmunity remains unclear. Toll-like receptors (TLRs) are the most well-characterized pattern recognition receptors and are a transmembrane protein coded by the Toll genes family. In addition to their protective role in immunity, it is also becoming clear that TLRs exhibit homeostatic roles. Toll-like receptors play potential roles in the development of disease and its maintenance. The objective of this study is to evaluate the distribution of TLR9 gene C/T (rs352140) polymorphisms and its possible association with clinicopathological finding in Egyptian adult primary ITP. This study was carried out at Internal Medicine Department, Menoufia University Hospital, Egypt, from August 2018 to January 2020. Eighty adults (≥ 18 years) were enrolled in the study; 40 patients with primary ITP and 40 healthy individuals as controls. Identification of the TLR9 C/T (rs352140) polymorphic variant was performed by polymerase chain reaction–restriction fragment length polymorphism. In our study, we excluded any other causes of secondary ITP. Distribution of the TLR9 C/T genotypes did not exhibit significant deviation between patients and controls. There was no significant difference between studied groups as regards allele (C and T) frequency. There was no significant difference regarding TLR9 gene C/T (rs352140) polymorphisms between Egyptian adult with primary ITP and controls. TLR9 gene C/T (rs352140) polymorphisms have no relation to any of the clinicohematological variables in primary ITP in Egyptians.

Keywords

immune thrombocytopenia, Toll-like receptors, polymorphism

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Introduction

Toll-like receptors (TLRs) are the main portal that transmit danger signals from different invading pathogens to the innate immune system and mediate its inflammatory response.¹ The TLRs are widely expressed on body cells, including dendritic cells, macrophages, lymphocytes, and endothelial cells.² Megakaryocytes and platelets variably express functional TLRs.³ At least 10 TLRs have been identified in humans.² Some TLRs are transmembrane receptors (TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10). The other group of TLRs is located intracellularly (endosome compartments; TLR3, TLR7, TLR8, and TLR9).¹ The TLR9 gene is located on chromosome 3 p21.3, with a length of ~5 kb, also it has 2 exons, and the major coding region is in the second exon. Twelve single-nucleotide polymorphisms have been identified for TLR9 gene, in which T>C (rs187084) located at the upstream of promoter may be an important one. The C genotype was

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associated with reduced TLR9 transcription activity when compared to T genotype, indicating humans with C genotype may have diseases related to TLR9 gene. TLR9 is expressed in different cells of the immune system such as dendritic cells, B lymphocytes, monocytes, and natural killer cells and produces cytokines, for example, interferon (type-I) and interleukin 12, which attribute to many biological actions such as apoptosis and antimicrobial activity. Inactive platelets express significant amounts of TLR9; it is markedly upregulated with thrombin but not lipopolysaccharides on activated platelet, suggesting that TLR9 also exists in intracellular compartments. After stimulation by a CpG motif or by thrombin, platelets overexpress TLR9 receptor. Platelet TLR9 was reported as binding for danger signal in cases of oxidative stress. Also, it promotes triggers aggregation and degranulation. Therefore, TLR9 on platelets appears to function as sensors of internal danger signals rather than external ones. Signaling of TLR has important roles in the establishment of disease and its progression. Environmental factors and genetic differences between people have an influence on TLR signaling in different human disease.

Toll-like receptors, portals for the innate immune system, are important in the recognition of pathogenic components. However, when inappropriately activated by self-molecular signatures, this may lead the occurrence of autoimmunity, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and systemic sclerosis. Systemic lupus erythematosus is an autoimmune disease characterized by the loss of tolerance to self-molecular antigens. For specific TLRs, overexpression of TLRs in patients and mouse models, TLR knockout mice being resistant to disease and TLR ligands exacerbating and TLR inhibitors alleviating disease. There are endogenous ligands for TLRs in human sera such as the nucleic acid–binding TLRs, including TLR7, TLR8, and TLR9. In patients with SLE, immune cells produce large quantities of autoantibodies against self-nuclear antigens which make immune complexes with self-nucleic acids and present in SLE serum. Data from literature on mouse models suggest that TLR7 have a pathogenic role and TLR9 have a protective one for signaling in the pathogenesis of SLE.

