Egyptian Journal of Medical Research

Print ISSN: 2682-4396 / Online ISSN: 2682-440X

Original article

New Aspects in Pathogenesis of Primary Immune Thrombocytopenia in Pediatric Patients

Taghreed Ahmed Abuzied[1], Dalia Saber Morgan[1], Rehab Muhamad Abdelkreem[2], Sahar Salah Abdelhalim[1]

[1]*Department of Pediatrics, Faculty of Medicine, Beni-Suef University, Beni-Suef, Egypt.*

[2]*Department of clinical and chemical pathology, Faculty of Medicine, Beni-Suef University, Beni-Suef, Egypt.*

Article Info

Article history:

Received 13 August 2024

Accepted 3 September 2024

Corresponding Author:

Taghreed Ahmed Abuzied

ahmednafady92@gmail.com

Keywords

Immune Thrombocytopenic Purpura, bleeding, platelets.

Abstract:

Background: Immune thrombocytopenia is an autoimmune condition marked by a reduced number of platelets in the blood, which can be caused by either a breakdown of platelets inside the blood vessels or the abnormal synthesis of platelets in the bone marrow. although autoantibodies have been shown as the principal factor in the pathogenesis of immune thrombocytopenia, also cellular immune modulation has been recognized to have a vital role in the pathophysiology of ITP. **Aim of the Work**: the pathogenesis of primary immune thrombocytopenia in pediatric patients. **Patients and Methods**. A Case-Control, analytical research that has been performed over one year after the approval of the research ethics committee on 70 participants (twenty-four) men & (forty-six) females with ages varying from (2 years) to (13 years) & an average age of (6.28±2.86) years old,

Cases underwent history taking & clinical investigation. Laboratory investigations included CBC, DPC, ESR, bleeding time, C- C-reactive protein, and single nucleotide polymorphism. **Conclusion**: consanguinity was associated with ITP. It is widely recognized that the pathogenesis of immune thrombocytopenia is significantly influenced by environmental & genetic factors. The susceptibility of cases to immune thrombocytopenia is influenced by single-nucleotide polymorphisms (SNP) of inflammatory cytokine loci, including IL-10, IL-17F, TNF-β, TNF-α, IL-6, TGF-β1, INF-γ, & IL0-1A. Additionally, various autoimmune disorders are related to genetic variants of inflammation-related genes, involving CD226, CD24,IL-2, FCRL3, ITGAM, IRF5,CARD8, NLRP3, SH2B3, PTPN22, TNFAIP3, TRAF1, & STAT4.

1. Introduction:

One of the most prevalent bleeding disorders is 1ry immune thrombocytopenia, which is distinguished by a decreased platelet count & an elevated probability of hemorrhage. ***(1).*** Autoantibodies opsonize platelets, which are subsequently eliminated by phagocytic cells in ITP, an acquired autoimmune disorder. The pathogenesis of ITP is characterized by a hyperactivated T-cell response, which is crucial to produce IgG & cell-mediated cytotoxicity. ***(2)*** Autoimmune disorders, involving systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome,

Evans syndrome, & antiphospholipid syndrome, are among the primary risk factors for secondary ITP. Secondary ITP is also reported to be caused by lymphoproliferative illnesses (particularly chronic lymphocytic leukemia), immunodeficiency (common variable immunodeficiency), chronic infections caused by viral or bacterial proteins (such ashepatitis C virus

(HCV), human immunodeficiency virus (HIV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), Helicobacter pylori), & drugs. ***(3)*** There are several primary mechanisms that regulate T-cell-mediated immune responses. One of these mechanisms is the immunological checkpoint, which involves signal pathways that entail both co-stimulation and co-inhibition. The signals of co-inhibition and co-stimulation, which are also referred to as "second signals," synergistically By altering the "first signal" provided by the T cell receptor (TCR) and MHC recognition, the fate of adaptive T cell immunity can be determined. (4) The co-stimulatory molecules of inducible costimulatory (ICOS),T cells are CD28, TNF superfamily member 4 (TNFSF4), and DNAMI (CD226), while the co-inhibitory molecules include TIM3, cytotoxic T-lymphocyte correlated protein 4 (CTLA4), programmed death - 1 (PD1), & CTLA4. These molecules represent the most extensively studied costimulatory processes. ***(5).*** The universal distribution & function of single nucleotide polymorphisms, the most prevalent pattern of genetic variations, have garnered attention. The development and therapy of immune thrombocytopenia may be influenced by SNPs such as TNFA,TNFAIP3, & CD28. ***(6).***

