

Sodium Potassium ATPase and Glucose 6 Phosphate Dehydrogenase Activity in Patients with Hyperthyroidism: A Comparison with Euthyroid Patients

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Sodium potassium ATPase (Na⁺-K⁺ ATPase) and Glucose 6 phosphate dehydrogenase (G 6 PD) activities in different tissues have been found to be stimulated by thyroid hormones. In erythrocytes, the activities of these enzymes were reported to vary. The aim of our study was to determine the sodium potassium ATPase and glucose 6 phosphate dehydrogenase activities in patients with hyperthyroidism and compare them with those of patients with euthyroid goiter.

Materials and Methods: After approval from the Institutional Ethics Committee and obtaining informed consent from all patients, 40 hyperthyroid patients (17 men, 23 women; mean age 44.75±2.6 years) and 50 patients with euthyroid goiter (13 men, 37 women; mean age 37.2±1.6 years) were included in the study. They were classified based on T3, T4, TSH measurements. Erythrocyte Na⁺-K⁺ ATPase and G 6 PD activity were measured using spectrophotometry.

Results: In hyperthyroid patients, Na⁺-K⁺ ATPase activity was significantly lower compared to euthyroid controls (134.98±3.78 vs. 164.34±3.85 nmol pi/hr.mg protein, p<0.001) and G 6 PD levels were significantly elevated when compared to euthyroid controls (19.19±0.438 U/g Hb vs. 11.505±0.385 U/g Hb, p<0.001).

Conclusion: Hyperthyroidism is associated with decrease in Na⁺-K⁺ ATPase activity and increase in G 6 PD levels when compared to patients with euthyroid goiter. The measurement of Na⁺-K⁺ ATPase activity could be used as an early marker for diagnosis of hyperthyroidism.

Key words: Sodium potassium ATPase, Glucose 6 phosphate dehydrogenase, Hyperthyroidism

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Introduction

Thyroid hormones include the iodinated amino acid derivatives T3 (3, 3', 5-triiodo-L-thyronine) and T4 (3, 3', 5', 5-tetraiodo-L-thyronine), the only iodinated hormones produced endogenously. T3 is a biologically active hormone, and is mostly produced from T4 in extra thyroidal tissues, while T4 lacks significant bioactivity and is a hormone precursor. With the possible exception of the adult brain, anterior pituitary, spleen, and testes, these thyroid hormones exert a thermogenic (calorigenic) effect and increase oxygen consumption and energy expenditure

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through their effect on ATP formation and breakdown.

In animal experiments, the activity of sodium potassium ATPase ($\text{Na}^+\text{-K}^+\text{ATPase}$) in different tissues was found to be stimulated by thyroid hormones. On the other hand, in erythrocytes of hyperthyroid patients the activity of this enzyme was reported to vary.^{1,2} The role of $\text{Na}^+\text{-K}^+$ ATPase for thyroid hormone dependent energy expenditure in cells remains unclear. It has been proposed that the activity of thyroid hormones at the cellular level maybe due to their influence on the $\text{Na}^+\text{-K}^+$ ATPase, which accounts for a major proportion of the energy requirements.³ The energy required for $\text{Na}^+\text{-K}^+$ ATPase transport in the erythrocyte is supplied by phosphoglycerate in glycolysis. Glycolysis in RBCs, under aerobic conditions, always ends with lactate due to the absence of mitochondria.⁴

Glucose 6 phosphate dehydrogenase (G6PD), a dimer of identical subunits, each with molecular weight of 60000 daltons, is the first enzyme in the pentose phosphate pathway. Since, the erythrocyte lacks the TCA cycle, it depends on the pentose phosphate pathway, for supply of NADPH, which is required to maintain intracellular concentration of reduced glutathione; G6PD catalyzes the dehydrogenation of glucose 6 phosphate to 6 phospho gluconate via the formation of 6 phospho gluconolactone. In RBCs, glycolysis and the pentose phosphate pathways are the predominant pathways of energy metabolism, which supply ATP for the membrane ion pumps and NADPH for the reduction of glutathione, which protects against oxidative injury;⁵ G6PD can usually make an adequate supply of NADPH under normal conditions.^{6,7} Earlier studies have shown that thyroid hormones do influence the levels of G6PD in the erythrocytes.^{8,9} In RBCs, the pentose phosphate pathway provides NADPH for the reduction of oxidized glutathione catalyzed by glutathione reductase, a flavoprotein containing FAD; reduced glutathione removes H_2O_2 in a

reaction catalyzed by glutathione peroxidase, an enzyme containing selenium analogue of cysteine at the active site. Accumulation of H_2O_2 may decrease the lifespan of erythrocytes by causing oxidative damage to the cell membrane, leading to hemolysis.¹⁰

