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The Oxidative Stress And Antioxidant Status Childhood With Immune Thrombocytopenic Purpura

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Introduction

The most common cause of acute onset thrombocytopenia in a healthy child is acute immune thrombocytopenic purpura (ITP). Thrombocytopenia is defined as a platelet count below 150x109 / L (1). Immune thrombocytopenic purpura is an autoimmune disease characterized by immune-mediated platelet destruction in the reticuloendothelial system (RES) (2). Other mechanisms that have been suggested to be responsible include impaired platelet production (3), complement-dependent mechanism thrombolysis (4), and antibody-dependent oxidant product hydrogen peroxide causing cellular damage (5).

Approximately 75-80% of the clinical cases of immune thrombocytopenic purpura have been classifiied as acute (self-limiting within six months) and 20-25% as chronic (lasting more than six months) (6)

The aim of the treatment is to inhibit the development of antibodies against platelets by suppressing the immune system and stop the breakdown of platelets in the spleen. Corticosteroids, intravenous immunoglobulin (IVIG), anti-D immunoglobulin and rituximab can be used in the treatment (7). High dose MP or IVIG is preferred as the initial treatment for childhood ITP. Since there is no difference in success rates in treatment, the choice is made on the basis of costs and side effects.

Oxidative damage plays a role in the pathogenesis of autoimmune diseases. Oxidative stress and free radicals have been suggested to be responsible for the pathogenesis and prognosis of ITP. Increased lipid peroxidation and decreased antioxidant capacity in ITP may play a significant role on antibodies bound to membrane lipids and platelet destruction (8).

In the literature, there is little information about oxidative stress and antioxidant defense mechanism in ITP (8). The aim of this study is to investigate the effects of oxidative stress level and different treatment options on antioxidant capacity in acute and chronic ITP and to show that whether the disease would be acute or chronic type can be predicted and the most appropriate choice of treatment can be defined by depending on the oxidative stress index (OSI) obtained during the diagnosis phase.

Materials and Methods

The study group consisted of 44 patients who were diagnosed with ITP in the outpatient clinic of the Department of Pediatric Hematology, Faculty of Medicine, Fırat University. The patients were divided into two groups as Group I: Acute ITP [n: 33] and Group II: Chronic ITP [n: 11]). According to the treatment, acute ITP group was divided into subgroups of Group Ia (MP [n: 21]), Group Ib (IVIG [n: 6]), Group Ic (MP + IVIG [n: 6]) and chronic ITP group was divided into subgroups of Group IIa (MP [n 5]), Group IIb (IVIG [n = 6]). Parents of the children diagnosed with immune thrombocytopenic purpura were informed about the study and their written consent was obtained. Approval was received by Fırat University

Clinical Research Ethics Committee.

Acute ITP was diagnosed by isolated thrombocytopenia (<150x109 / L), increased or normal megakaryocytes in the bone marrow, thrombocyte-associated IgG elevation, familial thrombocytopenia, drug intake, active inflammation, lack of blood transfusion or splenomegaly, and direct coombs test, and negative antinuclear antibody (22,23). Thrombocytopenia lasting longer than 6 months was defined as chronic ITP (2,4).

After the patients who admitted to the outpatient clinic had been diagnosed and given written permission, MP (30 mg / kg / day 3 days, 20 mg / kg / day 4 days, oral) and IVIG (1 g / kg / day 2 days) treatments were given (2,3). Drug preference was randomized. Total antioxidant capacity (TAOC) of the patients, who underwent treatment in our clinic, were measured before and after treatment.

The data were evaluated by using SPSS software. Data were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) and post-ANOVA tests were used to compare treatment modalities between groups and within groups, a value of $p < 0.05$ was considered statistically significant.

Results

Group I consisted of 33 cases, including 17 (53%) females and 16 (47%) males. Group II consisted of 11 cases, including 4 (36%) females and 7 (74%) males. The socio-demographic characteristics of the cases are given in Table I.

Pre- and post-treatment oxidative / anti-oxidative parameters of Group I, Group II and total cases are given in Table II. There were statistically significant differences between pre- and post-treatment levels of total peroxide, TAOC and OSI, in Group I ($p < 0.05$, $p < 0.001$, $p < 0.05$, respectively). There were statistically significant differences between pre and post treatment levels of total peroxide and OSI, in Group II ($p < 0.05$). There was a statistically significant difference between Group I and Group II in terms of pre-treatment levels of total peroxide ($p < 0.05$). There was a statistically significant difference between pre and post treatment levels of total peroxide, TAOC and OSI, in the total ITP group ($p < 0.05$, $p = 0.001$, $p = 0.001$, respectively).

