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LEVELS OF MALONDIALDEHYDE, GLUTATHIONE AND ASCORBIC ACID IN IDIOPATHIC THROMBOCYTOPAENIC PURPURA

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## LEVELS OF MALONDIALDEHYDE, GLUTATHIONE AND ASCORBIC ACID IN IDIOPATHIC THROMBOCYTOPAENIC PURPURA

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### ABSTRACT

**Background:** Idiopathic thrombocytopenic purpura (ITP) is an autoimmune disorder, which causes an acute or chronic thrombocytopenia, and may result in potentially life-threatening haemorrhage. Oxidative damage may be involved in the pathogenesis of autoimmune diseases. Antibodies to bind to membrane lipids and platelet destruction may play a role on lipid peroxidation in ITP.

**Objectives:** To investigate the possible role of lipid peroxidation and antioxidants in patients with ITP.

**Design:** The levels of plasma and erythrocyte malondialdehyde (MDA), erythrocyte glutathione and ascorbic acid were analysed in patients with ITP.

**Methods.** The MDA levels were performed according to the method of Bidlack WR. Plasma MDA, erythrocyte glutathione and ascorbic acid levels were carried out according to the methods of Ohkawa H, Beutler E and Bauer JD, respectively.

**Results:** The erythrocyte and plasma MDA levels in patients with ITP were found to be  $9.52 \pm 4.65$ ,  $3.03 \pm 1.44$  ( $p < 0.001$ ) and in control group were found to be  $2.49 \pm 0.57$ ,  $1.03 \pm 0.28$  nmol/ml ( $p < 0.001$ ), respectively. Erythrocyte glutathione was found to be  $3.71 \pm 0.82$ ,  $6.26 \pm 0.66$   $\mu$ mol/gr Hb ( $p < 0.001$ ). Ascorbic acid levels of these groups were  $1.09 \pm 0.25$ ,  $1.70 \pm 0.33$  mg/dl ( $p < 0.001$ ).

**Conclusion:** The oxidative damage is involved in the pathogenesis of ITP. In patients with ITP, the platelet destruction and bleeding may play significant role on elevation of lipid peroxidation and reduction of antioxidant capacity. Further studies on oxidant and antioxidant status of ITP are also needed to confirm these results.

### INTRODUCTION

Idiopathic thrombocytopenic purpura (ITP) is characterised by a variable reduction platelet counts due to the presence of platelet autoantibodies resulting in petechiae, purpura, and mucocutaneous bleeding(1). This disorder is a self-limited/in many acute forms that follows a viral infection in children, and a chronic disorder with no apparent predisposing cause in adults(1,2). ITP is believed to occur when platelets undergo premature destruction as a result of autoantibody or immune complex deposition on their membranes. Antibodies in ITP sera have been demonstrated to bind to glycoproteins, glycosphingolipids and cardiolipin(3-5).

Oxidative damage may be involved in the pathogenesis of major diseases. Inactivation and removal of reactive oxygen species depend on reactions involving the antioxidative system. The capacity is determined by a dynamic interaction between individual

components, which include ascorbic acid, reduced glutathione and several antioxidative enzymes(6,7). Monitoring free radicals can be performed by measuring the generation of free radicals in patients, as well as their antioxidant capacity or often the damages resulting of the stress(8). Antioxidant capacity of the subject can be estimated by measuring the status of each antioxidant of plasma or erythrocytes. Generally the evaluation of oxidative stress is performed by the determination of damaged biological products. A number of lipid derivatives can be measured: conjugated dienes, hydroperoxydes, aldehydes (malondialdehyde). The most controversial but the most used indicator is the determination of malonaldehyde(9). Lipid peroxidation in blood platelets or in certain tissues plays an important role in the platelet metabolism(10). The lipid molecules are arranged as a continuous double layer of the membrane surrounding cells and intracellular organelles(11). The oxygen molecule can attack polyunsaturated fatty acids and form lipid peroxy

radicals(12). Iron as a transient metal that is increased in many tissues after haemorrhage can be involved in lipid peroxidation. The iron ion-oxygen complexes are also claimed to initiate this pathology(13).

The oxidative stress and free radical status may be involved in the pathogenesis and prognosis of idiopathic thrombocytopenic purpura. In this study, the measurements of lipid peroxidation and related antioxidants were planned in samples which were taken from patients with ITP for explanation of their involvement in this disorder. We aimed to investigate the erythrocyte and plasma malondialdehyde (MDA), erythrocyte glutathione and ascorbic acid levels in patients with ITP.

#### MATERIALS AND METHODS

Blood samples were collected from 32 patients with diagnosed ITP (21 females and 11 males; age range, 21-42 years) and 31 controls (18 females and 13 males; age range, 25-46 years). Patients were excluded from the study where any of the following criteria were present: smoking, alcoholism, chronic diarrhoea, and malnutrition. A control group, from the same region that was approximately the same age as the patients in our study groups and subjectively appeared to be healthy, was selected randomly.

