Original Article

Access this article online

Quick Response Code:

Website: www.ijhonline.org

DOI: 10.4103/ijh.ijh_40_20

Interplaying of regulatory T-cells and related chemokines in immune thrombocytopenic purpura patients

Zeyad A. Shabeeb, Yusur F. Faraj, Majed M. Mahmood[1], Baan Abdulatif Mtashar

Abstract:

BACKGROUND: Chronic immune thrombocytopenic purpura (ITP) is an immune-mediated bleeding disorder, in which platelets are opsonized by autoantibodies directed against platelet surface membrane glycoproteins, and prematurely cleared and destructed by Fc-receptors on the surface of macrophages in the reticuloendothelial system.

OBJECTIVES: This work is designed to show the contribution of lymphocyte subsets and platelet destruction in adult chronic ITP and role of infection.

MATERIALS AND METHODS: Transforming growth factor-β1 (TGF-β1) was measured using ELISA, and the frequency of regulatory T-cell (Treg) profile (CD4+CD25+CD127−) was investigated by FCM in blood samples of 50 Iraqi ITP patients (35 on-treatment ITP patients and 15 newly diagnosed) along with 20 age-matched healthy people that act as controls, as well as all patients were breath tested for detecting *Helicobacter pylori* using urea breath test. The study was carried out in the National Center of Hematology, Mustansiriyah University.

RESULTS: The results showed that although there was a significant reduction in Treg number in ITP patients compared with the control individuals ($P < 0.001$), the effect of treatment has shown a restored count of Tregs in comparison to the newly diagnosed ones ($P = 0.002$), while the assessment of cytokine serum level revealed that TGF-β1 was significantly increased ($P = 0.001$) in the on-treatment group of patients (TGF-β1 = 3.24 ± 0.3 ng/μl) in comparison with the nontreated group of patients (TGF-β1 = 1.75 ± 0.2 ng/μl). However, it was still significantly ($P < 0.001$) less than their values in the apparently healthy individuals (TGF-β1 = 9.0 ± 0.2 ng/μ). Moreover, 25 out of 50 (50%) showed positive results for the presence of *H. pylori*.

CONCLUSION: The present study revealed that Treg and its cytokines may play a fundamental role in the pathophysiology of adult chronic ITP since they contribute to the maintenance of peripheral immune tolerance. However, a causal link between *H. pylori* infection and ITP diseases is considerable.

Keywords:

Flow cytometry, *H. pylori*, immune thrombocytopenic purpura, regulatory T-cells, transforming growth factor-β1

National Center of Hematology, Mustansiriyah University, [1]Department of Immunology, College of Science, Mustansiriyah University, Baghdad, Iraq

Address for correspondence:
Dr. Zeyad A. Shabeeb, National Center of Hematology, Mustansiriyah University, Baghdad, Iraq.
E-mail: dr.ziadahmed@gmail.com

Submission: 08-05-2020
Accepted: 16-09-2020
Published: 10-11-2020

Introduction

Immune thrombocytopenic purpura (ITP) is an autoimmune bleeding disorder characterized by thrombocytopenia with platelet counts <100 × 10^9/L, in which patient's immune system is activated by platelet autoantigens resulting in immune-mediated platelet destruction and/or suppression of platelet production. ITP affects people of both genders at all ages. It is estimated to affect approximately 3.3/100,000 adults per year, higher in women than in men worldwide. Two distinct clinical syndromes manifest as an acute condition in children and a chronic condition in adults. The acute form often follows an infection and spontaneously resolves

How to cite this article: Shabeeb ZA, Faraj YF, Mahmood MM, Mtashar BA. Interplaying of regulatory T-cells and related chemokines in immune thrombocytopenic purpura patients. Iraqi J Hematol 2020;9:138-44.

within 2 months. Chronic immune thrombocytopenia persists longer than 6 months, with a specific cause being unknown.[1,2]

Although ITP patients may be asymptomatic, clinical features of ITP, including skin petechiae and bleeding in the mucous membranes or internal organs, are easily manifested if the platelet count falls below (20×10^9/L). The diagnosis of ITP depends on clinical characteristics and the laboratory examinations conducted, as well as the ability of excluding other agents associated with thrombocytopenia.[3,4]

