

Erythrocyte glutathione peroxidase levels in hyperthyroidism—effect of treatment with methimazole

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Abstract

Background: The hypermetabolic state seen in hyperthyroidism causes increased oxygen consumption resulting in an increased formation of Reactive Oxygen Species leading to tissue injury. Increased oxidative stress characterised by elevated levels of free radicals and diminished antioxidant status has been described in animal models as well as in patients with hyperthyroidism. Thyroid gland is characterized by high activity of the antioxidant enzyme Glutathione Peroxidase (GSHPx) and this has been studied much reflecting the imbalance of antioxidant enzyme systems in thyroid dysfunction. However, contradictory results have been obtained regarding plasma and erythrocyte GSHPx levels in hyperthyroidism. Studies have also shown that antithyroid drugs like Methimazole and propyl thiouracil have antioxidant effects. How the treatment with these drugs affect the antioxidant status is also a matter of debate. Hence, the present study aimed to evaluate the erythrocyte Glutathione Peroxidase levels in hyperthyroidism in comparison with normal controls and evaluate the effect of methimazole treatment.

Methods: The study was carried out in patients attending the special investigation laboratory in the department of Biochemistry of our college for assessment of their thyroid function. The study groups included: Group 1 (n = 40) consisting of healthy euthyroid controls. Group 2: (n=30) clinically diagnosed hyperthyroid patients, 17 were untreated fresh cases (series1) and 13 patients were undergoing treatment with methimazole for the past one month (series2). Erythrocyte Glutathione peroxidase enzyme levels were analyzed in blood samples collected from these subjects along with thyroid function tests T3, T4 and TSH.

Results & Conclusion: Comparison of the mean values showed that the elevated levels of thyroid hormones have come down in the Methimazole (MMI) treatment group as expected. There were very low TSH levels in the untreated group whereas in treated group TSH levels were at the lower limit of normal range. The mean GSH Px levels in untreated hyperthyroid patients were significantly elevated ($6.970 \pm 0.302 \mu\text{moles/L}$) compared to normal controls ($6.117 \pm 0.133 \mu\text{moles/L}$). The GSHPx levels in the treated group were closer to the GSHPx values of the control group ($6.125 \pm 0.482 \mu\text{moles/L}$).

Keywords: Hyperthyroidism, Methimazole, Glutathione peroxidase, Oxidative stress, Antioxidant status, Reactive Oxygen Species

Introduction

Glutathione peroxidase (GSHPx) is a member of the antioxidant family and it plays a significant role in the thyroid gland where high amounts of H_2O_2 are produced during thyroid hormone biosynthesis.⁽¹⁾ Thyroid gland is characterised by high activity of GSHPx enzyme to scavenge this H_2O_2 and it is a major determinant of the antioxidant status of thyroid.⁽²⁾ Hyperthyroidism leads to increased rate of the basal metabolism due to associated increase in concentration of thyroid hormones and there is a rise in the total consumption of oxygen. As a result, in untreated hyperthyroid patients and also in animal models of hyperthyroidism, there is increased formation of reactive oxygen species (ROS) which leads to oxidative stress and damage to biomembrane lipids.^(3,4) To counter the effects of this, antioxidant defence mechanisms are activated. Results obtained from many studies indicated an enhanced generation of ROS and a marked increase in the intra cellular antioxidants in hyperthyroidism.^(5,6) Marcocci et al (2012) have stated that usually measured parameters of oxidative stress in serum/plasma or erythrocytes such as hydrogen peroxide, lipid peroxides, conjugated dienes, thiobarbituric acid-reacting substances (TBARS), MDA etc. are elevated in untreated hyperthyroidism compared with normal euthyroid subjects.⁽⁷⁾ However, there has been a discrepancy regarding blood levels of

antioxidants with some studies showing higher levels and some others demonstrating decreased levels of glutathione peroxidase and other enzymatic antioxidants in patients with hyperthyroidism. Whether the scavenging antioxidant enzymes get activated in an attempt to counter the oxidative stress thus causing increased blood levels or are they getting used up or exhausted leading to decreased blood levels needs to be examined further. Plasma and erythrocyte levels of glutathione peroxidase were found to be decreased in the studies by Janus Bednarek et al⁽⁸⁾ (2004) and Abdellah A et al⁽⁹⁾ (2011). In the study by Boguslavskaja et al⁽¹⁰⁾ (1997) GSHPx levels were found to be decreased in the thyroid tissue in patients with hyperthyroidism. Thus, controversial results have been found about the status of glutathione peroxidase enzyme in hyperthyroidism and its response to treatment.