2. Patients And Methods:

The current research was case-control research conducted on pediatric cases from a hematology outpatient clinic in Beni-Suef University Hospital, during the period from June 2021 (after the approval of the ethical committee to June 2022. **Approval no: FMBSUREC/06072021/Abo Zeid**

Patients: The children included in this study were selected randomly from Beni Suef University Hospital Pediatric Hematology outpatient clinic according to the following exclusion and inclusion criteria:

Inclusion criteria: Pediatric patients with ITP, Both genders were included, Acute and chronic patients and age: 2 years to 13 years of age.

Exclusion criteria: Age: less than 2 years and not more than 13 years, Hepatosplenomegaly or lymphadenopathy and The causes of thrombocytopenia are either congenital or acquired.

Sample size: The present study included 69 children divided into 2 groups as the following: -

- **Group (1):** Thirty-five children with primary immune thrombocytopenia were included in the research.
- **Group (2):** Thirty-five healthy children whose age and sex were involved in the research as a control.

All individuals involved in the investigaton have been exposed to: Full history taking, Full clinical examination (including liver, spleen, LN examination), Complete blood count, Direct platelet count, Bone marrow examination, Pelvis-abdominal ultrasound, Liver function tests, Kidney function tests, H. Pylori testing, Virology testing (HCV, HBV, HIV), C reactive protein and Anti-ds DNA, ANA, C3&C4, & Urine analysis, to exclude systemic auto-immune diseases.

Treatment: All cases have been administered steroids for therapy. The dosage & method of administration have been selected based on the count of platelet & presence of active bleeding. In emergency situations, infusions of methylprednisolone or dexamethasone have been utilized, while less severe cases have been managed with oral prednisone or intravenous prednisolone. A small number of cases were treated with Revolade. After the platelet count has increased, the dosage of steroid is slowly decreased while closely monitoring the potential for a recurrence.

Methodology: The samples have been digesting using Proteinase K in the provided Lysis Solution. Subsequently, the lysate has been mixed with ethanol & introduced into the purification column, facilitating the attachment of DNA to the silica membrane. The created Wash Buffers were used to efficiently eliminate impurities from the column through washing. The genomic DNA was subsequently extracted using the Elution Buffer, which had low ionic strength.

3. Results:

The current study was case-control research performed on pediatric cases with Immune Thrombocytopenia aged more than 2 years and less than 13 years old and matched the Healthy control group.

Table (1): Demographic Distribution among ITP Cases and Healthy Controls; (N=70):

		N (%)		Total Number = 70	p-value*
		ITP Patients Number = 35	Healthy Controls Number= 35		
Age in years (mean ±SD)		6.29±2.88	6.26±2.88	6.28±2.86	0.965
Sex	**Male**	12 (34.3)	12 (34.3)	24 (34.3)	1.00
	Female	23 (65.7)	23 (65.7)	46 (65.7)	
consanguinity	**Yes**	13 (37.1)	5 (14.3)	18 (25.7)	0.029*
	No	22 (62.9)	30 (85.7)	52 (74.3)	

**p-value >0.05 is considered non-significant by the Chi-Square test for sex and consanguinity and the age test.*

Table (1) demonstrates the age and gender distribution of the cases and the controls; the mean age among ITP cases was nearly equal controls for matching without significant difference. Of the ITP cases, 34.4% of them were males and 65.7% were females. There was statistically insignificant distinction between the cases & the control groups regarding sex (p-value> 0.05). Regarding consanguinity, nearly one-third of ITP cases had positive consanguinity with a statistically significant difference as p. value= 0.029.

Table (2): ITP Clinical Picture among studied cases; (N= 35):

		Frequency	Percent (%)
Clinical Picture (Yes)	**Purpura**	32	91.4
	Ecchymosis	32	91.4
	Pallor	20	57.1
	Epistaxis	21	60
	Bleeding	3	8.6

Table (2) demonstrated that purpura was the most common clinical finding in ITP studied cases (32 cases, 91.4%) had a positive history of purpura and ecchymosis and (21 cases, 60%) complaining of epistaxis (20 cases, 57.1%) had pallor. Three cases (8.6%) complained of bleeding.