Immune thrombocytopenia (ITP) is not an uncommon blood disease characterized by isolated thrombocytopenia. It may be primary or a secondary with another disorder that is usually immune or infectious. Primary ITP is defined as isolated thrombocytopenia (platelet count <100 × 10^3 /dL) in the absence of other causes or disorders that may be associated with thrombocytopenia. Patients with ITP have a bleeding time that is disproportionately short for the degree of thrombocytopenia. This is due to presence of a more hemostatically and larger competent platelet and microparticles. The exact cause for ITP is not well postulated; however, both genetic and environmental factors are thought to play a role in the development of the disease.

**Aim of the Work**

The aim of this work is to evaluate the distribution of TLR9 gene C/T (rs352140) polymorphisms and their possible association with clinicopathological finding in Egyptian adult primary ITP.

**Patients and Methods**

**Patients**

This cross-sectional study was carried out at Hematology Unit, Internal Medicine Department, Menoufia University Hospital, Egypt, from August 2018 to January 2020; it followed the ethical standards of our institution. Informed written consents were obtained from all the participants.

A total number of 80 adults were included in this study. Group (I) included 40 patients with ITP. Group (II) included 40 healthy individuals as age- and sex-matched controls.

**Methods**

All participants underwent full history taking, clinical examination, and laboratory investigations, including (1) complete blood count, blood films, and reticulocytes; (2) hepatitis C virus antibodies, HBsAg, HBeAb, and HIV antibodies; (c) ANA, C-reactive protein, and erythrocyte sedimentation rate (ESR); (4) *Helicobacter pylori* antibody in stool; (5) KFTs, LFTs, and coagulation profile; (6) thyroid-stimulating hormone; (7) TLR9 gene C/T (rs352140) polymorphism by polymerase chain reaction (PCR); and (8) abdominal ultrasound. Direct antiglobulin test and iron profile were done in all patients having anemia. In this study, patients with infections, other autoimmune diseases, malignant diseases, pregnancy, and any other causes of secondary ITP were excluded.

**Genotyping**

Venous blood samples of 5 mL were withdrawn from the cubital vein of every patient and then was transferred slowly into vacuated EDTA tube for isolation of DNA for genotyping. DNA was extracted using Gene JET TM whole-blood Genomic DNA purification Mini kit (Thermo Scientific EU/Lithuania).

The amplification reaction for TLR9 C/T (rs 352140) was performed in 25-μL volumes (10 μL DNA template + 15 μL Master Mix [containing 2.5 μL of 10× PCR buffer, 0.25 μL MgCl2 25 mM, 1.0 μL dNTPs mM, 1.0 μL forward primer [F5'-GCAGCACCTCAACTTAC-3'], 1.0 μL reverse primer [R5'-GGCTGTGATGTGTGGG-3'], 0.30 μL Taq polymerase 5(μL/μL, and 8.95 μL distilled water] using an initial denaturation (5 minutes at 95 °C), denaturation (1 minute at 95 °C), annealing (1 minute at 60 °C), extension (1 minute at 72 °C), number of cycles: 35 cycles, and final extension (7 minutes at 72 °C) using Perkin Elmer thermal cycler 2400.

The PCR product was then digested with BstUI (New England Biolabs) at 73 °C for 3 hours (2 μL 10× buffer, 1 μL BstUI, 7 μL distilled water, and 10 μL PCR product). The
BstUI digestive products were run by 2% agarose gel electrophoresis for 30 minutes and stained with ethidium bromide, and the bands were visualized under ultraviolet light. Digested PCR products yielded 360-bp bands in TT homozygotes, 133- and 277-bp bands in CC homozygotes, and all 3 bands in CT heterozygotes.

Statistical Analysis

Data were collected, tabulated, and statistically analyzed using an IBM personal computer with Statistical Package of Social Science (SPSS) version 22 (SPSS Inc). Descriptive statistics in which quantitative data were presented in the form of mean, standard deviation (SD), range, and qualitative data were presented in the form of numbers and percentages. Analytical statistics was used to find out the possible association between studied factors and the targeted disease. The used tests of significance included $\chi^2$ test, odds ratio, Fischer exact test, Student t test, Mann-Whitney U test (nonparametric test), Kruskal-Wallis test, and analysis of variance (f) test. $P$ value of $>.05$ was considered statistically nonsignificant, and $P$ value of $<.05$ was considered statistically significant.

Results

Demographic data of patients with ITP and control are detailed in Table 1. There was no significant difference between studied groups as regards age and gender. Mean age in group I was 39.7 ± 15.1. Mean age in group II was 35.5 ± 12.4. Group I (ITP group) showed female predominance with women constituting 87.5%.

