Zagazig University Medical Journal
www.zumj.journals.ekb.eg
https://doi.org/10.21608/zumj.2024.304491.3476 Volume 30, Issue 9, December. 2024
Manuscript id: ZUMJ-2407-3476
Doi: 10.21608/ZUMJ.2024.304491.3476

ORIGINAL ARTICLE

The Role of Lymphocyte Subsets in the Pathogenesis of Immune Thrombocytopenia in Children

Tamer Hassan[1], Asmaa Esh [2], Samah Abdel Hamid [1*], Ahmed Emam [1]
[1] Pediatrics Department, Faculty of Medicine, Zagazig University, Egypt
[2] Clinical Pathology Department, Faculty of Medicine, Zagazig University, Egypt

***Corresponding author:**
Samah Abdel Hamid
Email:
samahbassam39@gmail.com

Submit Date: 25-07-2024
Revise Date: 02-09-2024
Accept Date: 03-09-2024

ABSTRACT

Background: Immunological tolerance is compromised in children with immunological thrombocytopenic purpura (ITP), an autoimmune disease. The purpose of this study was to measure the levels of lymphocyte subsets in children with ITP and to assess how these subsets are related to the patient's chronic illness and responsiveness to treatment.

Methods: The pediatric hematology outpatient clinic at Zagazig University Hospitals served as the site of this case-control study. Sixty-four patients (16 with newly diagnosed ITP, 16 with persistent ITP, 16 with chronic ITP, and 16 healthy children as a control group) participated in the study. Using flow cytometry, all of the study's patients and controls had their lymphocyte subsets evaluated.

Results: CD3+, CD4+, and CD56+ lymphocytes were significantly lower in patients with ITP compared to controls while CD8+ and CD19+ lymphocytes were significantly higher in patients with ITP compared to controls. Apart from CD8+ lymphocytes which were significantly higher in patients with chronic ITP compared to other patients' groups, there was no significant difference among different patients' groups in relation to different lymphocyte subsets. Patients with newly diagnosed ITP who responded to 1st line therapy had lower CD4+ and higher CD8+ and CD19+ lymphocytes compared to those who did not respond to 1st line therapy.

Conclusion: We concluded that lymphocyte subsets play a significant role in the pathogenesis, chronicity, and response to treatment in childhood ITP.

Keywords: lymphocyte, Immune thrombocytopenia, children

INTRODUCTION

Autoimmune disorders are defined by several variables that lead to a collapse in self-tolerance, or the immune system's capacity to recognize oneself from non-self and avoid attacking oneself [1].

A platelet count of less than 100,000/microL with normal white blood cell and hemoglobin levels count, known as isolated thrombocytopenia, is the diagnostic feature of pediatric immune thrombocytopenia (ITP). Although the origin of ITP is still mostly unknown, other immunologic or environmental factors, such as viral infections, may be to blame [2].

ITP is caused by an aberrant autoantibody binding to circulating platelet membranes. The immune system's IgG, which is specific to one or more glycoproteins found on the platelet membrane, is frequently this autoantibody. Platelets coated with autoantibodies cause mononuclear macrophages to phagocytose them via the Fc receptor, mostly (though not only) in the spleen [3].

The pathophysiology of ITP is significantly influenced by anomalies in T cells, dendritic cells, natural killer cells, cytokines, oxidative stress, infection, pregnancy, and programmed cell death and medications, according to several recent researches [4].

Among these anomalies are the following: a rise in T helper 1 (Th1) cells; a decline in inhibitory ability of regulatory T cells (Tregs); and cytotoxicity of T cells leading to platelet destruction [4].

Additionally, in ITP patients, dysregulated T cells may encourage the development of platelet autoantibodies, directly damage platelets, and hinder megakaryocytes' ability to produce platelets [5].

T-cell-mediated immunological disorders have gained recognition as a significant contributor to

the pathophysiology of ITP in recent decades. T cell anomalies include a marked shift in favor of Th17 pro-inflammatory immune responses and Th1 cells [6], a reduction in the quantity or poor performance comprising cytotoxic T lymphocytes (CTLs) and regulatory T cells (Treg) that destroy platelets [7,8].