The oxidative / anti-oxidative parameters of Group I according to treatment modalities are given in Table III. There were statistically significant differences between the pre and post treatment levels of total peroxide, TAOC and OSI, in Group Ib ($p < 0.05$). There was no statistically significant difference between pre and post treatment levels of total peroxide, in the other groups.

The oxidative / antioxidative parameters of Group II according to the treatment modalities are given in Table IV. There were statistically significant differences between pre and post treatment levels of total peroxide, TAOC and OSI in Group IIa ($p < 0.05$). There were no statistically significant differences between the pre and post treatment levels of total peroxide, TAOC and OSI, in Group IIb.

Discussion

ITP is an autoimmune disease that results in acute or chronic isolated thrombocytopenia. Oxidative stress, defined as the deterioration of the balance between oxidant and antioxidants in favor of oxidants, may play a role in the pathogenesis of autoimmune diseases (8).

In our study we found statistically significant differences between the pre and post treatment levels of total peroxide, in acute and chronic ITP groups ($p < 0.05$). In acute IT group, post treatment levels of total peroxide and OSI were significantly decreased and TAOC levels were significantly increased when compared to the pre treatment levels ($p < 0.05$, $p < 0.001$). In

chronic ITP group, the post treatment levels of total peroxide and OSI were significantly lower ($p < 0.05$), while there was no statistically significant difference between the pre and post treatment levels of TAOC. When all patients were evaluated together, we found that the levels of total peroxide and OSI decreased ($p < 0.05$, $p = 0.001$, respectively) and TAOC levels increased significantly after treatment ($p = 0.001$).

Polat et al. (8) investigated levels of lipid peroxidation, glutathione and ascorbic acid, in adult ITP patients. They found that lipid peroxidation levels were higher and glutathione and ascorbic acid levels were lower in the study group than the control group ($p < 0.05$). Akbayram et al. (9) Found that malondialdehyde, total oxidant status and OSI were increased and TAOC decreased in children with acute and chronic ITP. Similarly, in our acute and chronic ITP cases and in our total patient group, oxidative parameters were significantly lower and anti-oxidative parameters were significantly higher after treatment.

When the acute ITP cases were compared according to the treatment modalities, we found that total peroxide levels decreased significantly after treatment in IVIG group ($p < 0.05$). Total peroxide levels decreased also in MP and MP + IVIG groups, however the difference was not statistically significant. There was a statistically significant increase in TAOC after IVIG treatment ($p < 0.05$), whereas the increase in TAOC in MP and MP + IVIG groups was not statistically significant. There was a statistically significant decrease in OSI ($p < 0.05$) after treatment in IVIG group, whereas the decrease was not statistically significant, in MP and MP + IVIG groups. Our results suggest that IVIG treatment is more effective than MP treatment in decreasing the oxidative parameters and increasing the antioxidant parameters, in acute ITP cases.

When we compared the chronic ITP cases according to the treatment modalities we found that total peroxide levels were significantly decreased in MP and IVIG groups, however the decrease was statistically significant only in the MP group ($p < 0.05$). OSI values also decreased after both modalities of treatment, however the difference was statistically significant only in MP group ($p < 0.05$). There was an increase in TAOC after treatment in both modalities, but the difference was statistically significant only in the MP group ($p < 0.05$). these results suggest that MP treatment is more effective than IVIG treatment, in decreasing oxidative stress and increasing antioxidant system, in chronic ITP cases.

Conclusion

In our study, we found that total peroxide and OSI levels in acute and chronic ITP were higher before treatment and we think that oxidative damage may play a role in the pathogenesis of ITP. We found statistically significant differences between total peroxide levels before and after treatment in acute and chronic ITP cases. In conclusion, we think we can predict whether the disease would be acute or chronic, by measuring plasma oxidant parameters at the beginning of the disease. We found significant decreases in the levels total peroxide and OSI and significant increases in TAOC levels with IVIG treatment in acute ITP and in with MP chronic ITP. We suggest to prefer IVIG treatment in cases we predicted to be acute ITP and MP treatment in cases we predicted to be chronic ITP.