Descriptive clinical, hematological, and laboratory data of patients with ITP (group I) are detailed in Table 2. In all, 85% of patients with ITP had no other comorbidities; 5% of patients with ITP had hypertension; 2.5% had diabetes mellitus; and 7.5% had both hypertension and diabetes mellitus. Of the patients with ITP, 77.5% have platelets less than 30 000, 5% have platelets more than 50 000, and 17.5% have platelets ranging from 30 000 to 50 000. According to World Health Organization standardized bleeding grade, 65% of patients with ITP had grade 1 bleeding (petechial bleeding), 27.5% had grade 2 bleeding (mild blood loss with clinically significance, eg, menorrhagia, vaginal bleeding, and so on), and 7.5% had grade 0 bleeding (no bleeding). Other laboratory data including hemoglobin (HB) range 6.50 to 15.6 gm/dL, this low HB level was due to severe vaginal bleeding and blood loss, white blood cells (WBCs) range 4-13 × 10^3/mm^3, ESR (range 5-55 mm/h), urea, creatinine, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) are detailed in Table 2. All patients with low HB level were females with menorrhagia, and all have negative direct antiglobulin test and low iron profile.

### Table 1. Demographic Data of the Studied Groups.

| Studied variable | Cases, N = 40 | Controls, N = 40 | Test of significance | P |
|------------------|--------------|-----------------|----------------------|----|
| Age, years       |              |                 |                      |    |
| Mean ± SD        | 37.9 ± 15.1  | 35.5 ± 12.4     | U = 1.25             | .211 |
| Range            | 18-75        | 19-70           |                      |    |
| Sex, n (%)       |              |                 |                      |    |
| Male             | 5 (12.5)     | 7 (17.5)        |                      |    |
| Female           | 35 (87.5)    | 33 (82.5)       |                      |    |

Abbreviations: $\chi^2$, chi square test; N, number; %, percentage; U, Mann Whitney U test.

*Significance level at $P < .05$.

### Table 2. Clinicohematological and Laboratory Findings Among the Studied Patients.

| Studied variables | Cases, N = 40 | Comorbidities, n (%) |
|-------------------|--------------|----------------------|
|                   |              | Hypertension         |
|                   |              | 2 (5.00)             |
|                   |              | Diabetes mellitus    |
|                   |              | 1 (2.50)             |
|                   |              | Hypertension and diabetes mellitus |
|                   |              | 3 (7.50)             |
|                   |              | No                   |
|                   |              | 34 (85.0)            |
|                   |              | Thrombocytopenia grades, n (%) |
|                   |              | Mild (PL < 50)       |
|                   |              | 2 (5.00)             |
|                   |              | Moderate (PLT = 30-50) |
|                   |              | 7 (17.5)             |
|                   |              | Severe (PLT < 30)    |
|                   |              | 31 (77.5)            |
|                   |              | Bleeding grade, n (%) |
|                   |              | Grade 0              |
|                   |              | 3 (7.50)             |
|                   |              | Grade 1              |
|                   |              | 26 (65.0)            |
|                   |              | Grade 2              |
|                   |              | 11 (27.5)            |
|                   |              | Hb, g/dL             |
|                   |              | Mean ± SD            |
|                   |              | 11.6 ± 1.86          |
|                   |              | Range                |
|                   |              | 6.50-15.6            |
|                   |              | WBCs, 10^3/mm^3      |
|                   |              | Mean ± SD            |
|                   |              | 7.33 ± 2.39          |
|                   |              | Range                |
|                   |              | 4-13                 |
|                   |              | PLT, 10^3/mm^3       |
|                   |              | Mean ± SD            |
|                   |              | 19.3 ± 12.5          |
|                   |              | Range                |
|                   |              | 4-52                 |
|                   |              | ESR, mm/h            |
|                   |              | Mean ± SD            |
|                   |              | 16.2 ± 12.7          |
|                   |              | Range                |
|                   |              | 5-55                 |
|                   |              | Serum creatinine, mg/dL |
|                   |              | Mean ± SD            |
|                   |              | 0.69 ± 0.32          |
|                   |              | Range                |
|                   |              | 0.30-1.10            |
|                   |              | BUN, mg/dL           |
|                   |              | Mean ± SD            |
|                   |              | 12.4 ± 3.16          |
|                   |              | Range                |
|                   |              | 8-19                 |
|                   |              | AST, IU/L            |
|                   |              | Mean ± SD            |
|                   |              | 27.9 ± 4.33          |
|                   |              | Range                |
|                   |              | 20-35                |
|                   |              | ALT, IU/L            |
|                   |              | Mean ± SD            |
|                   |              | 29.0 ± 3.38          |
|                   |              | Range                |
|                   |              | 21-37                |
|                   |              | Serum albumin, g/dL   |
|                   |              | Mean ± SD            |
|                   |              | 4.08 ± 0.42          |
|                   |              | Range                |
|                   |              | 3.5-5                |