Additionally, there have been multiple reports of naturally existing killer (NK) cells in peripheral blood from ITP patients at different concentrations, notwithstanding their functional deficiency [9].

The etiology of ITP in children is multifaceted. Recent findings indicate that the pathophysiology of this illness involves a sophisticated dysregulation of the immune system. Nevertheless, there aren't many publications that have addressed this problem, particularly in Egypt.

Our objectives were to ascertain the lymphocyte subset levels in children suffering from immunological thrombocytopenia (ITP) and to assess its relationship with response to treatment and disease chronicity in these patients.

METHODS

In the pediatric hematology outpatient clinic at Zagazig University Hospitals, a case-control study was carried out. It included 64 participants; group 1: 16 patients with newly diagnosed ITP (duration of disease less than 3 months), group 2: 16 patients with persistent ITP (duration of disease from 3 to 12 months), group 3: 16 patients with chronic ITP (disease duration greater than 12 months),4: 16 healthy children as a control group.

Individuals with immunological thrombocytopenia of both sexes and ages ranging from 1 to 18 years were eligible for inclusion. Individuals suffering from different forms of thrombocytopenia, those with secondary immune thrombocytopenia.

Less than 100,000 platelets/µL is in agreement with the 2011 American Society of Hematology (ASH) clinical practice guidelines used to diagnose ITP when there were no other possible causes or illnesses that could be linked to thrombocytopenia. ITP was categorized as chronic (lasting longer than 12 months), persistent (lasting from 3 months to 12 months), or newly diagnosed (diagnosed to 3 months) [10].

Every patient in this study underwent a complete medical history, a careful clinical examination, and standard investigations, such as CBC and ur local standards. In this study, flow cytometry was used to identify lymphocyte subsets in all patients and controls (CD3: T cell marker, CD4: T helper marker, CD8: T cytotoxic marker, CD 19: B cell marker, CD 56: Natural Killer cell marker).

Blood collection

On the day of collection, blood samples were processed in one hour while being kept at room temperature. An automated hematology analyzer (Sysmex XN1000, Japan) was used to collect whole blood aseptically into tubes containing potassium ethylenediaminetetraacetic acid for differential and total blood counts as well as immunophenotyping using flow cytometry. The analysis parameters included the number of white blood cells (WBC), percentages and numbers of lymphocytes, $CD3^+$ cells, $CD3^+CD4^+$ cells, $CD3^+CD8^+$ cells, $CD3^-CD19^+$ cells, and $CD3^-CD56^+$ cells.

Flow cytometric analysis

Premixed monoclonal antibodies (mAbs) in 12x75-mm capped polypropylene test tubes (BD PharmingenTM, San Diego, CA, USA) were filled with aliquots (50µl) of whole blood. The most effective dosage for each antibody was determined by repeatedly diluting the antibody in an initial titration experiment. The amount of each antibody that was determined was used to produce two sets of antibody cocktails. In one test tube, mice were conjugated Fluorescein isothiocyanate (FITC) and Allophycocyanin (APC) are utilized in conjunction with mice anti-human antibodies. CD45 mAb, phycoerythrin (PE) for anti-human CD8 mAb, and PE cyanin5 for anti-human CD4 mAb. Mice were conjugated with anti-human CD3 mAb (FITC), anti-human CD56 mAb (PE), mouse anti-human CD19 mAb (PE cyanin5), and mouse anti-human CD45 mAb (APC) in the other test tube. Following a 20-minute room temperature incubation period in a dark room with each set of antibodies, red blood cells were lysed for 15 minutes using 440µl of FACS lysing solution (BD Biosciences, San Jose, CA, USA). The results were immediately assessed using a flow cytometer (BD FACSCalibur, San Jose, CA, USA) after 50µl of CountBrightTM absolute counting beads (Molecular Probes, Carlsbad, CA, USA) were added to each cell-staining tube. The data was then evaluated utilizing the CellQuestTM pro software (BD, San Jose, CA, USA).

Ethics Considerations :

The Institutional Reviewer Board (IRB #5104-14-3-2019) of the Faculty of Medicine, Zagazig University Hospital, authorized this study ethically. The research was carried out adhering to The code of ethics for research involving human subjects established by the World Medical Association, known as the Declaration of Helsinki.

