The Relation between Serum Progranulin and Platelet Count in Immune Thrombocytopenia Patients

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Abstract:

Background: Immune thrombocytopenia (ITP) is an autoimmune disease with excessive platelet destruction and reduced platelet production. Progranulin (PGRN) has a protective effect against autoimmune diseases by competitive binding tumor necrosis factor-α receptors. Thus, we assessed the serum progranulin level in relation to platelet count in ITP patients, pre-and post-induction of corticosteroid therapy. **Methods:** A case – control study was conducted on 30 ITP patients and 30 apparently healthy subjects serving as a control group. Platelet count and serum progranulin levels were measured in newly diagnosed ITP patients before and 3−7 days after starting corticosteroid treatment. **Results:** The mean serum level of PGRN was significantly higher in ITP patients pre-treatment compared to healthy control subjects (P <0.001). After 3-7 days of treatment of ITP patients with corticosteroid; the platelets count increased significantly, whereas serum PGRN level decreased significantly (P <0.001 each). Serum PRGN showed a significant negative correlation with platelets count pre-steroid treatment and a significant positive correlation with platelets count post-steroid treatment.

Conclusion: The elevated serum PGRN levels in ITP patients supports its involvement in the disease pathogenesis. The inversely correlated serum PGRN levels with platelets count in ITP patients before and after treatment induction suggest that PGRN may be a useful biomarker that helps the diagnosis, treatment decisions and follow up of ITP patients.

Keywords: Progranulin, Immune Thrombocytopenia, Biomarker, Protective.

Introduction:

Immune thrombocytopenia (ITP) is an autoimmune disease characterized by excessive platelet destruction and reduced platelet production. [1] ITP is defined in a patient with generalized purpuric eruption, when peripheral blood platelet count is less than 100×10^9/L while total white blood cell count and hemoglobin level are normal. [2]

Platelet destruction in ITP is mediated by an immunoglobulin G autoantibody which develops against platelet membrane proteins. The antibody-coated platelets are cleared progressively by macrophages in spleen. T-cell mediated cytotoxicity is proposed to reduce platelet production where cytotoxic T cells attack megakaryocytes in the bone marrow. [3]

The guidelines of the American Society of Hematology (ASH) for a newly diagnosed adult with ITP recommend corticosteroids, intravenous immune globulin (IVIG) or Rh (D) Immune Globulin (RhIg) as first-line therapy. The fastest platelet count responses can be achieved with IVIG within 12–48 hours, then high-dose dexamethasone within 1–2 days and standard-dose prednisone within 2–4 days. [4]

Progranulin (PGRN) is a 593 amino acids glycoprotein. It is composed of repeats of granulin module like beads on a chain. PGRN has been shown to be involved in stimulating cell proliferation and regulating neuropathology, cancer cell proliferation, infection and wound healing. [5] It affects directly the growth, migration, and invasion of human colon cancer cells. [6]

Progranulin has a protective effect against autoimmune diseases by competitively binding tumor necrosis factor-α (TNF-α) receptors (TNFR-1 and -2). [7] However, the exact role of progranulin in ITP remains unclear. Thus, we aimed to assess serum progranulin level in relation to platelet count in ITP patients, pre-and post-induction of corticosteroid therapy.

Subjects and methods

Subjects

The present study was conducted at Internal Medicine department of Tanta University Hospital and Clinical and Chemical Pathology department of Benha University Hospital between November 2018 and May 2019 on a total of 60 subjects were enrolled in this case – control study, included 30 patients diagnosed with ITP according to the ASH criteria for diagnosis of ITP (platelet count less than 100 x 10^3/µl without morphologic evidence for dysplasia in the peripheral blood film) after exclusion of

other causes of primary and secondary thrombocytopenia. They were further subdivided into pre-treatment and post-treatment where they are newly diagnosed ITP patients before staring corticosteroid therapy and 3−7 days after starting corticosteroid treatment, respectively. Any case presented with diabetes mellitus or other autoimmune disorders was excluded from this study.

In addition, 30 apparently healthy volunteers without history of chronic, inflammatory, autoimmune or malignant diseases or under any kind of medical treatment were enrolled as control group. The study was approved by the local ethics committee on research involving human subjects in Faculty of Medicine, Benha University in agreement with the Declaration of Helsinki. [8] Informed consent was obtained from each subject prior to participation.

Methods

All patients underwent full history taking and comprehensive clinical examination by a clinical hematologist. Laboratory investigations included complete blood count and serum progranulin levels pre- and post-corticosteroid therapy induction. Serum progranulin (PRGN) was detected by a double-antibody sandwich ELISA Kit for research use ***(Cat#: E-02467HU, Cloud-Clone Crop., USA).*** The sensitivity by this assay was 10 pg/ml.

