An-Najah National University

Faculty of Graduate Studies

THE ASSOCIATION OF FCgRIIA AND FCgRIIIA POLYMORPHISMS AND ANTIPLATELETS AUTOANTIBODIES WITH PRIMARY IMMUNE THROMBOCYTOPENIA PATHOGENESIS AMONG PALESTINIAN CHILDREN

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This Dissertation is Submitted in Partial Fulfillment of the Requirements for the Degree of PhD in Clinical Laboratory Sciences, Faculty of Graduate Studies, An-Najah National University, Nablus, Palestine.

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In accordance with An-Najah National University Deans Council regulations for the award of Doctor of Philosophy, the following paper has been published after its extraction from the dissertation:

Amer K, Amer J, Taha AA, Baker W, Abuhamed A and Salhab A (2025) Combined *FCGR2A* (131H/R) and *FCGR3A* (158F/V) genotypes and their gender-specific association with chronic and refractory immune thrombocytopenia in Palestinian children. *Front. Med.* 12:1606953. doi: 10.3389/fmed.2025.1606953

Dedication

To my family, especially my parents, your support, love, and enduring faith in my path have made this possible. I am forever grateful for your guidance and prayers.

To my small, beloved children, Ward, Omar, and the cute Yasmina
Your smiles, small hands, and love have been my constant source of strength. You inspire me every day to be better, to work harder, and to dream bigger. This achievement is as much for you as it is because of you.

To Osama, for your endless support, patience, and belief in me during every step of this journey.

To my dear friends, your companionship, encouragement, and unwavering support have carried me through this journey. Thank you

To Palestinian children battling illness, your strength, suffering, and participation appreciated. This work is dedicated to your spirit, your dreams, and the hope that one day, every child in Palestine will grow up in health, safety, and peace.

To An-Najah National University, my academic home and the foundation of this journey, thank you for nurturing my passion, challenging my mind, and supporting my achievement. I am proud to be part of this institution and to carry its name forward.
With all my heart, this work is dedicated to you.

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Declaration

I, the undersigned, declare that I submitted the thesis entitled:

THE ASSOCIATION OF FCgRIIA AND FCgRIIIA POLYMORPHISMS AND ANTIPLATELETS AUTOANTIBODIES WITH PRIMARY IMMUNE THROMBOCYTOPENIA PATHOGENESIS AMONG PALESTINIAN CHILDREN

I declare that the work provided in this Dissertation, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name: Khitam Musa Diab Amer

Signature: *Khitam Amer*

Date: 15/3/3026

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Abstract

Background: Immune thrombocytopenia (ITP) is a common pediatric autoimmune disorder characterized by low platelet counts and bleeding risk. Fc gamma receptors (FcγRs), particularly FCGR2A (131H/R) and FCGR3A (158F/V), mediate immune responses and may influence ITP susceptibility. Gender-related genetic variation has been proposed but remains underexplored. In the current study, we aim to assess the prevalence and clinical relevance of FCGR2A and FCGR3A polymorphisms, including gender-based tendencies, in Palestinian children with ITP.

Methods: A multicenter case-control study included 40 proven pediatric ITP patients (20 males, 20 females; mean age (6.76▪ ±▪ 4.13 years) and 80 age- and sex-matched healthy controls. Genotyping was performed using PCR-RFLP and nested PCR, the antinuclear antibody (ANA) and the anti-double stranded DNA (A- dsDNA) were tested in 32 patient sample using (ELISA).

Results: No statistically significant differences in genotype distributions were observed or FCGR3A (FF: 25.0%, FV: 55.0%, VV: 20.0%) ($p > 0.05$). However, gender specific trends yielded noteworthy observations: FCGR2A-HH was numerically more frequent in male ITP patients (57.4%) than in females (42.8%), while HR was lower in males (48% vs. 52%). Similarly, FCGR3A-VV occurred in 62.5% of males versus 37.5% in females. Furthermore, the combined HR/FV genotype (32.5%) showed a non-significant trend of association with chronic ITP (69.2%), while the VV/HH genotype, although rare (5%), was linked to 50% of refractory presentations. The ANA was positive in 53% of tested samples, and a 3% were positive to both ANA and the A-dsDNA. ANA positive cases (70%) were in patients having the FV and HR genotypes.

Conclusion: This exploratory study found no statistically significant association between FCGR2A and FCGR3A polymorphisms and overall ITP susceptibility in the full cohort. However, the observed trends, with gender-based distribution of specific genotypes and the association of combined genotypes with case severity, suggest that these genetic markers may play a role in disease progression. Further investigation in a larger study is warranted to validate these findings.

Keywords: Immune thrombocytopenia (ITP); Fc gamma receptors (FcγRs); FCGR2A (131H/R); FCGR3A (158F/V); Polymorphisms. Antinuclear antibody (ANA), anti – double stranded DNA (A-dsDNA).

Chapter One

Introduction and Theoretical Background

Immune thrombocytopenia (ITP) is an acquired autoimmune disorder characterized by isolated thrombocytopenia, which can lead to increased risk of bleeding and negatively affect quality of life. While the majority of pediatric cases were acute and self-limited, a significant proportion progress to chronic ITP, requiring long-term management. There is little data available about ITP in children in the region, neither regional nor national guidelines for managing ITP in most Arab countries have been reported. In this part theoretical basis of the disease will be entangled, and research studies will be addressed.

1.1 Theoretical basis

Autoimmune diseases (ADs) is a group of rare heterogenous disorders, characterized by chronic inflammation, multiorgan and tissues involvement (Scherlinger et al., 2020). Usually, ADs are accompanied by immune system activation and impaired tolerance, with inability to differentiate in between self-antigens (Chen et al., 2022).

ADs and their importance were recognized in about 50 years ago by Macfarlane Burnett and his hypothesis of the ‘forbidden clone’ (L. Wang et al., 2015).

Epidemiological investigations revealed that ADs affect 3–5% of the population, with type I diabetes (T1D) and autoimmune thyroid disease being the more prevalent. There are about 100 autoimmune diseases identified which can be organ specific like the primary biliary cirrhosis (PBC) or with multiple organ involvement such as systemic lupus erythematosus (SLE) (Yu et al., 2014).

Changes in incidence and prevalence rate of autoimmune disease were difficult to assess, the lack of a database on national and international level, the absence of proposed criteria for proper case definition and the heterogenous characteristics of the disease make the estimation of actual registered cases more difficult and challenging (Miller, 2023).

The ADs may occur at any age, and differences are seen in different disease states. Ethnic and geographic differences were reported in incidence of specific autoimmune diseases; some groups may be at higher risk for some ADs and at lower risk for others.

And some of these may affect children more, while others more common among adults (G. S. Cooper & Stroehla, 2003).

Theories indicate that genetic predisposition, epigenetics phenomena, and environmental exposure are the major players that contribute to the development of autoimmune diseases. No definite genetic tools available that can be applied to predict ADs risk, but the identification of certain chemokines, and their receptors, were effective in blocking active inflammatory response by the generation of novel therapies (L. Wang et al., 2015)

In children autoimmune diseases represent a challenge to physicians in term of diagnosis, management, and treatment despite the ongoing research in this area their cause is not well defined (Carneiro-Sampaio & Coutinho, 2007).

The ADs were not well studied in children, but early onset ADs (diagnosed at < 5 years of age), have been linked to a genetic determinant which may alter cellular function, including variants with relation to inborne errors of immunity, human leukocyte antigen (HLA), and non-HLA variants (Tangye et al., 2022).

Environmental changes, climate, xenobiotics use, food habits, lifestyles, stress, and other changes all over the world may contribute to gradual increase in autoimmune disease development (Miller, 2023).

Large population- based study has estimated that up to approximately 10% of the population was impacted by ADs during the study period (from 2000 to 2019) (Conrad et al., 2023).

Immune thrombocytopenia (ITP) is an acquired autoimmune disorder (Schifferli et al., 2021)and the most common cause of isolated thrombocytopenia in children with a platelet count of (< 100 × 109/L) (Bergmann et al., 2010).

The first case of ITP was diagnosed in 1025, and in 1949 glucocorticoids were introduced as therapy of the disease. Several years after the recommended use of splenectomy, in 1950 the term “idiopathic” became obsolete, and the “I” in ITP stands for “immune” rather than “idiopathic,” in which the main problem in ITP was identified to be due to a specific serum factor (antibody) that destroy platelets in peripheral blood,

this is confirmed when William Harrington transfused himself with blood from an ITP patients, and directly his platelets fall to 3000 from 250,000 without any obvious change on his bone marrow megakaryocytes (Wilder-Smith & Mulholland, 2021).

ITP in children occurs with an incidence rate of 1.1 to 5.8 per 100,000 patient/years. (Kohli & Chaturvedi, 2019). Phases of the disease (ITP) were defined as newly diagnosed, persistent, and chronic according to a newly introduced terminology by the International Working Group (IWG); the newly diagnosed ITP: extends from the time of diagnosis up to 3 months; the Persistent: between 3 and 12 months; and finally, the Chronic ITP phase: with a disease duration of more than 12 months (Rodeghiero et al., 2009).

The pathophysiology of the disease is initiated by the production of autoantibodies directed against specific platelets glycoproteins leading to its opsonization, destruction, and impaired production (Kohli & Chaturvedi, 2019).

In children ITP is usually acute and seasonal; cases reported more in both fall and winter, indicating that environmental factors, and infectious agents: like the viral one, either acquired directly, or introduced by vaccination (especially measles, mumps, rubella vaccine) may evoke the immune system activity to attack platelets by platelets autoantibodies (Nugent, 2002).

Many cases in children remit spontaneously, most commonly within 6 months (Neunert et al., 2013). Approximately 20 to 30% of them develop the chronic form of the disease (Treutiger et al., 2007).

The acute form is usually secondary to viral infections in contrary to the chronic form, and many patients are asymptomatic, however, up to two-thirds of them may experience bleeding in skin and mucous membranes with epistaxis, gum bleeding, petechiae, and bruising (Praituan & Rojnuckarin, 2009). With the absence of splenomegaly, lymphadenopathy, and systemic symptoms in pediatric ITP (C. Neunert et al., 2011a). Patients with chronic ITP tend to bleed more and develop bruise when platelets count fall to $<30 \times 109/L$ (Neylon et al., 2003).

Bleeding tendency in patient's variable, such a variation despite the low platelets count among patients may indicate that, a protective mechanism may be initiated, studies

indicate that plate microparticles (PMP) formation may have a role on this (Boulware & Refaai, 2020).

The platelets microparticles, are small vesicles, released from apoptosis, and from the membrane of platelets upon activation, usually small with variable size of 0.2 to 2.0μm, and on its surface, it carries specific antigenic markers from its parent cells, it has the ability to activate the coagulation system, to carry factor V and tissue factor antigen (Westendorp et al., 2011).

Microparticles, involved in inflammation as confirmed in adult studies, the presence of PMP will reduce the bleeding tendency in thrombocytopenia cases, and its absence or impaired production will lead to sever bleeding as seen in Scott syndrome, it can be activated by platelets activation, exposure to agonists, by damage involving the vascular endothelium and other predisposing mechanisms (Piccin et al., 2007).

In pediatrics, bleeding rarely occurs despite of the low platelets count, high PMP level provides such a protection, patients need regular follow-up, monitoring, and evaluation because they are at risk to thrombosis in future, so care must be given after splenectomy to those with persistent activation of platelets (Tantawy et al., 2010).

Platelets production is variable in ITP cases, and this is correlated with the activity of the immune system and to what extent autoantibodies prevents their production, and when compared with other hematologic disorders like, the acute myeloblastic leukemia (AML), and the myelodysplastic syndrome (MDS), results indicate that there is more platelets activation, and increased immature platelet fraction (IPF) in ITP patients than others (Psaila et al., 2011).

In ITP pathogenesis and development different mechanisms are implicated, including the B-cells production of autoantibodies against platelets glycoproteins, complement system activation, the Fc gamma receptors mediated phagocytosis by splenic macrophages, also variations involving the T lymphocytes, and impaired megakaryopoesis (Evangelidis et al., 202) (Figure. 1).

Despite the idiopathic nature of the disease, a Defect in a regulatory T cell, and a loss of immune tolerance mostly accompanied by autoreactive B lymphocyte, that differentiates to antibody producing plasma cell is also indicated in ITP pathogenesis.

Glycoprotein IIb/IIIa is the target to splenic macrophages, and to antiplatelet antibodies by direct interaction with Both the humoral immunity, and the cell mediated cytotoxicity by cytotoxic T cell, it contributes to ITP pathogenesis by affecting both peripheral blood platelets, and megakaryocytes (Audia et al., 2021).

In 1991, the presence of reactive T lymphocytes was described by Semple and his colleagues, in which the T cells in ITP patient upon activation can release the (IL)-2 by autologous platelets (Semple et al., 1996).

Recently, in response to platelets those T cells with helper activity CD4+ were able to recognize the GPIIb/IIIa in ITP patients and induce the formation of IgG antibodies against it invitro, mainly to recombinant tryptic peptides of this receptor, produced only by bacteria not against the normal platelet receptor (Kuwana et al., 1998).

It is assumed that those tryptic peptides normally released early during the processing of the receptor, and produced in low levels, it is not recognized or ignored by the immune system, those peptides were recognized in ITP by antigen presenting cells (APCs) leading to T cell activation. GPIIIA cryptic epitopes were first described in a British ITP patient. The spleen is the source for the autoantibodies(Sander, 2000), and antigen presenting cells is the source for reactive T cell production, it remains unclear which APCs in the spleen are mainly involved in presenting cryptic peptide (Kuwana et al., 2009).

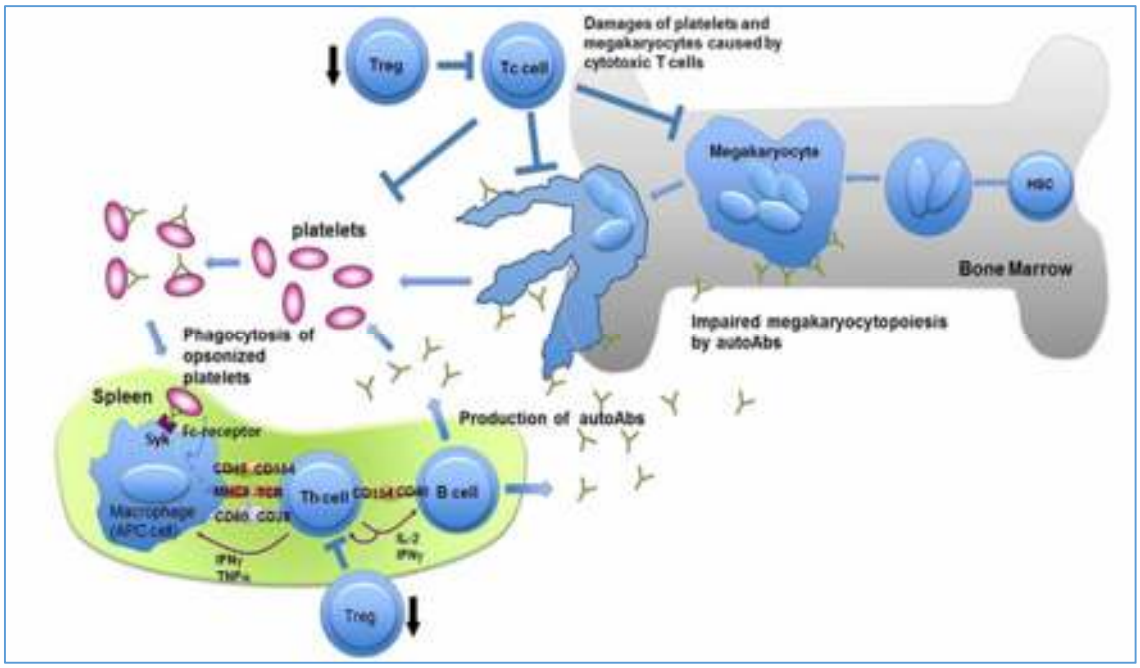
The Fc gamma receptors (FcγRs) proposed mechanism, is implicated when platelets get coated with anti GPIIb/IIIa antibody, and a non FcγR-dependent mechanism implicated in ITP pathogenesis when platelets destruction occurs by binding to the Ashwell-Morell receptor, and then cleared in the liver when it gets sensitized with an anti- GPIbα antibody, which leads to receptor desialylation upon platelet activation (Wilder-Smith & Mulholland, 2021).

Then this mechanism is implicated when platelets coated with antibodies against GPIbα not against the GPIIb/IIIa. GPIbα is the second most common receptor in platelets surface, and 60% of its composition is carbohydrate, it’s a glycosylated protein with high sialic acid content nearly 64% of the total platelets content of sialic acid, and its glycosylation results in hepatic platelets clearance (Okumura et al., 1976).

Glycan modification could enhance platelets clearance by an FcγR-independent mechanism, so as the corticosteroids intake is associated with side effects and the IVIG costly, these medications have to be given to those who can benefit from their intake, while for those with antibodies against GPIbα no benefits achieved from the intake of first line therapies and splenectomy and further is needed for the potential use of sialidase inhibitors(J. Li et al., 2015a)

Figure 1

Schematic illustration to the pathophysiology of chronic ITP (cITP)



Note: Kashiwagi & Tomiyama (2013).

The compliment, and its role in the pathophysiology of ITP is of particular interest since 1980s, its involvement has reached clinical development. In 50%- 60% of cases an activation of the classical complement pathway by the sera of ITP patients is observed (Peerschke et al., 2010).

This activation correlates with the presence of antiplatelet antibodies and their corresponding types. The percentage varied from 33% in cases where detection did not occur to 81% when detection took place; and greater tendency for compliment pathway activation by the anti-GPIIb/IIIa antibodies demonstrated compared to anti-GPIb/IX (73%–100% versus 40%–65%) (Audia et al., 2021).

One of several potential mechanisms that explain how a typical immune response to either an infection or a vaccination can generate cross-reactive antibodies that recognize and eliminate circulating platelets is the concept of "molecular mimicry," in which antigens on the platelets share similar protein sequences or resemble viral structures in their shape. A sequence homology of up to 220 amino acids were seen in between the common human DNA viruses and the platelet glycoprotein IIb/IIIa, adding to this High-rate of homologies were also seen with varicella zoster, herpes simplex, adenovirus, Epstein-Barr virus (EBV), and cytomegalovirus (CMV), also, high-homologies in sequence were also found in mumps, measles, and rubella, which are associated with acute ITP. In contrast to this is the Low scoring homologies found in Coxsackie, parvovirus, and the human immunodeficiency virus (HIV) (M. S. Elalfy & Nugent, 2016).

Individuals of all ages, races and either gender could be affected and may develop ITP but higher incidence rate were reported among adult females (Bussel et al., 2021).

ITP is further classified into two categories: the primary and the secondary. Primary ITP is an acquired immune disorder, while secondary ITP is usually triggered by another underlying disorders (Zitek et al., 2022).

Primary ITP is defined as a platelet count of less than (100 x109 /L) in the absence of any other causes or underlying conditions (Mithoowani & Arnold, 2019).