STATISTICAL ANALYSIS

SPSS version 20 (Armonk, NY: IBM Corp.) was used to verify, enter, and analyze the data. For quantitative variables, mean ± standard deviation was used to express the results; for qualitative variables, the number and percentage were used. The t-test for unpaired students, the chi-square test (X2), ANOVA (F test), and Pearson coefficient of correlation (r) were used as necessary. Significance is attributed to results with p-values < 0.05, whereas p-values < 0.001 are considered highly significant.

RESULTS

At diagnosis, the average age was 6.8 years. Compared to other patients and controls, people with persistent ITP were noticeably older. Gender matching was used to match patients and controls. Table 1 shows the demographic details of the research participants.

Regarding initial clinical presentation, purpura, ecchymosis, and wet bleeding were the most common presenting symptoms. When it came to the initial clinical presentation, there was not a noticeable distinction between the patient cohorts (Table 2).

The initial mean platelet count in our patients was 21.9 X103 /uL. As opposed to individuals with long-term ITP, those with recently diagnosed persistent ITP had considerably lower platelet counts (19.4, 18.9, and 27.5 $X10^3$ /uL respectively).

As regards the treatment lines, steroids were the most used 1st line therapy in all patient groups. Thrombopoietin receptor agonists (TPO-RAs) were the most used 2nd line therapy in individuals with chronic and ongoing ITP.

Regarding lymphocyte subsets, CD3+, CD4+, and CD56+ Lymphocyte levels were significantly reduced in patients with ITP compared to the control group. CD8+ and CD19+ lymphocytes were significantly higher in patients with ITP compared to controls (Table 3). Among patient groups, apart from CD8 which was not substantially different between patient groups with regard to various lymphocyte subsets, however, the incidence was much greater in individuals with chronic ITP as compared to other patient groups (Table 4).

There was no significant difference between different lymphocyte subsets and any of age, age at diagnosis, gender, or initial platelet count (P > 0.05).

Lower CD4 levels were seen in patients with recently diagnosed ITP who responded to first-line therapy and greater CD8 and CD19 compared to those who did not respond to first-line therapy, indicating a connection between lymphocyte subsets and response to first-line therapy (Table 5).

Table 1: Demographic characteristics of different study groups

Variable	Newly diagnosed ITP N=16	Persistent ITP N=16	Chronic ITP N=16	Controls N=16	Test	P value
Age Mean ± SD	6.81± 2.97	8.43± 2.53	11.56± 2.73	7± 3.65	F= 8.57	0 .00008
Age at diagnosis Mean ± SD	6.81± 2.97	8.43± 2.53	9.37± 2.06	7± 3.65	F= 2.9	0.04
Sex (n, %) Male Female	9 (56%) 7 (44%)	8 (50%) 8 (50%)	10 (62.5%) 6 (37.5%)	8 (50%) 8 (50%)	X^2=1.19	0.75

Table 2: Clinical characteristics of different patient groups

Variable	Newly diagnosed ITP N=16	Persistent ITP N=16	Chronic ITP N=16	Test	P value
Purpura (n, %)	13 (81.25%)	14 (87.5%)	14 (87.5%)	X^2=0.8	0.93
Ecchymoses (n, %)	12 (66.7%)	12 (66.7%)	10 (62.5%)		
Wet bleeding (n, %)	9 (56.25%)	10 (62.5%)	6 (37.5%)		

Table 3: Lymphocyte subsets in patients and controls

Lymphocytes subsets	Patients N= 48	Controls N=16	Test	P value
CD3+	59.4± 6.6	72.5± 1.8	t= -7.73325.	< 0.00001
CD4+	27.4± 5.6	33.3± 2.7	t=-3.94697	0.000102
CD8+	31.3± 5.5	22.3± 4.6	t= 5.58837.	<0 .00001
CD19+	22.5± 4.3	17.8± 1.5	t= 4.21438.	0. 000041
CD56+	5.7± 2.1	9.8± 3.6	t=-5.44972	<0 .00001

The data are expressed as mean + SD

Table 4: Lymphocyte subsets in different patient groups

Lymphocytes subsets	Newly diagnosed ITP N=16	Persistent ITP N=16	Chronic ITP N=16	Test	P value
CD3+	58.7± 7.1	61.5 ± 3.9	57.8± 8.2	F= 1.3	0.28
CD4 +	25.3± 6.3	27.9± 5.2	29± 5.1	F= 1.86	0.17
CD8 +	29.1± 4.8	30.4± 6.7	34.3±4.0	F= 4.1	0.02
CD19 +	23.1± 3.3	21.7± 5.4	22.8±4.4	F= 0.4	0.65
CD56+	5.9± 2.2	5.7± 2.5	5.6±1.7	F= 0.07	0.93

The data are expressed as mean + SD.