The mechanism of ITP is multifactorial. It has been found that the loss of tolerance resulting from a decreased number and defective function of regulatory T-cells (Tregs) plays an important part in the progression of the disease. Moreover, a role for cytotoxic T-cells in direct lysis of platelets and megakaryocytes in the bone marrow has been proposed.[5,6] The high-level expression of the CD4 and CD25 surface markers of Tregs and the production of transforming growth factor-β1 (TGF-β1) suppress the proliferation of many immune cell types including T- and B-cells, either directly through cell contact or indirectly through secretion of cytokines, thereby dampening inappropriate immune activation and autoreactivity.[7]

Since TGF-β1 is a critical regulator of thymic T-cell development and differentiation during the immune response as well as a crucial player in peripheral T-cell homeostasis and tolerance to self-antigens, therefore, its association in ITP is thought to be as a potent inhibitor of megakaryocyte maturation, and thus, its level has been inversely correlated with the disease activity.[8]

In order to evaluate the role of immune cells and their cytokines in the pathogenesis of ITP, this study was designed to investigate the level of CD4+, CD25+, and CD127-profile markers to determine the percentage of regulatory T-lymphocyte using FCM technique, TGF-β1 cytokine to determine the activity of Tregs using ELISA assay. In addition, the present study aimed to evaluate the association of ITP disorder with some infectious diseases like *Helicobacter pylori* infection.

Materials and Methods

A control-based study has been carried out on Iraqi patients with ITP in the National Center of Hematology, Mustansiriyah University, Baghdad. Fifty patients are enrolled in this study (39 females and 11 males), with age ranging from 15 to 70 years; 35 of them were diagnosed as chronic ITP, with a history of disease from few months to several years, while 15 were newly diagnosed. Along with the patient group, 20 healthy controls with matched gender and age were involved and considered as a control group. This study is approved by the Ethical Committee of the National Center of Hematology, Mustansiriyah University, and appropriate patient and research study participant consent is obtained.

Parameters of study

patients were subjected to full medical history and complete clinical examination and clinical signs of ITP. All patients were investigated by complete blood picture (Convergence, Germany) and blood film, immunophenotyping profile of Tregs (CD4+CD25+CD127−) and serum level of TGF-β1, as well as the detection of *H. pylori*.

Sample collection

Blood samples were collected from all individuals (healthy controls and patients). About 5–10 ml of blood was aspirated using peripheral vein punctures and divided into 2 aliquots; the first one is transferred into EDTA tube for direct examination of CD markers, complete blood picture, and blood film. The second was dispensed in a nonheparinized plain tube and left for 15 min at 4°C to clot; then, it was centrifuged at 3000 rpm for 10 min to collect serum which stored in −80°C until be used for determination of TGF-β1 cytokine. However, breath samples were obtained only from the (50) patients to identify infections by *H. pylori*.

Immunophenotyping

In this study, immunophenotyping CD4+, CD25+, and CD127− (BD Biosciences, Germany) expression were investigated using fully equipped desktop four-color flow cytometry (FCM) (Partec, Germany). CyFlow Cube features a modular optical concept. This allows using different lasers as light sources. The CyFlow Cube allows easy optimization of the optics for any application by simple exchange of optical filters and mirrors.

Antibody labeling

One hundred microliters (μl) of whole blood or isolated leukocytes was mixed with 10 μl of conjugated antibodies in a test tube, mixed thoroughly, and then incubated for 15 min in the dark at room temperature.

Leukocytes fixation

From reagent A, 100 μl was mixed and incubated for 10 min in the dark at room temperature.

Erythrocyte lysis

From reagent B, 2.5 ml were added and shaked gently and incubated for 20 min in dark at room temperature.

The sample was then analyzed by flow cytometer.

Calculation of results

Data acquisition, instrument control, and data analysis

are controlled and performed by the CyView software (Cylab, USA).