Secondary ITP is associated with all forms of immune-mediated thrombocytopenia, it may occur secondary to infections and other diseases, including systemic lupus erythematosus (SLE), antiphospho-lipid syndrome, and the immunodeficiency disorders like the common variable immunodeficiency, and the IgA deficiency, adding to this the chronic lymphocytic leukemia (CLL), lymphoma, large granular lymphocytic leukemia (LGL), and infection with the human immunodeficiency virus(HIV), hepatitis C virus (HCV), cytomegalovirus (CMV), H. Pylori; and can also develop secondary to drug exposure following heparin and quinidine intake (Ouglas et al., 2002a).

The diagnosis of primary ITP is one of exclusion (Rodeghiero et al., 2009) with no confirmatory lab test available to indicate it (Provan et al., 2019).

The initial evaluation includes physical examination, patient and family histories, the complete blood count and the reticulocyte counts, blood film examination, the direct antiglobulin test (DAT), blood group determination, immunoglobulin level measurement, Helicobacter pylori stool antigen test, bone marrow biopsies, in addition to this testing for hepatitis C virus and the human immunodeficiency virus, and further laboratory investigation for the evidence of hemolysis (Provan et al., 2019a).

The antiplatelet antibodies were produced by autoreactive B lymphocytes which bind to the platelet's surface, platelets get opsonized for splenic clearance, resulting in their low level in circulation (Anthony et al., 2008).

Autoantibodies against GPIb-IX complex, the Willebrand factor (VWF) receptor were detected in 70–80% of cases, adding to this in 20–40% of ITP patients, autoantibodies were directed against the fibrinogen receptor glycoprotein (GP) IIb/IIIa, and mainly detected in patients with active disease and non-splenectomized cases (Hou et al., 1995).

Studies have shown that inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), autoantibodies, and cytotoxic T cells may target megakaryocytes, leading to suppression of their maturation, production and release, (Provan et al., 2019a).

Moreover, sustained thrombocytopenia observed in ITP representing the inadequate compensatory production and the increased destruction, the recognition of antiplatelets antibodies is the diagnostic hallmark of ITP. Platelets Antibodies may recognize antigens derived from multiple or a single glycoprotein, detected in approximately 60% of patients, and this may be attributed to limited sensitivity issues of the test, or due to an additional implicated mechanisms of platelet loss or unspecified antigens (Kelton et al., 1982).

Direct and indirect antigen specific assays for the measurements of antibodies against platelet glycoproteins (GP) had emerged. Direct assays (measuring antibody on platelets) for anti-GP IIb-IIIa and/or anti-GP Ib-IX had moderate sensitivity (49–66%) and a specificity of (78–93%) while Indirect tests for the measurement of circulating antibodies were rarely positive (Warner et al., 1999).

The limited sensitivity issues of platelet antibody testing may be low, because of the heterogeneity of the ITP patient population in which they were studied, adding to this is the inability of current assays to detect other antibodies. Most commonly antibodies with known specificity to (GPIIb-IIIa and GPIb-IX) were recognized. Finally, impaired megakaryocytes production may support the hypothesis of underproduction and autoantibodies formation (Toltl & Arnold, 2011) as shown in cell culture techniques, the distribution of these autoantibodies in tissues and cells may limit their detection in a specific assay (McMillan & Durette, 2004).

The guidelines implemented in 2019 by the American Society of Hematology (ASH) for ITP management don't recommend testing to autoimmune diseases in absence of clinical signs, while the international guidelines prefer It. Antinuclear antibodies (ANAs) are autoantibodies directed against various components of the cell nucleus. In Europe about 33.3% of the population were ANA positive and increasing with time, as the titter increased more it indicates higher possibility to develop autoimmune disease (Akmatov et al., 2017).

Most of the studies investigating A possible association in between ANA and primary ITP were retrospective and show variability in terms of the study population (e.g., children vs. adults, newly diagnosed vs. chronic cases), methods applied and the criteria to define ANA positivity. In recent studies A 40% prevalence rate were estimated when using the threshold of ≥1:160 (Moulis et al., 2020).

Low titer estimates were 13% to 65% in children. ANAs were more common in adolescent girls than in younger children and boys (Agmon-Levin et al., 2014), adding to this as mentioned in previous retrospective studies an association confirmed in between ANA and the progression to chronic ITP, In a retrospective series of 365 ITP children, the percentage of patients with chronic ITP with a positive ANA dosage (≥1:80) was (18.7%) compared with those with 'acute' ITP (6.9%).

In this study the prevalence of ANAs is higher in patients compared with the general population, in children, and adults, studies argue the possible use of this as a potential biomarker for the progression of the case to chronicity, to evaluate response to some second line treatments, and progression to systemic lupus and thrombosis, which may

rarely accompany the ITP, higher tendency to develop lupus in females with high ANA titer and presence of anti- ENA/anti- DNA antibodies (Moulis et al., 2023).

A characteristic feature of connective tissue diseases (CTDs) such as systemic lupus erythematosus (SLE), Sjögren's syndrome, antiphospholipid syndrome (APS), and others is the presence of antinuclear antibodies. Also, it can be found in some ITP patients who do not meet CTD diagnostic criteria Thus, it has been assumed that positive- ANA primary ITP might be a distinct subgroup of primary ITP due to the risk of developing CTD, following the 2009 standardization guidelines (Lambert & Gernsheimer, 2017).

SLE is closely associated with the presence of both ANA and thrombocytopenia. A preclinical stage of SLE is presented in those having ANA positive primary ITP Patients. In this group of patient's treatment strategies, maintenance therapy, prognosis and impact on quality of life were all different than SLE. In nearly 5% of SLE patients, isolated thrombocytopenia is the first reported clinical manifestation, and it has been proven that patients with primary ITP are at increased risk of SLE development (Zhu et al., 2020).

This study affirmed that patients with primary ITP who are ANA positive had a 21.6-fold increased risk of developing a new connective tissue disease and a 48-fold higher risk of (SLE) development, in comparison with ANA-negative ITP patients (Fayyaz et al., 2015).

These results suggest that primary ITP with positive ANA represents a unique subgroup in terms of CTD risk. The highest risk for developing CTD was observed within the first four years after ITP diagnosis, and then after that the risk gradually declined. Overall, evaluating this subgroup could improve ITP management, facilitate earlier SLE diagnosis, and offer insights into the early stages of SLE pathogenesis (Y. Liu et al., 2021).

Cellular death can occur via different processes including apoptosis, exposure to ultraviolet light, drugs, and infections, when NETosis occur cells extrude the DNA and the neutrophil extracellular traps (NETs) (Mistry & Kaplan, 2017).

In apoptosis, apoptotic bodies contains the cleaved DNA, and normally the nuclear antigens as the dsDNA are not exposed to the immune system, and usually contained in the nucleus and degraded in the cytoplasm and the endosomes by the DNases (Magna & Pisetsky, 2016) adding to this the DNA microparticles released by cellular necrosis can be recognized by the anti- DNA antibodies with immune complexes formation which eventually will be recognized by immature dendritic cells (iDCs) via the FcγRs and later these cells will migrate to lymphatic organs from peripheral tissues for further maturation (Mackern-Oberti et al., 2015).

Different DNA structure can be recognized by the Anti- dsDNA antibodies including locked nucleic acids, DNA: RNA hybrids, ss-DNA and others like the modified DNA containing thymidine dimer which binds autoantibodies with higher affinity (Akberova, 2016).

Different subclasses of the Anti-dsDNA implicated and the one that correlates with disease in human is the IgA and the IgG and mainly with SLE and the IgM conferred protection by clearing apoptotic materials (Villalta et al., 2013)

The lack of a platelet response to all emerging therapies in pediatric ITP is termed refractory and represents a challenge and a high disease burden. These patients face a greater risk of persistent disease burden and warrant further assessment, alongside the exploration of clinical trials or innovative therapies. Future initiatives to characterize disease burden and treatment response will be carried out in partnership with the ITP International Working Group (Nakano et al., 2024).

According to data from the Intercontinental Cooperative ITP Study Group (ICIS) remission rate in which the (platelet count either ≥150 × 109/L or ≥100 × 109/L) occurred in 37% of patients exhibiting the disease between 28 days and 6 months, and it was 16% for those having the disease between 6 and 12 months and 24% for disease duration between 12 and 24 months (C. Neunert et al., 2011a).

More than 50% of children having the chronic form of the disease eventually achieved platelet remission. However, a minority of patients after a short period of remission experiences a recurrence of thrombocytopenia, known as recurrent ITP and the first time when recurrent ITP described it was in 1961, and defined as the recurrence of

thrombocytopenia after at least 3 months of re mission without treatment (Walker & Walker, 1961).

studies on recurrent ITP in children are limited, small series, Despite of the 60 years recognition period, (Yang et al., 2024). About 4%–6% of ITP cases in children were recurrent (Vranou et al., 2008).

Globally, women were more (ITP) affected than men, with a higher incidence were reported in older patients. However, across different countries limited data is known about the epidemiology and treatment (Bekadja et al., 2024).

During the last several years, proper recognition of the basic concepts in the pathophysiology of ITP has significantly improved but in the Asian population the epidemiology and clinical course of ITP have not been well investigated in the general population (Hamzah et al., 2022).

Genetic blood disorders are quite prevalent in the Arab region because of the high rate of consanguinity, but there are scattered data about Immune thrombocytopenia. In literature over the last decades, the published data were more retrospective studies. the International Cooperative ITP Study Group had been joined by national centers on their own (M. S. Elalfy, 2013a).

A retrospective study conducted in 1981 was the first to report about ITP in the Arab world. It suggested that ITP patterns in Arabs might differ from those in other countries, with over than 45% chronicity of ITP in four Arab countries, in comparison with a 30% rate of chronicity in Egyptian studies, which is comparable with the international reports, and confirmed recently in a newly published study (M. S. Elalfy, 2013a), a 10% lower chronicity rate was registered in a retrospective study in Lebanon (Moussalem & Yassine, 2003).

In low-income countries, the incidence of the disease is underestimate reflecting a lack of population-based data. in Nigeria in total hospital admissions a prevalence of 0.005% were reported in previous findings (Brændstrup et al., 2005). In contrast, in high income countries a 3-10% incidence rate is registered in children less than 16 years in age while the incidence in low-income countries is unknown (Rehman, 2007).

In children from high-income countries within 1 to 4 weeks following viral infections, patients typically presented with petechiae, sometimes mucosal bleeding, and bruising with absence of other systemic symptoms such as fever, pallor, weight loss, joint pain, hepatosplenomegaly, and lymphadenopathy. Mild splenomegaly is observed in approximately 5%–12% of symptomatic cases, in low-income countries clinical manifestations were comparable, However, anemia, splenomegaly, and lymphadenopathy may be more common in these regions, due to higher rate of infections and nutritional deficiencies. In a study in Nigeria in about 72% of cases iron deficiency anemia reported, and this reflects the burden of iron deficiency anemia in low-income countries (Rehman, 2007).

Both genders affected equally in high-income countries, whereas the female to male ratio is 2.3 to1.9 in low-income countries The peripheral blood smear examination, and the complete blood count (CBC), are the recommended lab tests by the ASH for ITP diagnosis, while, the DAT, antinuclear antibody, HIV testing, reticulocyte count are not recommended, in high income countries in about 75%- 90% of cases remission achieved, the bone marrow examination is not required, and can be ordered only when there is a doubt about the diagnosis in contrary to other published data which recommends the bone marrow examination in each a typical case (Rehman, 2007).

Isolated thrombocytopenia without a recognized predisposing causative agent is considered a primary type ITP, contrary to the secondary type ITP, that is accompanied with other coexisting systemic disease, so as no specific gold standard available to indicate it, primary ITP remains the exclusion of other causes of thrombocytopenia (Cines et al., 2009).

The human FCG receptors represent a group of receptors, distributed in all immune cells, and its gene is located on the long arm of chromosome 1 (De Wit1 et al., 1993). It exerts diverse functions through engagement with the Fc fraction of immunoglobulin G complexes, (Fleit et al., 1983)]. It becomes a key group of receptors, which are correlated with susceptibility or protection against autoimmune diseases. In addition, currently it is considered pharmacological targets of importance (Sepúlveda-Delgado et al., 2025). It allows responsiveness to all antigens opsonized with IgG. And by this the FCGRs have a big role in host defense and clearance of immune complexes. However,

an alteration in FcγR function could result in autoimmunity and impaired host defense (Sepúlveda-Delgado et al., 2025).

Autoimmune diseases have been associated with FCGR variants and copy number variation (CNV). Genetic and genome wide association studies have identified the participation of FCGR in the physiopathology of a wide variety of autoimmune diseases such as SLE (Bonegio et al., 2019), and rheumatoid arthritis (Ioan-Facsinay et al., 2002),

The human FCGR system is composed of 2 opposing families, the activating FcγRs (FcγRI, FcγRIIa, and FcγRIII) and the inhibitory FcγR (FcγRIIb)(X. G. Liu et al., 2011). Findings indicated that an altered FcγR balance might play a role in the pathogenesis of ITP. Both receptors play an important role in the phagocytosis of autoantibody coated platelets (Eyada et al., 2012a).

Within the human genome in between healthy individuals when compared, deletions and chromosomal duplications were seen, so copy number variations indicate that there are millions of nucleotides were different in their DNA, in addition to the reported single nucleotide polymorphisms, these copy number variations affect the gene expression and protein level (Linzmeier & Ganz, 2005).

This phenomenon is not new within the Fcγ R gene cluster, in both the FCGR2C gene and the FCGR3B gene it is reported and described, so genetic variations either SNPs or CNV contribute to susceptibility phenotype, making balance between the two Fcγ receptor families disturbed (Koene et al., 1998).

Several polymorphisms exist for both FCGRIII and FCGRII genes in humans. The importance of these variants as biological markers stands in the fact that these have altered affinities for IgG, leading to different rates of immune complexes clearance in patients who exhibit these variants (Pavkovic et al., 2018)

The FCGRIIa is the main phagocytic FCGR in humans and unique to higher primates It is with widespread in immune cells, its activity and ability to function depends on the type of the cells in which the receptor is expressed (Ramsland et al., 2011), in general

neutrophils and macrophages show high phagocytic activity through this receptor (Salmon et al., 1992).

Single nucleotide polymorphisms (SNPs), involving the FCGRIIa receptor, that affect affinity ligand binding have been studied. The one that is entangled more in research is the change in arginine (R) by histidine (H) at position 131. Individuals having the R allele of the FCGRIIa are at higher risk to develop autoimmune diseases and bacterial infections compared with those homozygous and heterozygous for the H allelic form (Shashidharamurthy et al., 2009).

The FCGRIIa-His131 binds with low affinity to IgG3, IgG1, and IgG2, while the FcgRIIa-Arg131 only binds IgG1 and IgG3 (Biezeveld et al., 2007).

Depending on ethnicity, the frequency of homozygous individuals for either the R131 or H131 variant of FCGRIIa varies between 25 to 35% (Lehrnbecher et al., 1999).

The FCGRIIIa is a transmembrane protein expressed on monocytes, dendritic cells, tissue specific macrophages, T cells, and the natural killer Its activity is recognized by the antibody-dependent cellular cytotoxicity (ADCC) which is important for antiviral immunity and antitumor therapies (Nimmerjahn & Ravetch, 2008).

The ADCC is initiated by NK cells through the binding of antibody-opsonized target cells by the FCGRIIIa, and cytotoxicity induced by the release of perforins and granzymes stored in intracellular granules. This mechanism contributes to the killing of tumor cells during immunotherapy. The ADCC is mainly triggered by IgG3 and IgG1 through interaction with the IgG-Fc-receptor FCGRIIIa (De Taeye et al., 2020).

Genetic polymorphisms affecting FCGRIIIa gene with T-to-G substitution at nucleotide 559 results in a change in amino acid at position 158, valine instead of phenylalanine, which lies in the immunoglobulin-binding domain and this substitution will be accompanied with increased binding capacity, more contribution in enhancing such an inflammatory response, and autoimmune diseases development (X. Li et al., 2009).

As a result, higher binding affinity in between the FCGRIIIa-V variant and IgG1 and IgG3 compared with the FCGRIIIa-F variant, indicating more contribution to ITP susceptibility, treatment response and its selection, while the FCGRIIa H allel is

significantly more reported in chronic ITP cases, adding to this, it is known that, mainly the platelet autoantibodies in ITP were IgG1 or IgG3 subclasses (Eyada et al., 2012a)

The effect of the FCGRIIa in human disease is understudied due to shortfalls in rodent models of the disease, and the wide complex effect conferred by the receptor polymorphisms, which exert an effect on activating platelets and other cells. taking in consideration that platelets express the FCGRIIa which triggers the release of secondary platelets mediators upon activation, future research interm of countering the effect of FCGRIIa in platelet specific manner is needed almost in patients exhibiting or at risk to develop autoimmune diseases and thrombotic complications (Qiao, Al-Tamimi, Baker, Andrews, Gardiner, et al., 2015).

Fall in platelets count were more severe in ITP patients with anti-GPIb-IX autoantibodies also, they are less responsive to conventional therapies (Swinkels et al., 2018a). Moreover, a new indicated mechanism which mediate hepatic platelets clearance by anti- GPIba antibody but not the GPIIbIIIa which is an Fc-independent one induced by a hepatic Ashwell–Morell receptors (Da Cunha-Bang et al., 2016) which may have a role in understanding the variations in response to splenectomy, further is needed to confirm a potential association (J. Li et al., 2015b)**.**

1.2 Literature review

There is conflicting data regarding the exact significance of the above-named polymorphisms in childhood ITP (Papagianni et al., 2013a). The association of FcgRIIa polymorphism with susceptibility to ITP development were investigated in research studies with conflicting results most probably relating to, differences in clinical definitions of ITP, ethnicity, and the small sample size (Qiao, Al-Tamimi, Baker, Andrews, Gardiner, et al., 2015).

Polymorphisms of the FCGR2/3 locus are associated with susceptibility to childhood ITP and progression to chronic disease. Genotyping of the FCGR2/3 locus may be helpful to determine prognosis and personalize treatment decisions (Schmidt et al., 2019).

To investigate the association in between the FCGR IIa and IIIa polymorphisms and ITP pathogenesis, a study was conducted including 98 children with ITP, who referred

usually to the Hospital for Sick Children, and 130 control healthy adults registered in the Canadian Blood Service in Toronto/ Canada, and the general participants characteristics were obtained from their medical records.

Among patients 27 of them have acute ITP with a mean age of (4.8 years old) at presentation, and 71 of them develop the chronic form with a mean age of (8.0 years old). Allele and genotype frequencies were compared between patients and controls.

The FCGRIIa-131H allele frequency was 0.600 in patients and 0.496 in controls and the FCGRIIa-131R allele frequency was 0.4 vs. 0.504 in control, and the three possible genotypes were significantly higher in patients than control with over presentation of the FCGRIIa-131HH genotypes. The FCGRIIIa-158F allele frequency was 0.577 vs. 0.690 in control, and V allele frequency was 0.423 vs. 0.310 respectively. Significant differences seen in terms of the three possible genotypes with significant trends in the FCGRIIIa VV genotype among patients.

Combined genotype analysis was performed and results showed that the combination of the high affinity alleles, The FCGRIIa-131H and the FCGRIIIa-158V alleles was more common in patients (61%) than control (46%) and the difference was statistically significant (P-value is 0. 02). Adding to this no difference is seen in between these polymorphisms based on case severity (acute vs. chronic), gender, and age at presentation (Carcao et al., 2003).

