Research Article

Evaluation of megakaryocytes number in thrombocytopenic patients

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Abstract

Background and objectives: The aim of the present study is to evaluate number of megakaryocytes in vivo in patients with thrombocytopenia. **Subjects and methods:** The study was carried out on 20 ITP(groupI) patients who were admitted to minia university hospital and 20 thrombocytopenic patients with myelodysplastic syndrome(groupII). Serial histological sections from bone marrow biopsies were stained for CD61. the number of bone marrow megakaryocytes were determined morphologically. **Results**: Megakaryocytes number was increased in ITP and MDS patients (group I & II) and there was no statistically significant difference between both groups (Mean ± SD 8.1 ±5.1 vs. 6 ±3.1 cells per HPF; p=0.271). **Conclusion**: researches in ITP should focus on ultrastructure of megakaryocytes and its relation to antibody.

Key Words: ITP, megakaryocytes, antibody specificity.

Introduction

The terms thrombocytopenia refers to a disorder in which there is a relative decrease of thrombocytes, commonly known as platelets, present in the blood. A normal human platelet count ranges from 150,000 to 450,000 platelets per microliter of blood[1].

Idiopathic thrombocytopenic purpura (ITP), characterized by a diminished peripheral platelet count ($<100 \times 10^9$/L) caused by platelet destruction with an increased risk of mucocutaneous bleeding, is a primary autoimmune disease[2] .primary ITP is characterized by isolated thrombocytopenia in the absence of any clear underlying cause or initiating factor[3]**.** Both clinical and experimental evidence support a dual mechanism of platelet destruction and platelet underproduction in ITP; however, the mechanism of platelet underproduction remains uncertain[4]**.**

Platelet autoantibodies to specific membrane glycoproteins mediate platelet destruction and are a major agent in the pathogenetic mechanism of ITP that includes antibody-mediated ,cell-mediated platelet destruction and the suppression of megakaryopoiesis[5].

With ITP, the immunoglobulin (Ig) G autoantibodies derived from auto-reactive B cells can recognize and bind to one or more glycoproteins (GPs) on the surface of platelets, including GP IIb/IIIa, GP Ib/IX, and GP Ia/IIa; approximately 75% are located on the platelet membrane glycoprotein GP IIb/IIIa or GP Ib/IX complex. These complexes can be easily swallowed and destroyed by the reticuloendothelial system by binding to Fcγ receptors expressed on monocytes and macrophages, leading to a diminished peripheral platele[6]**.**

Impaired platelet production in ITP is supported by evidence from radiolabelled autologous platelet survival studies demonstrating reduced platelet turnover, and by the success of thrombopoietin (TPO) receptor agonists[7]**.**

One possible mechanism for platelet underproduction is autoantibody-mediated megakaryocyte inhibition[8].In support of this hypothesis, previous experiments have demonstrated that autoantibodies and isolated immunoglobulin G (IgG) fractions from some ITP patients can inhibit megakaryocyte growth and maturation in vitro; and that antibodies from some ITP patients bind to target bone marrow megakaryocytes ex vivo[4]. In vivo studies investigating antibody binding in the bone marrow microenvironment are lacking[4].

Subjects and Methods

Study design:

The current study was carried out at Minia University Hospital in the period from december 2016 to December 2017. The study was performed on 20 patients diagnosed as primary ITP as group I and20 thrombocytopenic MDS patients as group II who were admitted to internal medicine department.They were 15 females and 5 males in group I and, 11 females and 9 males in group II. Their ages ranged from 4 to 68 years in group I and from 4 to 71 years in group II.

Both groups were subjected to Complete history taking then Clinical examination: Including: pallor, purpura, liver, spleen and lymph nodes enlargement. In addition to the laboratory investigations; bone marrow biopsy samples were collected, processed and fixed in the same manner.

Routine laboratory investigations:

CBC was determined by automated cell counter Sysmex KX-21N (TAO Medical Incorporation, Japan), Renal function tests (urea and creatinine), were assayed using fully automated clinical chemistry auto-analyzer system Konelab 60i (Thermo Electron Incorporation, Finland).

