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Original Article

Study of T cells and natural killer cells expression in patients with immune thrombocytopenic purpura

Ahmed Ahmed Allam, Eman Hassan Ali , Sara Mustafa Hashem Hamdan, Shereen Phillip Aziz

Department of Clincal and Chemical Pathology, Faculty of Medicine, Sohag University

Abstract

Immune thrombocytopenic purpura is an autoimmune bleeding disorder characterized by thrombocytopenia owing to increased platelet destruction and decrease platelet production.The hallmark of autoimmune diseases is the breakdown in self-tolerance which characterized by inability of the immune system to distinguish self from nonself-antigens .The abnormalities include increased number of the T helper 1 cells, decreased number or suppress function of regulatory T cells and the platelet destruction by cytotoxicity T lymphocytes.Dysregulated T cells in ITP patients may enable development of platelet autoantibodies, have a direct cytotoxic effect on platelets and inhibit megakaryocytes.

Objective: The purpose of this study was to evaluate the percentages of CD4+, CD8+, T helper cells, cytotoxic T cells and NK cells.

Patients and Methods: The study included (40) cases of ITP patients and (40) normal healthy participants. Investigations include peripheral blood samples from cases and controls were used to perform CBC, renal function tests, liver function tests,ESR,CD3,CD4,CD8,CD16,CD56,anti-nuclear antibody testing by immunofluorescence and bone marrow examinations only for cases

Results and conclusion : Data analysis by using SPSS. A p-value < 0.05 was considered statistically significant. This study show significant decrease in the T helper cell and significant increase in cytotoxic T cells in ITP patients when compared to control people.

Keywords: T cells; natural killer cells; immune thrombocytopenic purpura.

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Corresponding Author: **Sara Mustafa Hashem Hamdan** E.mail: sara011095@med.sohag.edu.eg

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Introduction

Immune thrombocytopenia (ITP) is an autoimmune bleeding disorder characterized by bleeding due to isolated thrombocytopenia with platelet count less than 100×10^9/L.[1] ITP is classified based on course of disease into acute (3– <12 months), and chronic (≥12 months).[2] As a main component of cellular immunity, T cells play an important role in body defense and peripheral tolerance. Changes in the number and function of these cells are linked to several disorders, including ITP.[3] It can be stated that CD4+ T cells are indirectly involved in ITP pathogenesis by inducing the increased activity of B cells during Auto-Abs production.[4] Numerous disorders, including ITP, are intimately connected with alterations in the quantity and function of cytotoxic T-cells.[4] NK cells can also modulate cellular immunity in ITP patients.[5]

Patients and Methods

This is a case control study conducted from October 2021 to November 2022 (after obtaining ethical approval), at Hematology unit in Clinical Pathology Department in Sohag University Hospital.The study was performed on 80 subjects; 40 patients with ITP diagnosed according to CBC and bone marrow findings and 40 healthy volunteers as control individuals The study include group I: age and sex matched healthy control individuals (40 subjects) and group II: available number of ITP patients (40 patients). "group IIa : acute ITP patients (32 patients) and group IIb : chronic ITP patients (8 patients)." All patients and controls are subjected to full history taking , good clinical examination and laboratory investigations which included : Complete blood count was performed on Sysmex Corporation (XN-1000), automated hematology analyzer With examination of leishman-stained PB smears and BM and examination of brilliant cresyl blue stained blood samples for reticulocytic percentage ,Immunophenotyping using BD FACSCalibur flow cytometer, liver function tests ,kidney function tests and erythrocyte sedimentation rate. All patients are subjected to Immunofluorescence testing for anti-nuclear antibodies.This research was revised by the Scientific Ethical Committee of Sohag University Hospital and An Informed written consent was taken from all patients and control groups.Data analysis by using SPSS statistical computer software package Version 20.The propability of error (P- value): P-value > 0.05 non-significant difference (NS).P-value ≤ 0.05 significant difference (S).