Results of this study were comparable to results by Foster et al. (2001), which involved a much smaller cohort of children with ITP. The FCGRIIa R/R is 25%, the HR 44%, and the HH 31% in control vs. 19%, 36%, and 44% in chronic ITP cases, while the FCGRIIIA FF, FV. And the VV possible genotypes were 50%, 39% and 11% respectively compared with that in patients 35%, 62%, and 3% (Foster et al., 2001).

However, three studies involving Caucasian childhood-ITP cases indicate that carriers of the 131H allele were at higher risk (Carcao et al., 2003; Foster et al., 2001).

Papagianni et al, studied the two SNPs in a group of patients (N. 53) with primary and chronic ITP their ages between 6 months to 15 years, who referred from March 2008 to June 2009 to the Pediatric Departments / Aristotle University of Thessaloniki, then patients were kept in follow-up until August 2010, among patients 30/53 develop newly

diagnosed ITP and recovered, 2/53 have persistent ITP and the remaining 21/53 were chronic, patient results were compared with a 45 adult healthy voluntary blood donors. Results indicate that allele frequencies for the FCGRIIa in both study groups were the same, the difference in FCGRIIIA F and V allele frequencies was statistically significant in between patient and control (0.547 and 0.453 vs. 0.655 and 0.344), and the ((P= 0.019), the FF, FV, and VV genotypes were exhibited in (11%,87% and 12%) of patients and in (33%, 64%, and 2%) respectively, the differences for the FF and the FV genotypes were significant. Adding to this no significant differences are seen between newly diagnosed and chronic cases (Papagianni et al., 2013b).

The two polymorphisms were also studied in adult ITP patients; in this study a total of 125 patients (90 female, 35 males) were included and 120 individuals (88 female, 32 male) were taken as a control group, the mean age of patients was 47 years old, and all participants were Macedonian orthodox of Caucasian origin, results indicate that, interm of allele and genotype frequencies, no statistical differences were observed regarding the FCGR2A-131H/R polymorphism in between patients and control, while a significant differences in allele not genotype frequencies of the FCGR3A polymorphism were noticed, the F allele frequency in patients compared with control was (52.8% vs. 62.%5) and the V allele frequency (47.2% vs. 37.5%) and the (p = 0.037).

In this study also patients were subcategorized into responsive and unresponsive treatment, and their status were correlated with their FCGR proposed polymorphisms, and a significant difference was observed within the FCGR3A not the FCGR2A polymorphism, the observed difference was in both allele and genotype frequencies.

Combined genotype analysis in this study indicates that the combination of the high affinity alleles the H and V were more in patients than control (55% vs. 40%; p = 0.024), and the low affinity alleles, the R and F allele combination were more in control than in patients (70% vs. 50.4%; p = 0.027) (Pavkovic et al., 2018a).

In a study of 116 ITP patient, 72/116 (48% males) were children and 44/16 were adults (15.9% males), genetic variations were studied by the use of anew assay Multiplex ligation-dependent probe amplification (MLPA) and PCR in between the two study groups, copy number variations to 3 copies of the FCGR3B allele were found doesn’t increase the susceptibility to ITP, no CNV observed in FCGR2A and FCGR2B, in

seven ITP patients 3 copies of the FCGR3Ballele were found, and in six patients 1 single FCGR3B allele was found, and these allelic variations linked to those linked to the FCGR2C gene which is expressed in18% of healthy individual's and in 34.4% of ITP patients, and these finding indicate its significance in susceptibility to hematologic autoimmune diseases particularly the ITP. With in the FCGR3A entity a gain CNV seen in four patients and also a loss of CNV in four patients seen, adding to this no significant difference observed in between patients and control regarding the Fc RIIa-131H/R polymorphism, while the difference was statistically significant regarding the Fc RIIIa-158F/V 55.2% and 44.8% and the (P.003) in the pediatric ITP group not the Adult ITP group in which the difference in comparison with the control group was statistically insignificant (Breunis et al., 2008).

In another study, in Netherlands,the relative contribution of (FCGR) to disease susceptibility and response to treatment (IVIg) were evaluated in a total of 122 children with Newly diagnosed ITP, 22 chronic children ITP cases, and 180 healthy controls, genotyping of both FCGR2/3 was performed, results indicate the distinct association of these SNPS with ITP susceptibility in both transient and chronic ITP cases and support the use of these in-patient treatment and prognosis. The FCGR2A*27W, FCGR2C*ORF and the FCGR2B promoter variant 2B.4 variants were more reported in patients with transient not chronic ITP, and in chronic cases the FCGR2C/FCGR3B, copy number region 1 deletion reported more in chronic cases with an odds ratio of 6.2 (95% CI, 1.8-24.7) (Schmidt et al., 2019).

In Egypt in 2011, a study among 92 ITP Egyptian children 48 females (52.2%) and 44 male (47.8%) (80 chronic (86.9) and 12 acute (13.1%)) attending a pediatric hospital in School of medicine/ Cairo University, their ages ranged 1-14 years, adding to this a control group of Ninety age and sex-matched normal individuals were included 50 females (55.6%) and 40 males (44.4%). Their ages are between 1 and 13 years, this study aimed to assess the frequency of those polymorphisms and their relative contribution to disease severity.

Results indicate that The V allele and FCGRIIIa FV heterotype were significantly higher in ITP patients and conferred increased ITP risk and the frequency of. FCGRIIIa gene polymorphism may contribute to susceptibility to ITP, and the combined genotype

analysis revealed that the HH/FV was significantly more in patients than controls with two fold increased ITP risk, no significant difference was noted in between patients and controls interm of the FCGRIIa polymorphism distribution, the FCGRIIa H allele was significantly higher among chronic ITP patients while the R allele is over represented in acute ITP cases, and these findings support the idea of the potential use of the H allele as a marker to predict chronicity of the case, the frequency of three possible FcgIIA131H/R genotypes were (70.6%) to HH, (8.6%) to HR and 20.7 to RR possible genotype.

Compared with other studies results were controversial correlated with some studies and opposite to others indicating the effect of ethnic differences among the studied groups (African American, Japanese, Caucasians, and the far east population), adding to this no observed association was seen in between the mutant, wild types of these SNPs and antiplatelets antibodies in ITP patients. Moreover, this study indicates these polymorphisms may offer protection or susceptibility to develop the ITP so medications effectiveness, and treatment selection, maybe conferred from the useful analysis of the FCGR polymorphisms in ITP patients, and those having the V allele will benefit more from by blocking this receptor by the use of anti- D and they are properly selected candidates to IVIG, and this have to be confirmed in further studies (Eyada et al., 2012a).

The high-affinity (158 V) FCGRIIIa variant is possibly indicated in disease susceptibility, but neither this nor the FCGR11A single-nucleotide polymorphisms seem to have a role on chronicity or therapeutic response to IVIG(Papagianni et al., 2013b) results by (Pavkovic et al., 2018b)suggested possible role of FCGR3A polymorphism in the etiology, development and clinical outcome of ITP, but there is a need to larger prospective studies to support these results (Carcao et al., 2003).

In a study in Egypt in 1.4-year follow-up period from 2018 until 2019, at the Pediatric Hematology Unit, Zagazig University Hospital. A Total of 80 patients with newly diagnosed ITP were kept under observation- and 86 healthy children were selected as a control group in this study patients with secondary, chronic and persistent ITP were excluded. Patients were (32 females and 48 males) with a mean age of (4.51) years at time of diagnosis, (80%) of them exhibit purpura, (65%) develop ecchymosis and (56%)

were seen with external bleeding, and in (61%) of them, acute ITP cases, a preceding viral infection were detected, corticosteroids were given to (60%, 48/80), while (17.5%, 14/80) of them given both intravenous immunoglobulin and corticosteroids, (5%, 4/80) treated only with the IVIG, and (17.5%, 14/80) were kept under observation. And during the follow up period (23.8%, 19/80) children develop chronic ITP.

Frequencies of the two single nucleotide polymorphisms were investigated, both the FCGRIIIa-158V and the FCGRIIa-131H were significantly over-represented in ITP patients. The same was noted interm of the combined FCGRIIa-131H and FcgRIIIa-158V allelic gene frequencies, allele frequencies for the FCGRIIa-131H and -131R were 51.3 % and 48.7%, respectively, in ITP children, while in controls75% and 25% and the differences were statistically significant ($p = 0.002$).

The compound heterozygous HR genotype was significantly higher in ITP patients ($p < 0.05$), and the FCGRIIIa-158F and -158V allele frequencies were 46.3% and 53.7%, respectively, in children with ITP, versus 70% and 30% in controls ($p = 0.002$). The combined HR/FV genotype was 47.5% in ITP patients, versus 10% in controls ($p <0.001$). No significant difference was noticed between children with newly diagnosed ITP and those who developed chronic ITP, regarding allele and genotypes frequencies for both the FCGRIIa and FCGRIIIa ($p > 0.05$) (Zakaria et al., 2021).

In Egypt, A case control study over a period of three months June- September 2016 in patients with ITP (27 females and 28 males) with a mean age of 6.29±3.76 years old were recruited from the outpatient clinic of the Pediatric Department of Beni Suef University Hospital, patients with SLE, type 1 diabetes, recent infections, secondary ITP and less than 6 months were excluded, also an age and gender matched control group was included (25 female, and 30 male) and their mean of age was 5.38±2.1 years old.

Analysis of the two proposed SNPSs showed that the FCGRIIa H and R allele frequencies were 56.4% and 43.6% respectively compared with 55.5% for the H allele and 45.5% for the R allele in control group, in which the difference is not statistically significant (P=0.891), allele frequencies for the FCGRIIIa (F vs. V) in patient groups were statistically insignificant when compared with the control group (47.3% vs. 38.2% for the F allel and 52.7% vs. 61.8% for the V allele), adding to this the FV variant were

more reported in ITP than control (54.5 % vs. 25.5%) and the difference is statistically significant, further on no association were found in between the FCGRIIa and FCGRIIIa SNPS, the severity of the case and bleeding tendency (Mohamed et al., 2019).

In Turkey, A recently published work in Turkish ITP children, the study included data about patients referred to a pediatric unit over a period of 13 years in a tertiary hospital in Turkey between 2006 and 2019, a total of 123 patients with a mean age of 6.4±4.0 years(range; 1-15 years), 49(39.8%) female, 74(60.2%) male were included,93 with acute ITP while 30 chronic, this study aims to assess any possible risk factors for the development of the chronic course of the disease results indicates that female gender. older age, and insidious onset of the disease at admission may predict chronic ITP in childhood findings indicate that mean age in acute cases were lower than chronic cases (5.0±2.9 vs. 10.7±3.7 years, $p<0.001$) and the chronic cases were common more in females (40.8% vs. 13.5) and the difference is statistically significant, adding to this girls were 4.1 times at higher risk of chronic ITP (95%CI: 1.5-10.3, $p=0.003$) in this study also about 30 (24.4) patients were tested for the antinuclear antibody, in about 43.3% of them (13/30) positivity confirmed but the difference in between acute and chronic cases were statistically insignificant (Alpdoğan & Gülen, 2023), In full agreement with what previously published (Elalfy, 2013b).

In another study in Turkey which included 201 Turkish ITP children (108 females and 93 male) in a follow up period of 22 months, cases from January 2000 to 2009 at the Department of Pediatric Hematology of Uludağ University Hospital were included in the study, epistaxis, gum bleeding, ecchymosis and petechia, (51%) of them given treatment, (65%) given IVIG and (35%) corticosteroids, and others (49%) kept under observation, among them findings indicate that, age and gender were found to be risk factors for the development of the chronic form of the disease, age above 10 years old and female gender, females above 10 years old tend more than males to develop chronic course, response to treatment were the same for both drugs and the ANA was positive in 10% of the chronic cases and the antiphospholipid antibody (APA) positive in 8% of them(Sezgin Evim et al., 2014a).

These Findings were contrary to data and findings by Yaprak et al, who studied a total of 350 children (164 males, 186 female) their ages between 6 months to 16 years old and did not find any gender preferences related to chronic ITP, in this study patients referred to the Pediatric Hematology Division, Tepecik Training and Research Hospital, İzmir, Turkey between 1990 to 2016, patients were evaluated interm of their presenting features, treatment response and duration of the disease, 63.8% of cases were acute,(29.1%) chronic, and (7.1%) recurrent, the study findings also indicate that children's who develop acute ITP were younger than those with chronic and recurrent ITP, and only (11.7%) of acute cases were in children whose ages above 10 years old, and there's two fold increased risk rate among those older than ten years to develop chronic ITP, for the chronic cases only (22.5%) of them achieve remission in an 18 month follow-up period (77.5%) were not responding to treatment and 27.8% of them were above 10 years old, splenectomy is recommended to non-responders and with severe symptoms and also they conclude that refractory ITP is self-limited and share the same clinical manifestation of acute ITP. (Yaprak et al., 2010).

In Saudi Arabia, variations in incidence and prevalence rate reported, reflecting the effect of genetic diversity combined with environmental triggers, and demographic distribution, creating a public health problem which needs further concern and awareness. Demographic data indicate that ITP were more common in adult females than males and variations in presentation of the disease were notable in between adults and children. Additionally, cultural factors may contribute to disease susceptibility and affect its prevalence rate, including food habits, the intake of certain traditional foods, lifestyle factors, and healthcare-seeking behaviors. Adding to this environmental exposure to specific infections in the region may contribute to secondary ITP development.

ITP diagnosis is usually by exclusion, to rule out any other underlying causes of thrombocytopenia, physical examination and the laboratory investigations which mainly include the complete blood count, certain specific assays, and the integration of advanced genetic study and testing for the potential use of specific genetic biomarker for ITP diagnosis and to discriminate in between primary and secondary cases and an ongoing study to identify autoantibodies associated with disease. Despite real advancements in this category, present which include the availability of resources,

expertise in this area, variations on health care setting and clinical practice guidelines (Anazi, 2025).

Usually, treatment directed toward preventing or limiting bleeding by increasing the platelet counts (>20–30 x109), and on improving their quality of life, Treatment must be individualized, taking in consideration the severity of the disease (Provan et al., 2019b).

Therapy with immunosuppressive agents, corticosteroids, rituximab and splenectomy has evolved over the previous years, with reduced effectiveness and undesirable side effects in some cases, which support or leads to the use of the second generation thrombopoietin receptor agonists (TPO-RAs) and recently, the fostamatinib(González-López & Provan, 2022).

For adult patients who develop a new course of the disease, the treatment approach involves the use of corticosteroids, typically prednisone or dexamethasone, and in cases of substantial bleeding the administration of intravenous immunoglobulin is usually needed (Song & Al-Samkari, 2021).

In general, the recommended therapy for subsequent treatment lines is TPO-RAs over splenectomy and rituximab and in less than 12 months about (80–90%) of ITP children recover, and in those who do not, there is a challenge in selecting treatment to young ages due to possible and serious side effects, despite of the use of the TPO-RAs which is proposed treatment of choice (González-López et al., 2024).

Current guidelines in cases with mild to moderate bleeding, a recommend strategy of treatment is watchful waiting. So, when bleeding requires immediate attention and recovery not achieved spontaneously with limited and restricted child activities pharmacological treatment is needed (C. Neunert et al., 2011a).

Chronic complicated cases with no response to treatment Splenectomy is indicated (Provan et al., 2019b). countries, and hospitals. Since the 2010 and 2011 guidelines updates by the International Working Group on ITP and the American Society of Hematologists, there is a growing number of patients with an applied strategy of watchful waiting (Sezgin Evim et al., 2014b).

Previous and recent studies have indicated that most patients receive medical treatment due to low platelet count, despite of the suggested guidelines in 2019 by the American Society of Hematology in which, if the platelet count is less than 30×103/mm3 and patients develops such a mucocutaneous bleeding or no symptoms corticosteroids have to be given (C. Neunert et al., 2019).

However. For patients with a platelet count greater than 30×103/mm3, the 2019 ASH guidelines strongly recommend observational "watch-and-wait" approach against corticosteroid therapy. While the World Health Organization (WHO) recommends the initiation of treatment for grade III and grade IV ITP based on bleeding symptoms without concern about the patient platelet count and watchful waiting in patients with severe thrombocytopenia (Rosthøj et al., 2003).

There is a debate in term of treatment initiation, in newly diagnosed ITP cases in children, the severity of the case, bleeding tendency and the case overall affect the decision to keep patients under observation or to make an intervention, when platelet counts above 20,000-30,000/μL it is enough to keep asymptomatic patients under observation (C. Neunert et al., 2011b).

Most children achieved spontaneous remission without therapy. If treatment is required first line therapies include intravenous immunoglobulin, corticosteroids and the anti-D. Most commonly Corticosteroids, such as prednisone, are used primarily for rapid platelet count increase; however, its proposed side effects due to long term intake may limit it use (Psaila & Bussel, 2007).

A more efficient First line therapy with minimal side effects is IVIG, it is usually used if patients experience severe bleeding and a rapid increase in platelets count is needed particularly before surgery. In non-splenectomized patients who are Rh-positive, the Anti- D may divert antiplatelet antibodies by inducing mild hemolysis (Ouglas et al., 2002b).

To manage Chronic ITP cases Second-line therapy is indicated when first line therapy is not efficient in maintaining safe count of platelets and the side effect accompanying its intake gets more intolerable, the anti CD20 monoclonal antibody, Rituximab, reduced autoantibody production by the B- lymphocytes is noticed and remission is achieved in

a minority of patients (J. Wang et al., 2005). And the same effect is initiated by the intake of both romiplostim and the eltrombopag which are considered as the Thrombopoietin receptor agonists (Thakur et al., 2024a).

When treatment has failed the traditional use of splenectomy for chronic ITP is recommended, with promising long-term remission rate. The Laparoscopic splenectomy instead of open surgeries is indicated also the splenic artery immobilization and the partial splenectomy were all possible in certain situations (Wood et al., 2010).

The decision to treat or not using standard therapies is complicated, due to the proposed potential side effects mediated by the drugs, behavioral and metabolic problems may limit the use of Steroids. Severe headaches may affect children receiving IVIG, preventing them from going to school, increased infection rate following splenectomy and rituximab treatment in patients with weak immune system were observed and a tendency more to develop malignancies with long term use, so both have to be used with caution (N. Cooper, 2014a).

Because of the desirable side effects and possible remission without treatment, current guidelines prohibit treatment of children until s significant sever bleeding exhibited by them (C. Neunert et al., 2011a).

However, others suggested that it is not ideal to keep children under observation until sever bleeding to develop patient and their families suffer in all aspects social and emotional, the low platelet counts and the unsuspected bleeding may restrict patients daily activities like going to school, travel and joining athletic activities, adding to this the feeling of anxiety among parents and the fear of bleeding (N. Cooper, 2014b).

There is an urgent need to manage patients with less invasive and less toxic approaches, particularly, for those having a persistent chronic ITP, who experience severe bleeding, and those who have impaired health care related quality of life (HRQol) because of continued medical attention, and emotional impact as a side effect (N. Cooper & Cines, 2019).

The food and drug administration approved the use of fostamatinib, spleen tyrosine kinase inhibitor (Syk) in April 2018, to treat chronic ITP cases not responsive to first lines of therapy to one of them at least (Newland et al., 2018).

