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The value of CD72 presence on memory B cell (CD19+ CD27+) in children with immune thrombocytopenic purpura (ITP)

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Abstract
Background: The hemodynamic profiles of cases with severe sepsis and septic shock vary considerably. Immune thrombocytopenic purpura (ITP), also known as idiopathic thrombocytopenic purpura, is an autoimmune disorder characterised by excessive production of a variety of auto-antibodies. The aim of this work was to investigate the value of CD72 presence on memory B cell (CD19+ CD27+) in children with ITP.
Methods: This prospective clinical research was carried out on 40 children aged less than 15 years old. Children were subdivided in to two equal groups: Group A: newly diagnosed ITP who were admitted to pediatric department, Tanta University hospital. These cases were diagnosed on the basis of clinical presentation, morphological and cytochemical smears of peripheral blood and bone marrow as well as immunophenotyping and group B (control group): apparently healthy children as a control group.
Results: There was significant association between CD72 (%) and both platelet count and platelet autoantibodies for group I. A notable association between CD72 (%) and sex for group I with P=0.037. ROC curve of CD72 (%) presence on memory B cells to diagnose ITP cases group I; excellent AUC was found (AUC = 0.965, p<0.001). At cut-off value >3.5%, sensitivity 70%, specificity 90%, PPV 90% and NPV 76.9%. Figure 1.
Conclusions: There was high significant rise in the presence of CD72% in children with ITP. A notable association between memory B cell (CD19, CD27) % and CD72 (%) in children with ITP. CD72 (%) presence on memory B cells can diagnose ITP.

Keywords: CD72, memory B cell (CD19+ CD27+), children, immune thrombocytopenic purpura

Introduction
Platelet count drops and bleeding becomes more likely in people with immunological thrombocytopenic purpura (ITP), also known as idiopathic thrombocytopenic purpura [1, 2].
The clinical signs of ITP are diverse and include, for example, the purple hue of skin where blood has "leaked" under the surface (bruise). ITP kids often have mysteriously huge bruises. Petechia, which are little red spots under the skin, epistaxis, which is bleeding in the mouth and/or around the gums, hematemisis, hematuria, and melena can all arise merely from moving the elbows and knees [3].
Autoantibodies targeting platelet membrane glycoproteins (GPs) may be found in the majority of ITP cases, suggesting a role for B lymphocytes in the pathogenesis of the disease [4]. Increased phagocytosis and destruction by macrophages as a result of Fc receptor (FcR)-mediated phagocytosis is a common consequence of these autoantibodies [5]. Active ITP is also reportedly lacking in regulatory B cells [6], which have the role of suppressing immunological responses.
The growth and differentiation of B lymphocytes are controlled by a dynamic equilibrium between signals transmitted by activating and inhibitory receptors expressed on the cells' surface. Membrane molecules such as CD19 up-regulate B CELL RECEPTORS signalling, whereas membrane molecules such as CD22 (cluster of differentiation) and CD72 negatively control B CELL RECEPTORS signalling and prevent B cell overstimulation [7]. Many of these coreceptors, such as CD72 and CD5, have been explicitly related to ITP in recent research [8, 9].

Memory CD19+CD27+ B cells and naïve CD19+CD27- B cells are the two main subgroups of B lymphocytes in the peripheral circulation [10].
Total CD27+ memory B cells declined in post-splenectomised ITP cases, demonstrating that the spleen is critical for the preservation of peripheral memory B-cell homeostasis in this disease. [11].
The aim of this work was to investigate the value of CD72 presence on memory B cell (CD19+ CD27+) in children with ITP.

Patients and Methods
Forty children younger than 15 were included in this prospective clinical trial at the paediatric department of Tanta University hospital.
The research project was conducted with the blessing of the Tanta Ethics board. Parents gave their informed, written approval.
Exclusion criteria were other causes of thrombocytopenia, age > 15 years old, cases with malignant disease, cardiac disease, inflammatory disease, autoimmune disease other than ITP and congenital disorders may resemble ITP.
Children were subdivided in to two equal groups: Group A: newly diagnosed ITP who were admitted to pediatric department, Tanta University hospital. These cases were diagnosed on the basis of clinical presentation, morphological and cytochemical smears of peripheral blood and bone marrow as well as immunophenotyping and group B (control group): apparently healthy children as a control group.
All children were subjected to: Complete history taking, thorough general examination, abdominal ultrasonography, routine laboratory investigations (Complete blood count, and bone marrow aspiration and examination), and specific laboratory investigation (CD72 presence on memory B cell (CD19+ CD27+) by flow cytometry).