Results

The age of control group ranged from (10 months to 55 years old) with a mean ± standard deviation (SD) of (21.150± 16.116) and age of ITP patients ranged from (1.5 to 55) years old with a mean age ± SD (17.047 ± 14.473) for group IIa and mean age ± SD (26.625± 19.906) for group IIb.The control subjects were 19 males (47.50%) and 21 females (52.50%) while ITP patients were 12 males(30%) and 28 females(70%) "group IIa : acute ITP patients(32 patients) and group IIb : chronic ITP patients(8 patients). "Shown in table (1)

Table(1) : Age and gender distribution between the Group I , Group IIa and Group IIb.

		Groups						ANOVA	
		Group I		Group IIA		Group IIB		F	P-value
Age (Years)	Range	0.83 - 55		1.5 - 55		2 - 55		1.355	0.264
	Mean ±SD	21.150 ± 16.116		17.047 ± 14.473		26.625 ± 19.906			
Chi-Square		N	%	N	%	N	%	X^2	P-value
Gender	Male	19	47.50	11	34.38	1	12.50	3.871	0.144
	Female	21	52.50	21	65.63	7	87.50		

By anova,cd4,cd8,cd4/cd8 ratio, T-helper cells and cytotoxic T-cells show significant difference between group I control subjects and group II ITP patients (P=0.041,P=0.047, P=0.008 ,P=0.030 and

p=0.035) respectively. By TUKEY'S Test, CD4 and T-helper cells (CD3+, CD4+) show significant decrease in group IIa acute ITP patients compared with group I control subjects(P=0.040 and P=0.024) respectively, significant decrease in group IIb chronic ITP patients compared with group I control subjects(P=0.05 and P=0.036) respectively and insignificant difference between group IIb Chronic ITP patients and group IIa acute ITP patients (P=0.990 and P=0.856) respectively. Also,CD4/CD8 ratio show significant decrease in group IIa acute ITP patients compared with group I control subjects(P=0.016),insignificant difference between group IIb chronic ITP patients and group I control subjects (P=0.072)and insignificant difference between group IIb chronic ITP patients and group IIa acute ITP patients (P=0.876).Besides that, CD8 and cytotoxic T cells show significant increase in group IIa acute ITP patients compared with group I control subjects(P=0.045 and P=0.047), Significant increase in group IIb chronic ITP patients compared with group I control subjects(P=0.031 and P=0.039) and insignificant difference between group IIa acute ITP patients and group IIb chronic ITP patients (P=0.057 and P=0.293) respectively. By ANOVA , gated cell, CD3 and NK cells show insignificant difference between group I control subjects and group IIa acute ITP patients, insignificant difference between patients and insignificant difference between group IIa acute ITP patients and group IIb chronic ITP patients (P=0.941,P=0283 and P=0.588) respectively shown in table (2).

Table (2): Immunophenotyping between the Group I, Group IIa and Group IIb.

		Groups			ANOVA		TUKEY'S Test		
		Group I	Group IIA	Group IIB	F	P-value	I&II A	I&II B	IIA&I IB
Gated cell	Range	7.8 - 64.9	7.1 - 59.7	5.33 - 51.6	0.061	0.941			
	Mean ±SD	26.704 ± 12.729	26.262 ± 15.870	24.791 ± 14.043					
CD3	Range	16.4 - 82.3	21.4 - 87.8	25.7 - 84.8	1.285	0.283			
	Mean ±SD	53.575 ± 18.952	57.544 ± 17.051	64.238 ± 18.720					
CD4	Range	0 - 46.2	0.06 - 46.3	1 - 33.5	3.343	0.041*	0.040*	0.05*	0.990
	Mean ±SD	22.936 ± 12.828	15.293 ± 13.228	15.978 ± 12.940					
CD8	Range	4.4 - 49.3	9.2 - 84.1	16.6 - 67	2.170	0.047*	0.045*	0.031*	0.057
	Mean ±SD	25.148 ± 9.918	28.025 ± 13.730	34.813 ± 16.536					
CD4/CD8 ratio	Range	0 - 3.341	0.003 - 2.437	0.015 - 0.895	5.150	0.008*	0.016*	0.072	0.876
	Mean ±SD	1.101 ± 0.805	0.634 ± 0.607	0.499 ± 0.326					
Percentage of T helper cells	Range	0 - 46.17	0 - 33.31	0.69 - 33.31	3.680	0.030*	0.024*	0.036*	0.856
	Mean ±SD	20.277 ± 11.696	13.052 ± 10.629	15.436 ± 12.386					
Percentage of cytotoxic T cells	Range	2.4 - 47.39	6 - 84.1	13.44 - 66.6	3.503	0.035*	0.047*	0.039*	0.293
	Mean ±SD	20.452 ± 10.434	25.197 ± 14.731	32.899 ± 16.399					
Percentage of NK cells	Range	0 - 10.51	0 - 4.71	0.03 - 6.19	0.534	0.588			
	Mean ±SD	1.150 ± 1.785	1.396 ± 1.451	1.790 ± 2.199					

