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## CLINICAL STUDY OF SODIUM-POTASSIUM ATPase ACTIVITY IN PATIENTS OF HYPO AND HYPER THYROIDISM

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### ABSTRACT

Thyroid hormone is known to modulate cell membrane sodium- potassium adenosine triphosphatase. The present study evaluates the sodium- potassium ATPase activity in human subjects with abnormal thyroid function. 180 hyperthyroid patients (mean age-  $41.63 \pm 10.34$  years), 220 hypothyroid patients (mean age-  $44.61 \pm 14.57$  years) and 200 euthyroid controls (mean age  $41.15 \pm 11.98$  years) were included in the study. The thyropropin, triiodothyronine and thyroxine levels of the patients were assayed by enzyme immunoassay method on ELISA Reader. Erythrocyte  $\text{Na}^+\text{-K}^+$  ATPase activity was measured in red cell lysates by the rate of release of phosphate from adenosine triphosphate in the presence and absence of ouabain. Patients were categorized as hyperthyroid, hypothyroid and euthyroids based on thyroid stimulating hormone (TSH), thyroxine (T4) and triiodothyronine (T3) measurements. Patients were diagnosed as having hyperthyroidism, if they matched one or more of the following criteria:  $\text{T3} > 2.10 \text{ ng/ml}$ ,  $\text{T4} > 12 \text{ } \mu\text{g/dl}$ ,  $\text{TSH} < 0.05 \text{ mIU/dl}$ . The hypothyroid patients exhibited decline in T3 and T4 concentration ( $\text{T3} < 0.80 \text{ ng/ml}$ ,  $\text{T4} < 4.5 \text{ } \mu\text{g/dl}$ ) and elevation in TSH level ( $\text{TSH} > 6 \text{ mIU/dl}$ ). In hyperthyroid patients, the activity of sodium- potassium ATPase was significantly lowered ( $93.16 \pm 7.29 \text{ nM/mg/hr}$ ,  $p < 0.0001$ ), whereas in hypothyroid patients its concentration was significantly elevated ( $166.62 \pm 22.66 \text{ nM/mg/hr}$ ,  $p < 0.0001$ ) in comparison to the euthyroid controls ( $137.29 \pm 7.64 \text{ nM/mg/hr}$ ). Sodium potassium ATPase activity showed significant direct relation with TSH ( $r = 0.69$ ) and significant inverse relationship with T3 ( $r = -0.85$ ) in hyperthyroid patients, while hypothyroid patients doesn't revealed any relationship between thyroid hormones and Sodium potassium ATPase activity. The results suggest that sodium- potassium ATPase is sensitive to subtle changes in thyroid function and it may reflect the overall peripheral metabolic state, regulated by thyroid hormone- dependant thermogenesis. Measurement of the activity of sodium-potassium ATPase could be used as a good biochemical marker of thyroid dysfunction.

**Keywords:** Sodium-potassium ATPase, TSH, T4, T3, Hypothyroid, Hyperthyroid

### INTRODUCTION

Thyroid hormones exert a thermogenic effect and increase oxygen consumption and energy expenditure through their effect on ATP formation and breakdown. Sodium- potassium ATPase is a membrane spinning protein complex responsible for extrusion of  $\text{Na}^+$  and absorption of  $\text{K}^+$  by most body cells to maintain the ionic gradients. Thyroid hormones are known to modulate cell membrane  $\text{Na}^+/\text{K}^+$ -ATPase and impairment of this enzyme has been reported in the presence of thyroid dysfunction (Ismail-Beigi and Edelman, 1971). It has been proposed that the activity of thyroid hormones at the cellular level may be due to their influence on the  $\text{Na}^+/\text{K}^+$  ATPase, which accounts for a major proportion of the energy requirements (Monti *et al.*, 1987). The activity of  $\text{Na}^+/\text{K}^+$  ATPase is increased in leukocytes (Khan and Baron, 1987) and platelets (Chan *et al.*, 2001) and in erythrocyte of hyperthyroid/hypothyroid subjects, the number of ouabain-binding sites and the activity of  $\text{Na}^+/\text{K}^+$ -ATPase is reported to be increased (Nicolini *et al.*, 2004) or decreased (Bildik *et al.*, 2002; Prasad and Nayak, 2009). 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 (Dasmahapatra *et al.*, 1985). The present investigation has been undertaken to demonstrate the level of erythrocyte sodium - potassium ATPase in patients of hyperthyroidism and hypothyroidism, and compare these with those of euthyroid controls.

