Role of platelet-associated immunoglobulin G in hypersplenism-associated thrombocytopenia

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Introduction
Hypersplenism is characterized by cytopenia, splenomegaly, and increased or normal bone marrow cellularity. Platelet-associated immunoglobulin G (PAIgG) is a class of platelet autoantibodies bound to the surface glycoprotein of the platelet and is mainly produced by the spleen. Significant higher PAIgG levels were found in patients with thrombocytopenic purpura and cirrhotic hypersplenism.

Aim
The aim was to assess the antiplatelet immunoglobulin G antibodies level in patients with hypersplenism-associated thrombocytopenia before and after, first, medical treatment (growth factors and corticosteroids) and second, surgical treatment (splenectomy).

Patients and methods
This study included 40 patients with hypersplenism and 10 age-matched and sex-matched healthy controls. Serum antiplatelet IgG was measured by ELISA technique using Human antiplatelet IgG (anti-PA IgG) ELISA Kit.

Results
In both the splenectomized and the medical groups, PAIgG had insignificant decrease during follow-up in comparison with baseline level (in case of the splenectomized group, 224.50 ± 51.32 vs 206.30 ± 69.82 μg/ml, $P = 0.33$, and in case of the medical group, 200.81 ± 55.41 vs 186.60 ± 72.29 μg/ml, $P = 0.07$).

Conclusion
An immune process may be mediated by PAIgG, and secondary hypersplenism due to portal hypertension is associated with thrombocytopenia in patients with liver cirrhosis. The authors also found that patients with splenomegaly subjected to splenectomy had a significant elevation of platelet counts and insignificant reduction in PAIgG levels.

Keywords:
associated immunoglobulin G, hypersplenism, platelet, thrombocytopenia

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Introduction

The spleen is an organ involved in the production of humoral antibodies, in the production and maturation of B and T lymphocytes and plasma cells, represents a blood filter by removing of undesirable particulate matter (e.g. bacteria, viruses, and old, malformed, or damaged red blood cells), and also acts as a reservoir for blood cells, mainly white blood cells and platelets. The palpable spleen is felt if it reaches at least twice the normal size [1].

Hypersplenism is a disease characterized by splenomegaly, cytopenia, increased or normal medullar cellularity, and elevated destruction of the involved blood element. Hypersplenism may occur owing to an increased demand for splenic function, infiltrative diseases of the spleen and splenic congestion owing to portal hypertension. Hypersplenism resulting from portal hypertension associated with congestive splenomegaly is usually due to liver cirrhosis [2].

The criteria to diagnose hypersplenism include splenomegaly, a peripheral cytopenia, a cellular bone marrow, and subsequent significant peripheral blood picture improvement following splenectomy [3].

There is no universally accepted hypersplenism therapy till now. Multiple blood transfusions may be required. The surgical treatment for hypersplenism includes open or laparoscopic splenectomy. An alternative treatment modality is the transcatheter ablation of splenic parenchyma [4].

Thrombocytopenia is found when platelet count is below 140 000/mm^3. One of the causes is splenic sequestration due to portal hypertension. If the medical treatment is unsuccessful, splenectomy is often considered as a last option to treat refractory thrombocytopenia [5].

However, the surgical treatment has perioperative and postoperative risks [6]. In patients with bad condition who cannot support general anesthesia, splenectomy is contraindicated and dangerous [7].

The spleen is the principal producer of antibodies meant for circulating blood cells but has been confirmed only for antiplatelet antibodies [8].

Platelet-associated immunoglobulin G (PAIgG) is a type of platelet autoantibodies bound to the platelet surface glycoprotein and primarily produced by the spleen. It was found that higher PAIgG was presented in patients with thrombocytopenic purpura and cirrhotic patients. PAIgG-bound platelets were easily trapped and phagocytized by macrophages while flowing in the spleen. PAIgG can also bind to and destroy megakaryocytes and their precursors, thus impeding their differentiation and platelet formation [9].

PAIgG has been involved as a thrombocytopenic factor mediated by an immune mechanism in patients with chronic liver disease. PAIgG binds to platelets and thus stimulates sequestration in the reticuloendothelial system. Numerous studies have reported that serum PAIgG levels are elevated in thrombocytopenic patients with hypersplenism [10].