Statistical Analysis

The collected data were tabulated and analyzed using SPSS version 21 software (Spss Inc, Chicago, ILL Company). Chi square test (X^2) or Monte Carlo exact test (MC) was used to analyze categorical variables. Quantitative data were tested for normality using Shapiro-Wilks test, assuming normality at P>0.05. Differences among two groups regarding parametric variables were analyzed by student t-test. Difference among two groups regarding non-parametric variables was analyzed by Mann Whitney test (U).

Relation between two parametric variables pre- and post-treatment was done by paired t-test. While, between two non-parametric variables were done by Wilcoxon signed rank test. Parametric correlations were assessed by Pearson's correlation coefficient (r).

ROC curve was constructed to detect cut off value of PGRN with optimum sensitivity and specificity in prediction of cases. The accepted level of significance in this work was stated at 0.05.

Results

The mean age of ITP patients 33.2±8.32 years was significantly lower than the mean age of healthy controls 45.03±7.3 years (P<0.001). The studied ITP patients were 14 males and 16 females who were matched with control subjects 20 males and 10 females (P 0.118). Platelets count was significantly decreased in ITP patients than controls (P <0.001) and hemoglobin level and total white blood cell count did not differ among studied groups before induction of corticosteroid therapy in ITP patients.

The mean serum level of PGRN was significantly higher in ITP patients pre-treatment compared to healthy control subjects (P <0.001). **Table (1)**

According to platelets count in studied ITP patients before starting corticosteroid treatment, bleeding symptoms did not differ significantly (P 0.333). While, serum PRGN level was significantly higher in ITP patients with platelets count less than or equal to 10 x 10^3/µl rather than that in patients with platelets count more than 10 x 10^3/µl (P 0.028). **Table (2)**

After 3-7 days of treatment of ITP patients with corticosteroid; the platelets count increased significantly (P <0.001), whereas serum PGRN level decreased significantly (P <0.001). **Table (3)**

It was found that serum PRGN level showed a significant negative correlation with platelets count pre-steroid treatment (*r*= -0.384, P 0.036) and a significant positive correlation with platelets count post-steroid treatment (*r*= 0.457, P 0.011). **Fig (1)**

The diagnostic performances of PRGN to discriminate between ITP patients and healthy control indicated that at a serum PRGN cut-off value >1592.5 pg/ml the sensitivity, specificity, positive predictive value and negative predictive value were 100% each with an excellent area under the ROC curve of 1.00 (P <0.001). **Fig (2)**

Table 1. Demographic and laboratory characteristics of the two studied groups (pre-treatment):

		ITP patients (n= 30)	Control (n=30)	Test	P
Age (yrs)		33.2± 8.32	45.03± 7.3	126.5*	**<0.001**
Sex	**Male**	14 (46.7%)	20 (66.7%)	2.443**	0.118
	Female	16 (53.3%)	10 (33.3%)		
Hb (g/dl)		11.7±1.12	11.8±0.85	0.272***	0.787
WBCs (x10^3/µl)		7.7 ± 2.68	7.6 ± 1.83	431.00*	0.779
Platelet count (x10^3/µl)		17.2 ± 5.17	242.2 ± 68.69	17.89***	**<0.001**
PGRN (pg/ml)		3655.00± 1373.21	858.1± 288.26	0.000*	**<0.001**

Data represented as mean ± SD and number (percentage).

*Mann-Whitney U-test, **Chi-square χ2-test, ***Student t-test.

Table 2. Relation between bleeding symptoms, PGRN level and platelets count in studied ITP patients pre-treatment:

	Platelet ≤ 10x 10^3/µl	Platelet >10x 10^3/µl	Test	P
Bleeding symptoms				
Ecchymosis, purpura (n=12, 40%)	1 (8.3%)	11 (91.7%)		
Bleeding per gum, nose, hematemesis (n=16, 53.3%)	2 (12.5%)	14 (87.5%)	2.596*	0.333
Unusual heavy menstruation (n=2, 6.67%)	1 (50%)	1 (50%)		
PGRN (pg/ml)	5417.5± 1876.53	3383.9± 1091.50	16.00**	**0.028**

Data represented as number (percentage) and mean ± SD.

*Monte Carlo exact test, **Mann-Whitney U-test.

