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The Expression Profile of MicroRNA LET-7a-5p in Children with Idiopathic Thrombocytopenic Purpura

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Abstract

Background: Paediatric immune thrombocytopenia (ITP) is an autoimmune condition whose cause is unclear and appears to include a number of different factors. **Methods**: In this investigation, quantitative real-time PCR was used to examine the expression profiles of the miRNA Let-7a-5p in blood samples from children with ITP and healthy controls (HCs). It was also examined whether miRNA Let-7a-5p was associated with ITP clinical characteristic parameters and whether it's potential usage as a non-invasive circulating biomarker for ITP diagnosis. The area under the curve was analysed after the receiver operating characteristic curve was generated. **Results**: When compared to HCs, ITP patients' levels of the miRNA Let-7a-5p were considerably higher ($P < 0.0001$). Furthermore, there was a strong positive correlation between the expression level of the Let-7a-5p biomarker and the patients' levels of the antiplatelet antibody glycoproteins GPIIIa, GPIIb, and GP1b/IX ($r = 0.637, 0.669$, and 0.486). For miRNA Let-7a-5p, the biomarker demonstrated excellent specificity (96.8) and sensitivity (100%) at cutoff values of > 18.5. Serum miRNA Let-7a-5p may be used as a possible biomarker to distinguish between HCs and children with ITP.

Keywords: Immune thrombocytopenic purpura (ITP), autoimmune disease, microRNAs, Let-7,Let-7a-5p

1. Introduction

Immune thrombocytopenia (ITP) is a highly complex autoimmune disease]**1, 2]. **ITP is the most frequent autoimmune dysfunction-related acquired hemorrhagic illness **[3].** Reduced platelet count in the peripheral blood is a hallmark of the autoimmune disorder; immune thrombocytopenia **[4], due** to autoantibody-mediated destruction of platelets or reduced platelet production **[5]**. Although the pathophysiology of ITP is still poorly understood, aberrant B and T lymphocyte function is the key factor that leads to the development of ITP**[6]**. Thelper cells (Th cells), such as Th1, Th2, Th3, Th17, Treg cells, etc., are important mediators of autoimmune responses **[7]**. It is believed that the autoantibodies produced by auto-reactive B-cells against self-antigens, particularly immunoglobulin G (IgG) antibodies against glycoprotein IIb (GPIIb)/IIIa and/or GPIb/IX, play a vital role **[8]**. ITP's etiology is convoluted. In ITP, auto-reactive B lymphocytes that secrete antiplatelet antibodies are typically thought to be the main immunologic abnormality **[6]**. New

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research has shown that the subsets of T cells take involvement in the development of ITP] **5]**.
Because of its variety and lack of diagnostic markers, immune thrombocytopenia (ITP) is a complex condition that makes treatment selection challenging **[9]**.Both children and adults can be afflicted with ITP. ITP comes in both acute and chronic forms. Seasonal and typically brief, the acute form of the disease is brought on by a minor viral infection or immunization **[10]**.

Children are mainly affected by acute but benign and self-limited forms of the disease that call for little treatment. Clinically, ITP can be classified into three phases, with the first phase, known as recently diagnosed, taking place in the first three months following diagnosis. In the second phase, known as persistent ITP, symptoms linger between three and twelve months, and in the third phase, known as chronic ITP, symptoms last for more than twelve months **[2]**.

Thrombocytopenia, a typical symptom of ITP, may appear as mucosal bleeding and purpura of the skin, or it may show no evidence of bleeding at all. Annual increases in the prevalence are observed **[3]**.
There are many different kinds of biological data included in the study materials and outcomes of bioinformatics analyses. Sequence alignment, gene recognition, gene recombination, protein structure prediction, gene expression, protein response prediction, and evolutionary modelling are among the often-used techniques **[11 , 12]**.
There is evidence that miRNAs are involved in the pathophysiology of human diseases, including cancer. MiRNA expression profiles are useful for classifying human malignancies **[13]** and autoimmune diseases as altered miRNA levels are observed in most autoimmune diseases and are recognised to influence autoimmunity through different mechanisms **[41]**.