Aim

The aim was assessment of the antiplatelet immunoglobulin G antibodies level in patients with hypersplenism-associated thrombocytopenia before and after medical treatment (growth factors and corticosteroids) and surgical treatment (splenectomy).

Patients and methods

This study was conducted on 40 patients with hypersplenism and 10 age-matched and sex-matched healthy controls. The patients' blood samples were selected from the Tropical Medicine and Gastroenterology Department, at Al-Rajhi Liver Hospital, Internal Medicine Department and Haematology Unit in the Assiut University Hospital. It was over a period of 1 year and 3 months from January 2018 to April 2019. Formal consent was obtained from patients and controls. The study was approved by the Ethical Committee of Faculty of Medicine Assiut University.

Classification of participants

The patients were classified as follows:

(1) Hypersplenism with splenectomized patients group (10 patients).
(2) Hypersplenism with medically treated patients group (30 patients).
(3) Control group included 10 apparently healthy sex-matched and age-matched individuals.

Sample collection, storage, and handling

Overall, 8 ml of venous blood was collected under complete aseptic conditions and divided into the following:

(1) 2 ml of venous blood was collected into EDTA-containing tube for a complete blood count.
(2) 2 ml of venous blood was collected into a plain tube without anticoagulant for antiplatelet IgG (anti-PA IgG).
(3) 4 ml of venous blood was collected into a plain tube without anticoagulant for hepatitis C virus and hepatitis B virus detection and liver function tests.
    Blood into the plain tubes was allowed to clot for 10–20 min at room temperature and centrifuged at the speed of 2000–3000 rpm for 20 min.
    Serum was collected and stored at −80°C till the time of assay of anti-platelet IgG (anti-PA IgG).
(4) A volume of 2 ml bone marrow sample was collected from either iliac or sternum of the patients; slides were prepared and stained by Leishman stain for assessment.
(5) Bone marrow trephine biopsy collected from posterior superior iliac spine from the patient.

Routine investigations

Complete blood count was done on ABX Pentra XL 80 (HORIBA Medical, France). Serum liver function tests were done on COBAS Integra 400 plus (Roche, Germany). Hepatitis C virus antibody and hepatitis B virus antigen tests were done on ARCHITECT i1000SR (Abbott, USA). Bone marrow aspirate examination and bone marrow trephine biopsy.

Special investigations

Serum antiplatelet IgG level was measured by a quantitative ELISA technique using Human anti-platelet IgG (anti-PA IgG) ELISA Kit, catalog no. SG-14187 (SinoGeneClon Biotech Co., China) and was read on Stat Fax-303 ELISA Plate Reader (Awareness Technology Inc, USA).

Statistical analysis

The data were collected and analyzed by using SPSS (Statistical Package for the Social Sciences, version 20; IBM, Armonk, New York, USA). The

continuous data were expressed in the form of mean ± SD or median (range), whereas the nominal data were expressed in the form of frequency (percentage).

χ^2-Test was used to compare the nominal data of different groups in the study, whereas Student's *t* test was used to compare the mean of different two groups and analysis of variance test for more than two groups. Baseline and follow-up data of the same group were compared with paired *t*-test. Level of confidence was kept at 95%, and hence, *P* value was significant if less than 0.05.

Results

Baseline complete blood picture in the studied groups

It was noticed that both the splenectomized and the medical groups had insignificant differences in the hemoglobin level, white blood cells, and platelet count. The control group had significantly higher hemoglobin level and platelet count in comparison with the medical group and the splenectomized group, but all groups had insignificant differences regarding the white blood cell count (Table 1, Figs. 1 and 2).

Baseline and follow-up complete blood picture in the studied groups

Both the splenectomized and the medical groups had insignificant differences regarding the baseline hemoglobin level, white blood cell, and platelet counts, but on follow-up, the splenectomized group had significantly higher white blood cell count (10.02 ± 6.07 vs 4.90 ± 2.90 × 10^3/ml; *P* = 0.01) and platelet count (206.30 ± 69.8 vs 186.60 ± 74.76 × 10^3/ml; *P* = 0.01) in comparison with those of the medical group, but both groups had insignificant difference regarding hemoglobin level.