All hematopoietic cells including platelets, macrophages and the B and T lymphocytes have the Syk. Activation of the Syk occurs secondary to the binding in between the Fcg receptor to its ligand with subsequent cytoskeletal rearrangement to Syk immunoreceptor motif leading to platelet phagocytosis, so it's a potential target for ITP treatment (Geahlen, 2014).

The intestinal alkaline phosphatase is responsible for the activation of The Syk inhibitor fostamatinib disodium (R788) to its active form R406 (Mcadoo et al., 2011) facilitating its absorption with a half-life of 15 hour it is is an adenosine triphosphate–competitive inhibitor of the Syk catalytic domain with multiple downstream effects on B cell receptors and the FcgR it is a novel therapy for chronic ITP cases, with limited response rate of response rate of 18% makes it unlikely to replace splenectomy, rituximab, or TPO mimetics (Connell & Berliner, 2019).

In 2018, few randomized trials,approve the effective and safe use of romiplostim in children with ITP, who are older than year, and have experience the disease with a duration of more than six months, improvement in child HRQol following treatment and reduced parental anxiety were observed, However, the safe long term use and sustained efficacy of the drug have to be confirmed to highlight its impact on the pathophysiology of the disease (Bussel et al., 2021).

Following treatment in some patients transaminitis and iron deficiency anemia may develop (C. Neunert et al., 2016) adding to this one concern, about the drug use, is that it may affect both megakaryocytes and bone marrow stem cells (N. Cooper & Cines, 2019).

In Arab countries wide variations in ITP management were observed from observation, to hospitalization, outpatient care and initiation of medical treatment(M. Elalfy et al., 2009). Admission to hospital is a habit when the platelet counts of $20x10^9$/L without regard to bleeding symptoms and patients receive platelets enhancing therapies (Al-Sayes et al., 2003).

In Egypt nowadays about 10% of acute cases admitted to hospitals, response to therapy responsive or not responsive have to be standardized in all Arab countries and

compliance to guidelines published by the American society of hematology is needed (C. Neunert et al., 2011a).

For initial patient management Both the IVIG and the anti-D immunoglobulin were usually used In the Arabian Gulf countries. However, the anti- D is not frequently used (El Alfy et al., 2006).

Corticosteroids, in the form of prednisolone or prednisone in the rest of the Arab countries is usually used, these patients are initially treated with prednisolone for two to three days depending on the dose or prednisone therapy for 2- 4 weeks. differences in treatment were attributed to regional guidelines and national income, which is more in the Arabian Gulf, while in Egypt national guidelines were in practice (M. Elalfy et al., 2009).

There are no published studies on management of chronic ITP in children in the Arab world, in Oman one study mentioned that 50% remission rate were noticed following the use of oral dexamethasone given 4 in 13 refractory ITP cases. and Splenectomy at 5 years were effective in inducing remission in 60% to 70% of cases in other countries, Kwait the remission rate was 75% and it was 80% in Iraq in which 40% of chronic cases in adults were splenectomized (Al-Aqabi et al., 2010).

In Egyptian children with chronic ITP splenectomy at 5 years were also effective in a study involving 112 patients During the period1980–1998 (El-Alfy et al., 2003).

The use of romiplostim and its efficacy were also reported in two studies involving Egyptian children, it was effective, tolerable with less undesired side effects maintaining a target platelet counts of 450 $x10^9$/L (M. S. Elalfy, 2013a).

According to the WHO quality of life is defined as “an individual’s perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns" and it is assumed that this concept is affected by many interfering factors including health, accommodation economic status, employment, and affection (Burckhardt & Anderson, 2003).

Mental, social, physical and school functioning are the main areas of interest according to the WHO that must be addressed to evaluate the impact of the disease on their quality of life, not the effectiveness of treatment (Nguyen et al., 2021).

In pediatric ITP prognostic factories varies factors like age at time of diagnosis, severity of case, and response to initial treatments are key players and determinant. A favorable prognosis is seen in children developing the acute form of the disease, most cases resolve within 6- 12 months. While, more complications and prolonged management is needed in chronic cases, poor outcomes were seen among those with severe thrombocytopenia, poor response to first line of treatment (Thakur et al., 2024b).

In 50% of chronic ITP cases, spontaneous recovery occurs within 5 years of diagnosis and a minority of children the case extends to many years later (Zhang et al., 2016).

Experts suggested that the finding and discovery of a potential diagnostic marker in ITP is only possible by new and powerful genetic studies, it can help in the confirmation of various types of common secondary ITP. These markers will vary from those seen in primary ITP. The micro-RNA (miRNA) and next-generation sequencing (NGS) are among the recently developed genetic tools, and the most intriguing for ITP diagnosis (González-López, 2025).

According to the population pyramid More than one-third of the Palestinians are less than 15 years, and representing 37.3% of total population,35.2% in West bank (WB) and 40.4% in Gaza strip (GS) the Palestinian population is young (Ministry of Health Health Annual Report Palestine 2023 State of Palestine PHIC, 2024)

In Palestine there are two retrospective studies entangled the ITP, both involving Gaza children with ITP, the first retrospective study aimed to define the incidence rate of (ITP) in both Alnasser hospital and Dr. A bed Alazeez AlRanteesy pediatric hospital, during the period of 2009- 2014. Patients with ITP were identified by the administrative code/ international classification of disease (ICD-69.3).

This study aimed to understand the incidence, features and clinical characteristics of the disease and treatment in use, A total number of 90 children below 12 years of age (54 males and 36 females) who were diagnosed with ITP during the study period, results indicate that the overall incidence of ITP in blood and oncology department/ Dr. Abed Alazeez AlRanteesy pediatric hospital is (3.72%), 10% of the studied children were acute cases, 63.3% were chronic, patients were almost male (60%) and (40%) of cases were females, the platelets count in about (17.5%) of them were less than20x10(9)/L at

the time of presentation, with (26.7) recovery rate. The study findings were in line with international reports, significant differences reported in platelets count, treatment response, and the patients age, the mortality rate (2.94%) was normal in comparison with the normal population, and more considerable in those who are treatment resistant. This study also recommends, period of five years follow up and screening to platelets count among those who experienced the disease (Saleh & DR Mohammed M Abu Selmia, 2017a).

The second study was a 10 year retrospective study involved admitted cases during the period between 2008- 20018 to assess the effectiveness of both the anti- D and intravenous immunoglobulin in treating ITP cases, a total of 134 cases were reported with a mean age of (5·8 years; range, 1·1–10·4 years) in which 68% of them received IVIG and 32% received the anti- D, results indicate the effectiveness of both in increasing the platelets count and the hemoglobin level following 5 days of treatment, but less side effects (vomiting, chilling, and headache) and shorter hospitalization were noticed in cases received the anti- D in comparison with the IVIG making it a good treatment alternative in acute cases (El-Habil, 2021).

To sum up, despite of the variations reported in the pathophysiology of the disease and the approval of TPO for its treatment, still there are unmet needs that require further investigations, ongoing trials for the development of new agents that is capable to change the course of the disease, and others willing to interfere with the underlying mechanisms for ITP development including the compliment system, the B lymphocyte maturation, and autoantibodies recycling, these agent represent a promising therapies for those who are unresponsive to therapy and those with refractory ITP, understanding the mechanism of autoimmunity in this aspect have a big role in finding a potential therapeutic target in future.

1.3 Problem statement

Patients, their families, society, and health care costs were all negatively affected by autoimmune diseases, and in the long term it may take place as one of the most common medical problems, and current projections suggest that ADs may soon take their place among the most prevalent medical disorders. Even though its rare and heterogenous, ADs prevalence and incidence rate must be entangled to enhance further

epidemiological investigations which may help in diagnosis, treatment, and management.

In terms of immune thrombocytopenia, which is the most common type of thrombocytopenia in children, cases may progress from acute to chronic and patients may have variable course of the disease, variations reported at the level of its clinical manifestations, duration, age, gender, and management. Patients may develop petechia, bruising, and sever bleeding following a viral infection. Although it's self-limited it may progress to chronic forms which need long term treatment and management.

There is a current lack of reliable platelets related tests relevant to the diagnosis or monitoring treatment in ITP, and usually diagnosis is made primarily by exclusion. Moreover, despite the presence of international guidelines, management practices vary between regions and additional challenges may clinicians experience in their work is the lack of standardized diagnostic protocols, inconsistent documentation, as well as the absence of epidemiological studies in the region which makes it difficult to validate an international guideline. There is a substantial gap in literature about ITP diagnosis and management across health care centers and lack of reliable data based on the proportion of children diagnosed or under-evaluated in West Bank.

It is assumed that finding a potential marker for accurate diagnosis is complicated by the diversity of the pathogenic antibodies, so the determination of the types of autoantibodies, leading to platelet destruction, not only provides solid evidence for diagnosis, but also helps to select an individualized strategy for ITP treatment. Also, there is conflicting data regarding the exact significance of the above-named polymorphisms in childhood ITP FCGRIIa [Histidine (H) instead of arginine (R) at position 131] and a single amino acid substitution in FCGRIIIa [valine (V) instead of phenylalanine (F) at position 158], which can both significantly affect antibody-binding capacity. This thesis aims to address these gaps among Palestinian ITP children in selected Palestinian hospitals.

1.4 Aims of the study

This study aims to achieve the following objectives:

1. Evaluate the burden of primary ITP in Palestinian children (2019–2023).
2. Assess FcγRIIa-131H/R and FcγRIIIa-158F/V polymorphisms as genetic risk markers.
3. Detect and characterize antiplatelet autoantibody subtypes.
4. Correlate FcγR polymorphisms and autoantibodies with ITP pathogenesis.
5. Explore their potential as diagnostic or predictive markers for pediatric ITP.

1.5 Importance of the study

There were scattered and limited data in the literature on ITP in children from the Arab region, neither regional nor national guidelines for ITP management in most Arab countries has been reported. Accurate diagnosis is complicated by the diversity of pathogenic antibodies. In addition to this, the relative contribution of platelet FcγrIIa and FcγrIIIa to platelet clearance and to reduced production of platelets in autoimmune thrombocytopenia remains unclear, in the future, physicians may be able to use genotyping to recommend an individualized treatment approach.

Overall, this study will provide comprehensive understanding of the burden of ITP among Palestinian children. The results are expected to contribute to knowledge about the usefulness of the FcRIIa-13H/R and FcRIIIa-158F/V polymorphisms as a diagnostic marker for ITP and to guide and monitor patient treatment.

Understanding the population burden of ITP requires knowledge of its prevalence. Knowledge of the prevalence of ITP is also important for the development of new treatments, as emphasized by recent pharmaceutical-supported studies of the epidemiology of ITP.

Knowledge will expand the area in this entity for future investigations and directions, that may pinpoint opportunities for development and improvement in all aspects concerning the ITP in the region, ultimately leading to improved health outcomes for those impacted by this condition.

By providing comprehensive analysis of epidemiology, pathophysiology, diagnostic criteria, and treatment strategies, we can promote collaboration among healthcare professionals, researchers, and policymakers in the regions. And by addressing challenges, we can improve the quality of care for individuals living with ITP and enhance their overall quality of life.

1.6 Study hypothesis

The burden and incidence rate of primary immune thrombocytopenia in the middle east and in Palestine particularly is rarely, or not entangled in research studies, and the majority of the conducted studies were retrospective, epidemiological one highlighting reported or registered ITP cases, the clinical practice in use, therapeutic intervention and the main presented features among patients, with no focus or concern about the pathophysiology of the disease, and the mechanisms implicated in impaired platelet production and its enhanced destruction.

Adding to this, the relationship in between platelets antibodies, the clinical picture of the disease (ITP), and the relative contribution of platelet FcγrIIa and FcγrIIIa polymorphisms to platelet clearance and to reduced production in ITP has been largely unclear.

In this study we hypothesize that these genetic polymorphisms involving the Fc gamma receptor gene were more reported in patients than healthy children, which definitely increase the risk to disease susceptibility, it is assumed that genetic variations exist in between cases, the newly diagnosed, the persistent and the chronic type of the disease which may have a correlation with the course of the disease and response to treatment, such a suggested assumption have to be investigated to confirm.

Finding a potential genetic marker may aid in diagnosis, and help in understanding the disease course, and improving patient s quality of life by reducing their suffering. Overall, this study will contribute to knowledge about the ITP in Arab countries, encouraging more research studies in this area.

Chapter Two

Methods

The study included children pre-diagnosed with primary immune thrombocytopenia between 2019 and 2024 under the age of 18. Data were retrieved from the hospital records and included demographic characteristics, observations, laboratory findings, treatment, and all possible patient outcomes.

2.1 Study design

This is a multicenter and retrospective case-control study was performed following IRB approval from An-Najah National University/Palestine [Mas. Nov.2023/30] and was approved by the Palestinian Ministry of Health / Education in Health and Scientific Research Unit. Adding to this approval was also obtained to conduct our research project at An-Najah National University Hospital by the Clinical Research Center Ref: CRC_2024_0225.

2.2 Study population

In the absence of any official data on the prevalence of ITP in Palestine, between February and May of 2024, we retrieved medical data from the hospital information system on documented cases of children with ITP and unspecified thrombocytopenia, who were referred to pediatric units at four different hospitals: the Martyr Dr. Khalil Suleiman Governmental Hospital in Jenin, the Palestine Medical Complex in Ramallah, Rafidia Governmental Surgical Hospital and An-Najah National University Hospital, both located in Nablus, between 2019 and May 2024.

Control samples were kindly obtained from Medicare labs in Palestine and from the Pediatric Unit of Rafidia Governmental Surgical Hospital in Nablus city. ITP cases were diagnosed based on appropriate history taking, clinical presentation, and laboratory findings to rule out other possible causes of thrombocytopenia. Patients with ITP were identified by the administrative code (ICD-69.3) or under the category of unspecified thrombocytopenia.

All demographic and clinical information regarding them was retrieved from the Hospitals' information system and afterward contact with patient's families were made.

Patients referred to Augusta Victoria Hospital in Jerusalem and Al-Istishari Arab Hospital in Ramallah were not included in the study as we previously planned, for sure due to the exceptional political situations during the study period that made travel difficult. Additionally, Al-Istishari Arab Hospital declined participation at the time of the study.

2.3 Study sample

By medical records investigation, and accessing the hospital information system during the designated study period, there were a total of 105 registered ITP cases (49 females and 56 males): 53 acute, 39 chronic, six persistent, and seven refractories, we are able to make contact with the majority of them but unfortunately some patient families refuse to participate and apologize, and we are not able to make contact with others, due to missing data on the system and some of them were from Gaza strip, and others travel outside and overall it was difficult to some families to participate by giving samples due to the unusual situations during the study period which make travel difficult.

Based on this in our study (case control study) a total of 120 participants were enrolled, including 40 ITP patients who accept to participate and give samples (representing 38% of the 105 registered cases) and 80 age- and sex-matched healthy controls.

The ITP group, with a mean age of 6.76 ± 4.13 years, consisted of an equal number of males (20; 50%) and females (20; 50%), while the control group included 44 males (55%) and 36 females (45%).

Inclusion criteria: approval to sign informed written consent, patients with newly, persistent and chronic diagnosed ITP, patients aged > 1 year and < 18 years and both sexes will be included.

Exclusion criteria: refusal to sign an informed written consent, patients with secondary immune thrombocytopenia and patients aged < 1 year or > 18 years. Secondary immune thrombocytopenia may develop due to other underlying diseases like CLL, SLE, and infections with HCV, HIV, H. Pylori infection, and others.

2.4 Study procedure

2.4.1 Blood sampling

From All ITP patients and the control group EDTA, blood samples (3-5 milliliters) were obtained for the purpose of genomic DNA extraction and molecular characterization of the proposed polymorphisms, in addition to this from all patients, plane tubes for serum collection collected, and then the serum separated and stored at -20°C until the time of use for further assessment and identification of antiplatelet antibodies. All the PCR-related techniques will be performed in the molecular biology laboratories/ Central Lab at An-Najah National University.

2.4.2 DNA Extraction.

The genomic DNA extraction was performed using the Gene JET^{TM} Whole Blood Genomic DNA purification Mini Kit (Thermo Scientific, catalog number K0782). The extracted DNA was stored at -20°C until the time of use.

The DNA was extracted by the following detailed test procedure, extraction was done according to manufacturer instructions, 20µL of Proteinase K Solution were added to 200µL of whole blood (buffy coat) which is previously separated and kept at -20°C until the time of use. The mixture (Buffy coat with proteinase K) then mixed by vortex. And then about 400µL of Lysis Solution 1 was added to the mixture and mixed thoroughly to obtain a uniform suspension.

After that samples are incubated at 56°C for 10 minutes with occasional vortex until the cells are completely lysed. After that 200µL of ethanol (96–100%) were added and mixed by pipetting. The prepared mixture was transferred to the spin column, then centrifuged at 6,000 × g for 1 minute. The collection tubes containing the flow-through solution discarded, then the column placed into a new 2 mL collection tube. 500 µL of Wash Buffer (WB I) which is previously prepared with ethanol, then centrifuge 8,000 × g for 1 minute.

The flow-through discarded and the column placed back into the collection tube. Then this step followed by the Addition of 500 µL of Wash Buffer II (WB2) (with ethanol added) to the column, then centrifuge at ≥20,000 × g for 3 minutes. collection tube

discarded and, new one used and the purification column placed in, and centrifuged at ≥20,000 × g for 1 minute, then collection tubes were discarded.

The genomic DNA was extracted by the addition of 200 µL of Elution Buffer. Tubes Incubated for 2 minutes at room temperature, then centrifuged at 8,000 × g for 1 minute and the extracted DNA kept at -20 until use.

2.4.3 FCGRIIA 131H/R gene genotyping using PCR and PCR-RFLP analysis

The FcγRIIa-131R/H genotyping was performed using the PCR-restriction fragment length polymorphism (PCR-RFLP) method. The primers for the FCGR2A gene were designed according to the protocol described by (Cartron et al., 2002), as specified in Table 1.

A PCR amplification reaction was performed in a total of 25µl reaction mixture with 3-5µl genomic DNA, 0.5µl of each primer, master mix and nuclease free water (NFW).

The first cycle consisted of 3 minutes at 94°C followed by 35 cycles (each consisting of 3 steps at 94°C for 15 seconds, 52°C for 30 seconds, and 72°Cfor 40 seconds) and 7 minutes at 72°C to complete extension.

For the RLFP, 5 µl of the amplified PCR product was digested at 37°C for 2 hours using 10U of the BstUI restriction endonuclease (Thermo- scientific), then the restriction enzyme was inactivated by incubation at 65°C for 20 minutes.