Table (3): Types of drugs among studied cases; (N= 35):

		Frequency	Percent (%)
Drug (Yes)	**Oral steroids**	33	94.3
	Imuran	0	0
	Revolade	5	14.3
	IV steroids	27	77.1

Table (3) demonstrates the drug intake among studied cases, (33 cases, 94.3%) treated by oral steroids, (27 cases, 77.1%) treated by IV steroids, (5 cases, 14.3%) treated by revealed. There were no cases needed Imuran in our study.

Table (4): Laboratory parameters Distribution among ITP Cases and Healthy Controls; (N=70):

Parameters		Patients N= 35	Healthy Controls N= 35	Total N= 70	p-value
Hb		***10.07±0.72***	10.76±0.36	10.42±0.66	**0.001***
platelets		***101.88±58.53***	322.22±37.095	***212.05±121.16***	**0.001***
TLC		*8.09±2.178*	7.64±0.93	7.86±1.67	0.276
ESR	**First hour**	***15.48±1.521***	11.00±0.34	13.24±2.510	**0.001***
	Second hour	***21.62±1.416***	13.45±0.918	17.54±4.28	**0.001***
Bleeding time		***7.61±0.493***	4.80± 0.405	6.18±1.487	**0.001***
DPC		***103.44±57.70***	322.22±37.095	214.42±120.18	**0.0001***

Table (4) demonstrated that ITP patient's Laboratory parameters had statistically significant differences regarding hemoglobin, platelets, ESR, Bleeding time, and DPC as mean values of hemoglobin, platelets and DPC were lower in ITP patients while mean values of ESR and Beeding time were higher in ITP patients. Other studied variables showed non-statistically significant correlation with RUNX1 expression levels among ITP patients, (p-values >0.05).

Table (5): CD28 gene polymorphism in immune thrombocytopenia cases as compared with healthy controls; (N=70):

CD28 gene polymorphism		N (%)		Total N= 70	p-value*
		ITP Patients N= 35	Healthy Controls N= 35		
	CT	**22 (62.9)**	14 (40)	36 (51.4)	0.049*
	CC	4 (11.4)	2 (5.7)	6 (8.6)	
	TT	9 (25.7)	**19 (54.3)**	28 (40)	

**p-value ≤0.05 is considered significant by one way a nova test.*

Table (5) demonstrates the CD28 gene polymorphism distribution of the cases and the controls; the ITP cases; 62.9% were CT and 54.3% of controls were TT type. There was a statistically significant distinction among the cases & the control groups (p-value≤ 0.05).

Table (6): TNFAIP3 rs 10499194 gene polymorphism in immune thrombocytopenia cases as compared with healthy controls; (N=70):

TNFAIP3 rs 10499194 gene polymorphism	N (%)		Total N= 70	P-value*
	ITP Patients Number= 35	Healthy Controls Number= 35		
CT	10 (28.6)	19 (54.3)	29 (41.1)	**0.049***
CC	23 (65.7)	16 (45.7)	39 (55.7)	
TT	2 (5.7)	0	2 (2.9)	

**p-value ≤0.05 is considered significant by Chi-Square test*

Table (6) demonstrates TNFAIP3 rs 10499194 gene polymorphism distribution of the cases and the controls; the ITP cases; of them 65.7% were CC and 28.6% of controls were TC type While in controls 55.7% were CC and 41.1% were CT. There was statistically significant variance among the cases & the control groups (p-value≤ 0.05).

4. Discussion:

One of the most prevalent bleeding disorders is primary immune thrombocytopenia, which is distinguished by a decreased platelet count & an elevated risk of hemorrhage. Autoantibodies opsonize platelets, which are subsequently eliminated by phagocytic cells in immune thrombocytopenia, an acquired autoimmune illness. The pathogenesis of immune thrombocytopenia is characterized by a hyper-activated T cell response, which is crucial to produce IgG & cell-mediated cytotoxicity. Consequently, the pathogenesis & development mechanism of immune thrombocytopenia may be revealed by examining T cell abnormalities in ITP cases. **(7).**