The aim of our study was to estimate the levels of erythrocyte sodium potassium ATPase and glucose-6-phosphate dehydrogenase in patients with hyperthyroidism and compare these with those of euthyroid patients.

Materials and Methods

In this cross-sectional study, a total of 90 patients, aged between 18 to 60 years (40 hyperthyroid and 50 euthyroid) were included in the study. The research protocol was approved by the Institutional Ethics Committee and informed consent was obtained from all patients. Subjects in the hyperthyroid group were sampled from patients seen in medical/surgical units in our hospital clinics. Patients were diagnosed as having hyperthyroidism if they matched one or more of the following criteria: $\text{T}_3 > 2$ ng/dL, $\text{T}_4 > 14$ $\mu\text{g/dL}$, $\text{TSH} < 0.05$ $\mu\text{U/mL}$. Individuals in the control group were selected from those who had goiter/thyroid nodules and normal thyroid function tests, and were not on any thyroid hormone medications; patients with any other co-morbid illness and those on any medication that alters the thyroid profile (e.g. amiodarone, lithium and digitalis) were excluded from in the study. Five ml of blood with anticoagulant (EDTA) was collected from the patients for estimation of erythrocyte $\text{Na}^+\text{-K}^+\text{ATPase}$ and G 6 PD levels.

Measurements:

Erythrocyte sodium potassium ATPase: $\text{Na}^+\text{-K}^+$ ATPase activity in the erythrocytes was measured as a release of inorganic phosphate from hydrolysis of ATP in the presence and absence of ouabain.^{4,11}

Sodium potassium ATPase activity was estimated by the Fiske and Subbaraw method¹² and the membrane protein was from the red blood cell ghosts estimated using the method adapted by Lowry et al.^{13,14}

Glucose-6-phosphate dehydrogenate (G 6 PD).¹⁰ G6PD catalyzes the oxidation of glucose 6 phosphate (G6P) to 6 phospho gluconate with a concurrent conversion of NADP⁺ to NADPH. The activity of G 6 PD was determined by measurement of the rate in increase in NADPH concentration. Whereas NADP⁺ is transparent to ultraviolet (UV) light, NADPH strongly absorbs UV light. Therefore, the rate of increase in absorbance at 340 nm was used as a measure of enzyme activity. The absorbance of the test solution at 540 nm was used to estimate the hemoglobin concentration. A reference range value of 8 – 16 U / gm Hb was considered.

$$G6PD \text{ activity } U/gHb = \frac{14.67 \times \Delta A_{340}}{\Delta A_{540}}$$

T3,¹⁵ T4¹⁶ and TSH¹⁷ were estimated by the Electro-chemiluminescence immunoassay “ECLIA” of Roche Elecsys and Modular Analytics.

Statistical analysis

All values of analyzed parameters were expressed as mean ± SEM. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS/PC; SPSS-13, Chicago, USA). Independent sample t- test was used to compare the mean values in the two groups followed by the non-parametric test Man-Whitney test; p<0.05 was considered statistically significant.

Results

The study included 90 patients, 50 of whom were euthyroid, and 40 hyperthyroid. All demographic parameters, except for age were comparable between the groups (Table 1). The gender distribution between the two groups and T3, T4 and TSH levels in euthyroid and hyperthyroid patients are shown in Table 1. Na⁺-K⁺ ATPase activity and glucose 6 phosphate dehydrogenase levels in euthyroid and hyperthyroid patients are depicted in figures 1 and 2. In hyperthyroid patients, Na⁺-K⁺ ATPase activity was significantly low when compared to euthyroid controls (134.98±3.78 vs 164.34±3.85 nmol pi/hr.mg protein, p<0.001). G6PD levels in hyperthyroid patients however was significantly elevated when compared to euthyroid controls (19.19±0.43 U/g Hb vs 11.50±0.38 U/g Hb, p<0.001).