MicroRNAs (miRNA or miRs) are small non-coding RNAs were initially identified in Caenorhabditis elegans (C. elegans) in 1993, and the first mammalian miRNA was identified seven years later. Their research introduced a new framework for the regulation of genes **[14]**.MiRNAs are thought to control about 30% of human genes **[15]**.
Short non-coding RNA molecules called microRNAs (miRNAs) prevent the target mRNAs' 30 untranslated regions from properly matching with bases, hence inhibiting the target mRNAs' ability to express genes **[13]**. These non-coding RNA molecules called microRNAs (miRNAs) inhibit the synthesis of mRNAs' proteins by binding to complementary sequences in the target mRNA's coding or 3' untranslated region (3'UTR)**[16]**.

MiRNAs are capable of regulating cell differentiation, apoptosis, and proliferation **[17, 18]**. Because of this, miRNAs frequently play important roles in controlling the expression of particular genes **[16]**. In physiological cell processes, MiRNAs perform crucial regulatory roles **[18]**, also in human diseases including diabetes, immune or neurodegenerative disorders, and cancer, as well as in development and metabolism **[19]**, and stem cell maintenance **[18]**.

MicroRNA (miRNA) the enzyme Drosha normally converts primary miRNAs, which are produced by RNA polymerase II, into 70-bp hairpin miRNA precursors (pre-miRNA) in the nucleus. Pre-miRNA is further converted into mature miRNA duplexes by Dicer1 in the cytoplasm. Via the miRNA-effector protein Argonaute2 (Ago2), which serves as a guide **[20]** .

In the past decade, inflammatory disorders have been found to be directly related to miRNA dysregulation. The immune system influences the development and progression of cancer, and members of the let-7 family of miRNAs have been identified as important immune response regulators in a variety of illnesses and tumours **[14]**.
Let-7 is one of the first two identified miRNAs which have been originally discovered in nematodes **[38]**.Let-7 is a second miRNA that was found in C. elegans that is highly conserved across many species, including humans. Let-7 studies have greatly grown since their discovery and paved the path for the ncRNA revolution, allowing the concept of miRNAs serving as tumour suppressors to develop and later as oncomiRs **[14]**.

The Let-7 family has twelve members that are spread over seven different chromosomes in the murine genome **[30]**. All of which share the same ‘‘seed sequence’’ **[27]**. The lethal-7 (let-7) protein is a key regulator of eukaryotic cell differentiation, pluripotency, and apoptosis. Let-7 is the largest miRNA family, and it is composed of 10 mature subtypes, including let-7a, let-7b, let-7c, let-7d, let-7e, let-7f, let-7g, let-7i, miR-98, and miR-202**[31]**. Despite being present in many different genetic

regions, all final let-7 miRs are similar in length, differing by only 0–3 nucleotides **[40]**.

Let-7b-5p is a member of the common let-7 miRNA family and is implicated in immune control and tumour resistance in a number of malignancies. According to research, let-7b-5p can directly target Toll-like receptor 4 to control cytokine production when Helicobacter pylori is present. Most recently, let- 7adf cluster was found to specifically target Hk2 and limit glucose activation in glycolysis, hence inhibiting IgM synthesis in B cells under T cell-independent antigen stimulation. **[13]**. Let-7a-5p expression and function in B cells of ITP patients have not, however, been discussed.

Human let-7 miRNAs target transcripts of the proto-oncogene RAS and are downregulated in a large proportion of lung cancer **[13]**.Let-7 miRNA has been linked to the TLR-mediated inflammatory response in many other diseases, such as coronary artery disease and in an oxygen-glucose deprivation model **[14]**.

These miRNAs play a role in the regulation of both innate and adaptive immune responses **[21]**. Additionally, studies have shown a connection between members of the let-7 family and the control of the innate immune response to pathogenic infection and stress. They all demonstrate that members of the let-7 family target TLR4 to control innate immunity, inflammation, and the regulation of diverse immune effectors in response to pathogen infection **[14]**. Let-7 has become a key player in controlling the homeostasis of the body's energy levels, and it exhibits amazing plasticity in how its metabolism reacts to nutritional availability and physiological activities **[22]**.