The objective of treatment is to prevent any active hemorrhage & prevent the occurrence of future hemorrhages. The American Society of Hematology's most recent clinical guidelines suggest that corticosteroids should be administered to newly diagnosed adult cases with a platelet count below 30.000. In the case of active hemorrhaging or cases who require a rapid enhance the platelet count, intravenous immunoglobulin (IVIG) should be added. Treatment options for cases who don't respond adequately to first-line therapy involve splenectomy, immune modulators like rituximab, or thrombopoietin-receptor agonists (TPO-RAs). **(8).**

By triggering the transcription of several pro-inflammatory genes, the nuclear factor-κB (NF-κB) signaling pathway is the most well-characterized molecular pathway for activating immune cells. Both human & experimental inflammatory illnesses have been associated with the excessive activation of NF-κB signaling in multiple cell types. **(9)**

In our research, the mean age of cases with ITP was 6.29±2.88 years and most of them were females (65.7%). The mean age and sex among ITP cases were nearly equal controls for matching without significant differences. Regarding consanguinity, nearly (37.1%) of ITP cases had positive consanguinity with statistically significant differences from controls. Two cases have a positive family history of ITP.

The investigation on Immune Thrombocytopenia (ITP) in Iraqi kids discovered no significant differences in the male to female ratio,

with a ratio of 1:1.5. Most of the cases (61.5%) were in the age range of two to ten years. Most cases (63), accounting for over eighty percent, did not have a family history of Immune thrombocytopenia. However, the family history status of six cases wasn't recorded. Fifty-five cases were kids of a father & mother who were closely related by blood.

Also, the study of **(11)** on pediatric cases with Immune Thrombocytopenic purpura showed that there were twenty-eight males (46.6%) & 32 females (53.3%) with ages varying from one to eighteen years old cases group showing positive consanguinity by (43.3%) and family history of the illness by (13.3%). In contrast to our findings, the investigation conducted by **(12)** on ITP in Egyptian kids reported that 53.2 percent of the participants were men & 46.8 percent were females. The median age at diagnosis was 5 ± 3.4 years, with most kids falling from the two to ten-year-old age group (72.7%). 4.2 percent of the cases exhibited consanguinity, while 3.9 percent of kids showed a positive familial history of ITP.

It has been shown that the disparity in the differentiation of Th2 & Th1 cells is a significant factor in the development & progression of ITP. Nevertheless, in recent times, it has been stated that abnormal differentiation of Th17 cells is also a contributing factor in the development of immune thrombocytopenia. A research investigation performed by **(13)** found no definitive correlation among consanguinity & ITP. However, consanguinity is prevalent in Asia & Africa, particularly in societies where Islam is predominant, as well as in Arab countries where there is a significant occurrence of genetic defects as well as autosomal illnesses.

As regards the clinical manifestations among our study participants, about 32 patients (91.4%) had a positive history of purpura and ecchymosis, 20 patients (57.1%) had pallor, 21 patients (60%) complained of epistaxis, and 3 patients (8.6%) complaining of bleeding.

Consistent with our findings, the investigation conducted by **(14)** demonstrated that bleeding symptoms ranged in severity from mild to severe. The prevalent manifestations of bleeding included petechiae, bruising, & mucosal hemorrhage. Additional instances of internal bleeding included melena, hematemesis, hematuria, & hemoptysis. Out of the seventy-one cases with an initial platelet count of 10x109 /l, ten cases (fourteen percent)

had internal bleeding consequently. Among these cases, four kids (5.6 percent) had intracranial bleeding.

Furthermore, an investigation examining the clinical characteristics of kids with Immune Thrombocytopenia found that subcutaneous & mucosal hemorrhage is a significant manifestation of ITP. All the cases in the research had hemorrhage of the skin. 47.3 percent of the cases had bleeding in epistaxis, and 57.9 percent had bleeding in gingival hemorrhage. None of the cases developed hemorrhage in the brain (intracranial hemorrhage) at the time of diagnosis or throughout the duration of follow-up.

A study conducted by **(16)** examined the predictive factors in kids' primary immune thrombocytopenia. The results revealed that Petechiae and/or bruises were the most prevalent (ninety two percent) clinical symptoms observed in kids diagnosed with ITP, followed by epistaxis (forty-four percent) & oral bleeding (thirty two percent).

In an investigation conducted by **(17)**, it was shown that eighty percent of Egyptian kids with immune thrombocytopenic purpura exhibited cutaneous hemorrhage in the form of ecchymosis or purpura. 36.7 percent of cases had hemorrhage from their gums or nose (epistaxis). Two cases with chronic immune thrombocytopenia had intracranial bleeding, accounting for 3.3 percent of the total cases. These cases had extremely low platelet counts.