Table 3. Comparison between platelets count and serum PGRN levels in ITP patients pre- and post-treatment:

	Pre-treatment	Post-treatment	Test	P
Platelet (x10^3/µl)	17.2 ± 5.17	66.9 ± 14.19	18.015*	**<0.001**
PGRN (pg/ml)	3655.00± 1373.21	1906.7± 720.86	4.783**	**<0.001**

Data represented as mean ± SD

* Paired t-test, ** Wilcoxon signed rank test.

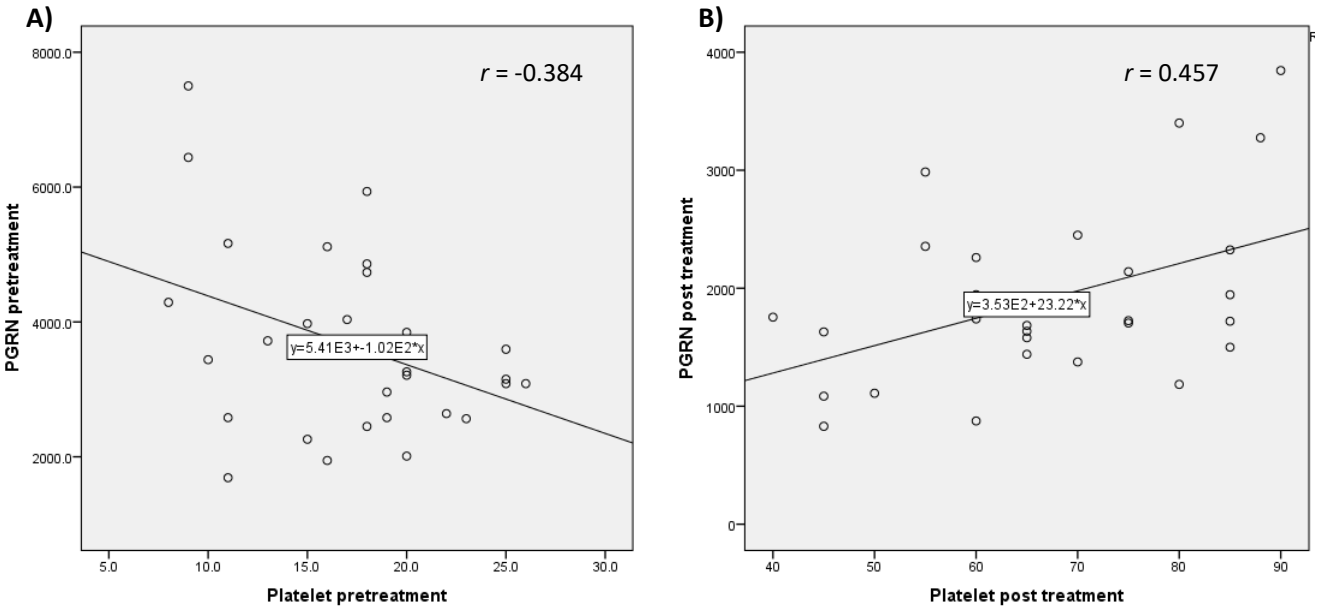

Figure 1. Correlation between serum PGRN level and platelets count in studied ITP patients; A) pre-treatment and B) post-treatment.

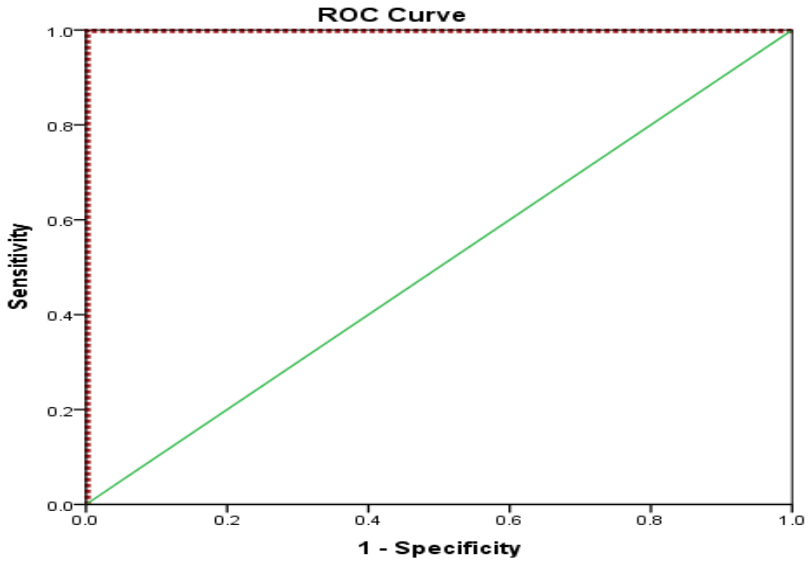

Figure 2. ROC curve for serum PRGN to diagnose ITP patients from healthy controls

