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Immunoglobulin A and Immunoglobulin M and Their Relation to Treatment Response in Adult Egyptian Immune Thrombocytopenic Purpura Patients

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ABSTRACT

Background: Immune thrombocytopenia (ITP) is a disorder of antibody mediated destruction and inhibition of production of platelets. In general, this is an idiopathic disease, and it is unclear what are the immune factors related to disease predisposition, severity, and especially response to treatment.

Objective: The aim of the work was to measure the level of Immunoglobulins M (IgM) and A (IgA) in patients with immune thrombocytopenic purpura (ITP) and to detect their relation to treatment response.

Patients and Methods: The serum level of both IgA and IgM were measured by ELISA assay in 60 newly diagnosed ITP patients received standard treatment in the form of steroids. Patients were aged from 16 to 45 years.

Results: Median level of IgA was higher in ITP patients at presentation in comparison to average normal population which was of no statistical significance while ITP patients had lower median level of IgM at presentation in comparison to average normal population with no statistically significance. No significant correlation could be detected between level of IgA and IgM and platelets count in ITP patients after one month and 3 months of treatment. This study indicated that non responder patients had IgA level higher than that of responder patients but of no statistically significant difference. (P value=0.536).

Conclusion: It could be concluded that statistically significant difference between responder and non-responder ITP patients as regard the level of IgM denotes that patients who had IgM level below the median were more resistant to steroids which is the standard treatment in ITP.

Keywords: ITP, IgA, IgM, Treatment response.

INTRODUCTION

Idiopathic thrombocytopenic purpura (ITP), also known as primary immune thrombocytopenic purpura is defined as isolated thrombocytopenia with normal bone marrow and in the absence of other causes of thrombocytopenia [(1)]. Immune thrombocytopenia (ITP) is a bleeding autoimmune disease due to decreased platelet production as well as accelerated platelet destruction mediated in part by autoantibody-based destruction mechanisms. Auto antibodies against platelet surface glycoproteins (GP), such as GPIIb/IIIa and GPIb/IX complexes, play major roles in both platelet destruction and impaired platelet production [(2)].

Immune dysregulation, as represented by elevated or decreased serum immunoglobulin (Ig) levels, may increase disease severity as represented by failure to respond to treatment. These alterations in Ig levels may represent an inflammatory or activated immune state that makes the disease more difficult to control with specific treatment [(3)]. Certain patients with known immunologic disorders have an increased the risk of ITP; supporting the concept that immune dysregulation may contribute to the development of ITP [(4)]. Although, it is unclear which immune factors related to disease predisposition, severity, and especially response to treatment [(3)].

The aim of the work was to measure the level of Immunoglobulins M (IgM) and A (IgA) in patients with immune thrombocytopenic purpura (ITP) and to detect their relation to treatment response.

PATIENTS AND METHODS

This cross-sectional study included a total of 60 newly diagnosed ITP patients received standard treatment in the form of steroids and 20 apparently healthy control of matched age and sex, attending at the Department of Clinical Hematology, Ain Shams University Hospitals.

Patients were subdivided into two groups according to treatment response; which is defined as platelet count ≥ 30 x109/L and a greater than two fold increase in platelet count from baseline measured on 2 occasion >7 days apart. **Group I**: steroids responder, and **Group II:** steroids non-responder.

Inclusion criteria: Patients diagnosed as ITP aged from 16- 65 years received standard treatment in the form of steroids.

Exclusion criteria: Patients with thrombocytopenia secondary to autoimmune disease or malignancy or viral infection, and pregnant females.

All patients were subjected to:

1. Full history taking.
2. Full clinical examination.
3. Complete blood picture (CBC) with blood film and blood chemistry including: (liver function tests, kidney function tests, serum electrolytes and LDH), coomb's test, autoimmune markers (ANA, Anti DNA, and C3&C4) and viral markers. (HCV ab, HBs Ag, HIV ab, EBV IgM & IgG and CMV IgM & IgG).

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4. At the time of diagnosis, serum IgA and IgM levels were determined by ELISA assay, using a commercially available ELISA kit (AUTOBIO DIAGNOSTICS CO., LTD).

Blood sampling:

For each subject, about 5 ml of venous blood was drawn under complete aseptic conditions and dispensed into a labeled vacutainer containing gel and clot activator, and serum was separated by centrifugation for 5 minutes, and then stored at temperature -20°C till assay for measurement of IgA and IgM.

Ethical approval and written informed consent:

An approval of the study was obtained from Ain Shams University academic and ethical committee. Every patient signed an informed written consent for acceptance of the operation.

Statistical analysis

Statistical presentation and analysis of this study was conducted, using the mean, standard deviation, Median, IQR, Chi-square, Kruskal-Wallis and Analysis of variance Mann-Whitney tests by using SPSS V17. ROC curve was plotted for determination of the cut-off point at which the relevant studied variable achieves best diagnostic performance. P value < 0.05 was considered significant.