In comparison of the baseline data with follow-up in both groups, it was noticed that platelet count and hemoglobin level were increased in both groups, but white blood cell count was significantly increased in the splenectomized group only (Table 2, Figs. 3 and 4).

Baseline PAIgG in the studied groups

In our results, all the studied groups had insignificant difference regarding baseline level of PAIgG, where it was 224.50 ± 51.32 μg/ml in the splenectomized group, 200.81 ± 55.41 μg/ml in the medical group and 167.71 ± 66.58 μg/ml in the control group (Table 3 and Fig. 5).

PAIgG in the studied patients at baseline and during follow-up

In both the splenectomized and the medical groups, PAIgG had insignificant decrease during follow-up in comparison with baseline level (224.50 ± 51.32 vs 206.30 ± 69.82 μg/ml, *P* = 0.33, in case of the splenectomized group, and 200.81 ± 55.41 vs 186.60 ± 72.29 μg/ml, *P* = 0.07, in case of the medical group) (Table 4 and Fig. 6).

Discussion

In our study, we evaluated the role of PAIgG in patients with hypersplenism-associated thrombocytopenia;

Table 1 Baseline complete blood picture of the studied groups

Parameters	Splenectomy group	Medical group	Control group	P_1	P_2	P_3
Complete blood picture						
Hemoglobin (g/dl)	9.27±2.12	9.45±2.54	14.32±1.53	0.45	0.03*	0.04*
White blood cells (×10^3/μl)	4.82±1.31	5.01±0.74	7.21±1.80	0.11	0.56	0.19
Platelets (×10^3/μl)	82.80±27.85	66.94±26.64	281.80±69.76	0.06	0.01*	0.01*

Data were expressed in the form of mean±SD. P_1 compared between splenectomy group and medical group; P_2 compared between splenectomy group and control group; P_3 compared between medical group and control group.

Table 2 Complete blood picture of the studied groups

Parameters	Splenectomy group		Medical group	
	Baseline	Follow-up	Baseline	Follow-up
Hemoglobin (g/dl)	9.27±2.12	10.48±2.41	9.45±2.54	10.42±1.92
White blood cells (×10^3/μl)	4.82±1.31	10.02±6.07	5.01±0.74	4.90±2.90
Platelets (×10^3/μl)	82.80±27.85	206.30±69.80	66.94±26.64	186.60±74.76
Significance	P_1	P_2	P_3	P_4
Hemoglobin (g/dl)	0.45	0.65	0.01*	0.03*
TLC (×10^3/μl)	0.11	0.01*	0.04*	0.11
Platelets (×10^3/μl)	0.06	0.01*	0.01*	0.01*

Data were expressed in the form of mean±SD. P_1 compared between splenectomy group and medical group at baseline data; P_2 compared between splenectomy group and medical group at follow-up; P_3 compared between baseline and follow-up data of splenectomy group; P_4 compared between baseline and follow-up data of medical group; TLC, total leukocyte count.

Table 3 Baseline platelet-associated immunoglobulin G of the studied groups

Parameters	Splenectomy group	Medical group	Control group	P_1	P_2	P_3
PAIgG (μg/ml)	224.50±51.32	200.81±55.41	167.71±66.58	0.56	0.10	0.09

Data were expressed in the form of mean±SD. P_1 compared between splenectomy group and medical group; P_2 compared between splenectomy group and control group; P_3 compared between medical group and control group; PAIgG, platelet-associated immunoglobulin G.

Table 4 Platelet-associated immunoglobulin G in the studied patients at baseline and during follow-up

Parameters	Baseline	Follow-up	*P*
Splenectomy group	224.50±51.32	206.30±69.82	0.33
Medical group	200.81±55.41	186.60±72.29	0.07

Data were expressed in the form of mean±SD. $P<0.05$, significant.

PAIgG levels have been reported to be in correlation with hypersplenism in thrombocytopenic patients [11].

PAIgG targets specific glycoproteins on the surface of platelets; however, antibody binding alone does not cause thrombocytopenia. In addition to the presence of PAIgG, an intact reticuloendothelial system is needed to cause thrombocytopenia [12].