MicroRNAs (miRNA) expression is evident in platelets and megakaryocytes, but miRNA activities in megakaryocyte differentiation and function have received little investigation. let-7a-5p and let-7g-5p were identified in the proliferation phase ofinduced pluriopotent stem cell (iPSC) -derived megakaryocytes Let-7 low megakaryocytes had an immune-skewed transcriptional signature and as the cells matured, interferon-responsive genes were enriched in let-7 low megakaryocytes, whereas platelet activation signaling was enriched in let-7 high cells. This data indicated an immune-skewed subpopulation of megakaryocytes in iPSC/embryonic stem cell-derived megakaryocytes that may be very significant as teams seek to make platelets in vitro from iPSCs and provide insights into the potential to limit their immune differentiation [43].

Let-7 has been shown to regulate the differentiation and function of natural killer T cells, a subset of T cells, as well as the maintenance and development of immature and mature immune cells **[23 - 25]**. Let-7 has also been linked to reports of the suppressive function of Tregs **[26]**. Furthermore, those members of the let-7 family inhibit B cell activation **[27, 14]** . Let-7a-5p miRNA is a significant factor in immunological responses, and this study identifies it as such. It has also been highlighted for its potential to serve as a non-invasive biomarker for ITP and its correlation with other parameters.

2. Subjects and Methods

2.1. Subjects

The study was conducted on 100 individuals attended the Paediatric Heamatology Department at Demerdash Hospital - Ain Shams University in the period from January 2020 to May 2021. After taking the approval of the research ethics committee of the faculty of medicine, Ain Shams University (No.FWA000017585) . An informed consent letter from all participants' parents was taken after informing him or her about the steps of the procedure and the expected effect before enrollment in the study**.** Group I:70 patients were diagnosed with idiopathic thrombocytopenic purpura. They were diagnosed on the basis of a complete blood picture. The diagnosis is confirmed by detection of anti-platelet antibodies against platelet glycoprotein GP IIb/IIIa (~70%) and/or the GP Ib-IX-V complex examination by Flow-Cytometry.

The 70 patients with ITP were studied for expressions and the potential value of the miRNA Let-7a-5p gene." Patients were selected respecting inclusion criteria: patients diagnosed with idiopathic thrombocytopenic purpura; age ranged from one year to 15 years old. And exclusion criteria: patients with any other types of autoimmune diseases; patients suffering from viral or bacterial infectious diseases.

Group II included 30 healthy subjects matched by age and sex. Individuals with another coexisting disease were excluded.

In this study, we have divided the main groups I and II into subgroups according to age (lower and higher than 5 years) and gender (males and females) to

investigate the relative expression variation of miRNA-let-7-5p in those subgroups.

The main group II has also been divided according to the severity degree of thrombocytopenia into a) mild thrombocytopenic patients (platelets count 100–<150 x10^9/uL) and b) moderate thrombocytopenic patients (platelets count <100 x10^9/uL).

2.1.1. Sample Collection

2.1.1.1. Blood Sampling

Peripheral blood samples were collected into vacutainer tubes containing EDTA-anticoagulant and into another vacutainer tube containing sodium citrate-anticoagulant. Sodium citrate samples were centrifuged, and plasma was collected and stored at -80 C until analysis.

2.2. Method

2.2.1. Complete Blood Picture Analysis

Whole blood samples (EDTA tubes) were analysed for a complete blood picture by using a hemology auto-analyzer (Sysmex XP-300, Kobe, Japan).

Blood films were prepared from EDTA-anticoagulated blood and then stained with Leishman stain. The blood films were examined and recorded as a percentage, where all differential white blood cells were counted in 100 white blood cells (Dacie and Lewis, 1995).

2.2.2. Extraction and purification of RNA, including miRNA, from plasma samples

Total RNA of the lymphocyte was extracted using Qiagene Reagents QIAamp spin columns technology for total RNA preparation. The reverse transcription reactions were carried out using miScript Primer Assays cDNA Synthesis MasterMix (Qiagen, Germany) following the manufacturer's procedure.