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; BUN, blood urea nitrogen; Hb, hemoglobin; ESR, erythrocyte sedimentation rate; N, number; %, percentage; PLT, platelet; SD, standard deviation; WBCs, white blood cells.

*Significance level at $P < .05$. 
There was no significant difference between studied patients’ TLR9 gene C/T (rs352140) genotypes as regards comorbidities. Patients with CC genotype had no comorbidity. Patients with CT genotype were 2 (8.30%). Patients with hypertension was 1 (4.20%). Patients with diabetes mellitus were 3 (12.5%). Patients with both hypertension and diabetes mellitus were 18 (75%). Patients with TT genotype had no comorbidity (Table 4).

Table 3 showed the distribution of the TLR9 C/T genotypes that did not exhibit significant difference between patients and controls. Frequency of the TLR9 T/T genotype in patients with ITP was 9 (22.5%). TLR9 C/T genotype was present in 24 (60%) patients, and 7 (17.5%) patients showed TLR9 C/C genotype. Frequency of the TLR9 T/T genotype in healthy individuals was 5 (12.5%). TLR9 C/T genotype was present in 22 (55%) healthy individuals, and 13 (32.5%) healthy individuals showed TLR9 C/C genotype.

There was no significant difference between studied groups as regards allele (C and T) frequency. In the patients group, allele frequency was 32 (40), but T allele frequency was 42 (52.5%). In the control group, allele frequency was 48 (60), but T allele frequency was 32 (40%); Table 3).

Dominate model (T/T and C/T vs C/C genotype) frequency showed no significant difference between studied groups. T/T and C/T genotypes presented in 33 (82.5%) patients and in 27 (67.5%) healthy individuals, respectively. C/C genotype presented in 7 (17.5%) patients and in 13 (32.5%) healthy individuals (Table 3).

Recessive model (T/T vs C/T and C/C genotype) frequency showed no significant difference between studied groups. T/T genotype presented in 9 (22.5%) patients and in 5 (12.5%) healthy individuals. C/C and C/T genotypes presented in 31 (77.5%) patients and in 35 (87.5%) healthy individuals, respectively (Table 3).

From Table 4, there was no significant difference between studied patients’ TLR9 gene C/T (rs352140) genotypes as regards age and gender. CC genotype had 7 (100%) patients aged below 60 years and no patients aged above 60 years. CT genotype had 21 (87.5%) patients aged below 60 years and 3 (12.5%) patients aged above 60 years. TT genotype had 7 (77.8%) patients younger than 60 years and 2 (22.2%) patients older than 60 years. CC genotype had 7 (77.8%) female patients but no male patients. CT genotype had 20 (83.3%) female patients and 4 (16.7%) male patients. TT genotype had 8 (88.9%) female patients and 1 (11.1%) male patients.
There was no significant difference between patients’ TLR9 C/T (rs352140) genotypes as regards clinicohematological findings (thrombocytopenia and bleeding grades). Patients with CC genotype had no patients with platelets more than 50 (10^3/mm^3), 2 (28.6%) patients with platelets 30 to 50 (10^3/mm^3), and 5 (71.4%) patients with platelets < 30 (10^3/mm^3). Among patients with CT genotype, 2 (8.30%) patients had platelets more than 50 (10^3/mm^3), 2 (8.30%) patients had platelets 30 to 50 (10^3/mm^3), and 20 (83.3%) patients had platelets less than 30 (10^3/mm^3). Patients with TT genotype had no patients with platelets more than 50 (10^3/mm^3), 3 (33.3%) patients with platelets 30 to 50 (10^3/mm^3), and 6 (66.7%) patients with platelets < 30 (10^3/mm^3). Patients with CC genotype had no patients with bleeding grade 0, 3 (42.9%) patients with bleeding grade 1, and 4 (57.1%) patients with bleeding grade 2. Patients with CT genotype had 3 (12.5%) patients with bleeding grade 0, 15 (62.5%) patients with bleeding grade 1, and 6 (25%) patients with bleeding grade 2. Patients with TT had no patients with bleeding grade 0, 8 (88.9%) patients with bleeding grade 1, and 1 (11.1%) patient with bleeding grade 2 (Table 4).
**Discussion**