The antithyroid drug methimazole (MMI) is used to treat patients with hyperthyroidism. The major action of MMI is to inhibit the synthesis of thyroid hormone. MMI also has antioxidant and immunomodulatory effects on thyrocytes and immune cells.⁽¹¹⁾ Treatment with methimazole resulted in normalisation of the free radical and the antioxidant activity indices. Studies show that methimazole therapy caused a relief in oxidative stress as reflected by significantly decreased TBARS levels, and a selective modification of the anti-oxidant profile in

hyperthyroidism.^(12,13) Achievement of euthyroidism leads to improvement or normalisation of the antioxidant enzymes. Contradictory results have also been found about the antioxidant response to treatment of hyperthyroidism.⁽⁵⁾ Antioxidant supplementation in the treatment of Graves disease and its effect on GSHPx activity was also examined by some investigators⁽¹⁴⁾ who found that the activity of whole blood GSHPx increased during treatment but the potential of such therapy needs to be explored further.

To fill such lacunae in knowledge about the antioxidant status in hyperthyroidism and its response to treatment, the present study was undertaken. The aim of this study was to evaluate the erythrocyte Glutathione Peroxidase levels in hyperthyroidism- in methimazole treated and untreated patients.

Materials and Methods

The study was carried out after getting approval from the institutional ethical committee in patients attending the special investigation laboratory, of our Medical College for assessment of their thyroid function. **Study Groups:** The present study was conducted on: Group 1 (n = 40) consisted of healthy euthyroid controls, Group 2: (n=30) clinically diagnosed hyperthyroid patients, 17 were untreated fresh cases (series 1) and 13 patients were undergoing treatment with methimazole (series2) for past one month. A detailed history of each subject which includes personal history, family history, past history, and treatment history was taken. Consent was obtained and blood collected by venepuncture, using disposable syringe and needle. One part of blood collected was transferred to bottle containing EDTA as anticoagulant for glutathione peroxidase enzyme assay, and rest to plain bottle, for determination of thyroid function tests. Blood was allowed to clot and serum was separated using a REMI centrifuge. Following parameters were analysed in each sample. In erythrocytes- Glutathione peroxidase enzyme, Serum-Thyroid function tests T3, T4, TSH. Commercially procured kits were used for the determination of these parameters. T3, T4 and TSH were determined using ELISA and GSHPx estimations were done in semi autoanalyzer.

Assay of glutathione peroxidase enzyme: Glutathione peroxidase (GSHPx) (GSH: H₂O₂ oxidoreductase, E.C.1.11.1.9): Glutathione peroxidase activity was assayed in erythrocytes, using cumene hydroperoxide as substrate. The method followed is as described by O' Brien and Little^(15,16) which is a modification of the method described by Paglia and Valentine⁽¹⁷⁾ (1967). Reagents: Tris HCl buffer, reduced glutathione, NADPH, EDTA, Cumene hydroperoxide and saponin. All the reagents were purchased from sigma. Deionized water was used for making all solutions. Reaction mixture (1ml) contained 0.120 mM NADPH, 0.1 M Tris –HCl buffer (pH 7), 0.25 mM GSH, 0.2 mM cumene hydroperoxide, 3mM EDTA, and a

large excess of GSSG reductase (which is contributed from the hemolysate). Glutathione peroxidase catalyses the reduction of hydroperoxides, such as hydrogen peroxide using reduced glutathione. In this procedure, the enzyme is coupled to NADPH via GSSG reductase and the rate of NADPH oxidation is measured spectrophotometrically at 340 nm. Analysis of the results was done by applying Student's t Probability Distribution.