Special investigations:

Formalin fixed bone marrow tissue blocks were deparaffinized,washed in xylene and rehydrated with graded washes of ethanol in water. Serial sections (2 - 4μm) were pretreated with target retrieval in a steamer for 45 minutes. Slides were washed and blocked in 1% normal goat serum for 20 minutes at room temperature. After washing, slides were incubated with anti-CD61 for 60 minutes. Following incubation, slides were washed and incubated with Envision™ FLEX Substrate for 20 minutes and counterstained with hematoxylin.

Statistical analysis

Data were coded and entered using the statistical package SPSS version 21. Data was summarized using mean and standard deviation for quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables. Comparison of non-parametric quantitative variables was done using Mann Whitney test. Chi square test used for comparison of qualitative data between the two groups.

Independent samples T test was used for parametric quantitative data between the two groups. P value <0.05 was taken as statistically significant.

Results

Megakaryocytes associated CD61 was increased in both ITP and MDS patients. (fig 1, 2).and There was no no statistically significant difference between both groups (Mean±SD 73.5±14.6 vs. 66.5±11.8%: p=0.104). (Table1). Median number of bone marrow megakaryocytes was increased in ITP and MDS patients (group I & II) and there was no statistically significant difference between both groups (Mean ± SD 8.1 ±5.1 vs. 6 ±3.1 cells per HPF; p=0.271). (table 2).

Table (I) Comparison between group I & II regarding the proportion of megakaryocytes associated with IgG, IgG binding and megagakaryocytes bound to CD61

		ITP N=20	MDS N=20	P value
Megakaryocytes bound CD61	Range Mean ± SD	(40-100) 73.5±14.6	(40-90) 66.5±11.8	*0.104*

Table (2): Comparison between studied groups regarding Hb,TLC,platelet count and number of megakaryocytes(per HPF):

		ITP N=20	MDS N=20	P value
Hb (1)	Range Mean ± SD	(7-13.8) 11.3±1.6	(5.5-14) 10±2	***0.034****
TLC (1)	Range Mean ± SD	(4.4-21.4) 8.8±4.1	(2.9-10.8) 6.4±2.4	***0.023****
Platelets (2)	Range Mean ± SD Median	(5-61) 22±14.8 17.5	(8-64) 38.3±14.4 38.5	***0.001****
Megs (2)	Range Mean ± SD Median	(2-18) 8.1±5.1 7	(2-12) 6±3.1 6	*0.271*

- *(1) Independent samples T test for parametric quantitative data beteen the two groups*
- *(2) Mann Whitney test for non-parametric quantitative data beteen the two groups*
- **: Significant difference at P value < 0.05*

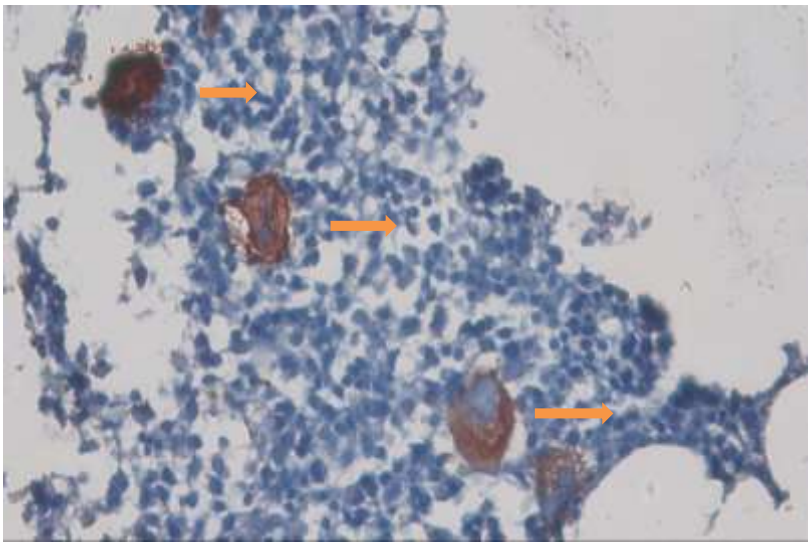

Figure1: immunohistochemical stains for CD61 of bone marrow biopsy specimens from a patient with ITP. . Arrows indicate megakaryocytes. Representative images are shown at 400X magnification