Table 5: Relationship between lymphocyte subsets and response to 1st line therapy in patients with newly diagnosed ITP

Lymphocyte subsets	Responders	Non-responders	Test	P value
CD3+	58.03± 9.04	59.3± 0.60	t=-0.29	0.39
CD4+	20.9±4.6	30.88±1.09	t=-4.41	0.0004
CD8+	32.6 ± 3.2	24.51± 1.12	t=5.15	0.0001
CD19+	24.65± 2.5	21.2± 1.47	t=2.6	0.01
CD56+	5.78± 2.66	6.2±0.98	t=-0.32	0.38

The data are expressed as mean + SD

DISCUSSION

ITP has a very complex and heterogeneous pathogenesis. T-cell-mediated immunological disorders have gained recognition as being just as significant in the etiology of ITP in recent decades [6-8].

The mean age at diagnosis in our study was 6.8 years. In the group of individuals who were recently diagnosed, 56% were males and 44% were females. Both males and females were equally impacted in cases of persistent ITP. Purpura was the most common initial clinical presentation in patients with ITP followed by ecchymoses and wet bleeding.

Our results matched with those documented in the literature, which indicates that children with ITP

are usually between the ages of two and seven. Both sexes are equally affected. Recent research, however, indicates that the male-to-female ratio was higher in infancy and decreased as people grew older. Almost all patients get petechial rashes and bruising as soon as the disease manifests [11]. In children with ITP, severe bleeding with a risk of death is uncommon (0.2–0.9%) [12].

Alternatively, **Hamed et al. [13]** found that 84.4% of their ITP patients were female and 15.6% of their patients were male. Additionally, investigations on individuals with ITP by **Del Vecchio et al. [14]** and **Talaat et al. [9]** demonstrated that ITP in adulthood affects girls more than males.

This could be clarified by the fact that our study focused exclusively on ITP-affected youngsters, whereas other research had adults with the condition as its subject.

The initial mean platelet count in our subjects was 21.9 $\times 10^3$/uL. Compared to individuals with chronic ITP, Individuals with persistent and recently diagnosed ITP had considerably lower platelet counts (19.4, 18.9, and 27.5 $\times 10^3$ /uL, respectively). It is important to consider that in children with ITP, a higher platelet count at the time of diagnosis is associated with a greater likelihood of developing a chronic condition.

Grimaldi-Bensouda et al. [15] observed that the only factor that may predict chronicity was a higher baseline platelet count [Odds Ratio 1.03; 95%CI: 1.00, 1.06], which provided good support for our findings. Similarly, individuals who acquired chronic ITP had a considerably increased platelet count observed on first diagnosis, as reported by **Heitink-Pollé et al. [16].** The mean difference was 5.27 (95% CI 2.69-7.86).

Although the proportion of female patients with chronic ITP exceeded that of male individuals who have been diagnosed recently versus those who are persistent was not statistically significant. In a comprehensive meta-analysis, **Heitink-Pollé et al. [16]** found that one of the predictors of chronicity was female gender (odds ratio [OR] 1.17, 95% confidence interval [CI] 1.04-1.31).

The age of the patients, the age at diagnosis, and the chronicity of the condition were all found to be significantly correlated in our study. This result was consistent with other earlier studies that found that among children with ITP, older people are more likely to have chronic illnesses **Heitink-Pollé et al. [16]** a key predictor of chronicity, according to their thorough Meta-analysis indicates that those who present at an older age are more likely to be affected (age ≥11 years; OR 2.47, 95% CI 1.94-3.15).

Our results showed that CD3+, CD4+, and CD56+ lymphocytes were substantially lower in individuals with ITP than with controls while CD8+ and CD19+ lymphocytes were Patients with ITP exhibited markedly elevated levels compared to the control group.