The current study revealed that both splenectomized and medical groups had insignificant differences regarding baseline hemoglobin level, white blood cell, and platelet counts, but on follow-up, splenectomized group had significantly higher white blood cell count (10.02 ± 6.07 vs $4.90 \pm 2.90 \times 10^3$/ml; $P = 0.01$) and platelet count (206.30 ± 69.8 vs $186.60 \pm 74.76 \times 10^3$/ml; $P = 0.01$) in comparison with those of medical group, but both groups had insignificant difference regarding hemoglobin level.

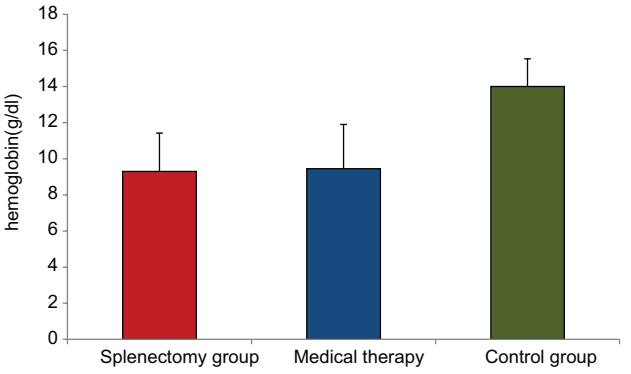
These results were in line with that of Kawanaka *et al.*[13] who reported that there was rapid enhancement in blood cell counts after splenectomy. Peripheral platelet and white blood cell counts were significantly higher in the splenectomized group than that in the nonsplenectomized patients ($P < 0.01$).

Moreover, these results were found to be in agreement with those of Voican *et al.*[14] who reported that the use of thrombopoietin agonists such as romiplostim and eltrombopag seems to be useful to elevate the platelet count.

In comparison of baseline data with follow-up data in both groups, it was noticed that platelet count and hemoglobin level were significantly increased in both groups, but the white blood cell count was significantly increased in splenectomized group only.

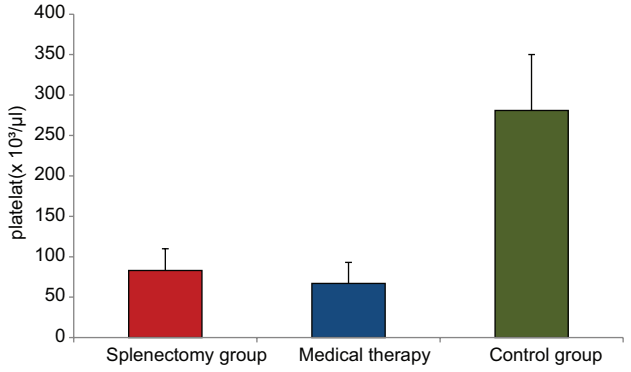
Our study revealed that in both splenectomized and medical groups, PAIgG had insignificant decrease during follow-up in comparison with the baseline level (224.50 ± 51.32 vs 206.30 ± 69.82 μg/ml, $P = 0.33$, in case of splenectomized group and 200.81 ± 55.41 vs 186.60 ± 72.29 μg/ml, $P = 0.07$, in case of medical group). However, Sekiguchi *et al.*[15] reported that PAIgG titers

Figure 1



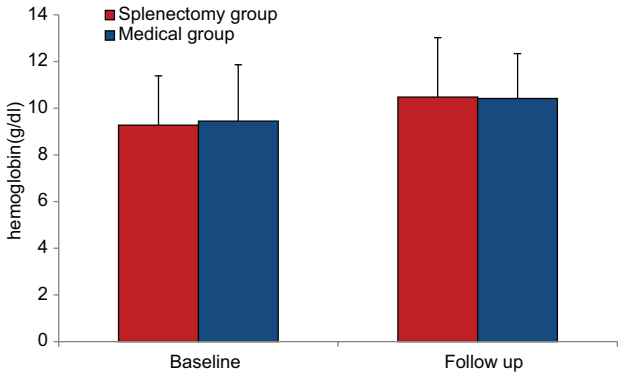
Mean hemoglobin of the studied groups.