Venous blood samples were collected from all individuals into tubes containing K3 EDTA. Erythrocytes were separated by cellulose colon (Sigma cell type 50, Sigma Chemical Company, St Louis, MO). All samples were rapidly taken to laboratory for analysis.

The levels of erythrocyte MDA were performed as described by Bidlack and Tappel(14). Plasma MDA, erythrocyte glutathione and vitamin C were estimated by using the methods of Ohkawa *et al*(15), Beutler(16) and Bauer(17), respectively.

**Determination of MDA:** The determination malondialdehyde (MDA) levels as an index of lipid peroxidation were determined by thiobarbituric acid (TBA) reaction. The principle of the method depends on measurement of the pink colour produced by interaction of barbituric acid with malondialdehyde elaborated as a result of lipid peroxidation. The coloured reaction 1,1,3,3,- tetraethoxy propane was used as the primary standard(15).

**Measurement of reduced glutathione:** Virtually all of the non-protein sulfhydryl of red cells is in the form of reduced glutathione (GSH). 5,5'-Dithiobis(2-nitrobenzoic acid)(DTNB) is a disulfide compound that is readily reduced by sulfhydryl compounds, forming a highly coloured yellow anion. The optical density is measured at 412 nm(16).

**Measurement of ascorbic acid:** The levels of ascorbic acid were measured in 30 minutes and measurement of this test depends on the fact that reduces the dye, 2,6 dichlorophenol into a colourless compound. The decrease in the colour intensity was monitored at 520 nm(17).

**Statistical analysis:** Data were expressed as mean and the standard deviation of the mean (SD). Statistical comparisons were made using analysis of Students' t test for MDA, glutathione and ascorbic acid content, with  $p < 0.05$  considered as significant. Further analyses were performed using SPSS 9.00 packed program for Windows.

#### RESULTS

The platelet counts of patients with ITP were lower than  $20 \times 10^9/L$  ( $8-18 \times 10^9/L$ ). The control results were between  $250-300 \times 10^9/L$ . Statistical significances were not found between WBC counts ( $7.13 \pm 2.86 \times 10^9/L$  and  $6.84 \pm 1.58 \times 10^9/L$ ), reticulocyte counts ( $0.72 \pm 0.33\%$  and  $0.65 \pm 0.42\%$ ), haemoglobin levels ( $14.0 \pm 2.7$  and  $15.6 \pm 2.2$  gr/dl) and total bilirubin levels ( $0.68 \pm 0.32$  and  $0.53 \pm 0.24$  mg/dl) between patients and control group. The erythrocyte and plasma MDA levels in patients with ITP were found to be  $9.52 \pm 4.65$ ,  $3.03 \pm 1.44$  ( $p < 0.001$ ) and in control group were found to be  $2.49 \pm 0.57$ ,  $1.03 \pm 0.28$  nmol/ml ( $p < 0.001$ ), respectively (Table 1 and Figure 1). Erythrocyte glutathione was found to be  $3.71 \pm 0.82$ ,  $6.26 \pm 0.66$   $\mu\text{mol/gr Hb}$  ( $p < 0.001$ ). Ascorbic acid levels of these groups were  $1.09 \pm 0.25$ ,  $1.70 \pm 0.33$  mg/dl ( $p < 0.001$ ) (Table 2 and Figure 2).

**Table 1**

*MDA levels in erythrocyte and plasma of patients and control group*

	Erythrocyte MDA (nmol/ml)	Plasma MDA (nmol/ml)
Patient group	$9.63 \pm 4.51$	$2.36 \pm 0.46$
Control group	$3.26 \pm 1.35$	$1.10 \pm 0.23$
Significance	( $p < 0.001$ )	( $p < 0.001$ )

The results are expressed in terms of arithmetic means (X) standard deviation (SD), P = values of significance with difference of each group.

**Table 2**

*Erythrocyte glutathione and ascorbic acid levels in patients and control groups*

	Glutathione ( $\mu\text{mol/gr Hb}$ )	Ascorbic acid (mg/dl)
Patient group	$3.83 \pm 0.86$	$1.12 \pm 0.22$
Control group	$6.45 \pm 0.61$	$1.75 \pm 0.28$
Significance	( $p < 0.001$ )	( $p < 0.001$ )

The results are expressed in terms of arithmetic means (X) standard deviation (SD), P = values of significance with difference of each group