Sampling: Peripheral blood sample: 3 ml venous blood samples from the anticubital vein were withdrawn and divided as follow; 1 ml was added to a tube containing EDTA for complete blood picture, and 2 ml were added on EDTA vacutainer for flowcytometric analysis. Peripheral blood films were stained with Geimsa stain for examination of differential leucocytic count.
Bone marrow sample: Bone marrow aspiration was done from anterior superior iliac spine under complete aseptic technique and added to EDTA tube for immunophenotyping. Films were prepared, stained with Geimsa stain for morphological research.

Flowcytometric Immunophenotyping
Flowcytometer consists of three main compartments: The fluid transports cells to the laser beam in a stream. Lasers are used to highlight particles in the sample stream, while optical filters guide the generated light signals to the proper detector. Photomultiplier tube (PMT) light signals are converted to computer-process able electrical signals by an electronic system.
In flowcytometry, scattered light is typically detected in two axes: forward light scatter and side light scatter. Flowcytometry employs several fluorochrome-conjugated antibodies to identify individual cells in addition to the natural light scattering capabilities of haemopoietic cells. These fluorochromes have specific excitation emission spectra. The argon laser is the mostly common in haemopoietic flowcytometry. It produces a 488 nm excitation which is able to excite many fluorochromes such as fluorescein isothiocyanate (FITC) and phycoerythrin (PE). These fluorochromes are frequently employed concurrently because they may be activated by the same beam of light yet light with distinct spectra.
When fluorochrome-conjugated antibodies are treated with haemopoietic cells that express the matching antigen on their surface, the antibodies attach to these cells. After incubation and washing to remove excess unbound antibody, the cells are analysed and counted using flowcytometry. As the cells with antibody-bound fluorochrome pass through the laser beam, the fluorochrome is excited, followed by light emission. Fluorochrome emits light with a specified wavelength. The signal of positive light emission is transformed to a digital signal and shown as a histogram. A set of mirrors and filters permit the separation of emitted light into distinct wavelength groups and the detection of fluorescence by various detectors.

Procedure
100 µl of EDTA peripheral blood were pipetted in the standard flowcytometric tube (Falcon tube, 5 ml polystyrene round bottom tube 12x25 mm non pyrogenic and sterile from BD immunocytometry system). Each tube was labled with the patient's name. 10 ul of labeled moAbs for CD19, CD27 and CD72 were added to the bottom of tubes. Vortex mixer was used at low speed for 3 seconds. Tubes were incubated for 30 minutes at room temperature and protected from direct light. 1 ml of FACs lysing solution was added to each tube, vortex mixer was immediately used at low speed for 3 seconds and incubate for 15 minutes at room temperature in the dark. After incubation, tubes were centrifuge at 3000/r.p.m for 5 minutes. Supernatant was aspirated leaving approximately 50µl of residual fluid to avoid disturbing the pellet. 0.5 ml of PBS was added to each tube for washing. The tubes were centrifuged at 3000/ r.p.m for 5 min. The supernatant was discarded, and the washing step was repeated once again. In the last wash, after discarding the supernatant, cells were resuspended in 0.3 ml of PBS which was added to each tube. Cells were ready for flowcytometric analysis.
Flowcytometric analysis: FACs caliber flowcytometry from Becton Dickinson utilised for analysis. Cell quest software was used for automated data collection and interpretation. The equipment was adjusted using manufacturer-supplied calibrated beads. Quality control measured were followed. 10,000 events were acquired. Light scatter, forward light scatter against long side scatter were used to delineate cell populations of interest (memory B lymphocytes) by bitamp gating. Using the cursor location from histograms for isotopic controls, gated fluorescence for positive cells was examined.

Statistical analysis: Statistical analysis of this research was conducted, using the mean, standard deviation and Student t-test [Unpaired], Chi-square and Kaplan-Meier test by SPSSV19 (Statistical Package for Social Studies). Kaplan-Meier method was defined as: A nonparametric method of compiling life tables or survival tables. Student t-test: used to test the differences between two mean values. For categorical variable the number and percentage were calculated and differences between subcategories were

tested by chi square test. P value ≤ 0.05 was considered statistically significant.