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### **MATERIALS AND METHODS**

#### **Subjects**

In the present study, a total of 600 subjects (180 hyperthyroid subjects, mean age-  $41.63 \pm 10.34$  years; 220 hypothyroid patients, mean age-  $44.61 \pm 14.57$  years and 200 euthyroid controls, mean age  $41.15 \pm 11.98$  years) were included. The research protocol was approved by institutional ethics committee and informed consent was obtained from all the patients.

#### **Endocrine tests**

The thyropropin, triiodothyronine and thyroxine levels of the patients were assayed by enzyme immunoassay method on ELISA Reader.

#### **Erythrocyte $\text{Na}^+$ - $\text{K}^+$ ATPase Activity**

$\text{Na}^+$ - $\text{K}^+$  ATPase activity was measured with a method based on the rate of release of phosphate (Pi) by hydrolysis of adenosine triphosphate in the presence and absence of ouabain as described by Raccach *et al.*, (1996).

The red cell pack extracted by centrifugation at 4°C were resuspended and diluted in 25 volumes of Tris-HCl buffer at pH 7.4. The hemolyzed cells were then centrifuged at 12,000 rpm at 4°C and the membrane pellet was suspended in 30 ml of 0.11 mol L<sup>-1</sup> Tris-HCl buffer. This centrifugation step was repeated three times. The final concentration of the membrane suspension was ~4 mg protein ml<sup>-1</sup> of Tris buffer. ATPase activity was measured in a final volume of 1 ml as follows: Membrane (400 ug) were preincubated for 10 minutes at 37°C in a mixture containing 92 mmol L<sup>-1</sup> Tris- HCl (pH=7.4), 100 mmol L<sup>-1</sup> NaCl, 20 mmol L<sup>-1</sup> KCl, 5 mmol L<sup>-1</sup> MgSO<sub>4</sub> .H<sub>2</sub>O and 1 mmol L<sup>-1</sup> EDTA. Assays were performed with and without 1mmol L<sup>-1</sup> Ouabain, a specific inhibitor of Na-K-ATPase. After incubation with 4 mmol L<sup>-1</sup> ATP (Vanadate free, Sigma) at 37°C for 10 minutes, the reaction was stopped by adding ice-cold trichloroacetic acid (5%). After centrifugation at 4°C, 5500 g for 10 minutes. The amount of inorganic phosphate in the supernatant was determined (Dryer and Tammes, 1957). Protein content of the erythrocyte lysate was determined by method of Lowery *et al.*, (1951)  $\text{Na}^+$ - $\text{K}^+$ -ATPase activity was calculated as the difference between inorganic phosphate released during the 10-minute incubation with and without Ouabain. Activity was corrected to a nanomolar concentration of inorganic phosphate released milligram<sup>-1</sup> protein hour<sup>-1</sup>.

#### **Statistical Analyses**

Results were presented as mean  $\pm$  SD. Comparison was made by ANOVA and post hoc multiple comparison (Tucky's HSD) test by using SPSS (19.0) statistics package. P value less than 0.001 was considered significant. Correlation between parameters was performed by correlation matrices analysis.

### **RESULTS AND DISCUSSION**

#### **Results**

The main clinical and hormonal data of the patients are listed in Table 1. The hyperthyroid patients showed significant increase in T4 ( $p < 0.001$ ) and T3 ( $p < 0.001$ ) levels, whereas the TSH concentration was significantly ( $p < 0.001$ ) suppressed. Hypothyroid patients exhibited decline in levels of T3 and T4, while TSH concentration was elevated.

One way ANOVA with posthoc analysis indicated a highly significant ( $F = 145.88$ ,  $p < 0.0001$ ) variance in the activity of sodium- potassium ATPase in hyperthyroid, hypothyroid patients and euthyroid controls. Tucky HSD multiple comparison test revealed that the activity of sodium-potassium ATPase was significantly lowered ( $q = -39.13$  to  $-68.46$ , 95% CI =  $-77.89$  -  $-29.53$ ,  $p < 0.0001$ ), whereas in hypothyroid patients its concentration was significantly elevated ( $q = 29.33$  to  $68.46$ , 95% CI =  $20.21$  -  $77.89$ ,  $p < 0.0001$ ) in comparison to the euthyroid controls.