2.2.3. Reverse transcription for mRNAs and miRNAs

Total RNAs were reversibly transcribed using the miScript II RT Kit (Qiagene,Hilden , Germany) (Cat No 218161) for cDNA synthesis according to the manufacturer's protocol.

2.2.4. Quantitative real-time Polymerase Chain Reaction (qRT-PCR) amplification of the miR-Let7-5p gene

Expression analysis of the miR-Let7-5p gene was detected through real-time PCR, which was performed with 2 SYBR Green PCR Master Mixes (Qiagene, Germany).

Using Bio-Rad/MJ Research models, the PCR component mix was briefly centrifuged, and the tubes were placed into the real-time cycler. The real-time cycler was then programmed according to an initial denaturation at 95 ºC for 10 min, followed by 40 cycles at 95 oC for 15 s, 40 oC for 30–40 s at 55 ^{oC} ,and then 40 cycles at 72 ºC for 30 s. The relative quantification (fold change) of gene expression was obtained by comparison with the relative expression of internal reference RUN6 houskeeping gene using the equation $2^{-\Delta\Delta Ct}$ test control where Relative quantitation (RQ) = $(2)^{-\Delta\Delta Ct}$, $\Delta\Delta CT = (C_{T\ gene} - C_{T\ reference\ gene})_{patient} - (C_{T\ gene} - C_{T\ reference\ gene})_{control}$

2.3. Statistical methods

Statistical analysis was performed with IBM® SPSS® Statistics Version 16. Categorical data were presented as frequencies and percentages and were analysed.

3. Results

3.1. Demographic data

The age of healthy control subjects ranged from 2.8 to 16.5 years with a mean value ±SD (10.34 ± 3.53 years), while that of ITP patients ranged from 1.5 to 15.6 years with a mean value ±SD (8.09±3.69 years). Around 51 (72.9%) patients with ITP were males, and 19 (27.1%) patients were females. While eighteen (60%) control subjects were males and 12 (40%) were females.

3.2. Comparative analysis for hematological parameters: WBCs, lymphocytes and Granulocytes in the studied groups

A non-parametric Kruskal-Wallis test was used to test for the significance difference between the two studied groups. Furthermore, applying a parametric Mann-Whitney test for biochemical and CBC parameter analysis.

Statistical analysis revealed that, no statistically significant difference was found between the two groups as regards Granulocytes, lymphocytes, and WBCs count as illustrated in Table 1.

Table 1: for hematological parameters: WBCs, lymphocytes and Granulocytes in control and ITP groups

Parameter	Control (N=30)	ITP (N=70)	P-value
WBCs (x10^3)/uL	(9541 ± 2568) #	(9580 ± 2700) #	0.373
Lymphocytes %	(39.8 ± 7.6) *	(40.7 ± 4.2) *	0.611
Granulocytes %	(60.1 ± 7.6) *	(59.2 ± 4.2) *	0.611

* Values are presented in Mean ± SD; # values are presented in median ± Interquartile Range IQR

3.3. Significant increase of miRNA FC (Let-7a-5p) relative expression in ITP with significant difference compared to control subjects

There was a significant increase in miRNA FC (Let-7-5p) expression in ITP paediatric patients versus healthy control groups (P<0.00) as shown in Table 2, and a significant difference in the expression of that gene was identified among patients with varying degrees of thrombocytopenia severity (mild thrombocytopenic ITP patient (platelets count 100–150) and moderate thrombocytopenic ITP patient (platelets count <100)) and (p values were 0.0 and 0.0, respectively) as shown in Table 3.

Table 2: Statistical analysis of Let-7a-5p concentration in control and ITP group.

	Group	N	Mean ±SD	P-value
FC (Let-7a-5p)	Control	30	2.18± 3.72	0.00
	ITP	70	67.78± 37.7	

Table 3: Association between microRNA- Let-7a-5p relative expression and ITP thrombocytopenia severity.