RESULTS

The studied groups were divided into different age groups: 53.33% (32 patients) aged from 16-35 years, 28.33% (17 patients) aged from 36-45 years, 18.33% (11patients) aged from 46-65 years with female predominance around 78.33%. About 66.66% of studied group were steroids responder and 33.34 % were non-responder. Bleeding at presentation constituted 52.5% in steroids responder group while in non-responder group it constituted 40% (Table 1).

Table (1): Demographic and descriptive data of studied group

		Number	%
Age (Years)	**16-35**	**32**	**53.33**
	36-45	**17**	**28.33**
	46-65	**11**	**18.33**
Sex	**Male**	**13**	**21.67**
	Female	**47**	**78.33**
Treatment response	**Responder**	**40**	**66.66**
	Non-Responder	**20**	**33.34**
Bleeding at presentation (Responder group)	**Yes**	**21**	**52.5**
	No	**19**	**47.5**
Bleeding at presentation (Non-Responder group)	**Yes**	**8**	**40**
	No	**12**	**60**

The median level of IgA was higher in ITP patients in comparison to normal population without statistically significant difference. **(p-value 0.491),** while the median level of IgM was low in comparison to normal individuals without statistically significant difference **(p-value 0.872) (Table 2).**

Table (2): **Comparison between IgA and IgM level in ITP cases and control group.**

Groups	N	IgA			Mann-Whitney Test	
		Range	Median	IQR	Z	P-value
Cases	60	23 - 773	186.50	117.00	0.689	0.491
Control	20	73 - 449	174.50	96.25		
Groups	**N**	**IgM**			**Mann-Whitney Test**	
		Range	**Median**	**IQR**	**Z**	**P-value**
Cases	60	30 - 512	125.50	92.00	0.161	0.872
Control	20	35 - 385	143.50	133.50		

There was no significant correlation could be detected between level of IgA and IgM and platelets count in ITP patients after one month and 3 months of treatment **(Table 3).**

Table (3): **Correlation between platelets count in ITP patients and both IgA ,IgM level after a month and 3 month of treatment**.

Correlations				
	IgA		IgM	
	r	P-value	r	P-value
PLT count after 1 Month	-0.090	0.494	0.010	0.941
PLT count after 3 Months	-0.135	0.303	0.042	0.752

As regard the median level of IgA, it was higher in unresponsive ITP patients than in responders without statistically significant difference **(p-value 0.536).** While, the median level of IgM was higher in responsive group than non-responders with statistically significance **(p-value 0.034) (Table 4).**

Table (4): Comparison between the level of IgA and IgM in ITP patients both responsive and nonresponsive to steroids as a standard treatment.

TTT Response	IgA			Mann-Whitney Test	
	Range	Median	IQR	Z	P-value
Responsive	23 - 428	185.50	105.00	0.619	0.536
Unresponsive	39 - 773	209.00	209.00		
TTT Response	**IgM**			**Mann-Whitney Test**	
	Range	**Median**	**IQR**	**Z**	**P-value**
Responsive	71 - 432	136.00	74.25	2.125	0.034*
Unresponsive	30 - 512	111.50	89.50		

52.5% of responsive patients had above median level of IgA while 55% of unresponsive patients had above median level of IgA with no statistically significant difference (**Table 5).**

Table (5) : Comparison between the level of IgA and treatment response.

IgA	TTT Response						Chi-Square	
	Responsive		Unresponsive		Total			
	N	%	N	%	N	%	X^2	P-value
Below Median	19	47.50	9	45.00	28	46.67	0.033	0.855
Above Median	21	52.50	11	55.00	32	53.33		
Total	40	100.00	20	100.00	60	100.00		

70 % of unresponsive ITP patients had below median level of IgM , while in responsive patients 55% have above median level of IgM with statistically significant difference **(P-value 0.044) (Table 6).**

Table (6): Comparison between the level of IgM and treatment response.

IgM	TTT Response						Chi-Square	
	Responsive		Unresponsive		Total			
	N	%	N	%	N	%	X^2	P-value
Normal Median	0	0.00	1	5.00	1	1.67	6.229	0.044*
Below Median	18	45.00	14	70.00	32	53.33		
Above Median	22	55.00	5	25.00	27	45.00		
Total	40	100.00	20	100.00	60	100.00		

By utilizing ROC curve : The cutoff value IgA ≤ 320, showed about 95% sensitivity, 30% specificity, accuracy about 54.9%, positive predictive value (PPV) 73.1%, negative predictive value (NPV) 75%, patients who had IgA level ≤ 320 were responsive to the standard treatment, while patients who had IgA level > 320 were unresponsive to treatment. The cutoff value IgM > 69, showed sensitivity 100%, specificity 40%, accuracy about 66.9%, PPV 76.9%, NPV 100%, patients who had IgM level > 69 were responsive to standard treatment, while patients who had IgM level < 69 were unresponsive to treatment **(Table 7 & Figures 1, 2).**

Table (7): ROC curve for IgA and IgM between responder and non-responder ITP patients.