There was a significant positive relationship existed between erythrocyte  $\text{Na}^+$ - $\text{K}^+$  ATPase activity and TSH in hyperthyroid ( $r = 0.69$ , Fig. 1) patients. A highly significant negative correlation between enzyme activity and triiodothyronine ( $r = -0.85$ , Fig. 2) was observed in hyperthyroid patients, while non significant relationship was found between thyroid hormones and  $\text{Na}^+$ - $\text{K}^+$  ATPase activity in hypothyroid patients (Figures 3,4).

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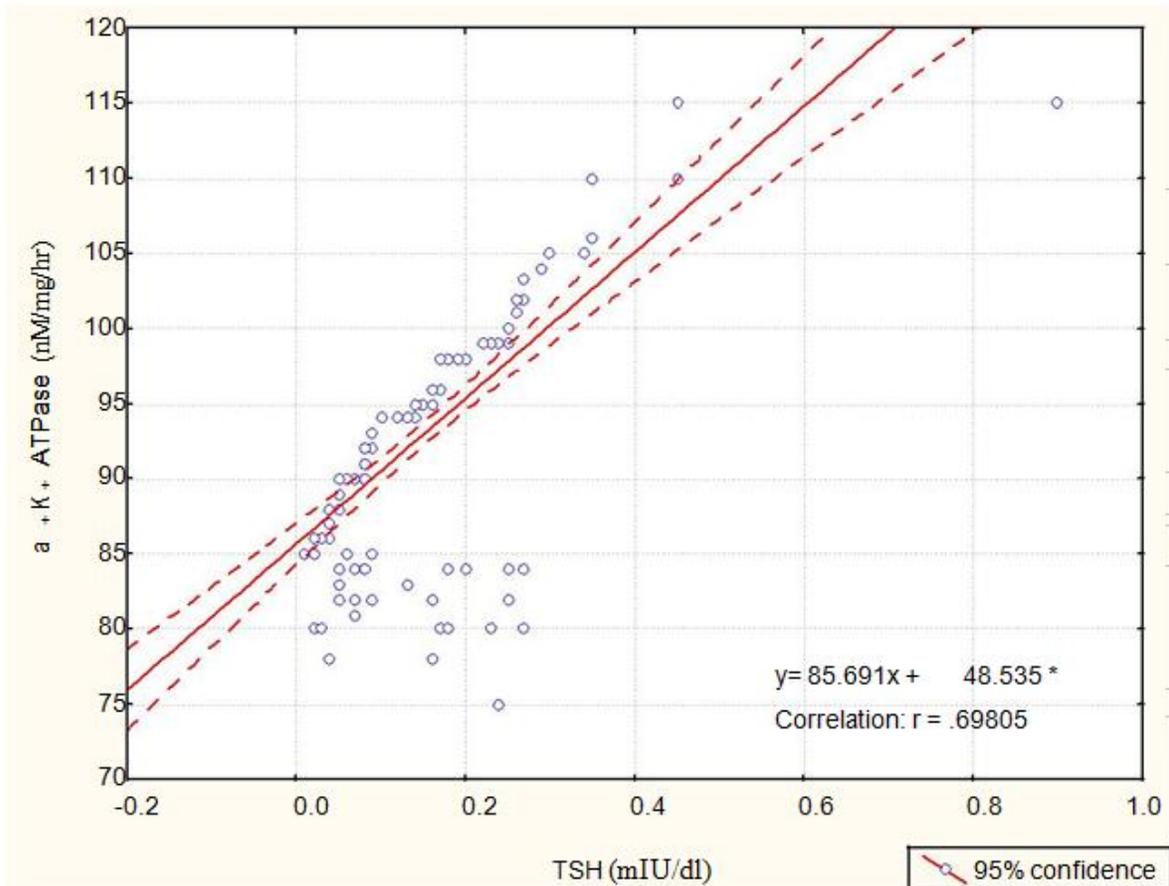
**Discussion**

Previous studies suggested that levels and activity of the sodium- potassium ATPase enzyme in RBCs of patients with thyroid dysfunction have not shown a consistent trend. Sato *et al.*, (1982) found that the Na<sup>+</sup>-K<sup>+</sup> ATPase activity was significantly reduced (p<0.01) in hypothyroid and also in hyperthyroid patients (p<0.01) which normalized after treatment, they suggested that Na<sup>+</sup>-K<sup>+</sup> ATPase activity can be used as a sensitive index of peripheral thyroid status. Arumanayagam *et al.*, (1990) documented that in erythrocytes of hyperthyroid patients the number of ouabain- binding sites and the activity of the Na<sup>+</sup>-K<sup>+</sup> ATPase are decreased whereas in erythrocytes of hypothyroid patients with overt disease they are increased. In contrast, with erythrocyte, other non thyroidal tissues showed an opposite trend between thyroid hormone concentrations and the Na<sup>+</sup>-K<sup>+</sup> ATPase status (Kim and Smith, 1984; Kjeldsen *et al.*, 1984).