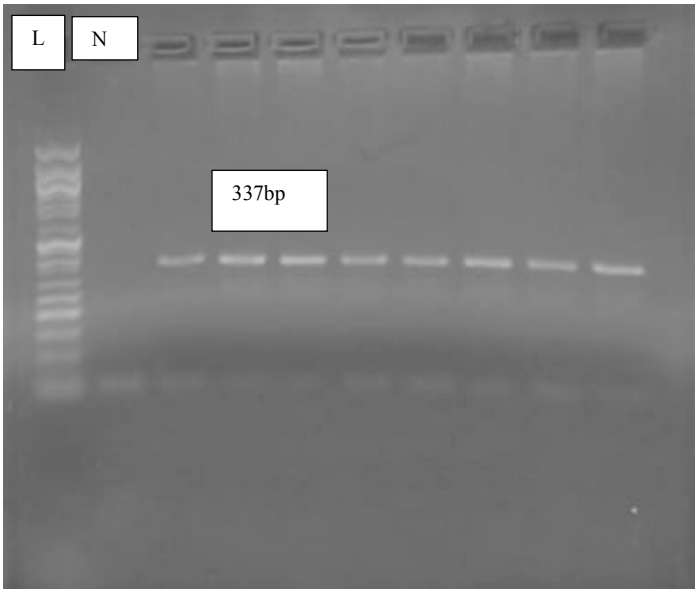
Digested products were electrophoresed on a 3.5% Metaphor agarose gel, for at least 4 hours. The R alleles generated two fragments of 316 and 21 bp, while the FCGR2A H allele was visualized as a 337 bp fragment (Fig. 2) as previously published by (Eyada et al., 2012b).

The Thermo Scientific Bsh1236I (BstUI) restriction enzyme recognizes CG^CG sites and cuts best at 37°C in R buffer, its cleavage site is shown below.

- 5' C G ↓ C G 3'
- 3' G C ↑G C 5'

Figure 2

Fc gamma receptor IIa131H/R amplified PCR product. Lane l is the 50bp ladder, lane. 1 negative control, lanes 2-8 different patient samples



2.4.4 FCGRA 158 V/F gene genotyping using an allele-specific restriction analysis based on nested PCR.

FCGR3A–158V/F genotyping was performed as previously described by Cartron *et al.* using nested PCR followed by allele-specific restriction enzyme digestion.

The initial PCR assay was to amplify a 1.2 kb fragment (Fig. 3), it was performed in a 25µl reaction mixture, with 4µl genomic DNA, 0. 5µl of 1:10 prediluted primers, 12.5µl master mix and 7.5µl nuclease free water.

The initial amplification step consisted of 10 minutes at 95°C, then followed by 35 cycles with three steps (step 1 at 95°C for 1 minute, step 2 at 57°C for 1.5 minutes, and step 3 at 72°C for 1.5 minutes), and finally to achieve complete extension 8 minutes at 72°C. Then, a second PCR reaction is done to amplify part (94bp) of the amplified DNA produced by the first reaction using primers shown in Table 1.

This PCR step was performed with 5µl of the amplified DNA, 0.5µl of each primer, 12.5µl master mix, and 6.5µl of nuclease free water. The first cycle consisted of 5 minutes at 95°C, then 35 cycles of three steps for each (step 1 at 95°C for 1 minute, then step 2 at 64°C for 1 minute, and the last step, step 3 at 72°Cfor 1minute), stage 3 and at

72°C for 9.5 minutes to complete extension, in this step a fragment of 94 bp amplified (Fig. 4).

The amplified DNA, about 5 µl was digested with 10U *NLaIII* restriction endonuclease (Thermo- scientific) at 37°C for 2h, then the restriction enzyme was inactivated by incubation at 65°C for 20 minutes.

The DNA fragments were visualized on 3.5% metaphor agarose gel, two fragments of 61 and 33 bp were generated by the V allele, while theFCGR3A F allele manifested as a 94 bp fragment (Fig.4) (Cartron et al., 2002).

The Thermo Scientific Hin1II (NlaIII) restriction enzyme recognizes CATG^ sites and cuts best at 37°C in G buffer its cleavage site is shown below.

- 5' C A T G ↓ 3'
- 3' ↑ G T A C 5'

Figure 3

Fc gamma receptor IIIa158F/V a 1.2Kb amplified PCR product. L is the 1500bp ladder, lanes 1-8 different patient sample

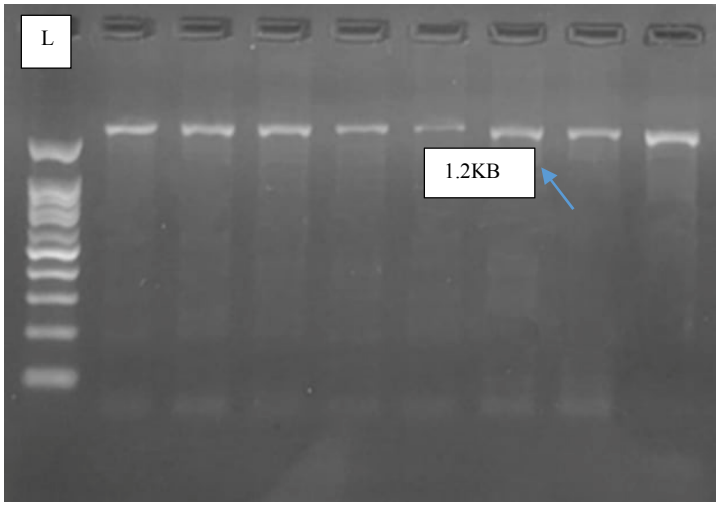
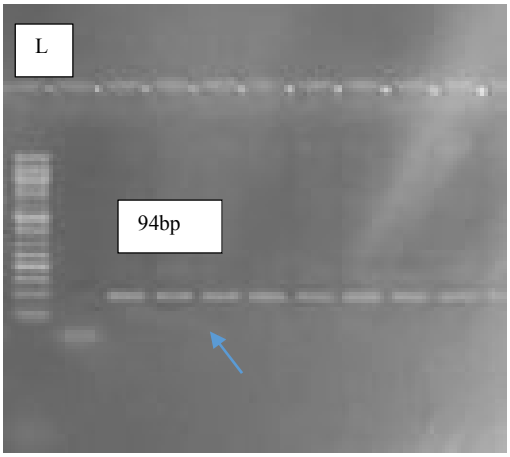


Figure 4

Fc gamma receptor IIIa158F/V a 94bp amplified PCR product. The L is the 50bp ladder,lane 1. Negative control, lanes 2-7 different patient samples



For both polymorphisms random samples selected and tested in duplicate to ensure the result, results read by two lab technicians and in each run positive or mutant sample was included.

Table 1

Primers used for genotyping FCGR3A & FCGR2AII

Genotyping FCGR3A158 V/F gene	Primer sequence	Band size bp.
Forward	5′- ATATTTACAGAATGGCACAGG-3′	1.2Kb
Reverse	5′-GACTTGGTACCCAGGTT GAA-3’	
Forward	5′-ATCAGATTCGATCCTACTTCTGCAGGGGGCAT-3′	94bp
Reverse	5′-ACGTGCTGAGCTTGAGTGATGGTGATGTTCAC-3′	
Genotyping FCGR2A 131H/R gene.		
Forward	5′-GGAAAATCCCAGAAATTCTCGC-3′	337bp
Reverse	5′-CAACAGCCTGACTACCTATTACGCGGG-3′	

2.5 Antinuclear antibody testing (ANA) and Anti-double stranded (A-dsDNA) investigation

Serum samples from a total of 40 patients were taken before the period July- September 2024, for the purpose of testing proposed autoantibodies in patient serum, at that time serum kept at -20 until the time of use from June- August 2025.

The ANA and the A- dsDNA to 32 of them (80%) were investigated using commercially available kits (Alegria®), all the testing procedures were done according to manufacture instructions, testing were done in Central Lab, Hadash Medical Organization (HMO)**.** Testing was done only to 32samples, in which 8 patient samples were insufficient or depleted, since we are trying at that time to investigate the antiplatelet autoantibodies by other techniques, and unfortunately, we are not successful.

The ANA screen, (REF. ORG 238) is an ELISA-based test system for the qualitative measurement of IgG class autoantibodies against Sjogren's Syndrome Related Antigen A60 (SS-A 60), Sjogren's Syndrome Related Antigen A52 (SS-A 52), Sjogren's Syndrome Related Antigen type B (SS-B), Ribonucleoprotein 70 (RNP-70), smith (Sm), Ribonucleoprotein/ smith (RNP/Sm), Anti Topoisomerase (Scl-70), centromere B, Anti-Histidyl Trna Synthetase(Jo-1) in human serum or plasma samples.

The Alegria® assay has a barcoded 8-well-microstrips, called Alegria® Test Strips. This strip is designed for a single determination of one patient sample. It holds a complete set of reagents. Included are enzyme conjugate, enzyme substrate, sample buffer and test specific control. Furthermore, each strip has two antigen-coated wells which serve as reaction wells for one control and one patient sample.

The determination is based on an indirect enzyme linked to immune reaction with the following steps: Antibodies present in positive samples bind to the antigen coated on the surface of the two reaction wells forming an antibody antigen complex. After incubation, a first washing step removes unbound and unspecific bound molecules.

Subsequently added enzyme conjugate binds to the immobilized antibody-antigen complex. After incubation, a second washing step removes unbound enzyme conjugate. Addition of enzyme substrate solution results in hydrolisation and color development

during incubation. The intensity of the blue color correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 650 nm.

The Alegria® Test Strip is based on the proprietary SMC®-Technology (Sensotronic Memorized Calibration): information about the assay, analysis and evaluation, and the lot-specific expiry date is contained on the barcode printed on each Alegria® Test Strip. The Alegria® Test Strip can be used with the diagnostic instrument Alegria® - a fully automated Random-Access Analyser. By means of SMC®-Technology data encoded on the barcode are transferred from the Alegria® Test Strip to the instrument and the assay is automatically processed and evaluated.

Anti-dsDNA IgG is an ELISA-based test system for the quantitative measurement of IgG class autoantibodies against double-stranded DNA in human serum or plasma samples. The test is used as an aid in the differential diagnosis of inflammatory autoimmune diseases, especially systemic lupus erythematosus (SLE). Autoantibodies to dsDNA are diagnostic markers for SLE and levels may be elevated during active disease. Evaluation of a test result should always consider all clinical and laboratory diagnostic findings. Highly purified double-stranded DNA (dsDNA) is bound to microwells.

The Alegria® assay features barcoded 8-well-microstrips, called Alegria® Test Strips. Each strip is designed for a single determination of one patient sample. The Alegria® Test Strip holds a complete set of reagents. Included are enzyme conjugate, enzyme substrate, sample buffer and test specific control.

Furthermore, each strip has two antigen-coated wells which serve as reaction wells for one control and one patient sample. V Alegria® Test Strips Wash Buffer System Fluid Ready to use 50 x concentrate 1000 x concentrate the determination is based on an indirect enzyme linked immune reaction Antibodies present in positive samples bind to the antigen coated on the surface of the two reactions well forming an antibody antigen complex.

After incubation, the first washing step removes unbound and unspecific bound molecules. Subsequently added enzyme conjugate binds to the immobilized antibody-antigen complex. After incubation, a second washing step removes unbound enzyme

conjugate. Addition of enzyme substrate solution results in hydrolisation and color development during incubation. The intensity of the blue color correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 650 nm.

This essay is used for initial diagnosis in cases of systemic lupus erythematosus and for differential diagnosis of systemic autoimmune diseases for which high titers of antibodies against antinuclear antibodies (ANA) are found. Autoantibodies against DNA and histones bind to single-stranded (ssDNA) and double-stranded (dsDNA). Antibodies against dsDNA are predominantly detected during the active stages of the disease and serve as activity markers for SLE. They are a prognostic indicator of severe, multisystemic disease progression.

2.6 Ethical Approval

All aspects of the study protocol, including access to patient's medical records throughout the hospital information system and the use of patient clinical information, were authorized by the Institutional Review Boards (IRBs) (Appendix 1) and the ministry of health (Appendix 2) before the initiation of this study. In addition, families of patients have then been contacted, informed of the significance of the study, and asked to participate. a written informed consent form was obtained from each patient Family (Appendix 3).

2.7 Statistical Analysis Methods

The Statistical Package for Social Science (IBM SPSS statistics 21 for Windows) was used to analyze the data. The range, mean, standard deviation, and median are the ways that numerical data are presented. Frequencies and percentages will be used to express qualitative data. The chi-square test was used to compare allele and genotype distribution between patients and controls for finding a potential significant difference. The odds ratio (OR) and 95% confidence interval (CI) were computed to estimate risk by combined genotype analysis. A probability value (P value) is less than 0.05 considered statistically significant.

Chapter Three

Results

This case control study is the first in West Bank, which investigate the prevalence of immune thrombocytopenia among Palestinian children during the period of 2019- 2024, since no published epidemiological studies about the disease have been found. In addition to this, the study highlights the diagnostic methods in use and reflects experience in clinical setting on how reported cases managed, and offering an overview of the current practices in pediatric units in Palestinian hospitals.

This study aimed to setup a potential diagnostic approach for finding a proposed genetic marker for ITP diagnosis by investigating the frequency of two genetic polymorphisms within the Fc gamma receptor gene, highlighting the ongoing challenges and future directions for research and clinical practice in this area in Palestine in which data availability is limited, giving this work significance, and novelty.

Adding to this the antinuclear antibodies and the anti-double stranded DNA will be tested to confirm such an autoimmune activation in these cases and concurrent SLE progression which may help in patient management and improving their quality of life.

In this chapter the key findings of our study will be presented from demographic data up to the level of finding significant differences in between the two study groups.

3.1 Demographic and Clinical Profile of Immune Thrombocytopenic Purpura

To understand the characteristics of immune thrombocytopenic purpura (ITP) cases within our clinical setting, it was essential to initially examine the broader patient demographics and clinical profiles. Table 2 summarizes the demographic and clinical characteristics of the registered ITP cases (N = 105) and details the subset of participants (N = 40) included in the study between 2019- 2024. Overall, among the 105 registered ITP cases, there were 56 males (53.3%) and 49 females (46.7%). In terms of disease duration, 53 cases (50.5%) were classified as acute, 39 cases (37.1%) as chronic, 6 cases (5.7%) as persistent, and 7 cases (6.7%) as refractory.

The registered cases were distributed as follows: Rafidia Hospital (33 cases, 31.4%), followed by NNUH (21 cases, 20.0%), PMC (17 cases, 16.2%), NNUH/Rafidia (16 cases, 15.2%), Jenin (15 cases, 14.3%), and NNUH/Jenin (3 cases, 2.9%) as shown in (Figure 5). Of these, 40 participants were selected for further analysis. Within the male group, 20 out of 56 cases (35.7%) were included, while 20 out of 49 female cases (40.8%) were selected. To this end, 120 participants were enrolled, including 40 ITP patients (representing 38% of the 105 registered cases) and 80 age- and sex-matched healthy controls.

The ITP group, with a mean age of 6.76 ± 4.13 years, consisted of an equal number of males (20; 50%) and females (20; 50%), while the control group included 44 males (55%) and 36 females (45%) see (Figure 6).

As the data implies that many cases were initially referred to Rafidia Surgical Governmental Hospital for management then some of them then referred to An- Najah National University Hospital as a referral hospital for bone marrow biopsies, management, and follow up. Few registered cases reported in other medical centers, and this may reflect the improper documentation issues in those hospitals, and the shortage in experienced Hematologists who can make accurate diagnosis.

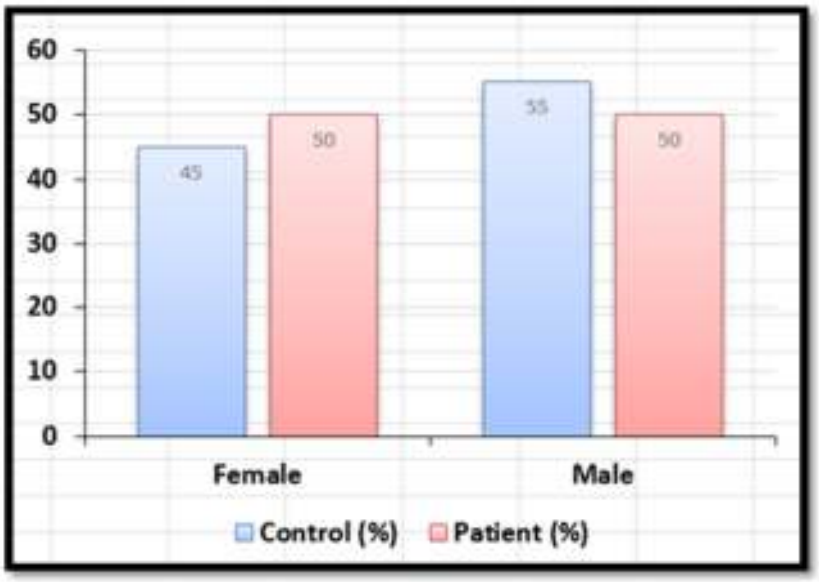
In term of patient general characteristics and their clinical manifestation at time of diagnosis, the majority of cases admitted with lower limb skin rash, which then extends to the trunk and spread all over the body, some patients develop petechia, ecchymosis, epistaxis, gingival bleeding, and in some females more bleeding exhibited when they start having their menstrual cycle.

By investigating the medical records, wide variations seen in documentation issues between hospitals, some medical records were detailed while others missing important clinical data, patients' diagnosis was made based on physical examination, laboratory findings, and the exclusion criteria. In all cases the platelets count was low and in most of them it was less than $20x10^3/\mu l$, and the blood film and the bone marrow biopsy showing evidence of platelets destruction. Steroids, and the IVIG were mainly used as first line therapies, some patients kept under observation, chronic cases were managed by rituximab and the Eltrombopag therapy, other patients were referred for Augusta

Victoria Hospital &(AVH)/ Jerusalem for follow up and treatment and to Chaim Sheba Medical Center at Tel HaShomer /Israel.

Figure 5

Gender distribution among the study groups



Among the ITP patients, the disease was categorized as acute in 45% of cases, chronic in 42.5%, persistent in 7.5%, and refractory in 5% (Table 2). Statistical analysis revealed no significant difference in gender distribution between the patient and control groups ($p = 0.605$), thereby confirming that the demographic characteristics of the two groups were comparable see (Table. 3).

In conclusion, the balanced distribution of demographic variables and the detailed categorization of ITP duration in the patient group provide a robust foundation for the subsequent analysis of FCGR2A and FCGR3A polymorphisms. This representativeness minimizes potential confounding factors and strengthens the validity of the genetic association analysis conducted in this study. So far, the genetic categorization for the two proposed single nucleotide polymorphisms has been investigated, and association studies were performed based on gender, and severity of the case. Results analyzed and comparative analysis in between patients and control performed to assess any statistical significance that may support our hypothesis of the study in finding specific potential genetic marker for ITP diagnosis.