Figure 2



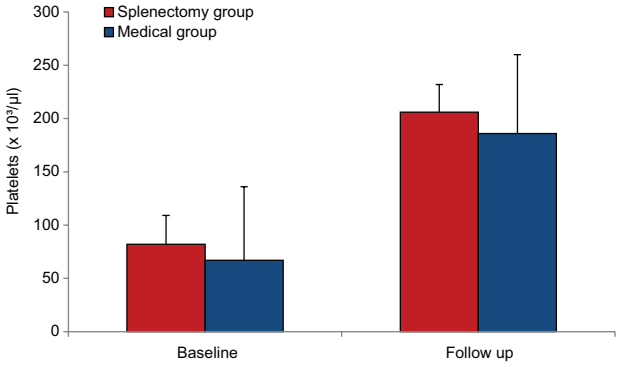
Mean platelets of the studied groups.

Figure 3



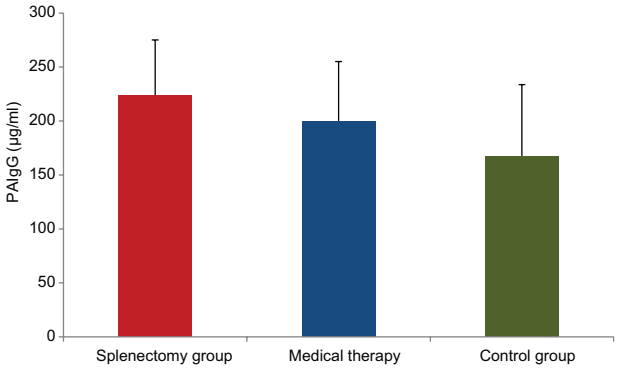
Baseline and follow-up level of hemoglobin in the studied patients.

Figure 4



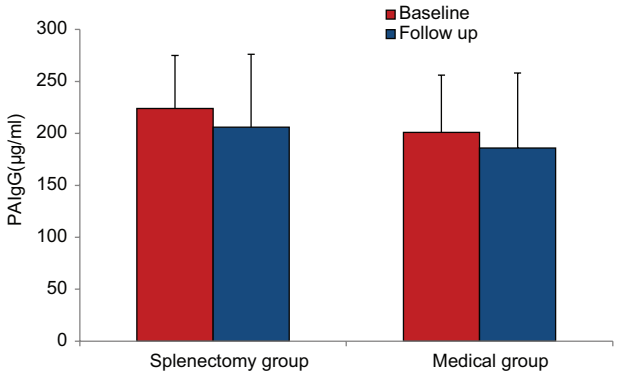
Baseline and follow-up level of platelets in the studied patients.

Figure 5



Mean platelet-associated immunoglobulin G of the studied group.

Figure 6



Baseline and follow-up level of platelet-associated immunoglobulin G in the studied patients.

were significantly higher in the cirrhotic patients with an intact spleen compared with the splenectomized patients (297.9 ± 197.0 vs 125.6 ± 87.8 µg/ml, $P < 0.05$). However, there was agreement that PAIgG levels were negatively correlated with platelet counts in both groups.

The conception that an immune-mediated process is involved in cirrhotic thrombocytopenia is not new. This idea was suggested based on the negative correlation between PAIgG and the platelet count in patients with liver cirrhosis. However, it is now widely accepted that the specificity of PAIgG for autoantibody-mediated thrombocytopenia is relatively low [11].

Definitive detection of PAIgG in thrombocytopenia and information concerning the biological implications of elevated platelet-bound immunoglobulin is not provided. Further studies with bigger groups may provide more conclusive proof of the role of PAIgG in hypersplenic patients with thrombocytopenia.

Finally, it was found that thrombocytopenia is a multifactorial phenomenon, and the relationship between the enlarged splenic volume, the stimulated PAIgG production, and the reduced platelet count is complex. So, more mechanism-oriented investigations are necessary.

Conclusion

(1) It was found that an immune process may be mediated via PAIgG, and secondary hypersplenism is associated with thrombocytopenia in patients with compensated liver cirrhosis.
(2) Presplenectomy and postsplenectomy studies elucidated the role of the spleen in platelet sequestration and destruction.
(3) Patients with splenomegaly subjected to splenectomy revealed a significant elevation of platelet counts and insignificant decrease in PAIgG levels.

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Conflicts of interest
There are no conflicts of interest.

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