**Table 1: Clinical and hormonal profile of hyperthyroid, hypothyroid and euthyroid controls**

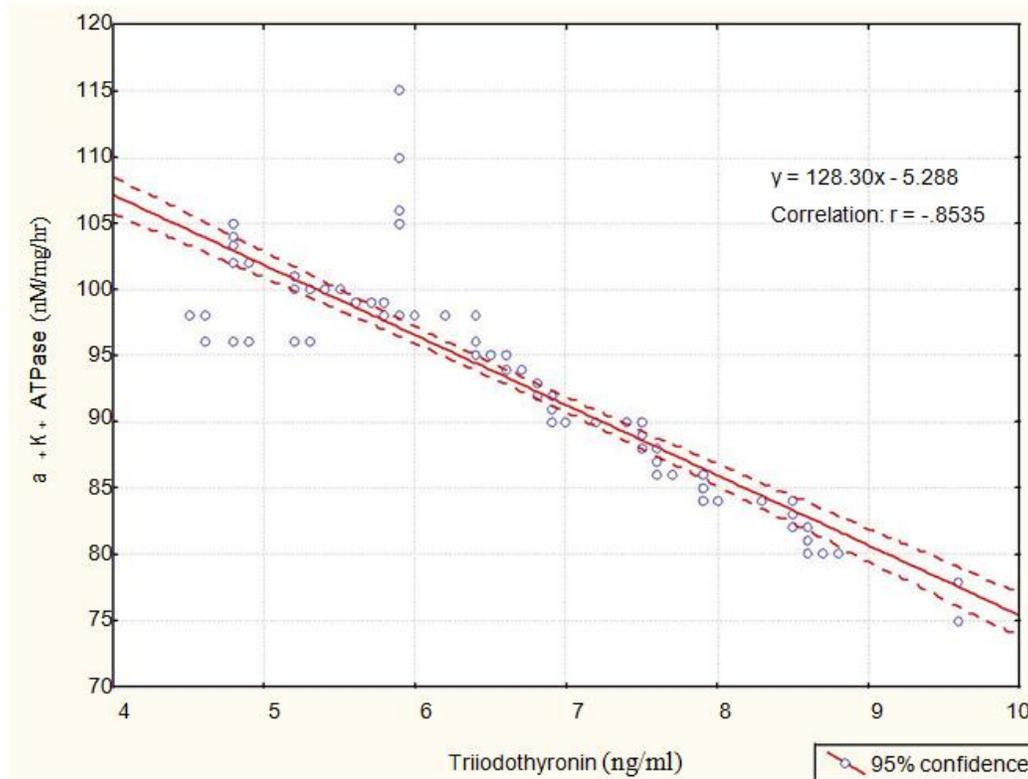
Type of disorder	Number of patients	Age (Years)	TSH (mIU/dl)	T3 (ng/ml)	T4 (µg/dl)
Hyperthyroid	180	41.6 ± 10.33	0.15 ± 0.11	6.69 ± 1.18	17.45 ± 1.28
Hypothyroid	220	44.6 ± 14.57	12.57 ± 4.68	0.17 ± 0.16	2.81 ± 1.11
Euthyroid	200	41.15 ± 11.98	3.38 ± 1.56	1.44 ± 0.33	8.57 ± 0.89