By ANOVA , there was a significant difference in red blood cells count , hemoglobin level , hematocrit level, Mean corpuscular volume, Mean corpuscular hemoglobin and platelet between group I control subjects and group II ITP patients (p=0.017, p=<0.001,p=<0.001,p=0.002,p= 0.003 and p=<0.001) respectively. By TUKEY'S Test,

there was significant decrease in red blood cells count , hemoglobin level , hematocrit level and Mean corpuscular volume in group IIa acute ITP patient compared with group I control subjects(P=0.027,P=<0.001,=P<0.001 and P=0.003) respectively. By TUKEY'S Test, there was significant decrease in Mean corpuscular hemoglobin and platelet in group IIa acute ITP patients compared with group I control subjects(P=0.003and p < 0.001) respectively and signify-cant decrease in group IIb chronic ITP patient compared with group I control subjects (P=0.034 and P< 0.001) respectively. Shown in table (3)

Table (3): Complete blood picture between Group I, Group IIa and Group IIb.

		Groups									ANOVA		TUKEY'S Test		
		Group I			Group IIA			Group IIB			F	P-value	I&IIA	I&IIB	IIA&IIB
WBC (10^3)	**Range**	5.53	-	15.49	2.96	-	18.5	4.72	-	14.29	0.865	0.425			
	Mean ±SD	8.768	±	2.11	7.994	±	3.274	9.043	±	3.597					
RBC (10^6)	**Range**	3.99	-	5.54	3.47	-	6.01	4.35	-	5.43	4.305	0.017*	0.027*	0.868	0.1
	Mean ±SD	4.698	±	0.394	4.405	±	0.567	4.79	±	0.349					
HGB (g/dl)	**Range**	9.8	-	15.6	8.2	-	14.8	9.7	-	13.6	16.255	<0.001*	<0.001*	0.151	0.265
	Mean ±SD	13.443	±	1.458	11.351	±	1.697	12.313	±	1.345					
HCT (%)	**Range**	28.3	-	53.2	26.9	-	41.8	31.9	-	40.7	18.194	<0.001*	<0.001*	0.16	0.175
	Mean ±SD	40.08	±	5.224	33.291	±	4.424	36.675	±	3.054					
MCV (fl)	**Range**	60.6	-	93.1	44.7	-	88.4	62.8	-	84.6	6.75	0.002*	0.003*	0.088	1
	Mean ±SD	83.628	±	8.725	76.888	±	7.887	76.8	±	6.684					
MCH (pg)	**Range**	22	-	31.3	13.6	-	31.8	19.1	-	29.2	7.115	0.001*	0.003*	0.034*	0.885
	Mean ±SD	28.618	±	2.304	26.353	±	3.251	25.825	±	3.44					
MCHC(g/dl)	**Range**	31.8	-	36.5	30.4	-	37.9	30.4	-	36.2	0.349	0.707			
	Mean ±SD	33.979	±	1.244	34.088	±	2.106	33.525	±	1.919					
PLT(10^3)	**Range**	180	-	412	1	-	43	3	-	54	386.844	<0.001*	<0.001*	<0.001*	0.955
	Mean ±SD	304.175	±	65.524	8.848	±	9.71	14.25	±	16.859					