Regarding the management of ITP patients in our study, 33 patients (94.3%) were treated with oral steroids, 5 patients (14.3%) were treated by roulette, and 27 patients (77.1%) were treated with IV steroids. There were no cases that needed Imuran in our study. 4 cases needed plasma and platelet transfusion and 6 cases needed blood transfusion.

Near results were found in the study of**, (18)** about the clinical features of ITP among Egyptian children. Their investigation revealed that a mere 10.5 percent of cases were not prescribed any medicine & were only treated by observation. The majority, 78.9 percent, got steroid treatment, with oral medication being the primary method for 57.9 percent of cases. Approximately 78.9 percent of cases received IVIG treatment, whereas around 73.7 percent received a combination of treatments. Revolade was administered to only two cases, & no cases received immunosuppressive medications.

In their study, researchers examined the association between a specific gene polymorphism called tumor necrosis factor-induced protein three & the risk of developing chronic primary immune thrombocytopenia in Egyptian kids. They found that all cases initially received corticosteroids as a treatment (one hundred), while a smaller percentage received intravenous immunoglobulin (ten percent) & platelet transfusion (five percent). As a maintenance treatment, (87.5%) were treated with oral corticosteroids, (60%) received Imuran, and (5%) received Revolade.

In the research, out of the total of fifty-two kids, 86.7 percent have been administered steroids, 16.7 percent received IVIG, and 13.3 percent of kids with chronic ITP have been treated with azathioprine (Immuran). Eighteen kids with chronic ITP (thirty percent) were administered eltrombopag (Revolade). Out of the total number of cases, four cases with chronic conditions had splenectomy, which accounts for 6.7 percent of the cases.

The objective of treatment is to cease any active bleeding & prevent future hemorrhage. According to the present clinical guidelines from the American Society of Hematology, it is recommended to treat an adult case who has been recently diagnosed with a platelet count below thirty thousand using corticosteroids. In addition, intravenous immunoglobulin should be added for cases who have active bleeding or for those who need a quick increase in their platelet count. For cases who don't have an adequate reaction to initial therapy, alternative choices for therapy involve thrombopoietin-receptor agonists (TPO-RAs), immunological modulators like rituximab, or splenectomy. **(21)**

Platelet & blood transfusions should be initiated as a routine only in the presence of acute bleeding or in the case of an emergency major operation. In some cases, it may be acceptable to administer a platelet transfusion for severe hemorrhaging that isn't quite critical, if there is evidence of clinical deterioration. **(22)**

In our study, the laboratory results among ITP patients showed statistically significant lower hemoglobin (10.07±0.72 Vs 10.76±0.36 gm/dl), platelets (101.88±58.53 Vs 322.22±37.095), and DPC (103.44±57.70 Vs 322.22±37.095) levels compared to controls. On the

other hand, the ESR (15.48±1.521 Vs 11.00±0.34 for the 1st hour and 21.62±1.416 Vs 13.45±0.918 for the 2nd hour), and the bleeding time (7.61±0.493 Vs 4.80±0.405) were significantly greater among ITP cases compared to the control group. The TLC count did not differ significantly between both groups (8.09±2.178 Vs 7.64±0.93).

Similar results were seen in the study of**, (23)** who investigated the tumor necrosis factor-induced protein three gene polymorphism & the susceptibility to chronic primary ITP in Egyptian kids & discovered that the mean platelet count was 124 ± 72.86 $\times 10^9$, the mean Hb level was 10.98 ± 1.45 gm/dl, and the mean TLC count was $10 \pm 4.32 \times 10^9$. Also, the study of**, (24)** about the presentation & management of 1ry immune thrombocytopenia in kids in Saudi Arabia showed that patients with ITP had a platelet count (10^9 /L) 18.11 ± 24.16 (1.00–98.00), hemoglobin (gram/dL) 11.14 ± 2.10 (5.70–17.00), and leukocyte count (10^3 /L) 8.85 ± 3.79 (3.30–27.06)

The TNFAIP3 rs 10499194 gene polymorphism distribution among our studied participants revealed that about 23 patients (65.7%) were CC compared to 16 cases (45.7%) in the control group, 2 patients (5.7%) were TT while no cases in control were TT, and 10 patients (28.6%) were CT compared to 19 cases (41.1%) in control group with a statistically significant variance among the cases & the control groups. Gene polymorphism had a statistically significant association with family history in two cases with positive family history one of them was CT and the other was TT as well as with platelets, ESR, bleeding time, and DPC laboratory parameters in studied participants. On the other hand, there was no statistically significant association with age, sex, consanguinity, or the clinical picture in the studied population.