ROC curve between Responsive and Unresponsive						
	Cutoff	Sens.	Spec.	PPV	NPV	Accuracy
IgA	≤320	95.00	30.00	73.1	75.0	54.9%
IgM	>69	100.00	40.00	76.9	100.0	66.9%

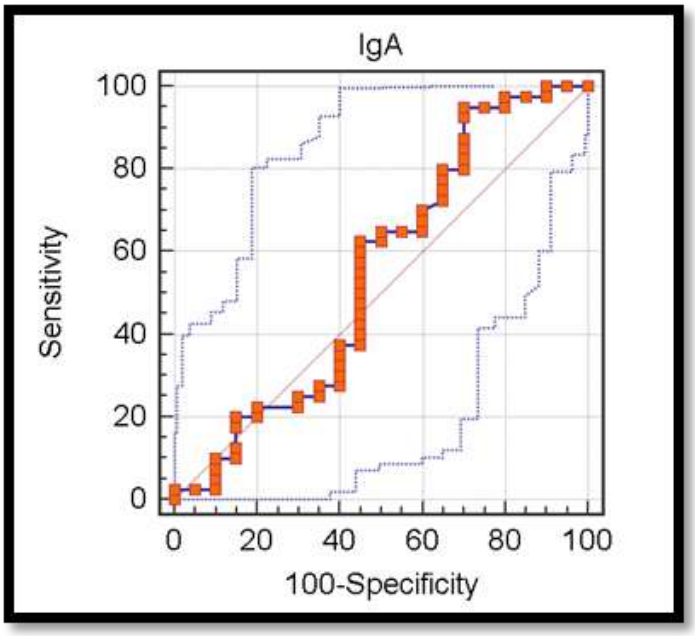

Figure (1): ROC curve of IgA between responder and non-responder ITP patients

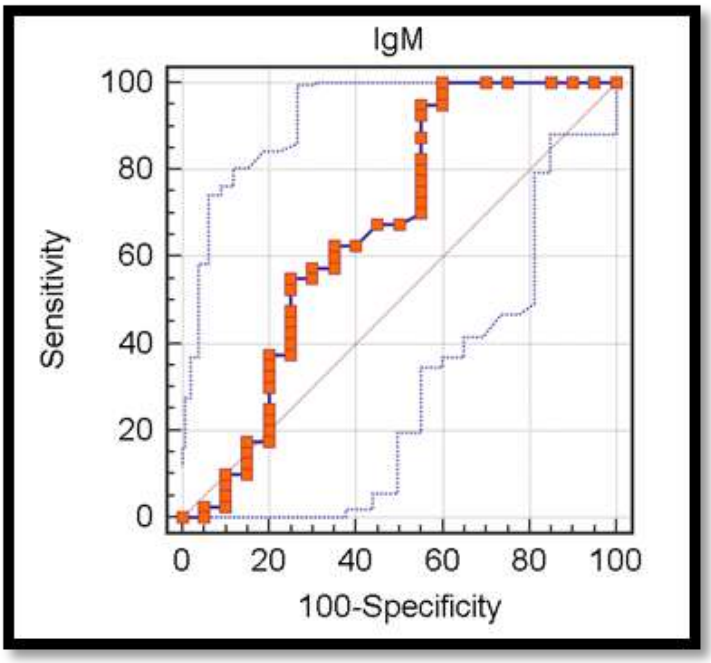

Figure (2): ROC curve of IgM patients between responder and non-responder ITP patients

DISCUSSION

ITP is a common autoimmune disorder resulting in isolated thrombocytopenia with normal bone marrow and the absence of other causes of thrombocytopenia [(5)]. Primary ITP is an acquired disorder where thrombocytopenia results from pathologic antiplatelet antibodies, impaired megakaryocytopoiesis, and T-cell mediated destruction of platelets, with each pathologic mechanism playing different roles in each patient [(6)].

Patients with active ITP have decreased regulatory T-cell population which may explain loss of tolerance, abnormal cytokine profiles, and an altered T helper 1/T helper 2 balance which suggest an underlying immune dysregulation and present a possible targets for novel therapies[(7)]. Patients with common variable immunodeficiency (CVID) with low IgG, IgA, and/or IgM levels have a 22% incidence of autoimmune diseases, such as ITP, supporting the association of abnormal Ig levels with ITP [(4)]. Elevated or decreased serum immunoglobulin (Ig) levels may increase disease severity as represented by failure to respond to treatment. These alterations in Ig levels may represent an inflammatory or activated immune state that makes the disease more difficult to control with specific treatments [(8)].