By ANOVA, there was significant difference in albumin and T. Bilirubin between group I control subjects and group II ITP patients (p=0.011 and p=0.029) respectively. By TUKEY'S Test , there was significant decrease in albumin in group IIa acute ITP patients when compared with group I control subjects (P=0.008). By TUKEY'S Test, there was significant increase in total bilirubin in group IIb chronic ITP patients compared with group I control subjects (P=0.022), insignificant difference in total bilirubin between group I control subjects and group IIa acute ITP patients and between group IIa acute ITP patients and group IIb chronic ITP patients (P=0.725 and P=0.076) respectively. T-test results showed a statistically significant rise in ESR (1-hour) betw-een group II ITP patients and group I controls (P=0.021).

In group II ITP patients there was a positive corr-elation between CD3 and ESR , T.Bilirubin , D.-Bilirubin and indirect bilirubin (p=0.024, p=0.016, p=0.044 and p=0.033) respectively .There was a positive correlation between CD4 and HGB (p=0.046) and negative correlation between CD4 and platelet count (p=0.046). There was positive correlation betw-een CD4/CD8 ratio and ALB (p=0.024) .There was negative correl-ation between percentage of NK cells (CD16+ CD56+) and T.P (P= 0.028).

There was negative correlation between CD4 and CD3 and platelet count. Shown in table (4) platelet count (p=0.046) but correlation between

Table(4): Correlations in group II ITP patients.

		CD3	CD4	CD8	CD4/CD8 ratio	Percentage of T helper cells	Percentage of cytotoxic T cells	Percentage of NK cells
WBC (103 /?l)	**R**	-0.011	0.123	0.195	0.066	0.159	0.149	0.244
	P-value	0.944	0.448	0.227	0.684	0.326	0.357	0.129
RBC (106/?l)	**R**	0.249	0.203	0.133	0.034	0.238	0.134	-0.097
	P-value	0.121	0.209	0.415	0.834	0.139	0.409	0.551
HGB (g/dl)	**R**	0.298	0.317	0.195	0.204	0.253	0.135	0.160
	P-value	0.062	0.046 *	0.229	0.208	0.116	0.406	0.324
PLT(103/?l)	**R**	0.041	-0.317	-0.018	-0.289	-0.262	0.033	-0.042
	P-value	0.800	0.046 *	0.913	0.071	0.103	0.838	0.796
ESR(mm/h)	**R**	0.357	0.078	0.255	-0.054	0.032	0.278	0.004
	P-value	0.024 *	0.631	0.112	0.741	0.844	0.082	0.982
ALT(u/l)	**R**	-0.047	0.241	-0.064	0.233	0.160	-0.105	-0.123
	P-value	0.774	0.134	0.694	0.148	0.324	0.518	0.450
AST(u/l)	**R**	-0.082	0.204	0.093	0.254	0.145	0.045	0.029
	P-value	0.615	0.208	0.570	0.114	0.371	0.781	0.860
T.P(g/dl)	**R**	-0.128	0.305	-0.150	0.274	0.188	-0.217	-0.348
	P-value	0.430	0.056	0.357	0.088	0.246	0.179	0.028*
AIB(g/dl)	**R**	-0.199	0.296	-0.021	0.357	0.256	-0.112	-0.165
	P-value	0.217	0.063	0.900	0.024*	0.111	0.490	0.310
T.Bilirubin (mg/dl)	**R**	0.380	0.135	0.285	-0.025	0.138	0.287	0.081
	P-value	0.016 *	0.405	0.075	0.881	0.396	0.072	0.619
D.Bilirubin (mg/dl)	**R**	0.319	0.093	0.376	-0.046	0.074	0.366	0.192
	P-value	0.044 *	0.567	0.062	0.779	0.648	0.063	0.234
Indirect Bilirubin(mg/dl)	**R**	0.339	0.126	0.196	-0.015	0.137	0.204	0.018
	P-value	0.033 *	0.437	0.226	0.929	0.399	0.207	0.911
Creat(mg/dl)	**R**	0.369	-0.132	0.168	-0.176	-0.216	0.174	-0.122
	P-value	0.072	0.416	0.300	0.276	0.180	0.283	0.452
Urea(mg/dl)	**R**	0.114	-0.222	0.196	-0.258	-0.316	0.199	-0.312
	P-value	0.483	0.168	0.225	0.109	0.067	0.219	0.062
Uric acid(mg/dl)	**R**	0.029	0.035	-0.048	0.089	-0.108	-0.091	-0.225
	P-value	0.857	0.829	0.768	0.583	0.505	0.576	0.163