Our findings can be explained as autoinflammatory disorders being distinguished by spontaneous, recurring or continuous inflammation without any indication of elevated levels of autoantibodies or antigen-specific T cells. Over twenty causal genes have been identified as the underlying cause of immune regulatory diseases, a significant number of which manifest with symptoms such as fever, systemic inflammation, & inflammation specific to certain organs. The proteins encoded by these genes are primarily expressed in cells of

the innate immune system & perform significant roles in the regulation of the inflammatory response. (**25)**

5. Conclusion:

We concluded that consanguinity plays a role in ITP devotement as nearly one-third of ITP cases had positive consanguinity with a statistically significant difference as p. value= 0.029. ITP patients' Laboratory parameters had statistically significant differences regarding hemoglobin, platelets, ESR, Bleeding time, and DPC as mean values of hemoglobin, platelets, and DPC were lower in ITP patients while mean values of ESR and Bleeding time were higher in ITP patients.

6. References:

1. **Lambert MP, Gernsheimer TB.(2017)** . Clinical updates in adult immune thrombocytopenia. Blood 129:2829–35. doi: 10.1182/blood-2017-03-754119.
2. **Morodomi Y , Kanaji S , Won E , Ruggeri ZM , Kanaji T .(2020).** Mechanisms of anti-GPlba antibody-induced thrombocytopenia, Blood; 135 (25): 2292-2301.
3. **Audia, S., Mahévas, M., Samson, M., Godeau, B., & Bonnotte, B. (2017).** Pathogenesis of immune thrombocytopenia. *Autoimmunity reviews*, *16*(6), 620-632.
4. **Chen L, Flies DB.(2013) .** Molecular mechanisms of T cell co-stimulation and inhibition. Nat Rev Immunol 13:227–42. doi: 10.1038/nri3484 .
5. **Lim EL, Okkenhaug K.(2019) .** Phosphoinositide 3-kinase delta is a regulatory T-cell target in cancer immunotherapy. Immunology (2019) 157:210–8. doi: 10.1111/ imm.13082 .
6. **Vernerova L, Spoutil F, Vlcek M, Krskova K, Penesova A, Meskova M, et al.(2016)** . ACombination of CD28 (rs1980422) and IRF5 (rs10488631) Polymorphisms Is Associated with Seropositivity in Rheumatoid Arthritis: A Case-Control Study. PloS One (2016) 11:e0153316. doi: 10.1371/journal.pone. 0153316.
7. **Wang, M. J., Yang, H. Y., Zhang, H., Zhou, X., Liu, R. P., & Mi, Y. Y. (2016).** TNFAIP3 gene rs10499194 and rs13207033 polymorphisms decrease the risk of rheumatoid arthritis. *Oncotarget*, *7*(50), 82933.
8. **Terrell, D. R., Beebe, L. A., Vesely, S. K., Neas, B. R., Segal, J. B., & George, J. N. (2010).** The incidence of immune thrombocytopenic purpura in children and adults: a critical review of published reports. *American journal of hematology*, *85*(3), 174-180