Table 1. Demographic parameters, T3, T4 and TSH levels (expressed as mean ± SEM) in the study groups

* p<0.05

| | Euthyroid (n= 50) | Hyperthyroid (n= 40) |
|----------------------------|----------------------|-------------------------|
| Age (yr) | 37.2±1.6* | 44.75±2.6 |
| Height (cm) | 156.36±0.9 | 156.53±1.5 |
| Weight (kg) | 57.66±1.19 | 54.93±1.4 |
| BMI (kg/m ²) | 23.59±0.4 | 22.57±0.6 |
| Sex distribution (M/ F) | 13/37 | 17/23 |
| T3 (ng/dL) | 1.23±0.32 | 2.69±1.55 |
| T4 (µg/dL) | 8.49±1.96 | 17.07±3.86 |
| TSH (µU/mL) | 1.68±0.87 | 0.03±0.05 |

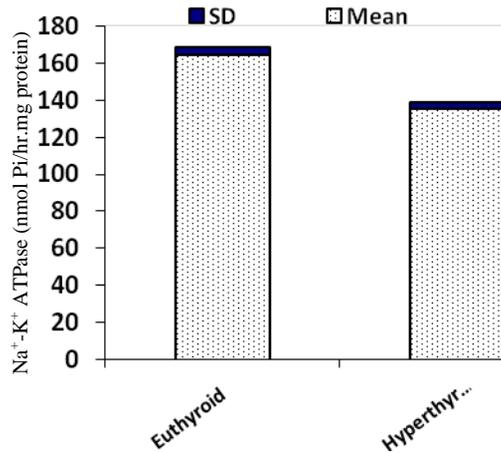


Fig.1. Mean Na⁺-K⁺ ATPase activity in euthyroid and hyperthyroid patients. The darker line depicts standard deviation

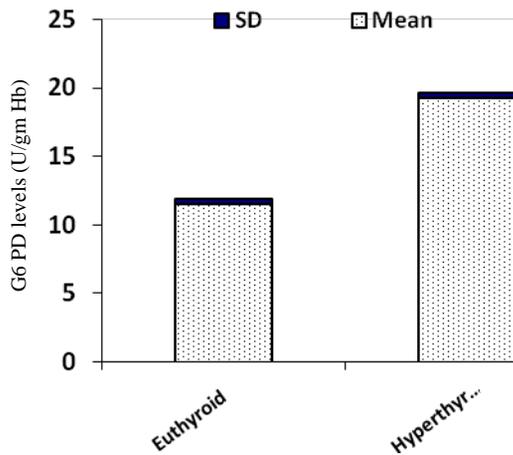


Fig.2. G6 PD levels in euthyroid and hyperthyroid patients

Discussion

Various studies investigating the activity of Na⁺-K⁺ ATPase in tissues have consistently shown that the activity is increased in hyperthyroidism.²⁴⁻²⁶ However, levels and activity of the enzyme in RBCs of patients with thyroid dysfunction have not shown a consistent trend. While Sato et al¹⁸ found that the activity of the enzyme decreased in both hyperthyroid and hypothyroid patients, DeLuise M et

al², Dasmahapatra et al⁴, and others^{6, 19, 20} found that the levels and activity of the enzyme decreased in hyperthyroid and increased in hypothyroid patients.

Sato et al¹⁸ found that the Na⁺-K⁺ ATPase activity was significantly reduced (11±4.6 vs 17.3±4.1 µg Pi/hr/mg protein, p<0.01) in hypothyroid and also in hyperthyroid patients (p<0.01) which normalized after treatment; they suggested that Na⁺-K⁺ ATPase activity

can be used as a sensitive index of peripheral thyroid status.