The aim of this study was to measure the level of Immunoglobulin M (IgM) and Immunoglobulin A (IgA) in ITP patients and to detect their relation to treatment response.

In our study, ITP patients had a higher median level of IgA at presentation in comparison to normal population which was of no statistically significance **(P value= 0.491).** This was consistent with study done by **Stasi *et al.*** [9] which reported that total concentration of IgG and IgA were all higher in patients with ITP than normal population.

Also we found that ITP patients had lower median level of IgM at presentation in comparison to normal population with no statistically significant difference. **(P value= 0.872)** and this was consistent with study done by **Psaila and Bussel** [10] which showed that the level of IgM not usually being high in ITP patients.

In contrary to study done by **McMillan *et al.*** [11] who found that there was statistically significant difference between level of IgM in ITP patients and normal population.

Correlation between platelets count after one month and 3 months of standard steroid treatment in ITP patients and level of IgA and IgM showed no statistically significance. This came in agreement with the study done by **Cines *et al.*** [3] which showed that no statistical significant difference in subjects with low, normal, or high IgA with platelet count or when analyzed in patients with an IgA level above or below the median, suggesting an alternative mechanism for bleeding beyond platelet count.

Although, no statistically significant correlation was detected , there were double logarithmic negative correlation found between IgA and platelet count after one month **(r= -0.090)** and after 3 months **(r= -0.135)** which matched with study done by **Panahi *et al.*** [12] who reported a statistically significant difference between number of platelets and between level of IgM, IgA, and IgG. All three Ig classes show highly significant correlations to the platelet counts **(P< 0.0001),** double logarithmic negative correlations have been found between IgG and platelet count **(r= -0.71)**, IgM and platelet count **(r= -0.84)**, and IgA and platelet count **(r= -0.79).** Statistical analysis using partial correlation and multiple regression methods showed that IgM is predominantly related to the platelet count, whereas IgG and IgA are only of secondary importance.

Another study performed by **McMillan**[13] showed that rare ITP patients have bleeding not due to change in platelet count but due to change in the platelet functions as the immunoglobulins affect the platelet function not the number and this matched with our study results.

We observed in our study that non responder patients had IgA level than that of responder patients but of no statistically significant difference**. (P value=0.536), (P value=0.855)**. This came in agreement with **Provan *et al.*** [14] results as he found that ITP patients who had IgA level above normal tend to be more resistant to standard treatment.

Also, **Wei and Hou**[15] study showed that high level of IgA is a bad index for treatment response to the standard therapy.

The median level of IgM was higher in responsive group than non-responders with statistical significance **(p-value 0.034),** 70 % of unresponsive ITP patients had below median level of IgM, while in responsive patients 55% had above median level of IgM with statistically significant difference **(P-value 0.044)** was detected in our study. This is similar to the results reported by **Provan *et al.*** [14] who showed that ITP patients with low IgM level tend to be more resistant to the standard treatment.

In contrary to the study performed by **Yazdanbakhsh *et al.*** [16] which concluded that low level of IgM is not a good indicator on treatment response to the standard treatment as there are a lot of reasons could affect the level of IgM.

ROC curve was done for determination the best cut off value of IgA and IgM which discriminate between responder and non-responder ITP patients.

ROC curve of IgA showed cutoff value ≤ 320, with 95% sensitivity, 30% specificity, accuracy about 54.9%, PPV 73.1%, NPV 75%. Patients who had IgA <320 (below median) were responsive to treatment while those had IgA >320 (above median) were non-responsive to treatment**.**

This consistent with study done by **Ma *et al.*** [17] which showed that high IgA level is a bad index for treatment response. But in the study done by **Stasi *et al.*** [18] showed that minor elevations in IgA represent an inflammatory state that may be more difficult to address with standard treatments for ITP.

our results were also matched with the results found by **Arnason *et al.*** [19] which showed that patients with above median IgA level is more likely to be resistant to steroids treatment.

ROC of IgM showed cutoff value >69, with100% sensitivity, 40% specificity, accuracy about 66.9%, PPV 76.9%, NPV 100%. Patients who had IgM >69 (above median) were responsive to treatment while those had IgM <69 (below median) were non-responsive to treatment.

This is consistent with a study done by **Cines *et al.*** [3] which showed that population of low IgM also appeared to have ITP more resistant to standard treatment and possibly to splenectomy.

Our results were also matched with a study done by **Arnason *et al.*** [19] which shows that non responder ITP patients tend to have low IgM.

CONCLUSION

It could be concluded that statistically significant difference between responder and non-responder ITP patients as regard the level of IgM denotes that patients who had IgM level below the median were more resistant to steroids, which is the standard treatment in ITP. On the other hand, non-responder patients had IgA level higher than that of responder patients but of no statistically significant difference**.**

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