Results are given as mean ± SD.  
 P<0.001

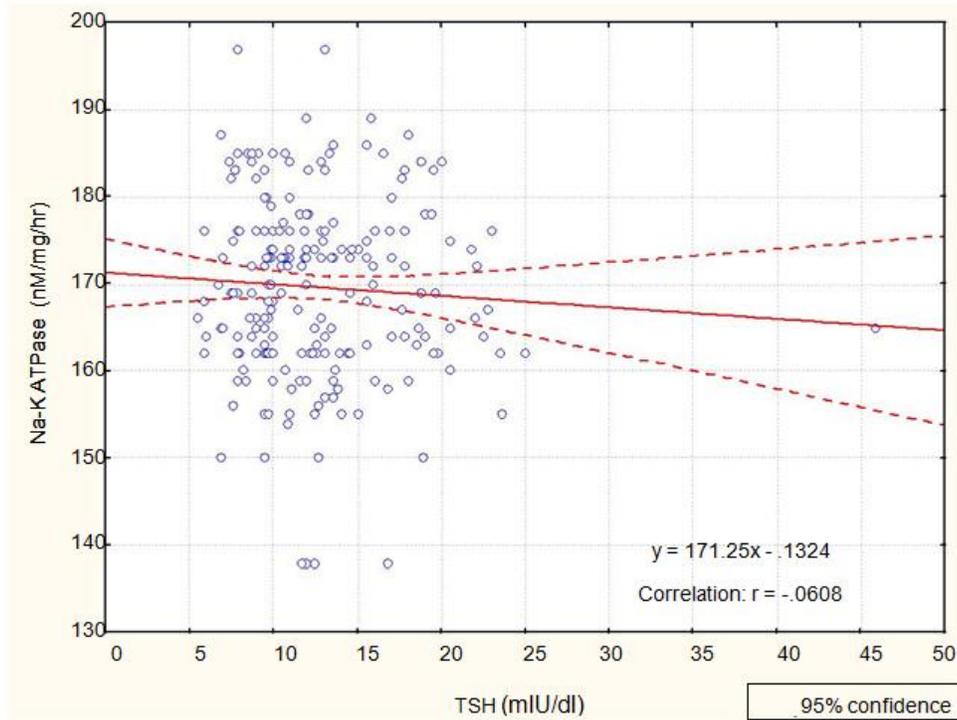


**Figure 1: Scatterplot showing correlation between serum TSH and Na<sup>+</sup> - K<sup>+</sup> ATPase activity in hyperthyroid patients**

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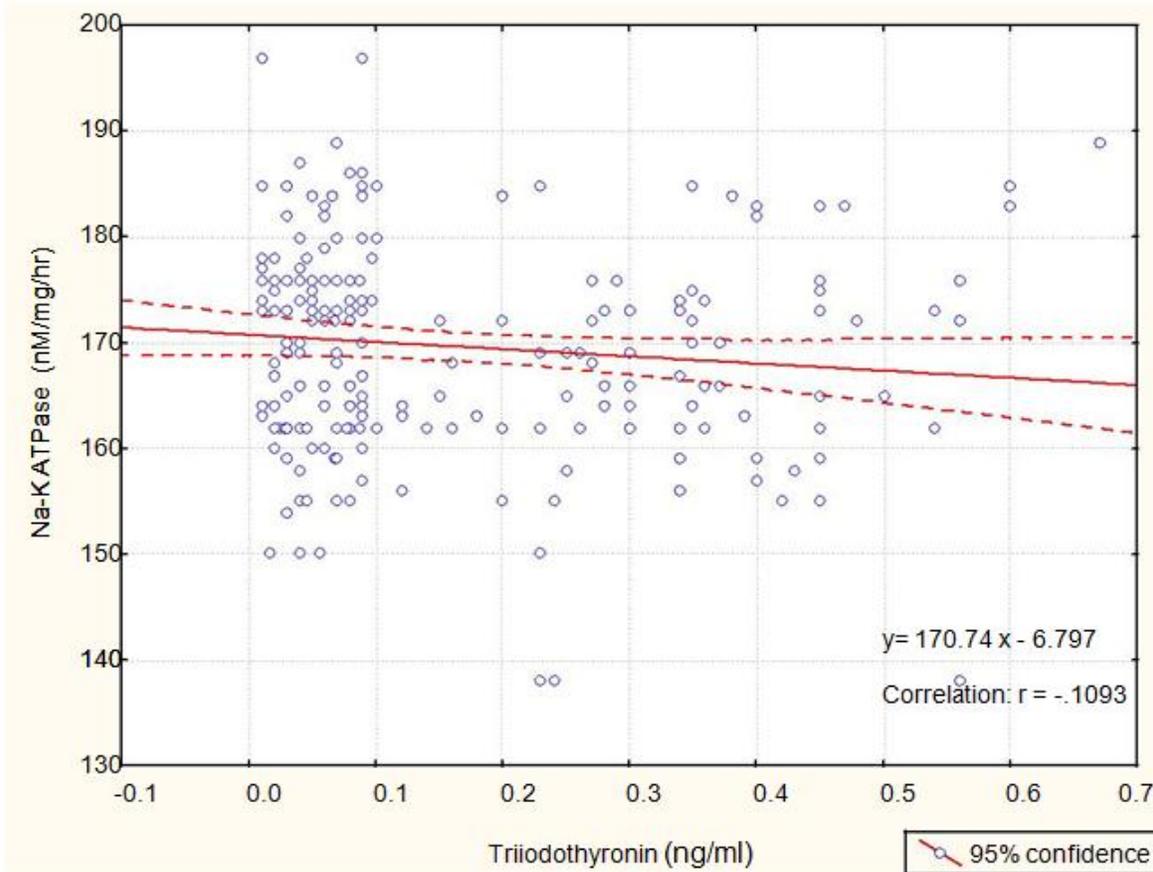