In our study, the levels of Na⁺-K⁺ ATPase were significantly decreased in hyperthyroid patients (134.98±3.78nmol Pi/mg.h vs 164.34±3.85 nmol Pi/mg.h). The results of our study were similar to those documented by Dasmahapatra et al⁴, who also found levels of the enzyme to be decreased in hyperthyroidism (307±30 nmol Pi/mg.h vs 380±24 nmol Pi/mg.h, p<0.05). The exact mechanism responsible for the reduction in the activity of RBC Na⁺-K⁺ ATPase pumps in hyperthyroidism is still uncertain. Various possible mechanisms have been proposed to explain the decrease in Na⁺-K⁺ ATPase in erythrocytes; Rubythron et al³ have suggested that thyroid hormones probably inhibit the synthesis of the sodium pump during the maturation in the bone marrow. However, this is unlikely as Aramunayam et al²¹ have shown in a preliminary study that Na⁺-K⁺ ATPase activity of an erythroid cell line was stimulated by T3. DeLuise and Flier et al² supported the hypothesis that the resultant change could be due to the degradation of Na⁺-K⁺ ATPase units in erythrocytes as thyroid hormones are known to accelerate catabolism of cell proteins, suggesting that this degradation may occur in the circulation during aging of the RBCs or during formation of the reticulocytes. Dasmahapatra et al⁴ have also postulated that the diminished degradation of erythrocyte pump units in hypothyroidism may be responsible for the increased enzyme activity in this group of patients. Since our study correlated well with their study, we think it is likely that the decreased erythrocyte Na⁺-K⁺ ATPase activity in hyperthyroidism may be due to an accelerated degradation of membrane proteins.

Many of the abovementioned studies have shown normalization of the enzyme activity following treatment of thyroid dysfunction.^{20,27,28} De Riva C et al²⁰ suggested that the decrease in enzyme levels after cessation of treatment could be used as an early indicator of recurrence of hypothyroidism. Since

we lost a number of patients on follow up, we could not assess the enzyme levels after treatment.

In view of the aforementioned data, we suggest that the measurement of activity of sodium potassium ATPase could be used as an early marker for the diagnosis of hyperthyroidism.

The intracellular redox potential is determined by the concentrations of oxidants and reductants. A critical modulator of the redox potential is NADPH, the principal intracellular reductant in all cell types. Glucose-6-phosphate dehydrogenase (G6PD), the rate limiting enzyme of the Pentose Phosphate Pathway (PPP), determines the amount of NADPH by controlling the metabolism of glucose via the PPP.²³ It has been traditionally thought that G6PD was a typical 'house-keeping' enzyme that was regulated solely by the ratio of NADPH to NADP.²⁹ But research suggests that this enzyme is highly regulated and plays important roles in a variety of cellular processes. G6PD is under close transcriptional, translational, and posttranslational control.²⁹⁻³¹ Research has demonstrated that growth factors like the thyroid hormone can rapidly activate G6PD and stimulate translocation of G6PD.^{30,31} In addition, G6PD activity plays a critical role in cell growth via its role in intracellular redox regulation.²³

In our study, G6PD activity was significantly elevated in hyperthyroid patients (p<0.001), findings which correlated well with those of Nehal et al.⁸ Bildik et al¹ also found a significant increase in the erythrocyte G6PD levels in hyperthyroid rabbits (P<0.05). However, Odcikin E et al²² found that the levels of G6PD was reduced in hyperthyroid patients when compared to euthyroid ones (10.19±1.87 U_g/Hb in the healthy group and 4.92±2.49 U_g/Hb in the patients).

The precise mechanism for the changes in the activities of red cell enzymes stimulated by T3 is not fully understood. It is obvious that thyroid hormones do have an overall effect on metabolism of responsive tissues.¹

Tian Wang-Ni et al²³ have shown that intracellular levels of G6PD are crucial in the balance of oxidant- antioxidant activity, over-expression of G6PD increased resistance to H₂O₂-induced cell death and that reduced levels predisposed the cell to oxidant induced cell death.²⁴ In view of the above findings, we postulate that increased levels of the enzyme activity in hyperthyroidism can help prevent RBCs from oxidant induced destruction.

To conclude, our findings show that erythrocyte Na⁺-K⁺ ATPase activity is decreased in patients with hyperthyroidism which may be due to an accelerated degradation of membrane proteins. Measurement of the activity of sodium potassium ATPase could be used as an early marker for the diagnosis of hyperthyroidism. Increased levels of the G6PD enzyme activity in hyperthyroidism can help prevent RBCs from oxidant induced cell destruction.

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