Discussion

Progranulin has protective effects toward autoimmune diseases. It enhances the Treg proliferation and decreases the inhibitory effect of tumor necrosis factor-α (TNF-α) on

them via its competitive binding with TNF-α receptors (TNFR1 and TNFR2). As a vital player in inhibiting autoimmune diseases, the role of PGRN in ITP remains unclear. [9]

This case−control study was conducted on 30 idiopathic thrombocytopenic purpura (ITP) patients, before and 3−7 days after starting corticosteroid therapy, and 30 apparently healthy subjects as control group. The mean age of ITP patients (33.2±8.32 years) was significantly lower than the controls (45.03±7.3 years) (P<0.001). Both groups were sex-matched (P=0.118). They were 14 males and 16 females ITP patients and 20 males and 10 females control subjects. In a previous Egyptian study, ITP patients and healthy controls were age and sex-matched (P 0.798 and 0.349, respectively) as the mean age of ITP patients was 31.37±5.99 years and of controls was 30.95±7.19 years with 53 female patients (88.3%) and 16 female controls (80%). [10]

The present study showed that at time of presentation, the bleeding symptoms in ITP patients were ecchymosis and /or purpura in 40%, bleeding per gum, nose and/or hematemesis in 53.3% and 6.7% of female patients had heavy menstruation. The relation between thrombocytopenia and bleeding is well documented. However, there is no clear evidence of a direct correlation between the degrees of thrombocytopenia and bleeding symptoms, especially at lower platelet counts [11]. It was demonstrated that most ITP patients manifest with hemorrhagic symptoms ranging from mild cutaneous bleeding to severe life-threating bleeding complications. [12]. Along with our results, a study had revealed that 47% of ITP patients had bled for a few days and 27% had bruised easily for several weeks or months "easy bruising syndrome". [13]

According to ASH guidelines for ITP diagnosis [4], ITP patients had a pre-treatment significantly lower platelets count compared with control group (P <0.001), while, no significant differences were detected in hemoglobin level and total white blood cell count (P >0.05 each).

In current work, mean serum PGRN level (3655.00 ± 137.21 pg/ml) was significantly elevated in newly diagnosed pre-treatment ITP patients compared to healthy control subjects (858.1 ± 288.26 pg/ml) (P <0.001). After 3−7 days of corticosteroid therapy induction, significant increase in platelets count was observed with a mean 66.9 ± 14.19 x 10^3/µl (range 40−90 x 10^3/µl). While, serum PGNR level was significantly decreased 1906.7 ± 720.86 pg/ml compered to patients before treatment (P <0.001 each). It was found that serum PRGN level has a

significant negative correlation with platelets count pre-steroid treatment (*r*= -0.384, P 0.036) and a significant positive correlation with platelets count post-steroid treatment (*r*= 0.457, P 0.011). This was in agreement with Yu et al.(2018) who reported an increased PGRN levels in ITP patient plasma, which were reduced after treatment. They found that plasma PGRN levels were negatively correlated with platelet count of ITP patients. [14]

PGRN was suggested to have an immunosuppressive effect on ITP being a protective regulator in ITP animal models, as PGRN deficiency led to fewer Treg cells in ITP mouse spleens. [14] ITP is a complex disease that is diagnosed mainly by exclusion. Up till now, no specific biomarker can support the differential diagnosis or treatment decisions for ITP.

Various studies have reported the impaired balance of T helper cytokines (Th1/Th2) and T regulatory cell cytokines in ITP pathogenesis; with controversial [15,16] and contradictory [17] data regarding Th1 cytokine profiles in ITP, whereas, the Tregs cytokine, transforming growth factor-β1 (TGFβ1), has been found to be down-regulated in ITP. [18] Lower levels of TGFβ1 correlate with defective Treg number and function in ITP patients. [19,20] PGRN has been proved to protect against autoimmune diseases such as rheumatoid arthritis [21,22], osteoarthritis [23], inflammatory bowel disease [24] and systemic lupus erythematosus. [25,26]

In conclusion, the inversely correlated serum PGRN levels with platelets count in ITP patients before and after treatment induction suggest that PGRN may be a useful biomarker that helps the diagnosis, treatment decisions and follow up of ITP patients. Furthermore, its evidenced effect on Treg proliferation might make PRGN a potential therapeutic strategy for management of ITP.

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