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Research article

TRACE ELEMENTS IN PATIENTS WITH THYROTOXICOSIS

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ABSTRACT : Studies have indicated a role for oxidative processes in the genesis of various degenerative diseases including hyperthyroidism-induced damage. In this study we investigated the effects of hyperthyroidism on five trace elements: selenium, manganese, copper, zinc and lead. Fourty hyperthyroid patients (mean age: 40.18±5.17 years) and thirty euthyroid, apparently healthy controls (mean age: 40.33±5.31 years) were used for this study. Plasma levels of thyroid hormones were measured using enzyme immunoassay method. The trace elements were measured using atomic absorption spectrophotometry (AAS). The results showed that patients with hyperthyroidism had higher levels of T_3 (6.24±2.94 nmol/L) and T_4 (245.43±77.87 nmol/L) but lower levels of TSH (0.16±0.08 MIU/L) when compared with those of controls (T_3: 1.49±0.26 nmol/L; T_4: 89.47±15.64 nmol/L; and TSH: 1.5±0.48 MIU/L; $P<0.05$) respectively. We also showed from our data, that hyperthyroid patients had a lower and significant plasma selenium, copper and zinc than the controls ($P<0.05$).Higher and significant levels of plasma lead was seen in the hyperthyroid patients when compared with the controls ($P<0.05$). Correlation between trace elements and the thyroid hormones showed no significant difference ($P>0.05$). From this study, it could be inferred that hyperthyroid patients had low plasma level of selenium, manganese, copper, zinc but high plasma lead. Measurement of these trace elements during the treatment of patients with thyrotoxicosis could be useful for adequate prognosis.

Key words: Trace elements, thyroid hormones, hyperthyroidism, micronutrients, oxidative stress.

INTRODUCTION

The normal thyroid gland produces the hormones triiodothyronine (T_3) and thyroxine (T_4) that play essential roles in development, energy metabolism and cellular differentiation. In the hyperthyroid state, the increased effect of these hormones on energy metabolism leads to accelerated oxidative metabolism (Seymen et al, 2004). The effects are accompanied by increased total oxygen consumption and an increased generation of reactive oxygen species (ROS), acceleration of free radical production and the occurrence of oxidative stress (Vcra et al 2004, Seymen et al, 2004). In this state of oxidative stress, an increased activity of antioxidative enzymes is induced by excessive production of free radicals. Evidence has shown a role for oxidative processes in the genesis of hyperthyroidism-induced damage (Seven et al, 1996). ROS and reactive nitrogen species (nitric oxide, nitrogen oxide) are known to attack various substrates in the body including, lipids, nucleic acids and proteins (Mayne, 2003), with a concomitant damage to the body cellularcomponents. In several experimental studies of hyperthyroidism, this free radical-mediated oxidative stress has been implicated in the genesis and exacerbation of degenerative diseases and loss of cellular integrity, including the cellular defense mechanisms. As thyrocytes produce hydrogen peroxide continuously throughout life, an effective defense system against hydrogen peroxide and reactive oxygen intermediates derives therefore, is essential for maintenance of normal thyroid function and protection of the gland (Kohrle, 1999). Trace elements are essential micronutrients that have been shown to play major roles in many physiological processes. They are also implicated in many pathological changes in the body (Al-Sayer et al, 2004). Many of these essential trace elements have been shown to influence the physiology of the thyroid gland (Zaichick et al, 1995). On the other hand, thyroid hormones are known to influence the metabolism of some of these trace elements (Henkin 1976, Al-Sayer et al, 2004). Imbalances in some of these elements have been associated with the development of some thyroid pathologies.

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The purpose of the present study is to determine the levels of selenium, manganese, copper, zinc and lead in hyperthyroid subjects and controls. We attempted to evaluate the relationship between the levels of these trace elements with T_3, T_4 and the thyroid stimulating hormone (TSH) levels, and also try to provide a scientific basis for the inclusion of measurement of these essential trace elements in the routine management of hyperthyroid patients

MATERIALS AND METHODS

Study Subjects

A total of 40 clinically diagnosed and randomly selected hyperthyroid subjects comprising of Grave's disease, thyrotoxicosis, toxic multinodular goitre, toxic goitre and simple multinodular goitre were included in this study. Their ages ranged between 34 and 52 years. 34 (85%) of the subjects were females while the remaining 6 (15%) subjects were males. The patients were drawn randomly from among those attending the Medical Out-Patient Department, of the University College Hospital, Ibadan, Nigeria. Neither of the study populations nor the control patients had diabetics, hypertension (all had normal fasting plasma glucose concentration of ≤126mg/dl and normal blood pressure reading) and any other disorders that could affect the parameters under evaluation in this study. Blood samples were obtained from the subjects in accordance with the guidelines of the Ethical Committee of the College of Medicine and the University College Hospital, Ibadan, Nigeria. The medical histories of the patients and the clinical aspects of their disease were evaluated from the case records by the attending consultant physician. 30 apparently healthy individuals whose ages ranged between 34 and 50 years were included in this study and served as controls. To be able to make a balanced comparison with our test subjects 25 (83%) of our controls were females, with the remaining 5 (17%) being males.