Results

In the present study, among the 30 hyperthyroid patients, 17 were untreated (series1) and 13 patients were undergoing treatment with methimazole (series2). Control group consisted of 40 healthy euthyroid patients.

Table 1: Mean Values of T3, T4 and TSH in the study groups

Group	Control	Untreated	MMI Treatment
N	40	17	13
Mean T3 ng/dL	113.93 ± 3.35	224.7 ± 35.82	187.36 ± 19.36
Mean T4 µg/dL	8.25 ± 0.291	18.74 ± 4.17	13.94 ± 1.81
Mean TSH µIU/L	2.49 ± 0.207	0.030 ± 0.09	0.545 ± 0.205

The mean GSH Px levels in untreated hyperthyroid patients were significantly elevated (6.970± 0.302µmoles/L) compared to normal controls (6.117 ± 0.133) as shown in Fig. 1.

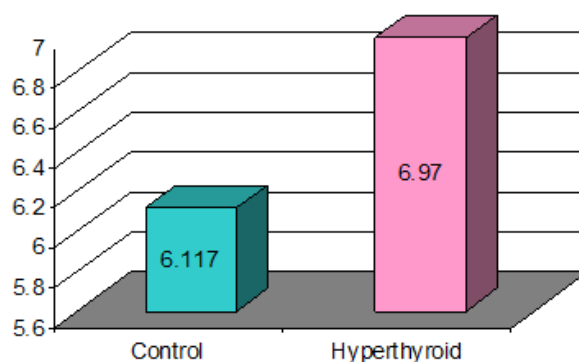


Fig. 1: Mean GSHPx levels in the study groups

Table 2: Mean Values of GSHPx in methimazole treated and untreated patients in comparison with normal controls

Group	Control	Hyperthyroid (Untreated)	Hyper thyroid (after MMI Treatment)
N	40	17	13
Mean GSH Px µmoles/L	6.117 ± 0.133	6.970± 0.302	6.125 ± 0.482

Discussion

In the present study, serum T3 and T4 levels were very significantly elevated in the hyperthyroid group when compared with that of the control group. The TSH levels were decreased very significantly. These findings of significantly increased T4 and T3 with low TSH values confirmed the clinical diagnosis of hyperthyroidism. Comparison of the mean values showed that the elevated T3 and T4 levels have come down in the Methimazole (MMI) treatment group. There were very low TSH levels in the untreated group whereas in treated group TSH levels were at the lower limit of normal range. Thus we can infer that methimazole treatment has been effective in normalising the thyroid function in these patients. The Glutathione Peroxidase levels were found to be increased in the group of untreated hyperthyroid patients whereas in the treated group it decreased and approached close to the values of the control group. The higher GSHPx values can be considered to mirror an increase in the activity of antioxidant defence enzymes, which occurs as an innate response to counteract the hyperthyroidism-induced increased ROS generation. From this we can infer the presence of an oxidative stress condition in hyperthyroidism which is getting alleviated by methimazole therapy.

Seven et al (1996) found that Superoxide dismutase (SOD), glutathione peroxidase (GSHPx) and glutathione (GSH) values were significantly increased in experimentally induced hyperthyroidism in rats compared to the control group.⁽¹⁸⁾ Vitamin E supplementation to these hyperthyroid rats induced a significant decrease in GSHPx activity and a significant increase in GSH levels which might be due to the antioxidant effect of vitamin E. They later reproduced the results in humans also- hyperthyroid patients were observed to have significantly higher levels of serum lipid peroxidation end products (TBARS) and antioxidant status parameters- glutathione and CuZnSOD than controls. These investigators have suggested that a selective modification of the antioxidant profile is happening in hyperthyroidism.⁽¹⁹⁾ In another study, the increase in cellular respiration mediated by the hormone thyroxine in hyperthyroidism, has been associated with a decrease in mitochondrial glutathione and increase in mitochondrial lipid peroxidation.⁽²⁰⁾ In hyperthyroidism, plasma levels of TBARS were also found to be increased, whereas vitamin E and coenzyme Q10 were reduced. Average levels of TBARS and antioxidant agents returned to normal in euthyroid patients, without differences in relation to the stoppage of thyrostatic therapy.⁽²¹⁾