Figure 1

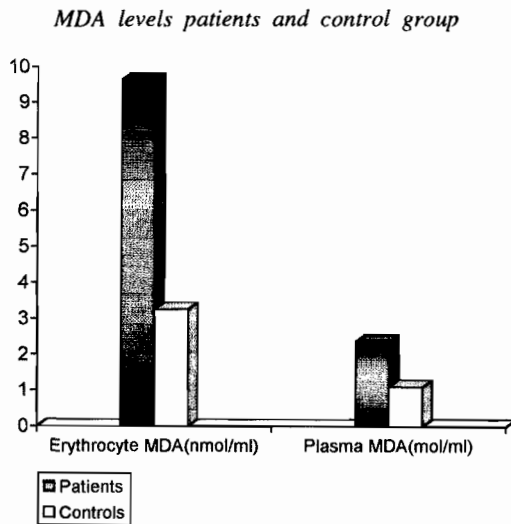
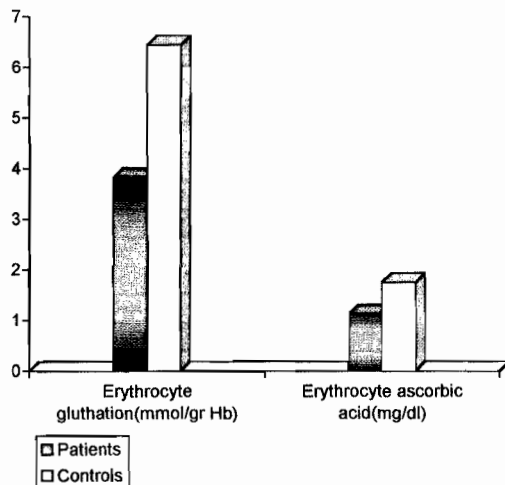


Figure 2

*Erythrocyte glutathione and ascorbic acid levels in patients and control groups*



## DISCUSSION

This study, investigated the levels of lipid peroxides, glutathione and ascorbic acid in patients with ITP. The malondialdehyde levels, product of lipid peroxidation, were found to be elevated in patient group when compared to control group. On the other hand, the glutathione and ascorbic acid levels were found to be lower in patient group when compared to control group.

Platelet-specific antibodies bind to platelets that are then rapidly cleared from the circulation cause the ITP syndrome. Thrombocytopenia occurs when platelets undergo destruction as a result of these antibodies or immune complex deposition on their membranes(2,3). Antibodies to glycoproteins, glycosphingolipids and cardiolipin of membranes have

been demonstrated in sera of patients with this disorder(4,5) Lipid-laden macrophages containing phospholipid, cholesterol and ceroid probably derived from phagocytosed platelets(18). The secretion of platelet contents by platelet destruction and phagocytosis of platelets by macrophages may cause some effects in the body.

Further it is known that oxidative damage may be involved in the pathogenesis of major diseases(19). The free oxygen molecules and oxidant agents can react with polyunsaturated fatty acids in membranes to form lipid peroxy radicals(12). Lipid peroxidation also normally occurs at a low level in all tissues(20). The cells have a number of ways to protect against the constant threat of the radicals produced in lipid peroxidation(8). These protection mechanisms include glutathione, nitric oxide, and several free radical scavengers(10,21,22). Glutathione, and ascorbic acid as free radical scavengers, play important roles in protecting cells against oxidative damage(22,23). We found reduced GSH and ascorbic acid levels in patients when compared to control samples. It is known that free radicals have several roles in pathogenesis of autoimmune diseases(24). In this study, presence of lipid peroxidation and reduced levels of glutathione and ascorbic acid indicated that free radicals play important roles on pathogenesis of ITP.

Haemorrhage represents the most serious complication and previous history of haemorrhage increase the risk of severe bleeding in ITP. If platelet counts are below  $20 \pm 10g/l$ , patients with ITP have an increased risk of life-threatening bleeding(25). After haemorrhage iron metal is increased in the involved tissues. Iron as a transient metal can be involved in lipid peroxidation. This process is mediated highly reactive hydroxyl radical. Several iron ion-oxygen complexes are also claimed to initiate peroxidation(13). In our investigation, we have found elevated levels of lipid peroxides in the erythrocyte and plasma samples of patients with ITP when compared to controls.

The platelet destruction and bleeding in patients with ITP(26) cause production of free oxygen radicals. Overproduction of these radicals results in lipid peroxidation and increase in malondialdehyde levels. Nevertheless, glutathione and ascorbic acid has been known to have reduction effects on free radical levels. In our study, glutathione and ascorbic acid levels in patients are reduced when compared to controls. The reduced levels of glutathione in patients are revealed that this compound has been oxidized by increased free radicals.

It may be suggested that free radicals have significant roles on pathogenesis of ITP. Ascorbic acid levels are not adequate as free radical scavenger in these patients. The levels of ascorbic acid may elevate, thus antioxidant responses may partly activate. Many oxidant agents are increased in ITP and this antioxidant compound is used in patients with ITP (27,28).

## CONCLUSION

We have found elevated levels of lipid peroxides in the erythrocyte and plasma samples of patients with ITP when compared to controls. The glutathione and ascorbic acid levels in patients are reduced when compared to controls. The oxidative damage may be involved in the pathogenesis of ITP. Antibodies that bind to membrane lipids and platelet destruction may play a role on lipid peroxidation. After haemorrhage iron metal may also increase and this transient metal can mediate highly reactive hydroxyl radical. Ascorbic acid levels are not adequate for its free radical scavenger function in untreated ITP patients. Further studies on oxidant and antioxidant status of ITP are also needed to confirm these results.

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