Results

No differences were found in the two groups regarding age, sex and WBCs. As regards Hb, HCT and platelet count, a significant decline was found in mean values of group I compared to group II. Absolute lymphocytes and platelet autoantibodies were significant rise for group I compared to group II. Table 1.

Table 1: Comparison between the two studied groups according to demographic data, CBC and platelet autoantibodies

		Group I (n = 20)	Group II (n = 20)	p
Sex	Male	7 (35.0%)	10 (50.0%)	0.337
	Female	13 (65.0%)	10 (50.0%)	
Age (years)		8.35±3.30	7.65±2.06	0.426
CBC				
Hb (gm/dl)		10.63±1.04	12.13±0.40	<0.001*
HCT (%)		32.17±2.75	36.36±1.41	<0.001*
Platelets (10^3/UL)		44.60±18.51	309.55±56.12	<0.001*
WBCs (10^3/UL)		9.24±2.83	8.55±1.37	0.329
Absolute lymphocytes (10^3/UL)		2.91±1.01	2.33±0.62	0.035*
Platelet autoantibodies		17(85%)	0 (0%)	<0.041*

Data are presented as mean ± SD or frequency (%), Hb: hemoglobin, HCT: Hematocrit, WBCs: White blood cells, *: Significant P value

Memory B cell (CD19, CD27) were insignificant differences in the two groups. CD72 was highly significant rise in the percent presence for group I in comparison to group II (p<0.001). a notable association between memory B cell (CD19, CD27) % and CD72 (%) for group I with P=0.041. Table 2.

Table 2: Comparison between the two studied groups according to memory B cell (CD19, CD27), CD72

	Group I (N = 20)	Group II (N = 20)	p
Memory B cell (CD19, CD27) %	52.25±14.28	48.85±9.09	0.376
CD72	7.54±6.63	1.43±0.89	<0.001*

Data are presented as mean ± SD, *: Significant P value

ROC curve of CD72 (%) presence on memory B cells to diagnose ITPcases group I; excellent AUC was found (AUC = 0.965, p<0.001). At cut-off value >3.5%, sensitivity 70%, specificity 90%, PPV 90% and NPV 76.9%. Figure 1

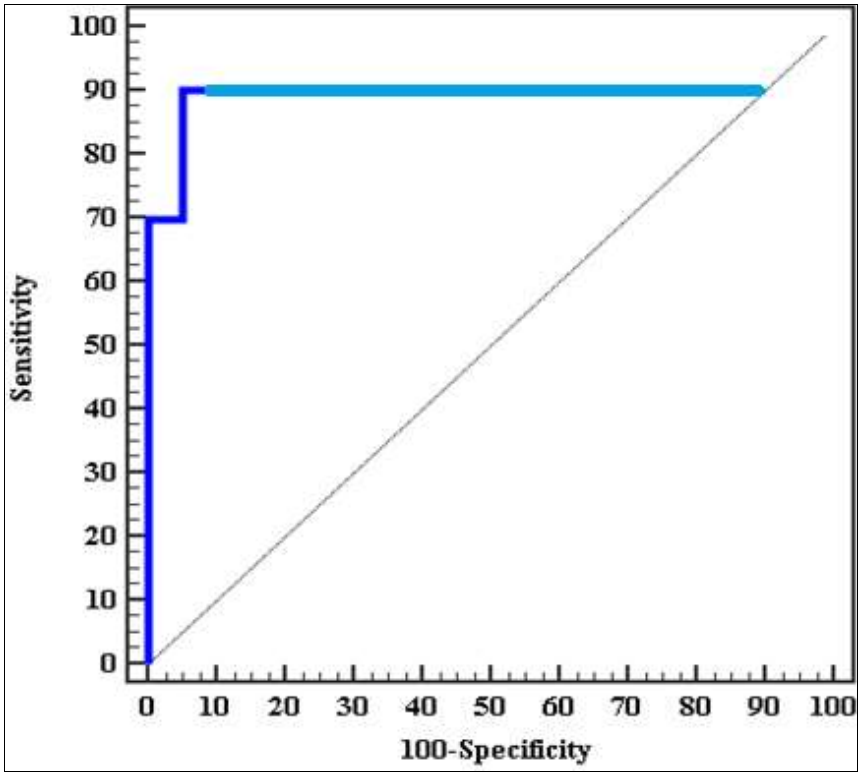

Fig 1: ROC curve for CD72 (%) to diagnose Immune thrombocytopenic purpura patients group (n = 20) from control cases (N=20)

There was no association between CD72 (%) and neither age, Hb, HCT, WBCs nor absolute lymphocytes for group I. While there was significant correlation between CD72 (%) and both platelet count and platelet autoantibodies for group I. A significant association was found between CD72 (%) and sex for group I with P=0.037. Table 3.