Kuang et al. conducted a study on 88 ITP children before treatment, 84 ITP children following treatment, and 45 normal controls. They discovered that all ITP children had significantly lower levels of Th helper (Th), The levels of Natural Killer (NK) cells, Th/Tc ratio and CD19+ B cells were significantly higher ($P<0.05$) than in the control group, while cytotoxic T cells (Tc) and natural killer (NK) were not as high **[17].**

In our study, apart from CD8+ lymphocytes which were significantly higher in patients with chronic ITP compared to other patients' groups, there was no significant difference among different patients' groups in relation to different lymphocyte subsets.

Our data were matched with **Lin et al.[18]** they discovered that the percentage of CD8 + cells in their study of 37 adult patients, 13 having recently been diagnosed with immune thrombocytopenic purpura (ITP), six individuals experiencing ongoing ITP, and 18 with chronic ITP chronic ITP was substantially greater than that of newly diagnosed ITP and healthy controls ($p < 0.05$, $p < 0.01$).

In our study, patients with recently discovered ITP who improved with first-line therapy had lower CD4 + lymphocytes and higher CD8+ and CD19 + lymphocytes in contrast to individuals who did not react to first-line treatment.

Similar to our research, **Rong et al. [19]** discovered that patients with higher CD4+ T cell counts had a lower propensity. The study found a positive correlation ($r = 0.69$, $P = 0.04$) between the effectiveness of corticosteroids and the identification of immune subsets that could be beneficial for ITP patients in terms of their prognosis.

On the contrary, **Žibřidová et al. [20]** on 35 ITP patients, found that those who responded to treatment had lower CD8+ T cell counts ($P = 0.02$), greater CD4/CD8 ratio The median value of 2.1 was seen in responders, compared to 1.5 in non-respondents, with a statistically significant difference ($P = 0.04$). Additionally, responders had a higher percentage of antiplatelet autoantibodies (median 58%) compared to non-responders (median 20%), also with a statistically significant difference ($P = 0.04$), before treatment.

The discrepancy among different studies could be attributed to the size of the patient sample, age of patients, whether children or adults, ethnic variations, and heterogeneity of the selected treatment plan, which results in different treatment outcomes.

CONCLUSION

We concluded that in childhood ITP, lymphocyte subsets are important for the pathophysiology, chronicity, and response to treatment. To validate these results, larger multicenter studies are still required.

Conflict of interest: The authors declare no conflict of interest.

Financial Disclosures: This study was not supported by any source of findings.

Sources of funding: No specific grant was obtained for this research from governmental, private, or nonprofit funding organizations.

Availability of data

Data supporting the results of this article are included within the article.

Author contributions

All the authors carried out this work **Tamer Hassan** and **Ahmed Emam** designed and directed the study. **Asmaa Esh** performed the investigations and analyzed the data.

All authors were involved in drafting the article and revising it for important intellectual content and all authors read and approved the final version to be published.

Acknowledgment

The authors would like to thank all the participants and the hospital staff who contributed to this study.