Estimation of transforming growth factor-β1

It was performed according to TGF-β1 ELISA kit (Kombiotech, South Korea) using ELISA reader (Linear, China).

Determination of *Helicobacter pylori*

The test was estimated using *H. pylori* analyzer with breath card (Shenzhen Headway, China). the method as follows: Patients swallowed urea labelled with an uncommon isotope, non-radioactive carbon-13. In the subsequent 15 minutes, the detection of isotope-labelled carbon dioxide in exhaled breath indicates that the urea was split; this indicates that urease (the enzyme that *H. pylori* uses to metabolize urea) is present in the stomach, and hence that *H. pylori* bacteria are present. The device principle is based on the production of β ray by the nuclide radioactive decay on sample card when reaches the detector, it creates electrical pulse signal. The system processes the signal and then gives out the diagnostic results (negative or positive). In the meantime, the results are displayed on LCD display and are printed out through the printer.

Results

The two groups of patients and controls were matched based on age, gender, and their family history, as shown in Table 1.

Two immunological parameters were investigated in all healthy and patient participants; the immunophenotypic profile of Tregs and TGF-β1. Changes in concentration of these parameters in different settings and their significance were recorded and are shown in Table 2.

Table 1: Characterization of controls and patients according to age, gender, family history, and treatment

Characters	Controls	Patients
n	20	50
Age range	18- 69	15- 70
Mean	34.30	40.64
SD	8.9	16.7
Gender (%)		
♂	6 (30)	11 (22)
♀	14 (70)	39 (78)
Family history		
Positive	0	1
Negative	20	49
Treatment		
Positive	0	35
Negative	20	15

SD: Standard deviation

To reveal whether inefficient production of Tregs contributes to loss of peripheral tolerance among patients with chronic ITP, immunophenotyping profile represented by circulating CD4+CD25+CD127-cells were investigated, and the results came to state that the frequency of Tregs was diminished significantly in ITP patients compared to their counterparts of the control group (0.92 ± 0.1 vs. 4.80% ± 0.3%, $P < 0.001$). Blood samples obtained from the on-treatment patients have shown a significant higher Treg percentages (1.24% ± 0.2%) in comparison to the nontreated levels (0.23% ± 0.1%, $P = 0.002$) [Figure 1].

As the platelet counts reflect the disease activity and it is closely related to platelet destruction, the correlation between Tregs and the platelet counts was examined. The result showed a positive nonsignificant relationship in the control group ($P = 0.13$, $r = +0.35$) and patient group ($P = 0.58$, $r = +0.08$) [Figure 2].

To estimate the possible role of immune regulatory cytokine, TGF-β1, the level of this cytokine was evaluated in the sera of ITP patients as well as in healthy controls. The presented results indicated that TGF-β1 (2.75 ± 0. 25 vs. 9.00 ± 0.21) ng/ml was significantly decreased in ITP patients compared to the control values ($P < 0.0001$) which could not be achieved even in treated patients (TGF-β1 = 3.24 ± 0.33 ng/ml) in spite of the significant increase in its level in comparison to the nontreated patients (TGF-β1 = 1.75 ± 0.69 ng/ml, $P = 0.001$), as shown in Figure 3.

All patients were breath tested to indicate the presence of *H. pylori* infection. Half of the patients (50%) were infected with bacteria and developed chronic ITP.

Discussion

The exact mechanism of the immune dysfunction in

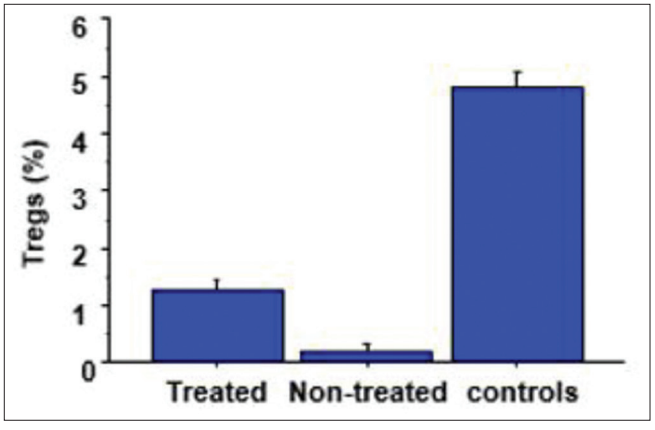

Figure 1: Percentages of regulatory T-cells in treated and nontreated groups of patients compared to healthy control group