Table 3: Correlation between CD72 (%) and different parameters for group I (Immune thrombocytopenic purpura), correlation between CD72 (%) and Platelet autoantibodies for group I (Immune thrombocytopenic purpura) and Correlation between CD72 (%) and sex for group I (Immune thrombocytopenic purpura) (N = 20)

		CD72 (%)	
		R_s	p
Age (years)		0.188	0.428
Hb (gm/dl)		-0.014	0.952
HCT (%)		0.033	0.891
Platelets (10^3/UL)		-0.509	0.043*
WBCs (10^3/UL)		-0.286	0.221
Absolute lymphocytes (10^3/UL)		-0.333	0.152
		X^2	p
Platelet autoantibodies		5.177	0.037*
Sex	Male	6.60±9.30	0.037*
	Female	8.04±5.04	

r_s: Spearman coefficient, *: Significant P value, X^2: Chi square test

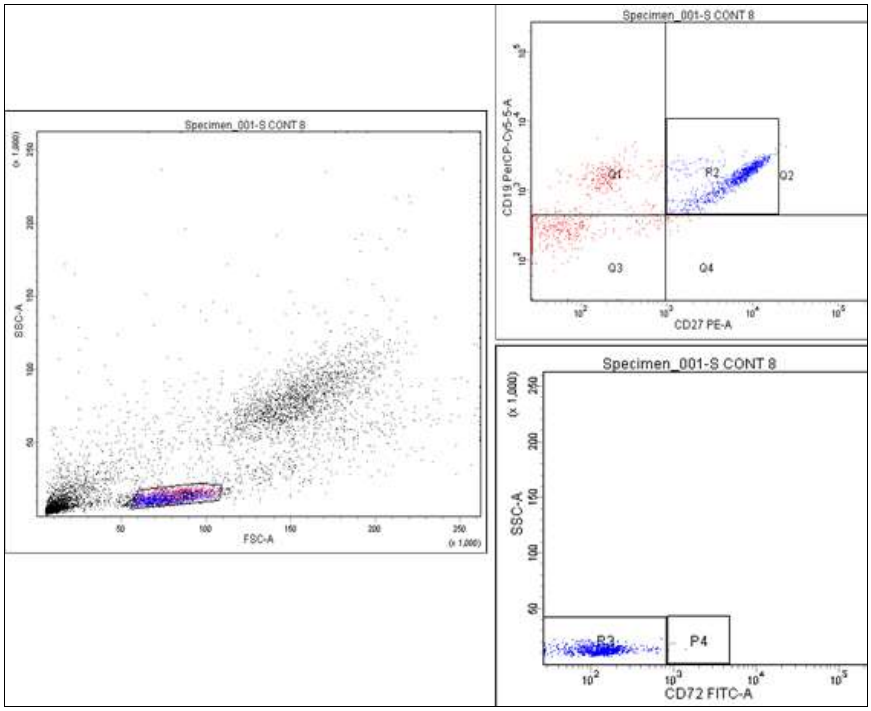

Fig 2: Control example

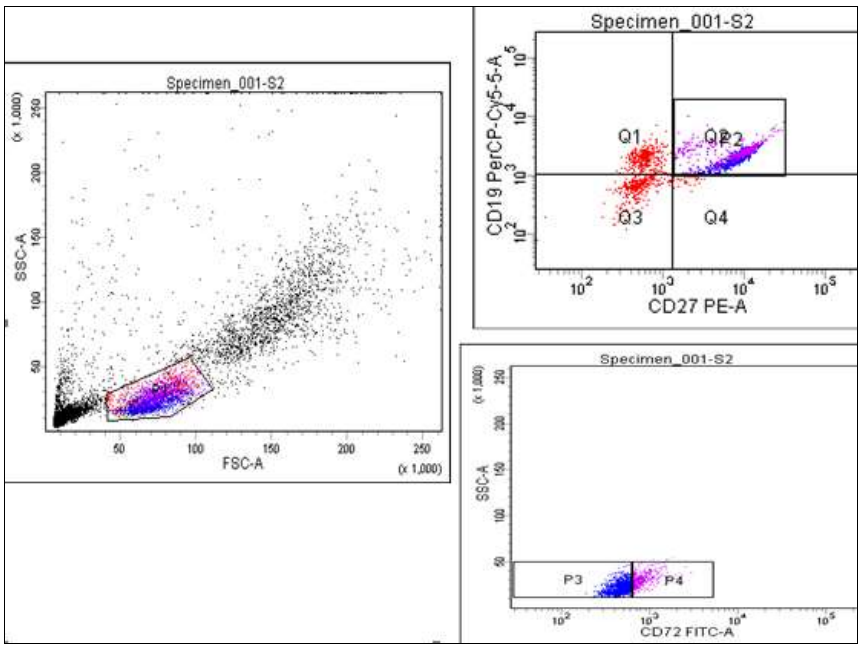

Fig 3: Patients example

Discussion

Autoantibody-mediated increased platelet destruction and dysmegakariocytopoiesis define the autoimmune illness known as primary immune thrombocytopenia (ITP) [12].

Consistent with the findings of Fang *et al*., [13], we found no significant difference in age or sex between the ITP group and the control group in this investigation.

Several autoimmune diseases have been linked to a decline in memory B cell numbers. Total CD27+ memory B lymphocytes were shown to be lower in Sjögren's disease cases than in healthy donors in a research by Hansen *et al*. [14]. (HD). More severe CD27+ B cell depletion was seen in individuals with chronic granulomatous disease compared to HD, as described by Bleesing *et al*. [15].

In this research, the Median memory B cell (CD19, CD27) for group I was 48.95 and for group II it was 47.05, with no statistically significant difference (P= 0.376), which contradicts the findings of Fang *et al*., [13] who discovered a significant rise in the frequency of bloodCD19+ CD27+ B memory cells in newly diagnosed cases versus that in cases in remission.

Lyu *et al*. [16] found a significant association between anti-platelet antibodies, platelet count, and CD72 dysregulation in CD27+ memory cells. These findings suggest that the increased presence of CD72 on the CD27+ memory B subset is associated with ITP disease activity and autoantibody production.