9. **Rui, L., Schmitz, R., Ceribelli, M., & Staudt, L. M. (2011).** Malignant pirates of the immune system. *Nature Immunology*, *12*(10), 933-940.
10. **Huang, J., Yang, Y., Liang, Z., Kang, M., Kuang, Y., & Li, F. (2015).** Association between the CD24 Ala57Val polymorphism and risk for multiple sclerosis and systemic lupus erythematosus: a meta-analysis. *Scientific reports*, *5*(1), 9557.
11. **Abdaljabbar, H. N., Faraj, S. A., & AL-Rubae, A. M. (2020).** Study of Immune Thrombocytopenia (ITP) in Iraqi children in Wasit Province. *EurAsian Journal of BioSciences*, *14*(2).
12. **Taha, G. E. M., Ahmed, A. B., & Mabrouk, A. G. (2022).** Chemokine 12 plasma level in pediatric patients with Immune Thrombocytopenic purpura and its relation to Disease Activity. *Egyptian Journal of Medical Research*, *3*(2), 213-225.
13. **Diab, A. M., Abouamer, A. A., Motaleb, G. S. A., Eid, K. A., & Abdelnaiem, H. I. (2021).** Prognostic evaluation of immune thrombocytopenia outcomes in Egyptian children: a retrospective single-center experience. *Вопросы гематологии/онкологии и иммунопатологии в педиатрии*, *20*(3), 26-30.
14. **Ali, A., Zahad, S., Masoumeh, A., & Azar, A. (2008).** Congenital malformations among live births at Arvand Hospital, Ahwaz, Iran-A prospective study. *Pakistan Journal of Medical Sciences*, *24*(1), 33.
15. **Ariawati, K., & Setiyawan, I. M. K. (2019).** Platelets level response after three days therapy in children with acute Immune Thrombocytopenic Purpura (ITP): a 10 yearsâ€™ experience at the tertiary hospital. *Bali Medical Journal*, *8*(3), 897-901.
16. **Mohammed, M. A., Ahmed, A. S., Zidan, A. A., & Abd EL-Hakam, A. M. (2022).** Study of Clinical Features and Laboratory Findings in Children with Immune Thrombocytopenia. *The Egyptian Journal of Hospital Medicine*, *87*(1), 2096-2100.
17. **AL-Zuhairy, S. H. (2013).** Evaluation of prognostic factors in newly diagnosed childhood primary immune thrombocytopenia (ITP): a two-year prospective study at Al-Sadder Hospital, Missan Province. *Med J Babylon*, *10*, 855-69.
18. **Ismail, A. M., Higazi, A. M., Nomeir, H. M., & Farag, N. M. (2021).** IL-23/Th17 pathway and IL-17A gene polymorphism in Egyptian

children with immune thrombocytopenic purpura. Italian Journal of Pediatrics, 47, 1-8.

19. **Mohammed, M. A., Ahmed, A. S., Zidan, A. A., & Abd EL-Hakam, A. M. (2022).** Study of Clinical Features and Laboratory Findings in Children with Immune Thrombocytopenia. *The Egyptian Journal of Hospital Medicine*, *87*(1), 2096-2100.
20. **El-hady, M. A., Mosallam, D. S., Anis, S. K., Mansour, B. S., & Yassa, M. E. (2021).** Tumor necrosis factor-induced protein 3 gene polymorphism and the susceptibility to chronic primary immune thrombocytopenia in Egyptian children: a case-control study. *Egyptian Journal of Medical Human Genetics*, *22*(1), 1-9.
21. **Fida, N. M., Hamed, E., Jaber, S., Al Najjar, S., Al Amawi, D., Al Siny, F., & Alharthy, B. (2021).** Presentation and Management of Primary Immune Thrombocytopenia in Children at the King Abdulaziz University Hospital, Jeddah, Saudi Arabia: A Retrospective Study. *Journal of King Abdulaziz University-Medical Sciences*, *28*(1), 1-9.
22. **El-hady, M. A., Mosallam, D. S., Anis, S. K., Mansour, B. S., & Yassa, M. E. (2021).** Tumor necrosis factor-induced protein 3 gene polymorphism and the susceptibility to chronic primary immune thrombocytopenia in Egyptian children: a case-control study. *Egyptian Journal of Medical Human Genetics*, *22*(1), 1-9.
23. **Ismail, A. M., Higazi, A. M., Nomeir, H. M., & Farag, N. M. (2021).** IL-23/Th17 pathway and IL-17A gene polymorphism in Egyptian children with immune thrombocytopenic purpura. Italian Journal of Pediatrics, 47, 1-8.
24. **Zitek, T., Weber, L., Pinzon, D., & Warren, N. (2022).** Assessment and management of immune thrombocytopenia (ITP) in the emergency department: current perspectives. *Open Access Emergency Medicine*, 25-34.
25. **Terrell, D. R., Neunert, C. E., Cooper, N., Heitink-Pollé, K. M., Kruse, C., Imbach, P., ... & Ghanima, W. (2020).** Immune thrombocytopenia (ITP): current limitations in patient management. *Medicina*, *56*(12), 667.