Discussion

Immune tolerance, the capacity to tell the difference between self- and non-self-antigens, may trigger dangerous autoimmune response ."Peripheral tolerance" refers to the elimination of mature reactive cells from the circulation by regulatory T cells (Tregs) after they have eluded negative selection in the thymus.[6] Antiplatelet self-reactive T cells and an imbalance in cytokine levels have been recently observed in patients with ITP. When regulatory T cells are absent, peripheral tolerance breaks down and autoimmunity might set in.[7] In this study we cleared that there was insignificant difference between acute and chronic ITP compared to control group as regards age and gender ($p > 0.05$), but in total acute ITP are insignificantly younger than chronic ITP patients. In this research, we found that individuals with acute and chronic ITP had significantly higher numbers of CD8 and cytotoxic T cells than the control group. As in El-Rashedi et al. 2017 and Zhang et al. 2018 discovered that CD8+ was noticeably greater in acute and chronic patients compared to the control group.[9,8] Our study showed that individuals with acute and chronic ITP had lower levels of CD4, CD4+/CD8+ ratio and T-helper cells than the control group. Additionally, individuals with ITP demonstrated a significant decline in CD4+ frequency and T-helper cell expression in both the Shan et al. 2014 study and Zahran et al. 2018 study.[10,11] Such decline increased along with the severity of the disease, indicating a potential role for CD4+ expression in the development and progression of ITP. El-Rashedi et al. 2017 also revealed that the CD4+ and CD4+/CD8+ ratios were considerably lower in acute and chronic patients compared to the control group. [9] However, Zhang et al. 2018 discovered that the 'newly diagnosed group had a much higher percentage of CD4+T cells than the controls. [8]In this study, we discovered no differences in CD4, CD8, CD4/CD8 ratio, T helper, or cytotoxic T cells between acute ITP patients and chronic ITP patients. Additionally, El-Rashedi et al. 2017 discovered that there is no difference between acute and chronic patients in terms of CD4, CD8, or CD4/CD8 ratio.[9]According to Dominguez-Garcia and Rodriguez-Moyado 2002, compared to chronic ITP, CD4 and CD4/CD8 levels decreased in patients of acute ITP. [12] In this study both acute and chronic ITP patients and control persons had similar numbers of NK cells. Ebbo et al. 2017, in ITP patients, the innate ability of NK cells to destroy antibody-coated cells was still present. [13] But, El-Rashedi et al. 2017 discovered that there was no significant difference between the chronic and control groups and that NK cell percentage was much lower in acute patients than in the control group.[9] We found that the number of RBCs dropped significantly between the acute and chronic ITP groups and the control group. A statistically insignificant difference in RBCs was detected between patients with acute ITP and controls in a study by Baraka et al.[14] Compared to the control group, patients with acute and chronic ITP had significantly lower hemoglobin, mean corpuscular volume ,mean corpuscular hemoglobin and mean corpuscular hemoglobin concentrations ($p < 0.001$).In accordance to Fahim and Monir, 2006 and Hamada et al. 2022 who observed that as compared to the controls, cases with acute ITP showed a substantial drop in the Hb concentration, which may be connected to bleeding.[15,16] Our results show that both acute and chronic ITP significantly lower platelet counts compared to the control group ($p < 0.001$). Studies by Mazzucco et al., Talaat et al., Zahran et al., Zhang et al., and El-Rashedi et al. showed that the mean platelet count was significantly lower in patients with ITP compared to control individual.[17,18,11,8,9] The ITP group had considerably lower albumin levels than the control group. Inhibiting albumin production, IL-6 plays a critical function in the immunological response (Tanaka et al.).[19]Total bilirubin was found to be significantly higher in the chronic ITP group compared to the control group. The antioxidant capabilities and immunomo-dulatory potency of bilirubin are at their strongest at physiologic or moderately increased amounts (Zhang et al .).[20] We showed in this thesis that acute and chronic ITP patients had significantly higher ESRs than the control group. Since immune cells and their related cytokines play a fundamental role in patients with ITP and are predictors of ITP progression, Hamed et al. 2017 found statistically significant differences between erythrocyte sedimentation rate levels across the groups. Patients with acute ITP had a higher erythrocyte sedimentation rate than those with chronic ITP compared with healthy controls.[21] ITP patients also had a considerably greater erythrocyte sedimentation rate in their peripheral