Table 2

General registered/ participants characteristics interm of gender, and duration of the disease

Variable.	Registered ITP cases (N= 105)		Participants (N= 40)	
Gender				
	Number	Percentage	Number	Percentage
Male	56/105	(53.3%)	20/56	35.7%
Female	49/105	(46.7%)	20/49	40.8%
Duration				
Acute	53	50.5%	18/53	34%
Chronic	39	37.1%	17/39	43.5%
Persistent	6	5.7%	3/6	50%
Refractory	7	6.7%	2/7	28.5%

Figure 6

Case distribution according to hospital

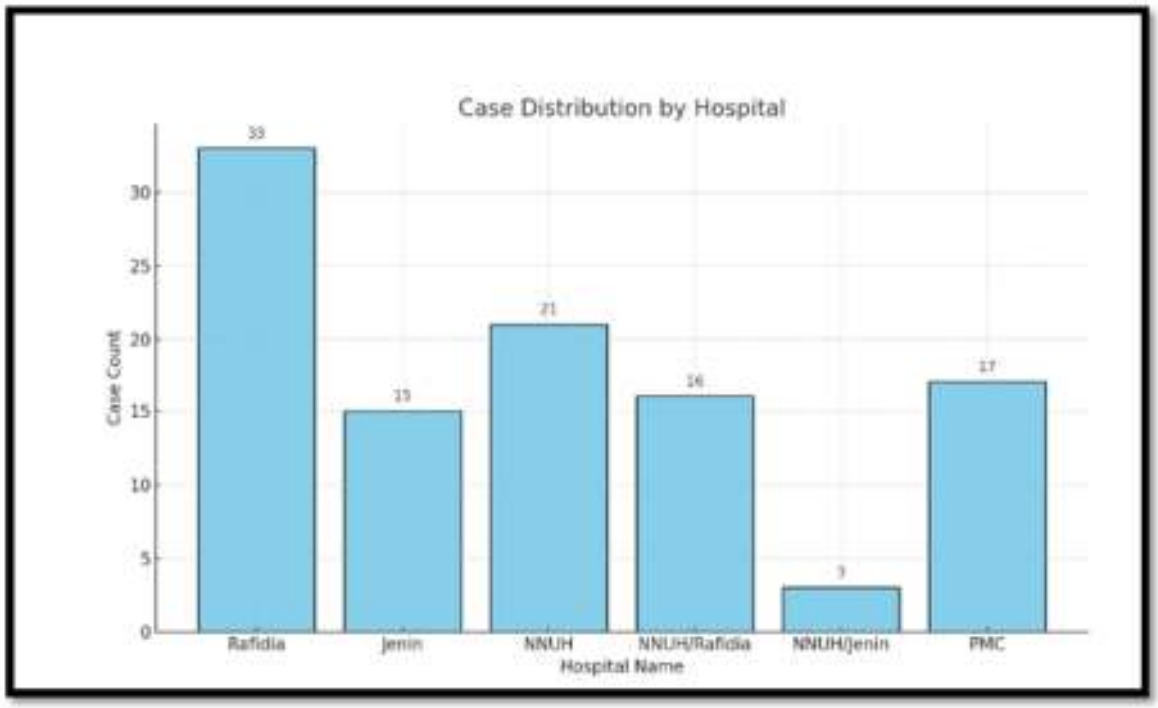


Table 3

General participants characteristics and the course of the disease

Variable		Total	ITP		Control group		Test	*P*
		N (%)	N	%	N	%		
Gender	Male	64(53.3)	20	50.0%	44	55.0%	.268	.605
	Female	56(46.7)	20	50.0%	36	45.0%		
Duration (ITP)								
	Acute	18	18	45.0%				
	Chronic	17	17	42.5%				
	Persistent	3	3	7.5%				
	Refractory	2	2	5.0%				

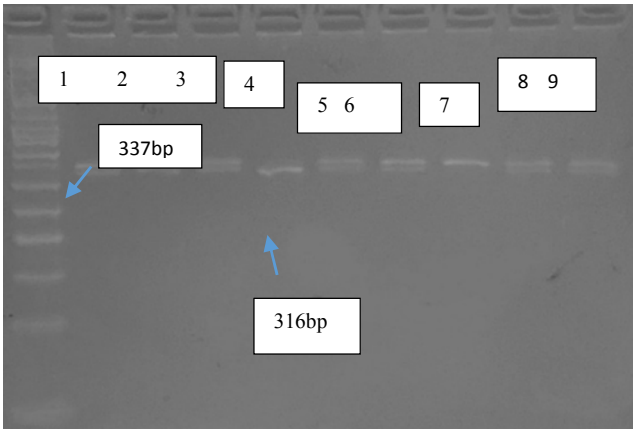
3.2 FcγRIIa H131R Genotyping by PCR-RFLP

Genotyping of the FcγRIIa H131R polymorphism (Fig. 7) was performed on the collected samples using the PCR-RFLP method. Figure 1 displays a representative image of the agarose gel electrophoresis results. Lane L contains a 50 bp DNA ladder, which was used as a molecular weight marker to confirm the size of the digested fragments. Subsequent lanes illustrate the three possible genotypes.

The FcγRIIa H allele was identified by a 337 bp fragment, while the R allele was identified by a 316 bp fragment following digestion with the BstUI restriction enzyme. The smaller 21 bp fragment resulting from the R allele digestion was not visible on the 3.5% agarose gel. The following distinct banding patterns corresponded to the different genotypes: • Heterozygous (HR): Lanes 1, 2, 3, 5, and 6 show two bands corresponding to the sizes of 337 bp and 316 bp. This pattern is characteristic of the heterozygous genotype, indicating the presence of both the H and R alleles. • Homozygous Mutant (RR): Lane 4 exhibits a single band at 316 bp. This pattern indicates a homozygous mutant genotype where both alleles are the R variant. • Homozygous Wild-type (HH): Lane 7 displays a single band at 337 bp. This pattern is consistent with the homozygous wild-type genotype, indicating the presence of two H alleles. In review the distribution of these genotypes was determined for the entire sample cohort, revealing a clear visual representation of each genotype.

Figure 7

The Fc gamma receptor IIa131H/R digested PCR products. The L is the 50bp ladder, Lanes 1, 2, 3, 5, and 6,8 and 9 for HR (337bp,316bp) lane 4 showing 1 band for the RR mutant type (316bp) and lane 7 for the HH wild type (337bp)

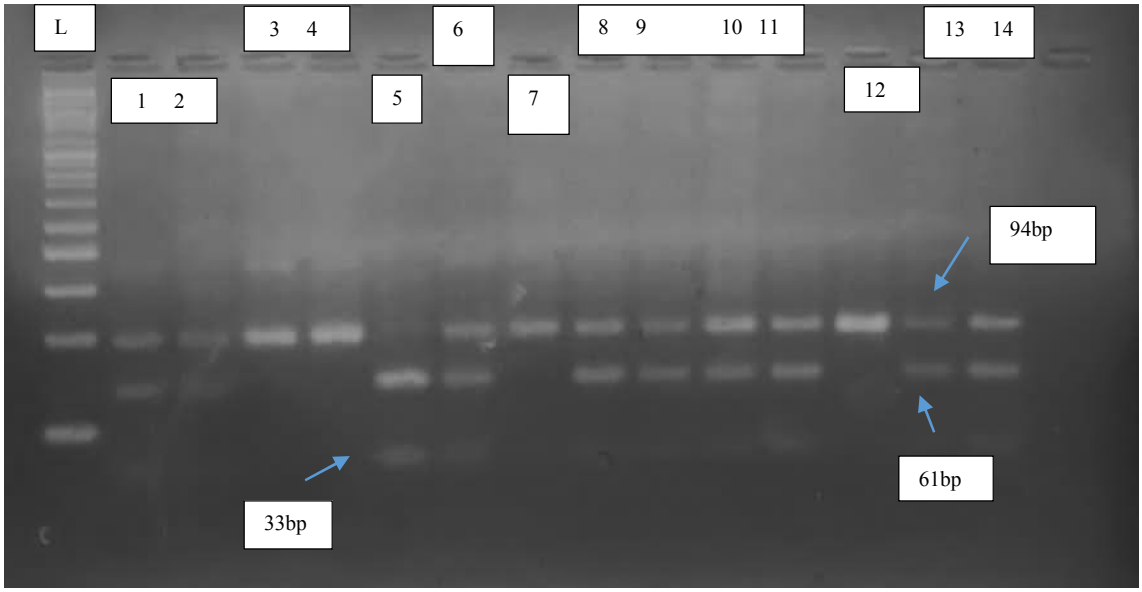


3.3 FcγRIIIa V158F Genotyping by PCR-RFLP

Genotyping for the FcγRIIIa V158F polymorphism was conducted using the PCR-RFLP method, and the results of the gel electrophoresis are presented in (Figure. 8). The leftmost lane (L) contains a 50 bp DNA ladder, which was used to determine the sizes of the DNA fragments. The analysis revealed three distinct banding patterns, corresponding to the FF, VV, and FV genotypes. • Homozygous Wild type (FF): As shown in lanes 3,4,7 and 12, this genotype is characterized by a single band at 94 bp. This fragment represents the undigested PCR product from the F allele, which lacks the restriction enzyme recognition site. • Homozygous Mutant (VV): Lanes 5 show a two-band pattern with fragments at 61 bp and 33 bp. This result is consistent with the homozygous mutant genotype. The V allele contains the restriction site, which leads to the digestion of the PCR product into these two fragments. • Heterozygous (FV): The heterozygous genotype is represented in lanes 6,8,9,10,11.13.14 and 6. These lanes display a three-band pattern, with fragments at 94 bp, 61 bp, and 33 bp. This pattern indicates the presence of both the F allele (uncut, 94 bp) and the V allele (cut, 61 bp and 33 bp). These distinct banding patterns enabled the successful classification of each sample into its corresponding FcγRIIIa V158F genotype.

Figure 8

Fc gamma receptor IIIa158F/V digested PCR products. L. 50bp Ladder, The FF (Wild) phenotype is represented by lanes 3,4,7 and 12 with one band 94bp, the VV(Homozygous) by lanes 5 (61bp, 33bp), and the FV(Heterozygous) by lanes 6,8,9,10,11.13.14 and 6 (94, 61, and 33bp)



3.4 Association Between FCGR2A and FCGR3A Polymorphisms and Clinical Course of ITP

Investigating FCGR2A and FCGR3A polymorphisms in this cohort were to determine whether variations in these IgG-binding receptors could influence ITP susceptibility and clinical presentation. Table 4 compares the genotype and allele frequencies of both polymorphisms between 40 ITP patients and 80 healthy controls. For FCGR2A, the frequencies of the HH, HR, and RR genotypes in the ITP patient group were 17.5%, 62.5%, and 20.0%, respectively, whereas in the control group, these genotypes were 25.0%, 52.5%, and 22.5%. No statistically significant difference emerged ($p = 0.543$). A notable observation was that the heterozygous HR genotype occurred in approximately 52% of female patients and in 52% of male controls. Allele frequencies (H and R) also showed no significant difference between groups ($p = 0.82$).

Table 4

Comparison of the genotypes and Allele Frequencies of the FCGR2A polymorphisms in patients and control groups

Variable	Alleles		Total		Patient		Control		χ^2 Test	*P*
			N	%	N	%	N	%		
FCGR2A	AA	Wild (HH)	27	22.5	7	17.5%	20	25.0%	1.221	.543
	GG	Homozygous (RR)	26	21.7	8	20.0%	18	22.5%		
	AG	Heterozygous (HR)	67	55.8	25	62.5%	42	52.5%		
	A	A(H)				48.75%	82	51.25%		0.82
	G	G(R)				51.25%	60	48.75%		

R, arginine; H, Histidine; ITP.

Similarly, for FCGR3A, the ITP patients exhibited FF, FV, and VV genotypes at 25.0%, 55.0%, and 20.0%, respectively, compared with 23.8%, 53.8%, and 22.5% in controls (p = 0.950). Although a higher frequency of the FV phenotype was noted in female patients (59.09%) compared with male controls (60.46%), this did not reach statistical significance. Allele frequencies (F and V) were comparable between the two groups (p= 0.88). In conclusion, FCGR2A nor FCGR3A polymorphisms showed a statistically significant association with ITP in this cohort. These findings suggest that, at least in this population, variations in these Fc gamma receptors do not appear to play a major role in determining disease susceptibility or phenotype.

Table 5

Comparison of the genotypes and Allele Frequencies of the FCGR3A and polymorphisms in patients and control groups

Variable	Alleles		Total		(ITP)		Control		Test	*P*
			N	%	N	%	N	%	X^2	
FCGR3A	TT	Wild (FF)	29	24.2	10	25.0%	19	23.8%	.102	.95
	GG	Homo (VV)	26	21.7	8	20.0%	18	22.5%		
	TG	Hetero (FV)	65	54.2	22	55.0%	43	53.8%		
	T	T (158F)				52.5%	56	50.63%		0.88
	G	G (158V)				47.5%	104	49.37%		

F, phenylalanine; G, Guanin; ITP, immune thrombocytopenia; T, Thymine; V, valine**.**

3.5 Gender-Associated Genotypic Trends in FCGR2A and FCGR3A Polymorphisms Among Pediatric ITP Patients

To determine if specific FCGR2A131H/R and FCGR3A158F/V genotype distributions are associated with increased susceptibility to ITP, particularly in relation to gender, potentially guiding personalized diagnostic approaches. Data represented in Table 5 demonstrated gender distribution analysis and revealed that the prevalence of FCGR2A131 HH genotype among male ITP patients was 57.4% as compared to 42.8% in their female counterparts in a ratio of 1.33:1 (M: F ratio).

In parallel, the prevalence of HH genotype among male healthy control was 35% as compared to 65 % in their female counterparts in a ratio of 0.53:1 (M: F ratio), indicating a higher frequency of HH allele variation of the FCGR2A131 among ITP male patients. The prevalence of FCGR2A131 HR genotype among male ITP patients was 48% as compared to 52% in their female counterparts in a ratio of 0.9:1 (M: F ratio). In parallel, the prevalence of HR genotype among male healthy control was 52.5% as compared to 40.47 % in their female counterparts in a ratio of 1.29:1 (M: F ratio), indicating a lower frequency of HR allele variation of the FCGR2A131 among ITP male patients. The RR population shows an equal distribution between male and female ITP patients and controls, with both groups having 50% of each gender in this category in a ratio of 1:1 (M:F ratio)

Table 6

The distribution of FCGR2A131H/R genotypes among patients with ITP and healthy controls

Genotype	ITP (N= 40)		Control (N= 80)	
FCGR2A131H/R	N	%	N	%
Total HH population	(7/40)	17.5%	(20/80)	25%
Male HH population	(4/7)	57.41%	(7/20)	35%
Female HH population	(3/7)	42.85%	(13/20)	65%
Total HR population	(25/40)	62.5%	(42/80)	52.5%
Male HR population	(12/25)	48%	(25/42)	52.5%
Female HR population	(13/25)	52%	(17/42)	40.47%
Total RR population	(8/40)	20%	(18/80)	22.5%
Male RR population	(4/8)	50%	(9/18)	50%
Female RR population	(4/8)	50%	(9/18)	50%

H, Histidine; R, Arginin

Moreover, analysis of the FCGR3A158F/V genotype revealed that the prevalence of FF genotype among ITP male was 30 % as compared to 70% in their female counterparts in a ratio of 0.42:1 (M: F ratio). In parallel, the prevalence of FF genotype among male healthy control was 36.8% as compared to 63.2 % in their female counterparts in a ratio of 0.58:1 (M: F ratio), indicating a similar frequency of FF allele variation of the FCGR2A131 among ITP male patients. On the other side, FV genotype among ITP male was 40.9% as compared to 59.09% in their female counterparts in a ratio of 0.69:1 (M: F ratio).

In parallel, the prevalence of FV genotype among male healthy control was 60.46% as compared to 39.3 % in their female counterparts in a ratio of 1.53:1 (M: F ratio), indicating a lower frequency of FV allele variation of the FCGR3A158F/V genotype among ITP male patients. Additional, when analyzing the frequencies of VV allele variation among male and females indicating 62.5% and 37.5%, respectively in the ITP patients in a ratio of 1.66:1 (M: F ratio). In parallel, the prevalence of VV genotype among male healthy control was 38.9% as compared to 61.1 % in their female counterparts in a ratio of 0.63:1 (M: F ratio), indicating a lower frequency of VV allele variation of the FCGR3A158F/V genotype among healthy male patients (Table 7).

In conclusion, the findings suggest that there is notable gender-specific differences in the distribution of FCGR2A131H/R and FCGR3A158F/V genotypes among ITP patients, with distinct variations observed between male and female subjects. Specifically, the higher frequency of the FCGR2A131HH genotype in male ITP patients compared to healthy male controls, along with a lower frequency of the HR genotype in male patients, indicates a potential association with increased susceptibility to ITP in males.

Similarly, the FCGR3A158F/V genotype analysis shows a trend of lower frequencies of FV and VV genotypes in male ITP patients compared to their healthy counterparts, further supporting the possibility of gender-specific genetic predispositions to ITP. These findings suggest that gender may play a significant role in the distribution of specific genotypes in ITP, potentially aiding in the development of more personalized diagnostic and therapeutic approaches for the condition.

Table 7

The distribution of FCGR3A158F/V genotypes among patients with ITP and healthy control

Genotype	ITP (N= 40)		Control group (N= 80)	
FCGR3A 158 F/V	N	%	N	%
Total FF population	(10/40)	25%	(19/80)	23.75
Male FF population	(3/10)	30%	(7/19)	36.8%
Female FF population	(7/10)	70%	(12/19)	63.2%
Total FV population	(22/40)	55%	(43/80)	53.75%
Male FV population	(9/22)	40.9%	(26/43)	60.46%
Female FV population	(13/22)	59.09%	(17/43)	39.53%
Total VV population	(8/40)	20%	(18/80)	22.5%
Male VV population	(5/8)	62.5%	(7/18)	38.9%
Female VV population	(3/8)	37.5%	(11/18)	61.1%

F, Phenyl Alanine; V, Valine.

3.6 Genetic Polymorphisms of FCGR3A 158F/V and FCGR2A 131H/R: Key Players in Chronic Immune Thrombocytopenia

Analysis of combined genotypes of FCGR2A and FCGR3A polymorphisms revealed variability in disease severity among the studied cases (Table 8). The genotype combination FV-HR was the most prevalent, representing 32.5% of cases, primarily associated with chronic presentations (69.2%), followed by acute (23.1%) and persistent (7.7%) cases, with no refractory occurrences. Conversely, combinations FV-RR and VV-RR exclusively correlated with acute cases (100%), though these genotypes had low absolute frequencies (10% and 2.5%, respectively). The VV-HH combination showed significant severity, with 50% refractory cases, indicating a potential association with treatment resistance despite its low prevalence (5%). FF-HR exhibited a diverse clinical profile, predominantly chronic (57.1%) but also associated with acute, refractory, and persistent presentations. No cases were recorded for the FF-HH genotype combination. These findings suggest that specific combinations of FCGR2A and FCGR3A polymorphisms are linked with distinct clinical outcomes, potentially guiding prognosis and tailored therapeutic approaches see (Table 8).