Thrombocytopenic severity of ITP patients (No. of platelets)	miRNA FC (Let-7a-5p)	
	Mean ±SD	P-value
Moderate thrombocytopenic ITP patient (platelets count <100)	0.04720 ± 0.026	0.00
Mild thrombocytopenic ITP patient (platelets count 100-<150)	7.1 ± 4.3	0.00

3.4. Relative gene expression of miRNA FC (Let-7a-5p) in relation to the other clinical parameters

Also, there was a significant positive correlation between FC (Let-7a-5p) and MCV, antiplatelet antibodies glycoproteins; GPIIIa,GPIIb,GP1b/IX (r = 0.741, 0.637, 0.669, 0.486), and a weak positive correlation between FC (Let-7a-5p) and Hb, RBCs (r = 0.002, 0.045), while there were negative significant correlations between FC (Let-7-5p) with each of the antiplatelet antibodies glycoproteins GP1b Platelets and HCT (r = -0.478, -0.478, and -0.045 respectively) as shown in Table 4.

Table 4: Statistical analysis of the correlation between miRNA FC (Let-7a-5p) and other clinical parameters.

Parameters	miRNA FC (Let-7a-5p)
	r
Platelets count(x10^9)/uL	-0.478
Hb (g/dL)	0.002
RBCs (x10^6)/uL	0.045
HCT	-0.045
MCV (fl)	0.741
GPIIIa	0.637
GPIIb	0.669
GP1b/IX	0.486
GP1b	-0.478

2. Diagnostic potential of miRNA FC (Let-7a-5p) in discriminating ITP patients from healthy controls

Test characteristics of serum FC (Let-7a-5p) for discrimination between the studied control and ITP groups are illustrated graphically by the ROC curve in Figure 1. For discrimination between control and ITP groups, ROC analysis estimated a sensitivity and specificity (100 and 96.8, respectively) at the best cutoff value of >18.5, which was determined by calculating the Youden index. The area under the curve (AUC) was 0.995 with a SE of 0.005 and a significant associated P-value < 0.000.

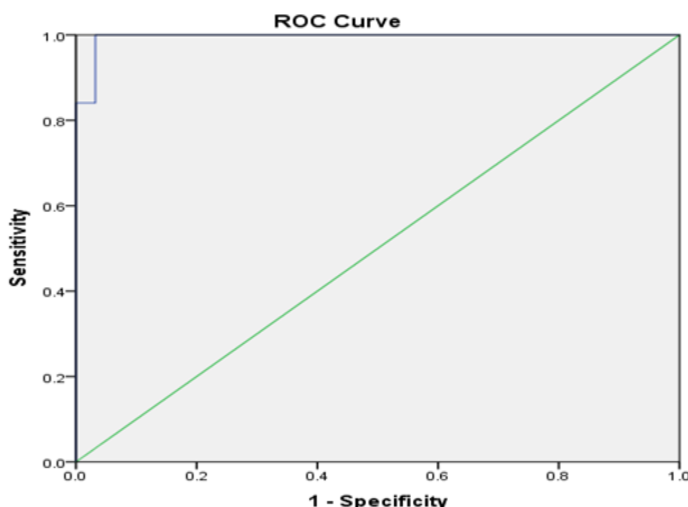

Figure 1: ROC curve for miRNA FC (Let-7a-5p) for pairwise discrimination between Control and ITP groups (optimal cutoff and AUCs were indicated).

3. Significant increase of miRNA FC (Let-7a-5p) relative expression in ITP with significant difference in subgroups

In the current study, subjects were divided according to age into > 5 years and < 5 years subgroups and according to gender into males and females subgroups. Among the studied subgroups in the present study, plasma Let-7a-5p levels showed a statistically significant difference between the studied groups (control and ITP included). as shown in Table 5.

Table 5: Association between miRNA FC (Let-7a-5p) relative expressions in subgroups

Subgroup		N	miRNA FC (Let-7a-5p)	
			Mean ± SD	P-value
Males	ITP	51	72.6 ± 40.5	< 0.000*
	Control	18	2.9 ± 4.6	
Females	ITP	19	54.8 ± 25.8	< 0.000 *
	Control	12	1.1 ± 1.3	
Children of age < 5 years	ITP	15	63.7 ± 37.1	< 0.034 *
	Control	7	0.77 ± 0.55	
Children of age > 5 years	ITP	52	68.9 ± 38.2	< 0.000 *
	Control	26	2.3 ± 3.8	