Table 2: Evaluation of immunological parameters (Tregs and TGF-β1) in all patent groups and controls

Parameters	G_0 controls (*n*=20)	G_1 nontreated patients (*n*=15)	G_2 patients on treatment (*n*=35)	G_3 total patients (*n*=50)	Significance
Tregs (%) M±SE	4.80±0.3	0.23±0.1	1.24±0.2	0.92±0.1	G_0 versus G_3. (S) G_1. versus G_2 (S) G_1. versus G_3.(S) G_2. versus G_3.(S)
TGF-β1 (ng/μl) M±SE	9.0±0.2	1.75±0.7	3.24±0.3	2.75±0.3	G_0 versus G_3. (S) G_1. versus G_2 (S) G_1. versus G_3.(S) G_2. versus G_3.(S)

S: Significant differences, NS: Nonsignificant differences, G: Group, M: Mean, SE: Standard error, TGF-β1: Transforming growth factor-β1, Tregs: Regulatory T-cells

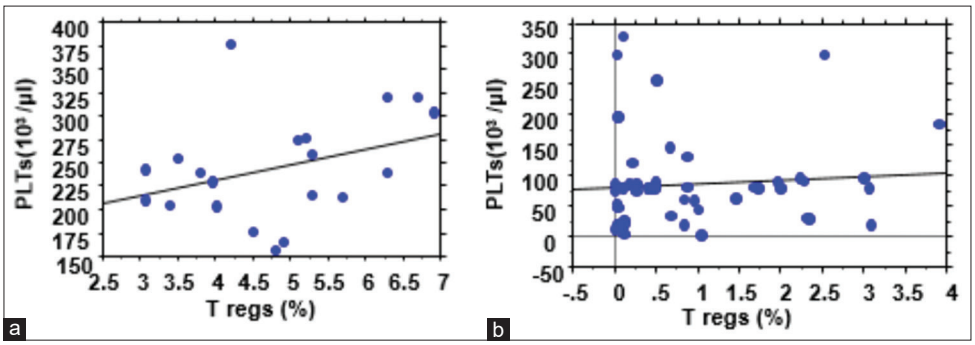

Figure 2: Correlative analysis of regulatory T-cell percentage and platelet counts (a) in control group (b) in patient group

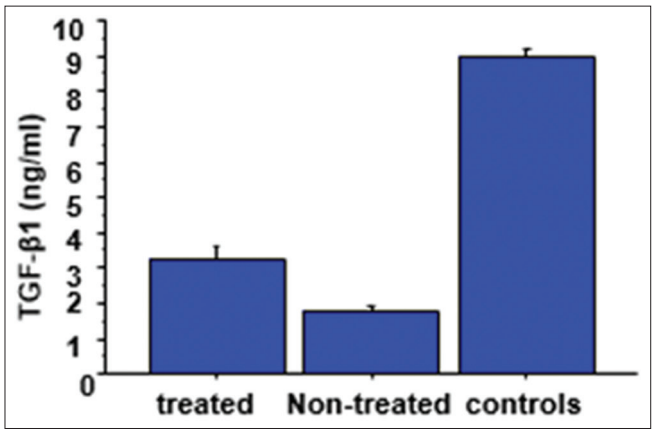

Figure 3: Levels of transforming growth factor-β1 in treated and nontreated groups of patients compared to healthy control group

ITP is generally not well known, but a number of T-cell abnormalities have been demonstrated in patients with ITP. These T-cell abnormalities may be characterized by abnormal numbers and functions of Tregs.[9,10] Tregs were expressing a panel of CD4+CD25+CD127−, and they secrete regulatory cytokines such as interleukin (IL)-10 and TGF-β1 to induce hemostasis and maintain peripheral immune tolerance.[11]