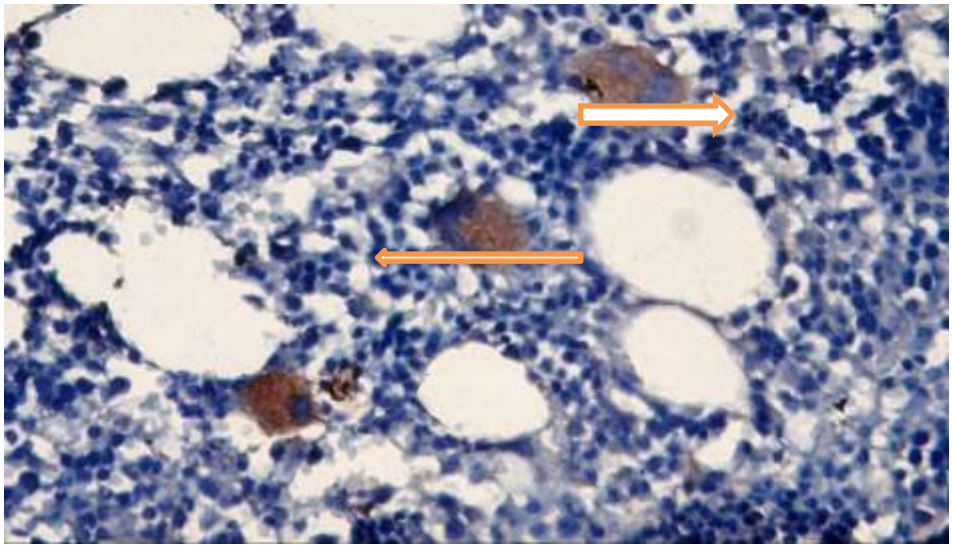

Figure2: immunohistochemical stains for CD61 of bone marrow biopsy specimens from a patient with MDS. . Arrows indicate megakaryocytes. Representative images are shown at 400X magnification.

Discussion

The aim of this study was to investigate number of megakaryocytes in bone marrow specimens of ITP patients. Our results indicate that high number of megakaryocytes occurs in immune (ITP), but also in MDS. Our findings provide a cautionary note about the need to consider antibody specificity in the pathogensis of ITP.

In our study platelet count is decreased in ITP patients in which downstream mechanism of impaired platelet production in ITP may be caused by upregulation of pro- or anti-apoptotic pathways in megakaryoctes; complement mediated megakaryocyte destruction; direct cellular effects on de novo platelet synthesis; as explained by Lev et al., (2014). The number of bone marrow megakaryocytes increased in our study in patients with ITP despite severe thrombocytopenia, in accordance with Mahabir et al., (2013). Who explained this by impairment of proplatelet formation by ITP autoantibodies.

Our study found that high IgG binding is increased in ITP patients. This is in agreement with Arnold et al., (2015) Who does not exclude the possibility that antibody-binding to megakaryocytes is a mechanism of disease. The amount of IgG bound to megakaryocytes increased as megakaryocyte numbers increased. These results are consistent with George, (1990) with data demonstrating that IgG is taken up by maturing megakaryocytes and ultimately stored in platelet αgranules. IgG uptake by megakaryocytes may be increased in conditions of thrombocytopenia due to the increased drive towards megakaryopoiesis.

Even though total IgG-binding to megakaryocytes did not differentiate immune and non-immune thrombocyte-penic conditions, our findings were in broad accordance with Arnold et al., (2015). Who reported that Megakaryocyte-specific antibody binding, even in small amounts, may be enough to disrupt normal megakaryopoiesis and thrombopoiesis. Thus, direct glycoprotein-specific assays, which could detect specific anti-GPIIbIIIa, anti-GPIbIX or other megakaryocyte antibodies are needed.

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