* Values are presented in Mean ± SD

Discussion

Immune thrombocytopenic purpura (ITP) is a rare hematological disorder with an autoimmune-mediated, often dramatic reduction of platelets in peripheral blood **Fillitz et al.[28]**and impaired platelet production **McCRAE [29]**. The pathogenesis of ITP remains unclear, although both antibody-mediated and/or T cell-mediated platelet destruction are key processes **Zufferey et al. [2]**. In the absence of a gold standard test, a certain diagn osis of ITP should rely on both laboratory and clinica l indices **Ayoub et al. [1]**

The purpose of this study was to investigate the relative expression of miRNA Let-7a-5p in ITP patients and discover it as a new predictive biomarker for ITP patients.

In the current study, the results showed that the expression level of the miRNA (hsa-let-7a-5p) gene was significantly increased nearly 25 times in ITP patients compared to its expression levels in the healthy control subjects.

Wang 2021 and others have found that miRNA profiles are largely altered in ITP. MicroRNA (miRNA) is one of many types of short non-coding RNAs that can bind to the 3′ untranslated region (UTR) of target mRNA to repress its translation and stability **Wang et al. [32]**.

Studies showing the regulation of miRNAs in the host to be related to infectious disorders and associated with the eradication or susceptibility of the infection highlight the crucial function of miRNAs in inflammation and immunological responses. During the development of the immune response, host-pathogen interactions cause signalling and physiological changes in host cells that lead to the miRNA-mediated post-transcriptional regulation of genes involved in the inflammatory response **Acuña et al. [21]**

Secreted miRNAs have recently been considered an emerging form of intracellular communication. Since both actively secreted miRNAs and passively leaked miRNAs can be released in a stable, cell-free form for circulation, they are also called circulating miRNAs. MiRNAs can enter the circulation through three pathways: (i) passive leakage from broken cells; (ii) active secretion via microvesicles, including exosomes and shedding vesicles; and (iii) active secretion in conjunction with the RNA-binding protein high-density lipoprotein (HDL) **Bao et al. [39]**.

As it was documented, no studies have talked about miRNA (hsa-let-7a-5p) with ITP, but many studies have talked about miRNAs with ITP, especially miRNA Let-7b-5b, which is a closer family member to miRNA Let-7a-5p, the marker predicted in the current study.

In **Wang *et al.,* 2021** study, they found that let-7b-5p expression as well as B cell survival were enhanced in patients with ITP compared with healthy controls. They said that MiRNA is a critical regulator involved in many physiological processes, including cell differentiation and the activation of immune cells. Their results suggest that let-7b-5p may enhance BAFF/BAFF-R-mediated signalling to promote B cell survival **Wang et al. [32]**.B cells have been demonstrated to play critical roles in the pathophysiology of ITP. B-cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL) are crucial cytokines supporting survival and differentiation of B cells, and dysregulation of BAFF/APRIL is involved in the pathogenesis of B-cell-related autoimmune diseases, including ITP **Liu and Hou [33]**.It was demonstrated that BAFF enhanced the survival of plasma blasts derived from memory B cells selectively and prevented the apoptosis of immunoglobulin (Ig)-secreting cells (ISC), thus increasing Ig synthesis **Ferraccioli and Gremese [34]**.

The pathogenesis of ITP includes the production of autoantibodies against platelet glycoproteins (IgG antibodies), which bind to platelets and mark them for phagocytic breakdown in the spleen and liver, as mentioned in **Zufferey et al. [2]** study that these autoreactive antibodies are secreted by plasma cells, which have been reported to be present at higher levels in patients with ITP, as well as the B cell regulator and B cell-activating factor (BAFF, also called B cell stimulator (BlyS)), which is an important factor in B cell selection, survival, and proliferation. So let-7b-5p plays an important role in ITP by promoting B cell activation and survival via BAFF/BAFF-R-mediated signaling. This could explain why Let-7a-5p levels increased in ITP patients.

Jiang & Wang, 2019 said that Let-7 miRs express widely in differentiated immune cells, including activated splenic B cells. They found that let-7a, let-7d, and let-7f were induced by lipopolysaccharide (LPS) in splenic B cells.