Table 8

Relationship between all possible combined genotypes of FCGR2A and FCGR3A polymorphism and severity of the case

FCGR3A 158F/V	FCGR2A 131H/R	N	%	Acute	Chronic	Refractory	Persistent
FV	HR	13	32.5	23.07	69.23	0	7.7
FV	RR	4	10	100	0	0	0
FV	HH	5	12.5	20	60	0	20
VV	HR	5	12.5	80	20	0	0
VV	RR	1	2.5	100	0	0	0
VV	HH	2	5	0	50	50	0
FF	HR	7	17.5	14.3	57.1	14.3	14.3
FF	RR	3	7.5	33.3	66.7	0	0
FF	HH	0	0	0	0	0	0

Combination of various polymorphic variants might have an effect. For the combined genotype analysis of the high affinity allele s (FCGR2A-131H or FCGR3A-158V), no

statistically significant differences between the two study groups, controls and patients were seen. Most ITP cases (32.5%) were heterozygous for both the FCGR (FV/HR) combined genotypes indicating a possible association even if it's not significant (OR) of 1.26 (95% CI: 0.56–2.89, p=0.57) compared with (27.5%) in control group. The (FF/HR) combination is in about 17.5% of them, and 7.5% were having the (FF/RR). The FV/HH and the VV/HR reported equally in a frequency of (12.5%), the rare HH/VV genotype was observed in 5% of patients versus 8.75% of controls (OR=0.55, 95% CI: 0.11–2.77, p=0.23), indicating no evidence of increased risk. Similarly, the RR/VV genotype appeared at very low frequencies (2.5% in cases vs. 3.75% in controls), with no significant association (OR=0.66, 95% CI: 0.07–6.54, p=0.36). see table 9

Table 9

Combined genotype analysis of FcγRIIA and FcγRIIIA among the study groups

Genotype	Absolute (n = 40)	Control (n = 80)	OR (95% CI)	p
HH/FF	0	1 (1.25%)	0.65 (0.026–16.42)	0.80
HR/FF	7 (17.5%)	12 (15%)	1.20 (0.43–3.34)	0.73
RR/FF	3 (7.5%)	6 (7.5%)	1.00 (0.24–4.22)	0.50
HH/FV	5 (12.5%)	12 (15%)	0.80 (0.26–2.48)	0.35
HR/FV	13 (32.5%)	22 (27.5%)	1.26 (0.56–2.89)	0.57
RR/FV	4 (10%)	9 (11.5%)	0.88 (0.25–3.04)	0.84
HH/VV	2 (5%)	7 (8.75%)	0.55 (0.11–2.77)	0.23
HR/VV	5 (12.5%)	8 (10%)	1.29 (0.39–4.28)	0.34
RR/VV	1 (2.5%)	3 (3.75%)	0.66 (0.07–6.54)	0.36

3.7 Prevalence of ANA and A-dsDNA antibodies in patients with primary immune thrombocytopenia

The ANA and A- dsDNA were both tested in 32 samples (80%) of the study population/ITP patients, 19 male,13 female, their ages between 2- 13.2 years old, about 17 case presented with acute ITP, 2 cases refractory, and 13 chronic, in addition to this 19 of them having the HR,6 with the HH, and 7 with the RR possible genotypes of the FcgRIIA131H/R polymorphism, and for the second FcgR polymorphism, the FcgRIIIA158F/V, 19 participant with the FV, 7 with the FF, and 6 having the VV genotype.

results, indicate that in about 53% of them (17/32) the ANA was positive and only 3% have positivity for both the ANA and the A-dsDNA (1/32).

Interm of disease duration, there is balanced ANA and A- dsDNA positivity in acute and chronic cases, no significant differences were found in between ANA positivity and ITP severity as shown in table 10.

Table10

The frequency of ANA positive cases in acute and chronic ITP cases

	Total	Acute		Chronic		χ^2	*P*
	N	N	%	N	%		
	30	17	56.7%	13	43.3%	1.97	0.16
ANA +	17	8	47%	9	69%		
ANA -	13	9	53%	4	21%		

ANA: Anti- nuclear antibody.

The association in between the ANA and the studied polymorphisms for the Fcg receptor were investigated, results indicates that the majority of ANA positive cases were predominant in the FV genotype of the FCgRIIIA (12/19, 63%) and the same estimates were seen with the HR genotype of the FcgRIIA (12/19, 63%), and these could be relevant polymorphisms in the pathogenesis of autoimmune thrombocytopenia.

all patients included in the study and tested for the ANA with the FF genotype (4/17,) were ANA positive and negative to the A- dsDNA, as well as the VV (1/17,) and the RR genotype (3/32, 9.3%) were ANA positive and one having the RR genotype who is six years old was positive to the A- dsDNA

Results also indicate that Anti-dsDNA is rarely positive, supporting the idea that SLE is not commonly coexisting or evolving in these patients, at least at this point. The only anti-dsDNA positive patient had a unique combination of FV/HH, which might warrant further investigation in a larger cohort.

Chapter Four

Discussions and Conclusions

The Immune thrombocytopenic is considered a very common and reportable disease; but there is a lack of definitive evidence regarding its incidence (Saleh & DR Mohammed M Abu Selmia, 2017b). key component of the immune pathophysiology of the autoantibody-sensitized platelets destruction is the Fc gamma receptors(Carcao et al., 2003). Most FCGRs are glycoproteins which bind to the Fc portion of the IgG activating the immune system (Swinkels et al., 2018b).

As reported in various research studies, alteration of the FCGRIIa and /or FCGRIIIa might result in altered receptor-binding affinity with the IgGs. The therapeutic use of intravenous immunoglobulin (IVIg) which may bind FCGRs by its Fc-portion provided further evidence that FCGR-mediated pathways are important in ITP pathogenesis. The IVIg is one of the effective treatments for ITP that increases platelet counts (Schwab & Nimmerjahn, 2013).

Ethnicity, limited sample size, and the exact clinical definition of immune thrombocytopenia have a role in making a final decision about the contribution of the Fc gamma receptors in ITP development, chronicity of the case, and response to treatment, results were conflicting in various research studies reported in literature (Qiao, Al-Tamimi, Baker, Andrews, & Gardiner, 2015). Adding to this is the lack in population data about the actual incidence of the disease particularly in low-income countries (Brændstrup et al., 2005).

So far, this current study has been conducted in a total of 120 children, 40 patients pre-diagnosed with primary immune thrombocytopenia based on exclusion criteria (45% Acute, 42.5%Chronic, 7.5% Persistent and5% Refractory), and 80 was taken as an age and gender matched control group.

In the current study we investigated the potential association of FCGR2A (131H/R) and FCGR3A (158F/V) polymorphisms with disease susceptibility, chronicity, and severity in pediatric immune thrombocytopenia (ITP), with a unique focus on gender-based genetic distributions. While no significant differences in overall genotype or allele frequencies were observed between ITP patients and healthy controls, the analysis of

combined genotypes and gender-specific patterns revealed insignificant results but trends observed.

For FCGR2A, the frequencies of the HH, HR, and RR genotypes in the ITP patient group were 17.5%, 62.5%, and 20.0%, respectively, whereas in the control group, these genotypes were 25.0%, 52.5%, and 22.5%. No statistically significant difference emerged ($p = 0.543$) A notable observation was that the heterozygous HR genotype occurred in approximately 52% of female patients and in 52% of male controls. Allele frequencies (H and R) also showed no significant difference between groups ($p = 0.82$).

Similarly, for FCGR3A no significant differences were noticed, Allele frequencies (F and V) were comparable between the two groups ($p= 0.88$). The ITP patients exhibited FF, FV, and VV genotypes at 25.0%, 55.0%, and 20.0%, respectively, compared with 23.8%, 53.8%, and 22.5% in controls ($p = 0.950$). Although a higher frequency of the FV phenotype was noted in female patients (59.09%) compared with male controls (60.46%), but it did not reach statistical significance. The study findings suggest that, these Fc gamma receptors do not appear to play a role in determining susceptibility to disease.

contrary to data reported by a study conducted in Egypt in 2011 by Zakaria et al who noticed that both allele and polymorphism frequencies were significantly over-represented in ITP patients compared to control. But in terms of case severity no significant differences reported in newly diagnosed and chronic ITP cases for both Fc gamma receptor polymorphisms (Zakaria et al., 2021).

In another study in Egypt, and in line with our findings Analysis of both SNPSs in patients and control showed that the FcgRIIa H and R allele frequencies were 56.4% vs. 55.5% and 43.6% vs. 45.5%) respectively and it was 47.3% vs. 38.2% for the F allel and 52.7% vs. 61.8% for the V allele and the differences were statistically insignificant. adding to this the FV variant were more reported in ITP than control (54.5 % vs. 25.5%) and the difference was statistically significant, while no association were found in between the FCGRIIa and FCGRIIIa polymorphisms with bleeding tendency and case severity (Mohamed et al., 2019).

Contrary to that, the most notable finding in our study was the high prevalence of the HR/FV combined genotype in ITP patients (32.5%), which was strongly associated with chronic disease (69.2%). In contrast, the RR/FV and RR/VV combinations were found exclusively in acute presentations, suggesting that certain genotype combinations may offer a protective effect against chronicity. Additionally, the rare VV/HH genotype (5%) was linked to refractory disease in 50% of cases, indicating a potential association with treatment resistance.

Gender-stratified analysis provided further insights, in particularly, the FCGR2A-HH genotype showing more frequent in male ITP patients (57.4%) than in females (42.8%), while the HR genotype was less common among males. FCGR3A-VV also showed prevalent in male ITP patients (62.5%) as compared to its incidence in females (37.5%). These patterns suggest that male patients may carry genotypes more closely associated with severe or persistent disease.

These results could be aligned with previous research suggesting gender-related differences in immune response and autoimmune disease expression (Cines & Blanchette, 2021; Park, Kim, & Lee, 2023). Moreover, our findings partially corroborate with earlier studies by Eyada et al. (2012) that reported a higher frequency of the FCGR3A-FV genotype in chronic ITP, while Papagianni et al. (2013) and Pavkovic et al. (2018) found no significant associations with disease susceptibility.

In Turkey in a study including 123 ITP patients,' 30 chronic and 93 acute, results indicate that older age, and female gender may predict chronic ITP development in children (Alpdoğan & Gülen, 2023).

These findings also confirmed in a second Turkish study which indicated that, age and gender were found to be risk factors for the development of the chronic form of the disease, age above 10 years old and female gender tend more than males to develop chronic ITP course (Sezgin Evim et al., 2014a).

These Findings were contrary to data and findings by Yaprak et al, who studied a total of 350 children (164 males, 186 female) their ages between 6 months to 16 years old and did not find any gender preferences related to chronic ITP (Yaprak et al., 2010).

But neither of these studies have examined the association of these risk factors with the Fc gamma receptor polymorphism.

Our findings in part could provide valuable insights by emphasizing the clinical significance of combined genotypes and uncovering sex-based genetic patterns that have been largely overlooked in pediatric ITP cohorts. These observations suggest a potential influence of sex on genotype distribution, which may reflect the modulatory effects of sex hormones or sex-linked immune mechanisms on Fcγ receptor function.

Adding to this several studies among adults and to less extent among children investigate the association in between immune thrombocytopenia and the Fc gamma receptor and there results were inconsistent, a meta- analysis of 17 published studies, were done to understand the contribution of the Fc gamma receptor in ITP susceptibility, six of these studies investigate both SNPs the FCGR2A H131R and FCGRIIIA F158V association in childhood ITP cases including patients from (Brazil, France, The Netherlands, Egypt, USA and Canada) and one more in Greec in 2013 which investigates only the contribution of the FCGRIIA F158V polymorphism.

In overall analysis a significant association in between the FCGR3A F158V polymorphism and the ITP observed, and no visible contribution seen with the FCGR2A H131R polymorphism, and in further analysis to subgroups based on ethnicity same results obtained in both Caucasians and Asians, a further analysis based on type of the disease showed that the FCGR2A H131R polymorphism were more significant with childhood -onset ITP, while the FCGR3A F158V polymorphism was significantly associated with both childhood□onset and adult□onset ITP. Overall, both may serve as potential genetic markers for ITP diagnosis in children and the FCGRA F158V for adults (G. Li et al., 2019), keeping inconsideration that the FCGRIIA is the only receptor expressed on platelets surface which reflects it significant association with ITP (Qiao, Al-Tamimi, Baker, Andrews, & Gardiner, 2015).

Small number of patients were included in these studies, which is comparable with our study regarding the sample size, and part of the findings. Indicating the need for further case control studies with more sample size involving children and adults for comparative issues and finding potential association, this study recommends that future studies have to investigate the association in between these polymorphisms and severity

of the case supporting our hypothesis and the importance of our study, giving potential to increase the awareness regarding this idea.

In a previous metanalysis for the same target including 10 case- control studies up to the year 2014, with a total of 741 ITP patients and 1092 healthy controls. Results proposed that FCGRIIIA F158V polymorphism is strongly correlated with predisposition to ITP in both children and adults، and Subgroup analysis revealed its contribution in both Caucasian and non-Caucasian populations to ITP tendency. while there is no significant correlation between the FCGRIIA polymorphism and ITP susceptibility in both ethnic groups (all P>0.05) (Xu et al., 2016).

For ANA testing, results indicate that in 53% of cases (17/32) the ANA was positive, and was reactive for both the ANA and the A-dsDNA (1/32) in 3% of case, there is fairly balanced ANA and A- dsDNA positivity, in acute and chronic cases, no significant differences were found in between ANA positivity, and ITP severity, ANA testing was not recommended in ASH 2011 and 2019 guidelines, but in terms of ITP management it could be used as a potential marker, and may contribute to chronicity(Moulis et al., 2023)

ANA positive ITP cases may progress to CTD, (risk ratio=12.43, 95% CI 7.91 to 19.55, p<0.00001), particularly the SLE in 4.0% of reactive cases (risk ratio=30.41, 95% CI 13.23 to 69.86, p<0.00001) (Y. Liu et al., 2021),

For association studies in between positive cases, and development of SLE among ITP patients, further is needed in larger cohorts, involving higher numbers of patients. results must be compared with healthy children within the same age group to conclude any possible association.

More is needed to understand the correlation between the ITP, the ANA, and the studied polymorphisms, in general the preliminary results give a general idea indicating that patients, particularly girls, need more follow-up, and continued evaluation.

The study has several notable strengths, particularly regarding its scope and focus on an underrepresented population: A significant strength is the investigation of FCGR polymorphisms in Palestinian children, a Middle Eastern pediatric cohort that is

generally underrepresented in immunogenetic research related to Immune Thrombocytopenia (ITP). This diversity of genetic backgrounds is crucial for a complete understanding of the disease.

The Investigation of Combined Genotypes: The study performed an exploratory analysis on the association of combined FCGR2A and FCGR3A genotypes with distinct clinical outcomes (severity, chronicity, and refractory disease). This approach moves beyond analyzing single polymorphisms and suggests that specific combinations may better guide prognosis and tailored therapeutic approaches.

The Gender-Specific Exploratory Analysis: The research included a focused, secondary analysis exploring gender-specific trends in the distribution of FCGR2A and FCGR3A polymorphisms. This is an underexplored area in ITP research, and the findings suggest that gender may play a significant role in the genetic predisposition and distribution of these genotypes, which could aid in developing personalized approaches. Finally, the study utilized a multicenter retrospective case-control design, drawing patients from four major hospitals in the West Bank/Palestine between 2019 and 2023. This approach enhances the representativeness of the ITP patient cohort within the region in which Detailed Phenotyping and Categorization of the disease documented, and cases categorized into acute, chronic, persistent, and refractory forms, allowing for correlation between genetic profiles and the clinical phenotype (disease course and severity).

4.1 Conclusion

While FCGR2A and FCGR3A polymorphisms alone were not significantly associated with ITP susceptibility, specific combined genotypes particularly HR/FV and gender-related variations appear to influence disease course and severity. These findings suggest that immunogenetic profiling, incorporating sex-based analyses, could enhance prognostic accuracy and personalize therapeutic approaches in pediatric ITP. New emerging therapeutic strategies that inhibit FcγR signaling, the neonatal Fc receptor or the classical complement pathway, will deeply modify the management of ITP soon.

ITP in children is a common heterogeneous bleeding disorder. There are still many questions to be answered regarding the natural progress of the clinical forms and the prediction of chronic or recurrent outcome, response to treatment and ultimate outcome.

Future directions should include larger multicenter studies to validate these genotype-phenotype associations and explore their mechanistic basis. Functional studies evaluating Fcγ receptor expression and downstream signaling in relation to these polymorphisms, particularly in a sex-specific context, are warranted. Understanding how these genetic variants influence response to therapies like IVIg or thrombopoietin receptor agonists could guide individualized treatment strategies.

The need for a multidisciplinary approach involving hematologists, immunologists, and other specialists to provide comprehensive care. Regular follow-up and individualized treatment plans based on disease severity, patient age, and response to therapy are essential for optimizing management and improving the quality of life for pediatric patients with ITP. Patients usually experience limitations in their activity, and have increased school absenteeism, depression, psychological stressors and social stigma related to their condition particularly those have chronic ITP. To address these issues effectively, support, including psychological counselling and educational interventions is needed. Adding to this, parents must understand that their children must avoid certain activities like sports, dental extraction, limited drug use like aspirin and ibuprofen, and have to know that they must attend hospital in case of an accident.

Most studies in the region and published reports are retrospective and there are few multicenter studies, even within the same country. Collaboration between facilities managing ITP children must be promoted and enhanced and the creation of a lacking group or ITP association must be encouraged. Additionally, it is essential to standardize practical guidelines for ITP management in the region is.

Future research should focus on better understanding the pathophysiology of ITP, refining diagnostic criteria, and evaluating long-term outcomes of various treatment strategies. As our knowledge of ITP continues to advance, it is crucial to integrate new findings into clinical practice to enhance the care and outcomes for children affected by this condition So, the buildup of regular follow-up plan and individualized treatment approaches will help improve their life expectancy with less complications and undesired negative consequences.

4.2 Study Limitations

On our study there are several restrictions and limitations that may affect the research work overall. The first of these, is the limited sample size, the research population is of relatively small number of patients who were selected from four medical centers in Palestine, all were referred to pediatric units at four different hospitals: the Martyr Dr. Khalil Suleiman Governmental Hospital in Jenin, the Palestine Medical Complex in Ramallah, Rafidia Governmental Surgical Hospital, and An-Najah National University Hospital, both located in Nablus, between 2019 and May 2024; therefore, care should be taken while interpreting and extrapolating these results.

Secondly, and adding to this is the retrospective nature of our study which is based on a review of patient's medical records. Existing data regarding patient's demographics, Clinical manifestations, the history of pre-admission infection, severity of the case, bleeding intensity and duration at admission, laboratory findings, and the kind of the given treatment were all extracted from patient's medical records. Of course, how the data were entered into and recorded by clinicians and the medical staff will absolutely affect its reliability.

The biological relevance of the identified variants remains uncertain, as functional assays assessing FCGR expression, IgG binding, or downstream effector functions were not performed. Furthermore, the PCR-RFLP methodology employed in this study detects only single nucleotide polymorphisms and cannot capture copy number variations, which are common in the FcγR gene cluster and have been shown to influence ITP susceptibility and therapeutic response.

These methodological limitations reflect constraints in both budget and available laboratory techniques at our institution. Despite these limitations, the study provides preliminary insights into combined genotype associations and sex-specific patterns in a pediatric Middle Eastern cohort, a population that is underrepresented in current immunogenetic research. Adding to this, is the lack of communication in between patients and clinicians and follow up problems, patients referred to different hospitals, and missing data were noticed concerning them, more is needed in this area, collaboration have to be encouraged in between hospitals and academic institution to support and facilitate more research work, the data concerning patients have to be

updated throughout the creation of a data base with solid background for future directions.

4.3 Recommendations

- Establish national or regional ITP registries to better define incidence and treatment outcomes.
- Promote collaborative research between Middle Eastern countries to address region-specific risk factors.
- Improve awareness and training among healthcare providers to recognize and manage ITP appropriately.
- Incorporate standardized diagnostic and management protocols across the region.
- Studies also recommend that further is needed to investigate gene to gene interaction and haplotype analysis since the mutant alleles contribute to immune dysfunction and the development of autoimmune diseases particularly the (ITP), by affecting the interaction in between the Fc receptors and the Immunoglobulin making the diagnosis more complex.
- The advanced diagnostic techniques must be integrated; flow cytometric analysis and molecular studies will both enrich the area of diagnosis and patient management issues. Regarding management issues, an approach is needed particularly to cope with the social and psychological aspects concerning living with the chronic case of the disease.