Immune thrombocytopenia is an immune disease characterized by thrombocytopenia and increased risk of bleeding. The initial event(s) leading to its autoimmunity remains unclear.\(^{20}\) Toll-like receptors are pattern recognition receptors and are coded by the Toll genes family. The TLRs play a crucial role not only in the detection of many PAMPs and DAMPs but also in the activation of the adaptive immune system.\(^{21}\) Disordered expression and activity of such receptors can lead to the development of inflammation as well as immune disorders.\(^{22}\)

This study showed that distribution of the TLR9 C/T (rs352140) genotypes did not exhibit significant deviation between patients and controls. There was no significant difference between studied groups as regards allele (C and T) frequency. Dominate model (T/T and C/T vs C/C genotype) frequency showed no significant difference between studied groups, and recessive model (T/T vs C/T and C/C genotype) frequency showed no significant difference between studied groups.

A study\(^ {23}\) reported that prevalence of the TLR9 C/T (rs352140) genotypes did not exhibit significant deviation between patients with SLE and controls. Another one\(^ {24}\) demonstrated a significant association of (rs352140) gene variants with the susceptibility to SLE and suggested that the T allele at position of (rs352140) was significantly associated with the susceptibility to SLE in dominant model but not in recessive model. They also showed that individuals with CT genotype had greater susceptibility to SLE than those without.

In this study, there was no significant difference between studied patients’ TLR9 gene C/T (rs352140) genotypes as regards age and gender, comorbidities, laboratory findings (Hb, PLTs, WBCs, ESR, serum creatinine, BUN, AST, ALT, and albumin), and clinical–hematological findings (thrombocytopenia and bleeding grades).

As far as we know, no research until now described TLR9 gene C/T (rs352140) polymorphism in Egyptian adult primary ITP pathogenesis or any TLR9 polymorphism, but there are some research that described TLR9 polymorphisms in other autoimmune diseases such as SLE and RA.

Only one study on patients with SLE in Egypt\(^ {25}\) reported that there was no statistically significant difference in the distribution of the AA genotype between Egyptian patients with SLE and the control group; however, the GA heterozygous patients were 3 times more likely to develop SLE. Another study on Egyptian patients with RA found association between TLR9 gene polymorphism and susceptibility to disease development.\(^ {26}\)

rs187084_rs5743836 TLR9 haplotypes studied in 529 persons living in the south of Brazil diagnosed as ankylosing arthritis and psoriatic arthritis (PsA) concluded that these polymorphisms appear to be involved in the development of PsA and can be a possible therapeutic target for the immune disorders.\(^ {21}\)

**Limitation**

Small number of patients with ITP in this study was an important limiting factor.

**Conclusion**

There was no significant difference regarding TLR9 gene C/T (rs352140) polymorphisms between Egyptian adults with primary ITP and controls. TLR9 gene C/T (rs352140) polymorphisms have no relation to any of the clinical and hematological variables in primary ITP in Egyptians.

**Declaration of Conflicting Interests**

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**References**

1. O’Neill LA, Golenbock D, Bowie AG. The history of Toll-like receptors—redefining innate immunity. Nat Rev Immunol. 2013;13(6):453-460.
2. Kang JY, Lee JO. Structural biology of the Toll-like receptor family. Annu Rev Biochem. 2011;80:917-941.
3. Aslam R, Speck ER, Kim M, et al. Platelet Toll-like receptor expression modulates lipopolysaccharide-induced thrombocytopenia and tumor necrosis factor- production in vivo. Blood. 2006;107(2):637-641.
4. Chen Z, Wang W, Liang J, Wang J, Feng S, Zhang G. Association between toll-like receptors 9 (TLR9) gene polymorphism and risk of pulmonary tuberculosis: meta-analysis. BMC Pulm Med. 2015;15:57.
5. Selvaraj P, Harishankar M, Singh B, Jawahar MS, Banarekha VV. Toll-like receptor and TIRAP gene polymorphisms in pulmonary tuberculosis patients of South India. Tuberculosis (Edinb). 2010;90(5):306-310.
6. Hashemi-Shahri SM, Taheri M, Gadari A, Naderi M, Bahari G, Hashemi M. Association between TLR8 and TLR9 gene polymorphisms and pulmonary tuberculosis. Gene Cell Tissue. 2014;1(1):e18316.
7. D’Atri LP, Schattner M. Platelet toll-like receptors in thromboinflammation. Front Biosci (Landmark Ed). 2017;22:1867-1883.
8. Cognasse F, Nguyen KA, Damien P, et al. The inflammatory role of platelets via their TLRs and Siglec receptors. Front Immunol. 2015;6:83.
9. Sabroe I, Parker LC, Dower SK, Whyte MKB. The role of TLR activation in inflammation. J Pathol. 2008;214(2):126-135.
10. Cook DN, Pisetsky DS, Schwartz DA. Toll-like receptors in the pathogenesis of human disease. Nat Immunol. 2004;5(10):975-979.
11. Farrugia M, Baron B. The role of toll-like receptors in autoimmune diseases through failure of the self-recognition mechanism. *Int J Inflamm*. 2017;2017:8391230.