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Table 1: Sociodemographic characteristics of the patients

	Group I (acute)	Group II (chronic)	Total
Age(mean ± SD, months) (low-high)	66.80±7.48 (2-192)	82.45±18.99 (2-168)	70.71±7.30 (4-192)
Gender n (%)			
Male	16 (47)	7 (74)	23 (52)
Female	17 (53)	4 (36)	21 (48)
Duration of the disease (months) Low- High	1.37±0.17 0.5-5	36. 27±5.35 16-60	10.10±2.64 0.5-60

n: number, mean: arithmetic mean, SD: Standard Deviation

Table 2: Oxidative/Antioxidative parameters of the patients

	GROUP I (Acute ITP)			GROUP II (Chronic ITP)			TOTAL		
	Pre-treatment	Post-treatment	p	Pre-treatment	Post-treatment	p	Pre- treatment	Post-treatment	p
Total peroxide ($\mu molH_2O_2/L$)	49.70±2.78*	42.00±3.60	<0.05	60.80±3.77*	49.38±3.76	<0.05	52.49±2.38	43.90±2.88	<0.05
Low-High	21.89-84.00	10.00-79.48		28.50-72.50	23.00-71.37		21.89-84.00	10.00-79.48	
TAOC (mmolTroloxequivalent/L)	0.99±0.01	1.13±0.03	<0.001	1.04±0.01	1.07±0.03	>0.05	1.00±0.01	1.12±0.02	0.001
Low- High	0.73-1.18	0.93-1.73		0.93-1.16	0.96-1.29		0.73-1.18	0.93-1.73	
OSI(AU)	5.13±0.34	3.92±0.37	<0.05	5.83±0.35	4.67±0.43	<0.05	5.31±0.27	4.10±0.30	0.001
Low- High	2.07-9.78	0.81-8.00		2.50-6.90	2.18-7.41		2.07-9.78	0.81-8.00	

*: Comparison of pre-treatment levels of total peroxide in Group 1 and Group II ($p<0.05$)

TAOC: Total Antioxidant Capacity, OSİ: Oxidative Stress Index

Table III. Oxidative / anti-oxidative parameters in patients with acute ITP

	Pre treatment			Post treatment			P<0.05
	MP(Ia)	IVIG(Ib)	MP+IVIG (ıc)	MP (Id)	IVIG (Ie)	MP+IVIG (If)	
Total peroxide (µmol H2O2/L)	52.59±5.27	46.82±3.80	50.12±5.38	47.66±5.85	31.26±4.30	51.28±8.02	Ib-Ie
Low- High	21.89-84	24.00-71.00	35.48-69.64	10.00-79.48	11.78-61.27	19.17-71.00	
TAOK (mmolTrolox equivalent/L)	1.02±0.02	0.98±0.03	0.95±0.03	1.07±0.36	1.22±0.05	1.06±0.05	Ib-Ie
Low- High	0.89-1.18	0.73-1.18	0.82-1.06	0.93-1.36	1.02-1.73	0.96-1.30	
OSI (AU)	5.23±0.58	4.95±0.55	5.35±0.76	4.58±0.61	2.68±0.41	5.37±0.83	Ib-Ie
Low- High	2.07-9.49	2.43-9.75	3.51-8.52	0.96-8.05	0.81-5.89	1.48-6.97	

Table 4. Oxidative / anti-oxidative parameters in patients with chronic ITP

	Pre treatment		Post treatment		P<0.05
	MP (IIa)	IVIG (IIb)	MP (IIc)	IVIG (IId)	
Total peroxide (µmol H2O2/L)	60.43±8.14	61.27±2.72	45.00±6.05	53.03±4.66	IIa-IIc
Low- High	28.50-72.57	53.00-59.00	23.00-58.00	39.00-71.30	
TAOK (mmolTrolox equivalent/L)	1.07±0.02	1.02±0.02	1.11±0.05	1.04±0.04	IIa-IIc
Low- High	1.00-1.16	0.93-1.10	0.99-1.29	0.96-1.25	
OSI (AU)	5.62±0.77	6.00±0.24	4.05±0.57	5.18±0.58	IIa-IIc
Low- High	2.58-6.90	5.18-6.69	2.18±5.61	3.45-7.41	