B cell activating factor (BAFF) and B cell receptor (BCR) stimulation can induce let-7b-5p expression in B cells in vitro. Overexpression of let-7b-5p in B cells upregulated the expression of surface BAFF-R and promoted B cell survival. Mechanistically, let-7b-5p enhanced the phosphorylation of NF-κB2 p100 and upregulated the expression of the survival factor Bcl-xL after BAFF induction. These results suggest that let-7b-5p may contribute to the hyperreactivity of B cells in ITP by enhancing BAFF/BAFF-R-mediated B cell survival. It is suspected the role of let-7-5p in ITP. That let-7b-5p may be a functional miRNA regulating antibody production and B cell survival **Wang et al. [32]**.As follow suggest that let-7a-5p role in ITP.

Gilles & Slack, 2018 highlighted let-7 as a new target for immunotherapy. Other studies show that let-7 modulation can be a tool to suppress tumour and immune reactions. They suggest that the let-7 family can regulate or inhibit immune responses and promote tumour immune evasion. They identified the role of let-7 in CD8 T cell differentiation and function. CD8 T cells promote rapid clearance of virus-infected cells.

Previous studies showed and explained how miRNAs, especially Let-7, have a significant role in ITP and other haematological diseases, cancers, especially all types of leukaemia, cardiovascular diseases, and autoimmune diseases like SLE, as mentioned in the following different previous studies. In particular, let-7a can induce a significant effect on vascular function, as it appears to regulate post-stroke angiogenesis through a transforming growth factor beta 3 (TGF-β3)-dependent mechanism **Bernstein et al. [40]**.

Undi *et al*., 2016 found that out of 88 miRNAs involved in stem cell development, let-7b was the only miRNA down-regulated (~10-fold) in neonates compared to adult MKs. These miRNAs might engage in systemic inflammation. The third group included six miRNAs, which were only down-regulated in ITP. Their results showed the inhibitory effect of let-7b on wnt signalling pathway by regulating Fzd4 (frizzled family receptor 4) and thereby regulating proliferation as well as differentiation.

Many studies also identified a new role for let-7 in CD8 T cell differentiation and function. CD8 T cells promote rapid clearance of cancer cells or virus-infected cells. They found that B cell survival was significantly enhanced in ITP patients, which may contribute to B cell hyperreactivity and autoantibody production in ITP **(Gilles and Slack [14]and Wang et al. [32]**.

Gilles & Slack, 2018 said also Let-7 has been reported to control maintenance and development of immature and mature immune cells but also to control differentiation and function of natural killer T cells, a subset of T cells. These miRNAs might be correlated with the organ-specific destruction of thrombocytes **Undi et al. [35]**

MiRNA, specifically let-7c, has been reported to play a role in regulating macrophage plasticity. Briefly, macrophages have the ability to switch between different functional phenotypes that are called M1 or

M2. M1 macrophages are critical to counteract external infections by bacteria or viruses, whereas M2 macrophages are found in the inflammatory zone and participate in many remodelling biological processes **Gilles and Slack [14]**.
An abnormal expression of miRNA Let-7 was found to lead to defects in cell proliferation and differentiation in AML cells. It was shown that high Let-7a-2-3p expression could be used alone as a biomarker for the prognosis of patients with AML. Furthermore, it was shown that downregulation of Let-7a-5p significantly increased the rate of apoptosis or necrosis of HL60 cells **Chen et al. [37]**.
Other diseases, like cardiovascular diseases,let-7 have been found to be highly expressed in all major types of cardiovascular cells, including vascular smooth muscle cells (VSMC), endothelial cells (EC), cardiomyocytes, and coronary arterial smooth muscle cells **Bao et al. [39]**.

Conclusion:

MicroRNA-Let -7a-5p may have a role in ITP due to their significantly increased relative expression levels in ITP individuals contrasted to controls. In patients with ITP, there was a significant positive relationship between miRNA- Let -7a-5p relative expression and other clinical parameters such as platelets and antiplatelet antibodies (GPIIIa, GPIIb, and GP1b/IX).

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