In the current study, the results have demonstrated that Tregs were reduced in number in ITP patients compared to healthy individuals, while the on-treatment group of patients has shown higher levels in comparison to the nontreated (newly diagnosed) individuals. Consistent with these results, there are many reports describing reduced numbers of Tregs in adult ITP patients.[9,12-14] Furthermore, many studies reported a highly significant decrease in the percentage of Tregs in children with acute ITP compared with controls.[15,16] Possible reasons for decreased Treg numbers can be due to impaired development, survival, proliferation, and/or stability of Tregs,[5] while Wu *et al*.'s study has shown that the percentages of CD4+CD25+CD127− cells were almost stable when determined by flow cytometry between ITP patients and healthy controls.[17]

Moreover, Yu *et al*. also found a comparable frequency of circulating CD4+CD25+Foxp3 + Tregs between the patients and the controls, so they suggested that functional defects, not the frequency, in Tregs contribute to the breakdown of self-tolerance in patients with chronic ITP.[18,19] The defects in Treg function may be explained by failed cell contact-dependent suppression or reduced secretion of cytokines including IL-10, TGF-β1, or IL-35 that mediate suppression.[20] Reduced Treg activity may also be due to increased resistance of effector T-cells to suppression.[18] Whereas many other studies concluded that both Treg frequency and their functional characteristics were defective in ITP patients and this might be responsible for loss of self-tolerance and subsequently destructive immune responses observed in ITP patients.[21] Meanwhile, researchers[5] mentioned that impaired regulatory compartment, including Tregs and Bregs, has been reported leading to immune dysregulation

in ITP patients. In response to dexamethasone therapy, a similar increase in Treg percentages was highlighted by others.[22] The same treatment-induced upregulation was found by Chun-Yan *et al*.[23] but with a higher level than the healthy controls, whereas using another protocol of treatment (rituximab), another study recorded that the elevation was not significantly different between patients in remission and controls.[24] All these results were contradicted with what was studied by Wu *et al*. who did not reach a significant change among the three groups (pretreatment patients, posttreatment patients, and healthy control).[17]

In contrast to this, Bakara *et al*. have found a significant positive correlation between Treg percentage and platelet counts in acute ITP patients indicating a close association between Treg percentage and the parameters known to reflect the degree of platelet destruction.[16] Meanwhile, defective Treg function and number may be explained by reduced secretion of cytokines that mediate suppression including IL-10, TGF-β, or IL-35.[20] In the current study, TGF-β1 was not significantly correlated with platelet counts, and then, it could affect the relation between Tregs and platelet counts, as shown in Figure 3. These findings raise the possibility that Tregs may regulate the disease phenotype, particularly in relation to the degree of thrombocytopenia. Furthermore, Zhang *et al*. found that the percentage of circulating Tregs may be decreased during active disease and the extent of this decrease correlates with the severity of the disease.[25]

TGF-β1 is a central player in maintaining the immune response balance, which belongs to regulator T-cell cytokine.[26] TGF-β1 was found to be an important inhibitor of B-cell proliferation and autoantibody production. It also suppresses some Th1 and Th2 cell-mediated autoimmune diseases.[27,28] The dominant function of TGF-β1 is to regulate peripheral immune homeostasis. This cytokine is considered as an additional mechanism responsible for peripheral tolerance. Accordingly, it seems that abnormal production of TGF-β1 by Tregs may represent additional mechanisms responsible for deleterious immune reactions occurring in ITP patients.[29]

This result may suggest that TGF-β1 low levels might be inversely correlated with the disease progression, and its protective effects against ITP development cannot be ignored, this might give a hope for a new strategy in the ITP treatment since it has no cure, and relapses may occur years after seemingly successful medical or surgical management.