**Figure 2: Scatterplot showing correlation between serum triiodothyronin and Na<sup>+</sup> - K<sup>+</sup> ATPase activity in hyperthyroid patients**



**Figure 3: Scatterplot showing correlation between serum TSH and Na<sup>+</sup> - K<sup>+</sup> ATPase activity in hypothyroid patients**

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**Figure 4: Scatterplot showing correlation between serum triiodothyronin and Na<sup>+</sup> - K<sup>+</sup> ATPase activity in hypothyroid patients**

**Table 2: Na<sup>+</sup>-K<sup>+</sup> ATPase activity in hyperthyroid, hypothyroid and euthyroid controls**

Type of disorder	Number of subjects	Na <sup>+</sup> -K <sup>+</sup> ATPase (nM/mg/hr)	F-Ratio
Hyperthyroid	180	98.16±6.05*‡	145.88***
Hypothyroid	220	166.62±22.*†	
Euthyroid	200	137.29±7.64†‡	

Results are given as mean ± SD.

\*\*\* P<0.0001

\*P<0.0001 vs. Euthyroid, † P<0.0001 vs. hyperthyroid, ‡ P<0.0001 vs. hypo

During present investigation activity of sodium- potassium ATPase is found to be significantly decreased in hyperthyroid patients in comparison to euthyroid control subjects. Moreover in hyperthyroid patients a significant positive correlation was found between enzyme activity and TSH concentration, a highly significant negative relationship was found between enzyme activity and triiodothyronin and thyroxine levels. Various studies investigating the activity of Na<sup>+</sup>-K<sup>+</sup> ATPase in tissues have consistently shown that the activity is increased in hyperthyroidism (Ogasawara and Nishikawa, 1988). Our results were similar to those recorded by Prasad and Nayak (2009) who also found levels of the enzyme to be decreased in hyperthyroidism (134.98±3.78 vs 164.34±3.85 nmol Pi/mg.h, p<0.05).

In our study, hypothyroid patients showed a significant increase in activity of Na<sup>+</sup>-K<sup>+</sup> ATPase in comparison to euthyroid controls, however thyroid hormones showed a non significant relationship with

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enzyme activity. Accordingly, the same behaviour has been observed in the thyroid gland where the Na<sup>+</sup>-K<sup>+</sup> ATPase is stimulated dramatically by hypothyroidism (LeGraw *et al.*, 1999). Nicolini *et al.*, (2004) observed a significant increase in the number of ouabain-binding sites and in Na<sup>+</sup>-K<sup>+</sup> ATPase activity in subclinical hypothyroid patients in comparison to control and hyperthyroids. In contrast to our results they found a positive relationship between the number of ouabain –binding sites and the plasma TSH concentration.

The exact mechanism responsible for the alteration in the activity in the activity of RBC Na<sup>+</sup>-K<sup>+</sup> ATPase pumps in thyroid dysfunction are still unclear. Rubython *et al.*, (1983) have suggested that thyroid hormones probably inhibit the synthesis of the sodium pump during the maturation in the bone marrow. De-Luise and Flier (1983) supported the hypothesis that reluctant changes could be due to the degradation of Na<sup>+</sup>-K<sup>+</sup> ATPase units in erythrocytes as thyroid hormone 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. As circulating erythrocytes are not nucleated, it has been suggested that impaired Na<sup>+</sup>-K<sup>+</sup> ATPase activity in thyroidal disease occurs during bone marrow maturation, or as consequence of a non genomic action of thyroid hormones on the plasma membrane (De Riva *et al.*, 1992; DeRiva and Vircici, 1998).

The results suggest that sodium- potassium ATPase is sensitive to subtle changes in thyroid function and it may reflect the overall peripheral metabolic state, regulated by thyroid hormone- dependant thermogenesis. Measurement of the activity of sodium- potassium ATPase could be used as a good biochemical marker of thyroid dysfunction.

### **ACKNOWLEDGEMENT**

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### **Conflict of Interest**

The authors declare that they have no conflicts of interest.

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