CD72 mRNA presence was considerably lower in cases with active disease (median 1.664, range 0.184-9.659) compared to cases in remission (median 3.491, range 0.266-47.38, p = 0.0290) and controls (median 3.251, range 0.289-19.15, p = 0.0296), as determined by Zhou *et al*. This contradicts Lyu *et al*. [16].'s results, which (median 1.20, range 0.70 –2.15).

In addition, Lyu *et al*. [16] found that the prevalence of CD72 was considerably lower in cases in remission and healthy controls than in cases with active ITP. They also observed that individuals with active ITP had elevated levels of CD72 on B cells. This CD72 up regulation was detected only in CD27+ memory B cells and not in any other B subsets. The CD72 presence level of CD27 B cells was either average or marginally increased.

In the current research, the ROC curve of CD72 (percent) was used to diagnose group I ITP cases. A remarkable AUC was discovered (AUC = 0.96, p0.001). At a threshold value of >3.5, the sensitivity was 70%, the specificity was 90%, the PPV was 90%, and the NPV was 76.9%.

In this research, there was no correlation between CD72 (%) and age, sex, Hb, HCT, WBCs, and absolute lymphocytes for group I, which was consistent with the findings of Zhou *et al*. [17], who found no statistically significant correlation between CD72 mRNA level and clinical parameters including age, gender, and disease duration.

Also, Lyu *et al*. [16] reported that there was no link between CD72 presence on B cell subsets and gender or age in individuals with active ITP, which is consistent with our findings.

In this research, A significant association was found between CD72 (%) and both platelet count and platelet autoantibodies for group I, which is consistent with Lyu *et al* [16].'s findings of a highly significant correlation between CD72 (%) and platelet count and platelet autoantibodies, but contradicts Zhou *et al*. [17] found that there was no statistically significant correlation between CD72 mRNA level and platelet count.

Conclusions

There was significant rise in the presence of CD 72% in children with ITP. A significant association was found between memory B cell (CD19, CD27) % and CD72 (%) in children with ITP. CD72 (%) presence on memory B cells can diagnose ITP with cut-off value >3.5%, sensitivity 70%, specificity 90%, PPV 90% and NPV 76.9%

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Author's Contribution

Not available

Conflict of Interest

Not available

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