These findings were in concordance with several previous reports which have shown that the levels of TGF-β1 cytokine were reduced in ITP patients,[8,26,30,31] while Panitsas *et al*. showed that although patients tended to have lower circulating TGF-β1 levels, the difference was not significant.[32]

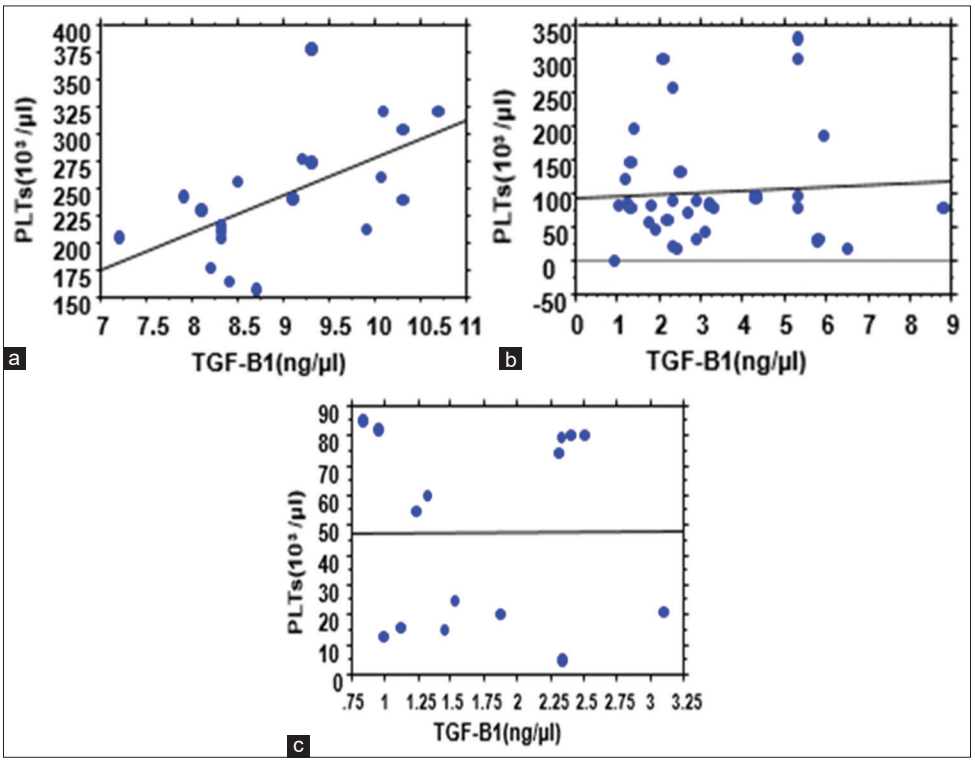

Figure 4: Correlative analysis of transforming growth factor-β1 levels and platelet counts (a) in control group (b) in nontreated patients (c) in the on-treatment group of patients

In respect to the response to treatment, treated patients show significantly higher levels of TGF-β1 compared to nontreated ones, yet they were significantly lower than the healthy controls. Such findings support some other results found by Guo *et al*. who recorded similar patterns of response of TGF-β1 levels, yet Li *et al*. have stated that the treatment-induced increment was significantly higher than the healthy group.[31,33] This inversely related relationship between TGF-β1 and the disease progression might provide an idea of producing drugs that stimulate TGF-β1 secretion for ITP treatment.

This study showed that circulating TGF-β1 levels are strongly correlated with the platelet counts in the healthy control group (P = 0.01, r = +0.59), and this correlation turned out to be weak and nonsignificant in patient groups (newly diagnosed and on-treatment patients) (P = 0.95, r = +1.52; P = 0.7, r = 0.07), respectively [Figure 4].

While, in a study done by Bao *et al*., the correlative analysis indicated a strong positive correlation between the levels of TGF-β1 and the degree of improvement in platelet counts.[34]