blood than controls did ((p <0.05), as shown by research by Baraka et al. in 2014.[14]Repeated blood loss through epistaxis, ecchymosis and petechiae, in addition to the presence of autoimmune antibodies to other hematopoetic precursors, may explain the positive connection between CD4 and HGB identified by (Katemba et al., 2018).[22] There was an inverse relationship between CD4 and platelet count. In the ITP population as a whole, Vrbensky et al. 2020 discovered an inverse relationship between CD4 expression and platelet count.[23] However, Namutebi et al. 2013 observed no statistical correlation between CD4+ cell numbers and anemia.[24] In this study, we discovered positive correlation between CD3 and ESR, T.Bilirubin, D.Bilirubin, and indirect bilirubin. Zhang et al. discovered that participants with high ESR titres had significantly higher frequencies of expressing CD3+T lymphocytes in patients with ITP because immune cells and the cytokines they produce play a crucial role in patients with ITP and are indicators of the disease's progression.[25]

Conclusion

This study's conclusion listed some characteristics of immunological dysregulation found in ITP patients. When compared to the control group, these patients had lower T helper cells, more cytotoxic T cells, high level of ESR, low level of albumin (in acute patients) and high level of T.bilirubin (in chronic patients)but neither of these parameters could be used to predict the disease's chronicity only.

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Author contributions:

A.A.A., E.H.A., S.M.H., S.P.A. conceived and designed the experiments. A.A.A., E.H.A., S.M.H., S.P.A. performed the experiments. A.A.A., E.H.A., S.M.H., S.P.A. analyzed the data and drafted the manuscript; A.A.A., E.H.A., S.M.H., S.P.A. writing, review and editing. All authors have read and agreed to the published version of the manuscript.

Reference

1. **NEUNERT, Cindy, et al.** American Society of Hematology 2019 guidelines for immune thrombocytopenia. Blood advances, 2019, 3.23: 3829-3866.
2. **PROVAN, Drew, et al.** Updated international consensus report on the investigation and management of primary immune thrombocytopenia. Blood advances, 2019, 3.22: 3780-3817.
3. **WEI, Yu; HOU, Ming.** T cells in the pathogenesis of immune thrombocytopenia. In: Seminars in hematology. WB Saunders, 2016. p. S13-S15.
4. **ZHAO, Zhenhua, et al.** Contributions of T lymphocyte abnormalities to therapeutic outcomes in newly diagnosed patients with immune thrombocytopenia. PLoS One, 2015, 10.5: e0126601.
5. **ZHANG, Xian, et al.** CD70-silenced dendritic cells induce immune tolerance in immune thrombocytopenia patients. British Journal of Haematology, 2020, 191.3: 466-475.
6. **OU, Yang, et al.** Relationship between the IL-10 (− 1082 A/G) polymorphism and the risk of immune/idiopathic thrombocytopenic purpura: A meta-analysis. Cytokine, 2020, 125: 154820.
7. **METREVELI, SOPHIO, et al.** CD4+ CD39 T CELLS IN THE PERIPHERAL BLOOD AND SPLEEN OF PATIENTS WITH IMMUNE THROMBOCYTOPENIA. Experimental and Clinical Medicine Georgia, 2022, 5.
8. **ZHANG, Jiakui, et al.** Immune dysregulation in primary immune thrombocytopenia patients. Hematology, 2018, 23.8: 510-516.
9. **EL-RASHEDI, Farida Hussein, et al.** Study of CD4+, CD8+, and natural killer cells (CD16+, CD56+) in children with immune thrombocytopenic purpura. Hematology/oncology and stem cell therapy, 2017, 10.1: 8-14.
10. **HAN, Ning-ning, et al.** Decreased Tim-3 and its correlation with Th1 cells in patients with immune thrombocytopenia. Thrombosis Research, 2014, 133.1: 52-56.
11. **AHRAN, Asmaa M., et al.** Clinical significance of T-cell immunoglobulin mucin 3 expression on peripheral blood mononuclear cells in pediatric acute immune thrombocytopenia. Clinical and