List of Abbreviations

Abbreviation	Meaning
ADCC	Antibody Dependent Cellular Cytotoxicity
ADs	Autoimmune diseases
A- dsDNA	Anti Double Stranded Deoxy Nucleic Acid
A-ENA	Anti- Extractable Nuclear Antigen
AML	Acute Myeloblastic Leukemia
ANA	Anti-Nuclear Antibody
APA	Anti- Phospholipid Antibody
APCs	Antigen Presenting Cells
ASH	American Society of Hematology
AVH	Augusta Victoria Hospital
BSTUI	Restriction Enzyme
CBC	Complete Blood Count
CD4	Cluster of Differentiation 4
CD20	Cluster of Differentiation 20
CI	Confidence Interval
cITP	Chronic Immune Thrombocytopenia
CLL	Chronic Lymphocytic Leukemia
CMV	Cyto-megalo Virus
CRC	Clinical Research Center
CTDs	Connective Tissue Diseases
DNA	Deoxy Nucleic Acid
DAT	Direct Antiglobulin Test
EBV	Epstein Bar Virus
EDTA	Ethylene Diamine Tetra Acetic Acid
F	Phenylalanine
FCγRs	Fc Gamma Receptor
G	Guanin
GP	Glycoprotien
GS	Gaza Strip

H	Histidin
HBV	Hepatitis B Virus
HCQ	Hydroxychloroquine
HCV	Hepatitis C virus
Hin 1 II	Restriction Enzyme
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HMO	Haddasah Medical Organization
HRQoL	Health care Related Quality of Life
ICD	International Classification of Disease
ICIS	Intercontinental Cooprative ITP Study Group
iDCs	Immature dendritic cells
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IL	Interleukin
IPF	Immature Platelet Fraction
ITP	Immune Thrombocytopenia
IVIG	Intravenous Immunoglobulin
IWG	International Working Group
Jo- 1	Anti- Histidyl Trna Synthetase
LGL	Large Granular Lymphocytic Leukemia
MDS	Myelo-dysplastic Syndrome
miRNA	Micro- RNA
MLPA	Multiplex ligation-dependent probe amplification
NETs	Neutrophil Extracellular Traps
NGS	Next Generation Sequencing
NNUH	Najah National University Hospital
OR	Odds Ratio
P	Probability Value
PBC	Primary Biliary Cirrhosis
PCR	Polymerase Chaim Reaction

PCR- RFLP	Polymerase Chain Reaction, Restriction Fragment Length Polymorphism
PHIC	Palestine Health Information Center
PMP	Platelet Microparticles
R	Arginin
RNP	Ribonucleoproteins
RNP/Sm	Ribonucleoprotien and smith (Sm) Protien
SCI-70	Anti Topoisomerase
SLE	Systemic Lupus Erythmatosis
SNPs	Single Nucleotide Polymophisms
A-Sm	Anti- Smith
SPSS	Statistical Package for the Social Sciences
SSA	Sjogren's Syndrome Related Antigen A
SS-B	Sjogren's Syndrome Type B
SyK	Spleen Tyrosine Kinase Inhibitor
T	Thymine
T1D	Type 1 Diabetes
TNF- α	Tumor Necrosis Factor- Alpha
TPO	Thrombopoietin
TPO- RAs	Thrombopoietin Receptor Agonists
V	Valine
VS	Versus
WB	West Bank
WB1	Wash Buffer 1
WB2	Wash Buffer 2

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Appendices

Appendix A

IRB Approval lettert

An-Najah National University
Faculty of Medicine & Health Sciences
Institutional Review Board

جامعة النجاح الوطنية
كلية الطب وعلوم الصحة
لجنة اخلاقيات البحث العلمي

Ref: Mas. Nov. 2023/30

IRB Approval Letter

Title of Research:

The association of Fcγrlla and Fcγrllla Polymorphisms and Antiplatelets Autoantibodies with Primary Immune Thrombocytopenia Pathogenesis among Palestinian Children"

Submitted by:
Khitam Musa Diab Amer

Supervisor
Adham Abu Taha

Aproved:
13th Nov. 2023

Your Study "The association of Fcγrlla and Fcγrllla Polymorphisms and Antiplatelets Autoantibodies with Primary Immune Thrombocytopenia Pathogenesis among Palestinian Children" reviewed by An-Najah National University IRB committee and was approved on 13th Nov. 2023

Hasan Fitian, MD

IRB Committee Chairman

IRB

Nablus - P.O Box :7 or 707 | Tel (970) (09) 2342902/4/7/8/14 | Faximile (970) (09) 2342910| E-mail : IRB@najah.edu

Appendix B

Ministry of Health education in health and Scientific Research Approval

State of Palestine Ministry of Health Education in Health and Scientific Research Unit	دولة فلسطين وزارة الصحة وحدة التعليم الصحي والبحث العلمي

Ref.: ....
Date:..................

الرقم: عح/٥٥/٢٠٢١
التاريخ: ١٥/١/٢٠٢١

عطوفة الوكيل المساعد لمجمع فلسطين الطبي المحترم،،،

عطوفة الوكيل المساعد لشؤون المستشفيات والطوارئ المحترم،،،

عطوفة الوكيل المساعد للمهن الصحية المساندة المحترم،،،

تحية واحترام..

<u>الموضــوع: تسهيل مهمة طالبة دكتوراه</u>

يرجى تسهيل مهمة الطالبة: ختام موسى ذياب عامر – طالبة دكتوراه علوم مخبرية سريرية/ جامعة النجاح، في عمل بحث الدكتوراه، بعنوان:

"The association of Fcgr11a and Fcgr111a Polymorphisms and Antiplatelets Autoantibodies with Primary Immune Thrombocytopenia Pathogenesis among Palestinian Children""

حيث تحتاج الطالبة لجمع معلومات من داخل المشفى ودون الحصول على معلومات للتواصل خارج المشفى، وذلك في:

- مجمع فلسطين الطبي
- مستشفى رفيديا – مستشفى جنين

مع العلم ان مشرف الدراسة د. ادهم ابو طه.

بشرط موافقة ادارة المشفى على البحث.

على ان يتم تزويد الوزارة بنسخة PDF من نتائج البحث، والتعهد بعدم النشر لحين الحصول على موافقة الوزارة على نتائج البحث.

مع الاحترام..

د. عبد الله القواسمي

رئيس وحدة التعليم الصحي والبحث العلمي

نسخة: نائب الرئيس للشؤون الاكاديمية المحترم/ جامعة النجاح

Telfax.:09-2333901 scientificresearch.dep@gmail.com تلفاكس: 09-2333901

Appendix C

AN- Najah National University Hospital Approval Letter

Approval date: 2024-01-21
Ref: CRC_2024_0225

Subject: Approval to conduct a research project at An-Najah National University Hospital

Dear Mrs. KHITAM AMER,

I am writing this letter to grant you permission to conduct your research project titled "The association of FcγRIIa and FcγRIIIa Polymorphisms and Antiplatelets Autoantibodies with Primary Immune Thrombocytopenia Pathogenesis among Palestinian Children"". I hope your study will provide new insights and contribute the advancement of knowledge and evidence. Furthermore, I would like to emphasize the importance of adhering to the ethical guidelines set forth by the hospital throughout the research process.

On behalf of An-Najah National University Hospital, I extend my best wishes and support for your research endeavors.

Sincerely,

Sa'ed H. Zyoud, Ph.D.

Clinical Toxicology

Director of Clinical Research Center

CC:

Chief Medical Officer

Chief Nursing Officer

Appendix D

Consent Form

نموذج طلب موافقة على المشاركة في بحث علمي

عنوان الدراسة: ارتباط تعدد أشكال Fcγ والأجسام المضادة الذاتية للصفائح مع التسبب في نقص الصفائح المناعي الأولي لدى الأطفال الفلسطينيين.

اسم الباحث الرئيسي: حتام عامر
المشرفين على البحث: د.أدهم ابو هنه (مشرفاً اكاديمياً) د. جوني عامر(مشرفاً اكاديمياً) .

ملخص البحث: تقوم بهذه الدراسة استيفاءاً لمتطلبات التخرج من برنامج الدكتوراه في العلوم المخبريه السريريه في جامعة النجاح الوطنية وهي دراسة تهدف لتحديد نسبه الاطفال الفلسطينيين المصابين بنقص الصفائح المناعي ونسبه انتشار بعض الطفرات الوراثيه لديهم وتعدد اشكالها Fcγ مقارنه بالاطفال الاصحاء ودورها بالاضافه لدورالأجسام المضاده مع التسبب في نقص الصفائح المناعي الأولي.

معلومات عن العينة المستقاة والفترة الزمنية المقررة لاستكمال المقابلة أو الاستبيان:
تم اختيار الأطفال دون سن الثامنه عشره كعينه لهذا البحث وهي حالات مرضيه يتم علاجها في مستشفى المطلع/ المستشفى الاستشاري/ مستشفى النجاح/ واقسام الاطفال العامه في كل من مستشفى رفيديا ورام الله الحكومي ومستشفى الشهيد خليل سليمان وسيتم الشروع بخطوات البحث حين استكمال الاجراءات اللازمه.

المخاطر المتوقعة والخصوصية:
ليست هنالك اي مخاطر للدراسة سواء نفسية او جسدية. سيتم حفظ خصوصية ملفاتكم في حال الموافقة على المشاركة بالدراسة وسوف يتم التكتم على هويتها وسيبقى الاسم على الكتمان والمكان الوحيد الذي سيتم ذكر الاسم فيه هو نموذج الموافقة على المشاركة في الدراسة. سوف يتم التعامل مع العينة والمعلومات الخاصة بملفاتك بطريقة الترميز حماية للخصوصية، ويحق لكم الانسحاب من البحث متى شئتم من دون أن يأثر ذلك على ملفاتك او على الرعاية الطبية التي يتلقاها.

المنافع المتوقعة: الطفرات الوراثيه التي سيتم الكشف عنها وايضا فحوصات الاجسام المضاده تلعب دور مهم في تحديد حدة المرض والية الاستجابه للعلاج ومستقبلا ممكن استخدامها لتشخيص هذه الحالات بشكل دون الحاجه لاجراء عدد كبير من الفحوصات المخبريه.

طريقة التواصل مع الباحث:
إذا كانت لديكم اي سؤال او استفسار عن الدراسة يمكنك التواصل مع الباحث (حتام عامر) بكل رحابة وفي اي وقت عن طريق (الهاتف 0599549229) أو البريدالإلكتروني(Khetam.amer@aaup.edu).

اقرار المشاركة في البحث:
لقد حصلت على شرح مفصل عن الدراسة وأهدافها وإجراءاتها، ومنافعها، والمخاطر المحتملة. ولقد فهمت كافة المعلومات التي قدمت لي وتمت الإجابة على كل أسئلتي. لذا فأنني أوافق وبمحض ارادتي على مشاركة ابني/ابنتي في هذه الدراسة.

الاسم:----------

التوقيع:----------

التاريخ:----------

Appendix E

The Published Article

OPEN ACCESS

EDITED BY
Alfred Hyoungju Kim,
Washington University in St. Louis,
United States

REVIEWED BY
Xu Hannah Zhang,
City of Hope National Medical Center,
United States
Mariele Gatto,
University of Turin, Italy

*CORRESPONDENCE
Johnny Amer
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†These authors have contributed equally to this work and share first authorship

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Amer K, Amer J, Taha AA, Baker W, Abuhamed A and Salhab A (2025) Combined *FCGR2A* (131H/R) and *FCGR3A* (158F/V) genotypes and their gender-specific association with chronic and refractory immune thrombocytopenia in Palestinian children.
Front. Med. 12:1606953.
doi: 10.3389/fmed.2025.1606953

Combined *FCGR2A* (131H/R) and *FCGR3A* (158F/V) genotypes and their gender-specific association with chronic and refractory immune thrombocytopenia in Palestinian children

Khitam Amer[1†], Johnny Amer[1*†], Adham Abu Taha[1], Wisam Baker[2], Awad Abuhamed[3] and Ahmad Salhab[1]

[1]Department of Allied and Applied Medical Sciences, An-Najah National University, Nablus, Palestine, [2]Martyr Dr. Khalil Suleiman Governmental Hospital, Jenin, Palestine, [3]Department of Pediatrics, Rafidia Government Surgical Hospital, Nablus, Palestine

Background: Immune thrombocytopenia (ITP) is a common pediatric autoimmune disorder characterized by low platelet counts and heightened bleeding risk. Fc gamma receptors (FcγRs), particularly *FCGR2A* (131H/R) and *FCGR3A* (158F/V), mediate immune responses and may influence ITP susceptibility and progression. Gender-related genetic variation has been proposed but remains underexplored, particularly in Middle Eastern pediatric populations. This study aimed to perform an exploratory assessment of the prevalence and potential clinical relevance of *FCGR2A* and *FCGR3A* polymorphisms, including gender-based tendencies, in Palestinian children with ITP.

Methods: A multicenter case-control study included 40 proven pediatric ITP patients (20 males, 20 females; mean age 6.76 ± 4.13 years) and 80 age- and sex-matched healthy controls. Genotyping was performed using PCR-RFLP and nested PCR. Genotype frequencies were correlated with disease phenotype and sex.

Results: No statistically significant differences in genotype distributions were observed between ITP cases and controls for either *FCGR2A* (HH: 17.5%, HR: 62.5%, RR: 20.0%) or *FCGR3A* (FF: 25.0%, FV: 55.0%, VV: 20.0%) ($p > 0.05$). However, a secondary, exploratory analysis for gender-specific trends yielded noteworthy observations: *FCGR2A*-HH was numerically more frequent in male ITP patients (57.4%) than in females (42.8%), while HR was lower in males (48% vs. 52%). Similarly, *FCGR3A*-VV occurred in 62.5% of male ITP patients versus 37.5% in females. Furthermore, the combined *HR/FV* genotype (32.5%) showed a non-significant trend of association with chronic ITP (69.2%), while the *VV/HH* genotype, although rare (5%), was linked to 50% of refractory presentations.

Conclusion: This exploratory study found no statistically significant association between *FCGR2A* and *FCGR3A* polymorphisms and overall ITP susceptibility in the full cohort. However, the observed trends, particularly the distinct gender-based distribution of specific genotypes and the association of combined

جامعة النجاح الوطنية
كلية الدراسات العليا

ارتباط تعدد أشكال FCgRIIA AND FCgRIIIA والأجسام المضادة الذاتية للصفائح مع التسبب في نقص الصفائح المناعي الأولي لدى الأطفال الفلسطينيين

إعداد
ختام موسى ذياب عامر

إشراف
د. جوني عامر

قدمت هذه الأطروحة استكمالاً لمتطلبات الحصول على درجة الدكتوراه في العلوم المخبرية السريرية، بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس – فلسطين.

2026

ارتباط تعدد أشكال FCgRIIA AND FCgRIIIA والأجسام المضادة الذاتية للصفائح مع التسبب في نقص الصفائح المناعي الأولي لدى الأطفال الفلسطينيين

إعداد

ختام موسى ذياب عامر

إشراف

د. جوني عامر

الملخص

نقص الصفائح المناعي يعد من الاضطرابات المناعيه عند الاطفال ويمتاز بانخفاض ملحوظ بعدد الصفائح الدمويه مما يزيد من خطر الاصابه بنزف الدم تلعب بعض المستقبلات المناعيه ك مستقبلات الغاما المناعيه دورا اساسيا في تنظيم رده فعل حهاز المناعه في الجسم ومن الممكن ان يكون لها دور في قابليه الاصابه بالمرض وتطوره وهناك كما يشار تباين جيني مرتبط بالجنس الا ان هذه الجزئيه غير مدروسه بشكل كافي في هذه الفئه العمريه (الأطفال) وتهدف هذه الدراسه الي استكشاف ماهيه انتشار هذه الطفرات FCGR2A(131H/R) و FCGR3A(158F/V) لدى الاطفال الفلسطينيين المصابين بالمرض. شملت الدراسه 40 مريضا اطفالا مشخصين بالمرض (20 ذكرًا، 20 أنثى ب متوسط العمر 6.76 ± 4.13 سنوات) وتمت مفارنتهم مع 80 طفلا اصحاء متوافقين بالعمر والجنس مع المرضى ونم تحديد الانماط الحينيه باستخدام كل من PCR-RFLP و Nested PCR بالاضافه لذلك تمت دراسه الاحسام المضاده للنواه (ANA)والحمض النووي (A-dsDNA) في 32 عينه.

لم تظهر النتائج فروقًا ذات دلالة إحصائية بين مرضى نقص الصفائح المناعي ومجموعه الضبط وكانت FCGR2A (HH: 17.5%، HR: 62.5%، RR: 20.0%) FCGR3A (FF: 25.0%و FV: 55.0%، VV: 20.0%) ($p > 0.05$). فيما يتعلق بالجنس تم الكشف عن ملاحظات هامه ؛ إذ كان النمط الجيني FCGR2A-HH أكثر شيوعاً لدى الذكور (57.4%) مقارنة بالإناث (42.8%)، في حين كان HR أقل شيوعاً عند الذكور (48%) مقابل (52%). كما ظهر النمط الجيني FCGR3A-VV بنسبة أعلى لدى الذكور (62.5%) مقارنة بالإناث (37.5%). كذلك أظهر النمط المشترك HR/FV (32.5%) اتجاهاً غير دال إحصائياً

للارتباط مع الشكل المزمن من (69.2%) ITP، بينما ارتبط النمط النادر (5%) VV/HH بنسبة 50% من الحالات المقاومة للعلاج.

سجلت إيجابية ANA في 53% من الحالات، في حين ظهرت الإيجابية المزدوجة لكل من ANA و A-dsDNA في 3% فقط. وأشارت النتائج إلى أن إيجابية (70%) ANA كانت أكثر شيوعاً لدى حاملي النمط الجيني (FV/H)، مما يدل على أن هذه التعددات الجينية قد تكون ذات صلة بآلية تطور نقص الصفيحات المناعي الذاتي.

لم تثبت هذه الدراسة وجود ارتباط ذي دلالة إحصائية بين تعدد أشكال FCGR2A و FCGR3A وبين القابلية العامة للإصابة بـ ITP في عينه الدراسه. ومع ذلك، فإن ما تم ملاحظته، وخاصةً التوزيع المختلف للأنماط الجينية تبعاً للجنس والارتباط بين الأنماط الجينية المركبة مع حده الحاله، تشير إلى أن هذه العلامات الجينية قد يكون لها دور في تطور المرض. وتؤكد ان هناك حاجة إلى إجراء دراسات أكبر وأكثر قوة لتأكيد هذه النتائج.

الكلمات المفتاحية: نقص الصفائح المناعي (ITP)؛ مستقبلات الغاما المناعية (FcγRs)؛ FCGR2A (131H/R)؛(158F/V) FCGR3A؛ تعدد الأشكال الجينية؛ الأجسام المضادة للنواه والأجسام المضادة للحمض النووي.