Conclusion

The present study revealed that Treg and its cytokines may play a fundamental role in the pathophysiology of adult chronic ITP since they contribute to the maintenance of peripheral immune tolerance. However, because of the high ratio of patients revealing positive urea breath test, a causal link between *H. pylori* infection and ITP can be suggested.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1. Yu L, Zhang Ch, Zhang L, Shi Y and Ji X. Biomarkers for immune thrombocytopenia. Biomark Res 2015;3:19.
2. Matzdorff A, Meyer O, Ostermann H, Kiefel V, Eberl W, Kuhne T, *et al*. Immune thrombocytopenia Current diagnostics and therapy: Recommendations of a joint working group of DGHO, ÖGHO, SGH, GPOH, and DGTI. Oncol Res Treat 2018;41 (suppl 5):1-30.
3. Sun L, Yu Z, Bu Y, Su J, Wang C, Cao L, *et al*. The clinical studies of 51 patients with thrombotic thrombocytopenic purpura. Zhonghua Xue Ye Xue Za Zhi 2014;35:147-51.
4. Arnold DM, Nazy I, Clare R, Jaffer AM, Aubie B, Li N, *et al*. Misdiagnosis of primary immune thrombocytopenia and frequency of bleeding: Lessons from the McMaster ITP Registry. Blood Adv 2017;1:2414-20.
5. Yazadanbakhsh K, Zhong H, Bao W. Immune dysregulation in immune thrombocytopenia (ITP). Semin Hematol 2013;50:S63-7.
6. Behzad MM, Asnafi AA, Jalalifar MA, Moghtadaei M, Jaseb K, Saki N. Cellular expression of CD markers in immune thrombocytopenic purpura: Implications for prognosis. APMIS 2018;126:523-32.
7. Sakaguchi S, Miyara M, Costantino CM, Hafler DA. FOXP3+regulatory T cells in the human immune system. Nat Rev Immunol 2010;10:490-500.
8. Andersson PO, Olsson A, Wadenvik H. Reduced transforming growth factor-beta1 production by mononuclear cells from patients with active chronic idiopathic thrombocytopenic purpura. Br J Haematol 2002;116:862-7.
9. Ling Y, Cao X, Yu Z, Ruan C. Circulating dendritic cells subsets and CD4+Foxp3+regulatory T cells in adult patients with chronic ITP before and after treatment with high-dose dexamethasome. Eur J Haematol 2007;79:310-6.
10. Aslam R, Hu Y, Gebremeskel S, Segel GB, Speck ER, Guo L, *et al*. Thymic retention of CD4+CD25+FoxP3+T regulatory cells is associated with their peripheral deficiency and thrombocytopenia in a murine model of immune thrombocytopenia. Blood 2012;120:2127-32.
11. Shi Z, Xue Y, Xue Y, Zhi Z. Expression and significance of CD4+CD25+CDl27low regulatory T Cells, TGF-β and Notch1 mRNA in patients with idiopathic Thrombocytopenic Purpura 2015;23:1652-6.
12. Liu B, Zhao H, Poon MC, Han Z, Gu D, Xu M, *et al*. Abnormality of CD4(+) CD25(+) regulatory T cells in idiopathic thrombocytopenic purpura. Eur J Haematol 2007;78:139-43.
13. Nishimoto T, Kuwana M. CD4+CD25+Foxp3+regulatory T cells in the pathophysiology of immune thrombocytopenia. Semin Hematol 2013;50 Suppl 1:S43-9.
14. Dana N, Brooks B. Pathophysiology update and diagnostic dilemmas. Vet Clin Pathol 2019;48:17-28.
15. Zahran AM, Elsayh KI. CD4+CD25+High Foxp3+regulatory T cells, B lymphocytes, and T lymphocytes in patients with acute ITP in Assiut Children Hospital. Clin Appl Thromb Hemost 2014;20:61-7.
16. Bakara A, Boria M, Hesham MA, Almalky MA. Study of T-regulatory cells in patients with acute, idiopathic thrombocytopenic purpura. Egyp J Haematol 2014;39:37-41.
17. Wu B, Zhan Y, Li F, Cheng L, Zou SH, Cheng Y. Functional plasticity in CD4+CD25+CD127- Treg population in primary immune thrombocytopenia. Blood 2015;126:3470.
18. Yu J, Heck S, Patel V, Levan J, Yu Y, Bussel JB, *et al*. Defective circulating CD25 regulatory T cells in patients with chronic immune thrombocytopenic purpura. Blood 2008;112:1325-8.
19. Zhu Y, Zhu H, Xie X, Zheng Z, Ling Y. MicroRNA expression profile in Treg cells in the course of primary immune thrombocytopenia. J Investig Med 2019;67:1118-24.
20. Buckner JH. Mechanisms of impaired regulation by CD4(+) CD25(+)FOXP3(+) regulatory T cells in human autoimmune diseases. Nat Rev Immunol 2010;10:849-59.
21. Arandi N, Mirshafiey A, Tehrani MJ, Shaghaghi M, Sadeghi B, Abolhassani H, *et al*. Alteration in frequency and function of CD4+CD25+FOXP3+ regulatory T cells in patients with immune thrombocytopenic Purpura. Iran J Allergy Asthma Immunol 2014;13:85-92.
22. Huang WY, Sun QH, Chen YP. Expression and significance of CD4+CD25+CDl27low regulatory T cells, TGF-β and Notch1 mRNA in patients with idiopathic Thrombocytopenic Purpura 2015;23:1652-6.
23. Chun-Yan W, Xiao-Juan Z, Ming H, Yan S, Jun P, Jian-Zhi S, *et al*. Effect of high-dose dexamethasone on BAFF and tregs in patients with immune thrombocytopenic purpura cite as. Chin J Hematol 2010;31:164-7.
24. Stasi R, Cooper N, Del Poeta G, Stipa E, Laura Evangelista M, Abruzzese E, *et al*. Analysis of regulatory T-cell changes in patients with idiopathic thrombocytopenic purpura receiving B