Sample Collection

Blood samples were collected from each subject into lithium heparin anticoagulant bottles, spun, and the plasma separated into sterile plain bottles and stored frozen at -20^0C, until analyzed.

Analytical Methods

Enzyme immunoassay (EIA) techniques were used for the quantitative measurements of T_3 and T_4 (competitive) as well as TSH (sandwich) hormones. The kits for the assays were supplied by Immunometrics (UK) Limited. The coefficient of variation of the kit is 0.01%, with minimum quantitation limit of 0.02 for all the hormones. Plasma level of manganese, copper, zinc and lead, was measured using furnace atomic absorption spectrophotometry (AAS), on Bulk Scientific Atomic Absorption Spectrophotometer Model 210 (East Norwalk, Connecticut), with the use of specific hollow cathode lamps for each element. The measurement of selenium was by the electrothermal AAS, Model 703 equipped with selenium hollow cathode lamp (Perkin Elmer Oak Brown, Illinois, USA). Prior measurements, plasma samples were diluted one in two with a 0.1% nitric acid solution, in order to reduce the effect of sample matrix on the flame and to enhance aspiration of the samples.

Statistical Methods

The mean and standard deviation of the cases (patients with hyperthyroidism) and control (euthyroid patients) were calculated. The correlations between variables were performed using the Pearson method. Test of significance for the differences between the means of the two groups was assessed by T-test. A probability of $P < 0.05$ was considered statistically significant.

RESULTS

The female: male ratio of hyperthyroid patients in this study was 5.7:1, with 34 women and 6 men being the subjects in this study. The mean age of incidence in females was 40.0±5.0 years, while that of the men was 39.2±6.7 years (Table 2). The mean concentration values for the thyroid hormone T3, T4 and TSH levels in both the cases and controls were shown in Table 1. Significant higher T3 and T4 values ($p<0.05$) and a significant low TSH values ($p<0.05$) characterized the results of the subjects as compared with the controls.

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Table 3 shows the normal values of the trace elements. It also shows the mean and standard deviation (S.D.) of these elements in the cases and corresponding controls. We observed that the hyperthyroid subjects had lower and significant plasma selenium, manganese, copper and zinc levels ($p<0.05$) as compared to what obtained in the controls. On the other hand, the hyperthyroid patients had a higher and significant mean lead concentration compared to what obtained in the control group ($p>0.05$). We also observed that male cases had significantly raised plasma zinc compared to the females subjects (Table 2). A weak positive or a weak negative correlation existed between the concentration of the hormones and the trace elements ($p>0.05$).

Table 1: ***Comparison the mean ± S.D of analytes in both cases and control in reference to reference values of analytes***

Parameters	Cases n = 40 Mean + S.D.	Control n = 30 Mean + S.D.	T-value	P-value	Sig.	Ref range
Age (years)	40.18 + 5.17	40.33 + 5.31	0.28	P>0.05	NS	N/A*
"Se (µmol/L)"	1.0 + 0.29	1.81 + 0.48	5.31	P<0.05	S	0.58 -1.82
Mn (µmol/L)	1.12 + 0.44	2.70 + 0.83	8.04	P<0.05	S	0.07 -0.28
Cu (µmol/L)	12.61 + 1.88	16.64 + 4.46	9.11	P<0.05	S	11.2 - 23.2
Zn (µmol/L)	12.09 + 4.18	15.09 + 3.28	6.49	P<0.05	S	8.4 – 22.9
Pb (µmol/L)	9.31 + 4.46	3.09 + 1.32	13.72	P<0.05	S	2.4 -3.9
T3 (nmol/L)	6.24 + 2.94	1.49 + 0.26	16.57	P<0.05	S	1.0-3.25
T4 (nmol/L)	245.43 + 77.87	89.47 + 15.64	99.24	P<0.05	S	65-175
TSH (MIU/L)	0.16 + 0.08	1.5 + 0.48	9.99	P<0.05	S	0.5-6.5

N/A Not Applicable S = statistically significant*

Table 2: Sex comparison of variables in cases.

Parameters	**Females N=34 Mean± SD**	**Males N=6 Mean ± SD**	**P value**
Age	40 ± 5.0	39 ±6.7	P>0.05
"Se (µmol/L"	0.99 ± 0.3	1.23± 0.2	P>0.05
Mn (µmol/L	1.12±0.4	1.12±0.5	P>0.05
Cu (µmol/L	12.63±1.9	12.52±1.6	P>0.05
Zn (µmol/L	11.65±3.9	14.57±5.0	P<0.05*
Pb (µmol/L)	9.50± 4.4	8.24±5.4	P>0.05
T3 (nmol/L)	6.28±3.0	6.02±2.8	P>0.05
T4 (nmol/L	249.6±76.2	222.0±90.6	P<0.05*
TSH (Miu/L)	0.17±0.1	0.15±0.1	P>0.05

**Significant level.*

TABLE 3: Correlation (r) between levels of trace elements with level T_3,T_4 and TSH in the study population