12. Duffy L, O’Reilly SC. Toll-like receptors in the pathogenesis of autoimmune diseases: recent and emerging translational developments. *Immunotargets Ther*. 2016;5:69-80.

13. Wu YW, Tang W, Zuo JP. Toll-like receptors: potential targets for lupus treatment. *Acta Pharmacologica Sinica*. 2015;36(12):1395-1407.

14. Hosseini AM, Majidi J, Baradaran B, Yousefi M. Toll-like receptors in the pathogenesis of autoimmune diseases. *Adv Pharm Bull*. 2015;5(suppl 1):605-614.

15. Celhar T, Magalhaes R, Fairhurst AM. TLR7 and TLR9 in SLE: when sensing self goes wrong. *Immunol Res*. 2012;53(1-3):58-77.

16. Rodeghiero F, Stasi R, Gernsheimer T, et al. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. *Blood*. 2009;113(11):2386-2393.

17. Kristensen AT, Weiss DJ, Klausner JS. Platelet dysfunction associated with canine immune-mediated thrombocytopenia (ITP). *J Vet Intern Med*. 1994;8(5):323-327.

18. Giltay JC, Brinkman HJ, Vlekke A, Kiefel V, van dem Borne AE. The platelet glycoprotein Ia-IIa-associated Bralloantigen system is expressed by cultured endothelial cells. *Br J Haematol*. 1990;75(4):557-560.

19. Rocha AM, De Souza C, Rocha GA, et al. IL1RN VNTR and IL2-330 polymorphic genes are independently associated with chronic immune thrombocytopenia. *Br J Haematol*. 2010;150(6):679-684.

20. Swinkels M, Rijkers M, Voorberg J, Vidarsson G, Leebeek FWG, Jansen AIG. Emerging concepts in immune thrombocytopenia. *Front Immunol*. 2018;9:880.

21. Oliveira-Toré CF, Moraes AG, Martinez GF, et al. Genetic polymorphisms of toll-like receptors 2 and 9 as susceptibility factors for the development of ankylosing spondylitis and psoriatic arthritis. *J Immunol Res*. 2019;2019:1492092.

22. Pérez-Cano FJ, Massot-Cladera M, Rodríguez-Lagunas MJ, Castell M. Flavonoids affect host-microbiota crosstalk through TLR modulation. *Antioxidants*. 2014;3(4):649-670.

23. Piotrowski P, Lianeri M, Wudarski M, Olesińska M, Jagodziński PP. Contribution of toll-like receptor 9 gene single-nucleotide polymorphism to systemic lupus erythematosus. *Rheumatol Int*. 2013;33(5):1121-1125.

24. Xu CJ, Zhang WH, Pan HF, Li XP, Xu JH, Ye DQ. Association study of a single nucleotide polymorphism in the exon 2 region of toll-like receptor 9 (TLR9) gene with susceptibility to systemic lupus erythematosus among Chinese. *Mol Biol Rep*. 2009;36(8):2245-2248.

25. Shahin RM, El Khateeb E, Khalifa RH, El Refai RM. Contribution of toll-like receptor 9 gene single-nucleotide polymorphism to systemic lupus erythematosus in Egyptian patients. *Immunol Invest*. 2016;45(3):235-242.

26. Auf A, Neseem N, Al-Harrass M. Toll-like receptor (TLR9)-1486 T/C (rs187084) gene polymorphism in Egyptian patients with rheumatoid arthritis. *Egypt Rheumatol*. 2019;41(3):173-176.