REFERENCES

1- Tao JH, Cheng M, Tang JP, Liu Q, Pan F, Li XP. Foxp3, regulatory T cell, and autoimmune diseases. Inflammation. 2017 Feb;40:328-39..
2- Rodeghiero F, Stasi R, Gernsheimer T, Michel M, Provan D, Arnold DM et al. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. Blood, Clin J Am Soc Hematol. 2009 Mar 12;113(11):2386-93.
3- Audia S, Mahévas M, Samson M, Godeau B, Bonnotte B. Pathogenesis of immune thrombocytopenia. Autoimmun Rev. 2017 Jun 1;16(6):620-32.
4- Yang ZZ, Novak AJ, Ziesmer SC, Witzig TE, Ansell SM. Attenuation of CD8+ T-cell function by CD4+ CD25+ regulatory T cells in B-cell non-Hodgkin's lymphoma. Cancer Rese. 2006 Oct 15;66(20):10145-52.
5- Zhan Y, Hua F, Ji L, Wang W, Zou S, Wang X, et al. Polymorphisms of the IL-23R gene are associated with primary immune thrombocytopenia but not with the clinical outcome of pulsed high-dose dexamethasone therapy. Ann Hematol. 2013 Aug;92:1057-62.
6- Rocha AM, Souza C, Rocha GA, de Melo FF, Clementino NC, Marino MC et al. The serum levels of the cytokines involved in the Th17 and Th1 cell commitment are increased in individuals with borderline thrombocytopenia. J hematol oncol. 2013 Dec;6:1-3.
7- Sakakura M, Wada H, Tawara I, Nobori T, Sugiyama T, Sagawa N et al. Reduced Cd4+ Cd25+ T cells in patients with idiopathic thrombocytopenic purpura. Thrombosis Rese. 2007 Jan 1;120(2):187-93.
8- Chow L, Aslam R, Speck ER, Kim M, Cridland N, Webster ML, et al. A murine model of severe immune thrombocytopenia antibody- is induced by antibody-and CD8+ T cell-mediated responses that are differentially sensitive to therapy. Blood. J Amer Society Hematol. 2010 Feb 11;115(6):1247-53.
9- Talaat RM, Elmaghraby AM, Barakat SS, El-Shahat M. Alterations in immune cell subsets and their cytokine secretion profile in childhood idiopathic thrombocytopenic purpura (ITP). Clinical Experimental Immunol. 2014 May;176(2):291-300.
10- Neunert C, Lim W, Crowther M, Cohen A, Solberg Jr L, Crowther MA. The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia. Blood, J Amer Soc Hematol. 2011 Apr 21;117(16):4190-207.
11- Fogarty PF, Segal JB. The epidemiology of immune thrombocytopenic purpura. Curr Opin Hematol. 2007 Sep 1;14(5):515-9.
12- Bolton-Maggs P. Severe bleeding in idiopathic thrombocytopenic purpura. J Pediatr Hematol Oncol. 2003 Dec 1;25:S47-51.
13- Hamed H, Moussa M, Fathey H, Tolba H. Role of measurement of interleukin 10 in idiopathic (immune) thrombocytopenic purpura. J Pediatr Hematol Oncol. 2017 Oct 1;42(4):148-54.
14- Del Vecchio GC, Giordano P, Tesse R, Piacente L, Altomare M, De Mattia D. Clinical significance of serum cytokine levels and thrombopoietic markers in childhood idiopathic thrombocytopenic purpura. Blood Transfusion. 2012 Apr;10(2):194.
15- Grimaldi-Bensouda L, Nordon C, Michel M, Viallard JF, Adoue D, Magy-Bertrand N et al. Immune thrombocytopenia in adults: a prospective cohort study of clinical features and predictors of outcome. haematol. 2016 Sep;101(9):1039.

16- Heitink-Polle KM, Nijsten J, Boonacker CW, de Haas M, Bruin MC. Clinical and laboratory predictors of chronic immune thrombocytopenia in children: a systematic review and meta-analysis. Blood. J Amer Soc Hematol. 2014 Nov 20;124(22):3295-307.
17- Kuang W, Zheng M, Li W, Yang H, Li R. Immune dysfunction of regulatory B cells and lymphocyte subsets in children with primary immune thrombocytopenia. J Chinese Physician. 2021:1321-5.
18- Lin X, Xu A, Zhou L, Zhao N, Zhang X, Xu J, et al. Imbalance of T lymphocyte subsets in adult immune thrombocytopenia. Int J Gen Med. 2021 Mar 18:937-47.
19- Rong W, Yan-xiang Z, Shan-shan X, Ju-mei S. Lymphocyte subsets in primary immune thrombocytopenia. Blood Coagulation Fibrinolysis. 2014 Dec 1;25(8):816-9.
20- Žibřidová K, Souček O, Krčmová LK, Jankovičová K, Gančarčíková M, Pejková MA et al. Lymphocyte subpopulations: a potential predictor of a response in patients with immune thrombocytopenia. Hematol. 2024 Dec 31;29(1):2304486.

Citation

Hassan, T., Esh, A., Abdel Hamid, S., Emam, A. The Role of Lymphocyte Subsets in the Pathogenesis of Immune Thrombocytopenia in Children. ***Zagazig University Medical Journal*****, 2024; (4523-4529): -. doi: 10.21608/zumj.2024.304491.3476**