cell-depleting therapy with rituximab. Blood 2008;112:1147-50.
25. Zhang XL, Peng J, Sun JZ, Liu JJ, Guo CS, Wang ZG *et al*. De novo induction of platelet-specific CD4(+)CD25(+) regulatory T cells from CD4(+)CD25(-) cells in patients with idiopathic thrombocytopenic purpura. Blood. 2009;113:2568-77. doi: 10.1182/blood-2008-03-148288. Epub 2008 Dec 3. PMID: 19056692.
26. Ma L, Liang Y, Fang M, Guan Y, Si Y, Jiang F, Wang F. The cytokines (IFN-γ, IL-2, IL-4, IL-10, IL-17) and Treg cytokine (TGF-β1) Levels in adults with immune thrombocytopenia. Pharmazie 2014;69:694-7.
27. Fujio K, Okamura T, Yamamoto K. The Family of IL-10-secreting CD4+T cells. Adv Immunol 2010;105:99-130.
28. Zhong H, Liu Y, Xu Z, Liang P, Yang H, Zhang X, *et al*. TGF-β-induced CD8+CD103+ regulatory T cells show potent therapeutic effect on chronic graft-versus-host disease lupus by suppressing B cells. Front Immunol 2018;9:35.
29. Malhotra N, Kang J. SMAD regulatory networks construct a balanced immune system. Immunology 2013;139:1-0.
30. Shevach EM, Stephens GL. The GITR-GITRL interaction: Co-stimulation or contrasuppression of regulatory activity? Nat Rev Immunol 2006;6:613-8.
31. Guo C, Chu X, Shi Y, He W, Li L, Wang L, *et al*. Correction of Th1-dominant cytokine profiles by high-dose dexamethasone in patients with chronic idiopathic thrombocytopenic purpura. J Clin Immunol 2007;27:557-62.
32. Panitsas FP, Theodoropoulou M, Kouraklis A, Karakantza M, Theodorou GL, Zoumbos NC, *et al*. Adult chronic idiopathic thrombocytopenic purpura (ITP) is the manifestation of a type-1 polarized immune response. Blood 2004;103:2645-7.
33. Li W, Wang X, Li J, Liu M, Feng J. [A study of immunocyte subsets and serum cytokine profiles before and after immunal suppression treatment in patients with immune thrombocytopenia]. Zhonghua Nei Ke Za Zhi 2016;55:111-5.
34. Bao W, Bussel JB, Heck S, He W, Karpoff M, Boulad N, *et al*. Improved regulatory T-cell activity in patients with chronic immune thrombocytopenia treated with thrombopoietic agents. Blood 2010;116:4639-45.