Variable	**T3**	**T4**	**TSH**
CU	- 0.06	0.30	0.01
ZN	-0.15	-0.20	-0.21
PB	0.26	0.14	-0.02
MN	0.10	0.15	0.19
SE	0.03	0.03	-0.10

Correlation (r) between levels of trace elements with levels T_3,T_4 and TSH in the study population showed no significant correction(p⍰0.05)

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DISCUSSION

Our findings of significantly increased T4 and T3 and a very low concentration of TSH values in our subjects confirmed the establishment of hyperthyroidism. We observed a female preponderance over that of males. Since our selection was random, this thus goes to confirm earlier suggestions that hyperthyroidism is more common in females than their male counterparts. The non-significant level of correlation between the hormones and the trace elements in this study, we assume may be a result of the small sample size. Thyroid hormones, of which T3 is the major active form, exert a multitude of physiological effects on the regulation of various body homeostasis. The increased circulating levels of the thyroid hormones are generally associated with modifications of the whole organism; the reflection being enormous as found with weight loss, increased metabolic rate and temperature. Like thyroid hormones, trace elements also play vital roles in normal body metabolism, and it has been shown that thyroid hormones do influence the metabolism of these elements (Leblondel & Allain, 1989, Al-Sayer et al. 2004), with changes in their concentration associated with the development of various thyroid pathologies and functional derangement. Experimental studies and epidemiological data inferre that hyperthyroidism is associated with a general increase in tissue oxidative stress. In the present study, we found the serum levels of both Copper and Zinc to be significantly decreased in hyperthyroidism. This is in conformity with the findings of Hawk et al (2003). These two trace elements are important for the activity of the enzyme superoxide dismutase (SOD) which has an active site for both elements in its structure. Copper is also an integral component of tyrosinase, an enzyme that is involved in tyrosine metabolism, which is very relevant in thyroid hormone biosynthesis (Schwarz, 1996). In hyperthyroidism, the increased rate of body metabolism, may lead to the presence of the superoxide radicals that are formed by univalent reduction of oxygen in tissues. Excessive generation of these free radicals and the scavenging action of Cu-Zn superoxide dismutase can possibly lead to depletion of both copper and zinc store. Furthermore, our results showed that men had significantly raised zinc level when compared to their female counterparts. Chatterjea and Shinde (2002) reported that basal metabolic rate (BMI) was higher in men than in women. It is therefore not unlikely that the higher BMI observed in men could result to more catabolic processes in men since thyroid hormone influences general body metabolism. The increased catabolic processes in men could lead to large turnover of tissues with resultant increase in plasma zinc since it is an intracellular cation. This however requires further experimentation considering our smaller sample size for men.

Our finding of a low Selenium concentration is consistent with the finding of Aliciquzel et al (2001). Selenium is a biologically active element in the form of selenocysteine in the active centre of selenoproteins. It is a structural component of two important enzymes, glutathione peroxidase and iodothyronine deiodinase (Lockith, 1996, Frier et al,1999 and Duffield et al,1999), which are essential for the activity of the deiodinase complex that converts T4 to T3 (Berry & Larsen, 1992). The increased levels of thyroid hormones and the concomitant increased production of hydrogen peroxide by the thyrocytes, give glutathione peroxidase, the required substrate, which may be the cause of selenium depletion in the blood. Manganese is an essential trace element that is an integral component of many metalloenzymes as well as playing a major role as an enzyme activator. Experimental studies have shown increased oxidative stress in hyperthyroidism as a result of increased catabolism and oxidant generation (Adali, et al 1999; Givelek et al, 2001; Konukoglu et al 2001 and Seven et al 2001). As a consequence, manganese-superoxide dismutase, thus, converts potentially toxic superoxide radical into hydrogen peroxide. As increased metabolism persists, increased toxic superoxide radical produced, require the scavenging action of manganese-superoxide dismutase, which may lead to the depletion of the manganese store and thus low level of plasma manganese in hyperthyroid subjects as found in this study. A major endogenous source of lead is bone (Chuang, et al, 2001). Hyperthyroidism is one of the endocrine disorders that increases bone turnover and thus favors lead mobilization. From this study, it was observed that hyperthyroid patients had a significant higher plasma lead levels than the controls. There was also a very weak positive, non- significant correlation between lead, T_3 and T_4. Our finding was in conformity with the findings of Klein et al (1998) who reported high lead levels in patients with hyperthyroidism. The weak positive, non significant correlation observed in this study could be due to the small sample size.

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This requires further evaluation using larger patients pool.In summary, of all the trace elements studied, we observed a decrease in the concentrations of selenium, manganese, copper, and zinc. Form this study, it could be inferred that since these trace elements were found to be lower in patients with hyperthyroidism, supplementation with these elements could be beneficial in the management of hyperthyroid patients. This however requires further investigation with a well designed longitudinal follow-up study with anti-thyroid treatment and trace elements/placebo supplementation in patients with thyrotoxicosis. Measurement of these trace elements during the treatment of patients with thyrotoxicosis could also be useful for adequate prognosis. Furthermore, the relevance of increased lead turnover in hyperthyroid patients may have implications that need further investigation.

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