Applied Thrombosis/Hemostasis, 2018, 24.6: 936-943.

12. **OMÍNGUEZ-GARCÍA, Ma Victoria; RODRÍGUEZ-MOYADO, Héctor.** Cellular and biochemical mechanisms involved in physiopathogenesis of autoimmune thrombocytopenic purpura. Gaceta Medica de Mexico, 2002, 138.5: 461-472.

13. **BBO, M., et al.** NK cell compartment in the peripheral blood and spleen in adult patients with primary immune thrombocytopenia. Clinical Immunology, 2017, 177: 18-28.

14. **ARAKA, Ahmad, et al.** Study of T-regulatory cells in patients with acute, idiopathic thrombocytopenic purpura. The Egyptian Journal of Haematology, 2014, 39.2: 37-41.

15. **AHIM, NEHAL MA* & MONIR, Eman**.** Functional role of CD4+ CD25+ regulatory T cells and transforming growth factor-betal in childhood immune thrombocytopenic purpura. Egyptian journal of immunology, 2006, 13.1: 173-187.

16. **AMADA, Sarah S.; AL DIWANY, Ola I.; SHAHIN, Radwa S.** Immunological markers changes in pediatric immune Thrombocytopenic Purpura. The Scientific Journal of Al-Azhar Medical Faculty, Girls, 2022, 6.1: 85-90.

17. **AZZUCCO, Karina LM, et al.** Assessment of regulatory T cells in childhood immune thrombocytopenic purpura. International Scholarly Research Notices, 2013, 2013.1: 143687.

18. **ALAAT, R. M., et al.** Alterations in immune cell subsets and their cytokine secretion profile in childhood idiopathic thrombocytopenic purpura (ITP). Clinical & Experimental Immunology, 2014, 176.2: 291-300.

19. **ANAKA, Toshio, et al.** A new era for the treatment of inflammatory autoimmune diseases by interleukin-6 blockade strategy. In: Seminars in immunology. Academic Press, 2014. p. 88-96.

20. **HANG, Hui, et al.** Correlation between total bilirubin, total bilirubin/albumin ratio with disease activity in patients with rheumatoid arthritis. International Journal of General Medicine, 2023, 273-280.

21. **AMED, Hanan, et al.** Role of measurement of interleukin 10 in idiopathic (immune) thrombocytopenic purpura. The Egyptian Journal of Haematology, 2017, 42.4: 148-154.

22. **ATEMBA, Crispus, et al.** Hematological abnormalities in HIV-antiretroviral therapy naïve clients as seen at an immune suppression syndrome clinic at Mbarara Regional Referral Hospital, southwestern Uganda. Journal of blood medicine, 2018, 105-110.

23. **RBENSKY, John R., et al.** Increased cytotoxic potential of CD8+ T cells in immune thrombocytopenia. British Journal of Haematology, 2020, 188.5.

24. **AMUTEBI, A. M. N.; KAMYA, M. R. K.; BYAKIKA-KIBWIKA, P.** Causes and outcome of hospitalization among HIV-infected adults receiving antiretroviral therapy in Mulago hospital, Uganda. African health sciences, 2013, 13.4: 977-985.

25. **HANG, Wenming; QIAN, Xiaodan; CHEN, Wei.** Evaluation of CD4/CD8 ratio in children with immune thrombocytopenic purpura (ITP) after treatment with intravenous immunoglobulin (IVIG). Cellular and Molecular Biology, 2022, 68.5: 186-191.