Similar results to our study were also obtained in the study by Komosinska – Vassev K et al⁽⁶⁾ (2000) who found an increase of erythrocyte GSHPx. However, though the erythrocyte antioxidants such as GSHPx, SOD and catalase were increased, total antioxidant status and serum glutathione reductase were not so, in 30

patients with Graves disease compared with age-matched controls. Here the erythrocyte antioxidant levels were increased whereas the serum levels were decreased in hyperthyroid patients. This discrepancy might be due to the fact that serum antioxidants get easily exhausted and can also be considered to reflect the oxidative stress indirectly. Bednarek et al⁽⁸⁾ (2004) showed that in infiltrative Graves disease patients with ophthalmopathy, hydrogen peroxide (H_2O_2), lipid hydroperoxides, thiobarbituric acid-reacting substances (TBARS) and ceruloplasmin levels along with superoxide dismutase (SOD) and catalase (CAT) activities were increased, whereas glutathione peroxidase (GSHPx) and glutathione reductase (GR) activities were reduced. In another detailed study on oxidative stress and antioxidant status in hypo- and hyperthyroidism, Mirela Petrulea et al (2012) suggested that in order to correct the impairment of the antioxidant system Vitamin E supplementation could be beneficial in hyperthyroidism and also can lead to diminution of thyroid hormone levels. Other antioxidant treatments such as selenium might also be helpful in reducing the oxidative damage due to hyperthyroidism.⁽²²⁾ In yet another study showing decreased GSHPx levels, the decrease was not significant, however this too normalised after methimazole therapy.⁽²³⁾

Study by Ho Kim et al(2001) has postulated that antioxidant and immunomodulatory effects of methimazole (MMI) involves the interferon γ (IFN- γ) signalling pathway in thyroidal cells. MMI rapidly eliminate H_2O_2 produced by IFN- γ treatment in thyroid cells and thus inhibits the H_2O_2 mediated phosphorylation of tyrosine 701 in STST1. MMI also eliminates H_2O_2 in vitro. MMI facilitates electron transfer from NADPH to H_2O_2 using thioredoxin or glutathione. MMI prevents the IFN- γ induced Janus Kinase (JAK/STAT) signalling pathway in thyroid cells. These results may in part explain the antioxidant and immunomodulatory effects of MMI in thyroid cells of hyperthyroid patients.⁽¹²⁾ Study by Sewerynek et al (2000) show that methimazole therapy caused a relief in oxidative stress and can protect against oxidative processes induced by hyperthyroidism.⁽²⁴⁾ Ademoglu et al(2006) reported that hyperthyroidism can enhance the lipid peroxide content which can increase glutathione S-transferase activity leading to decrease GSHPx activity.⁽²⁵⁾ The restoration of euthyroid status after methimazole therapy (10 mg/day) caused normalisation of GSHPx activity.

So, the achievement of the normal balance of the thyroid hormone status in hyperthyroidism by the antithyroid drugs favours the improvement of the GSHPx enzyme activity towards the normal. Thus we could hypothesize that thyroid hormones could regulate the activities of the antioxidant enzyme systems and restoration of thyroid balance by methimazole therapy in hyperthyroidism helps to relieve the oxidative stress in that condition. Also there could be a possible role for

selenium supplementation since GSHPX is a selenium containing enzyme.

Conclusion

Erythrocyte antioxidant parameter GSHPx levels were increased in hyperthyroidism as a response to counteract the elevated oxidative stress induced and this gets normalised by methimazole therapy. Further studies are warranted to study the effect of selenium supplementation along with methimazole in an effort to normalise the antioxidant status in hyperthyroidism.

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