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Comparison of selenium and deiodinase enzyme among healthy controls and hypothyroid patients-A pilot study

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Abstract---Background: Selenium an essential trace element plays an important role in human growth and development. Selenoproteins are involved in thyroid hormone metabolism and recent reports suggest there is increasing prevalence of subclinical hypothyroidism when compared to hypothyroidism. Objectives: In this paper, we aimed to analyse whether the altered thyroid profile observed in subclinical hypothyroidism is due to the deficiency of selenium or due to the defect in activity of deiodinase enzyme involved in thyroid hormone metabolism. Materials and methods: The study included the patients attending the outpatient department of General Medicine of VMKV Medical College and Hospitals, Salem. The study participants were classified into 3 groups based on their thyroid profile as Group 1- Control (n=20), Group 2: Patients with Subclinical hypothyroidism (n=20), Group 3: Patients with hypothyroidism (n=20). After obtaining informed consent from the study participants, blood sample was collected for Selenium and deiodinase assay. Selenium was performed in mass spectrophotometer using whole blood and deiodinase activity was measured in serum by ELISA method. Result: Our results showed a normal but statistically significant difference in the level of selenium and activity of deiodinase 2 enzyme in subclinical hypothyroidism and hypothyroidism when compared to healthy controls. Conclusion: The enzyme deiodinase which is a selenoprotein is involved in the conversion of T₄ to T₃ in the thyroid gland. Decrease in selenium level in the body may affect the activity of the enzyme resulting in decreased synthesis of T₃. The increase in DIO2 may be due to the reduced ubiquitination and proteosomal degradation induced by T₄ to maintain T₃ levels. Hence, to maintain thyroid level and overall

health the physiological concentration of selenium should be maintained through a balanced diet or through supplementation.

Keywords---Selenium, deiodinase 2 enzyme (DIO2), subclinical hypothyroidism, hypothyroidism.

Introduction

Selenium a micronutrient, plays an important role in human growth and development (1)(2). The thyroid gland has the highest concentration of selenium among the human tissues(3). Although selenium is not essential for plant growth the plants take up selenium to incorporate it in their aminoacids and proteins through which it exerts its functions. The selenoproteins are involved in cellular antioxidant defence systems and redox reactions. The glutathione reductase and thioredoxin reductase family are involved in the protection of the thyroid gland from excess hydrogen peroxide and reactive oxygen species, produced by the follicles during biosynthesis of thyroid hormones. The deiodinases are involved in the activation and inactivation of thyroid hormones(4)

It has been observed that Selenium deficiency subjects are more susceptible to health problems(5). Recent literature reveals that there is an increasing prevalence of subclinical hypothyroidism when compared to hypothyroidism in iodine sufficient regions(6)(7)(8). A study by Rebecca et al in 2009 in Puducherry showed that among 505 women examined, 9.5% were found to have subclinical hypothyroidism (9). Another recent study among the seafood consuming population in Kerala, it was observed that 15% had subclinical hypothyroidism(10). Hence there is a need to analyze, whether the elevated TSH observed in subclinical hypothyroidism is due to the deficiency of selenium or due to the impaired activity/synthesis of deiodinase enzyme involved in thyroid hormone metabolism.

Materials and Methods

Type of study: Cross sectional comparative study

Study population

The study population includes adult patients aged between 25 to 50 yrs attending the outpatient department of the VMKV Medical College with signs and symptoms of thyroid disease. Based on the laboratory data of thyroid function tests the study subjects are grouped as

Group 1- Control (n=20)

Group 2: Patients with Subclinical hypothyroidism (n=20)

Group 3: Patients with hypothyroidism (n=20)

Inclusion criteria

Patients with subclinical hypothyroidism (TSH between 4.5 to 10.0mIU/L) and hypothyroidism (TSH- >10.0 mIU/L).

Exclusion criteria

Hypothyroid patients on medication, patients on antioxidant medication, other metabolic diseases.

Sample collection

After obtaining informed consent from the study subjects, 5 ml of blood samples was collected by venipuncture. 3ml of whole blood is transferred to a heparinized tube for selenium assay and stored at - 20⁰ C. To measure the deiodinase enzyme activity, 2ml of blood is centrifuged at 1000 rpm for 20 minutes, serum separated and stored at -20⁰C till assay.

Measurement of Selenium

Inductively coupled plasma Mass spectrometry (ICP-MS) after microwave digestion(11) .

Measurement of Human Deiodinase 2 (DIOII) by ELISA method

Principle: The microplate provided in this kit has been precoated with an antibody specific to human DIO2. Standards or samples are then added to the appropriate wells with a Biotin- conjugated antibody specific to DIO2. Then Streptavidin conjugated to Horseradish Peroxidase is added to microtitre plate and incubated. After TMB substrate solution is added, only those wells which contain DIO2, Biotin conjugated antibody and enzyme- conjugated Avidin will exhibit a change in color. The enzyme substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured at a wavelength of 450nm. The concentration of DIO2 in the sample is then determined by comparing the OD of the samples to the standard curve.

Statistical Analysis

Statistical analysis was performed using SPSS version 16. Normality assumption was tested. Mean, median and standard deviation was calculated for continuous variables. Correlation coefficient was calculated for all the variables.

Results

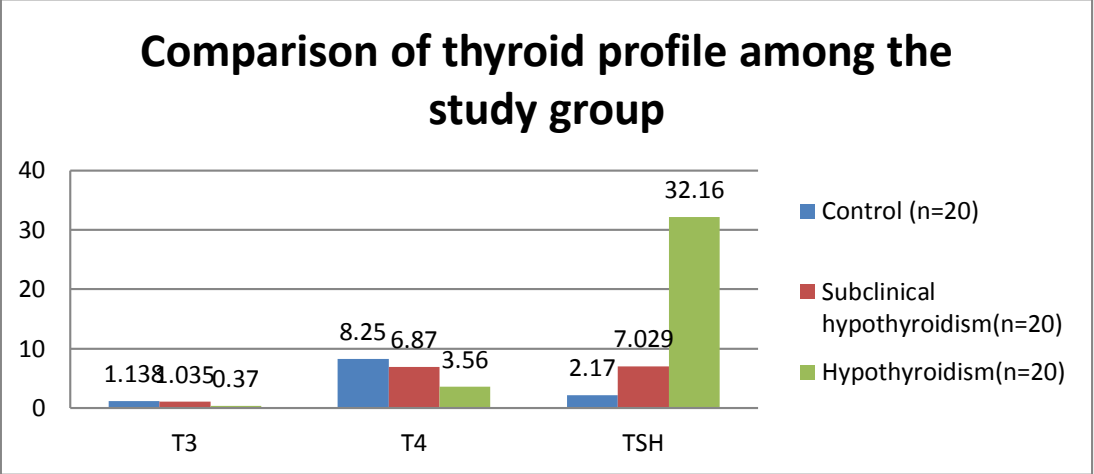


Figure 1: Thyroid profile of control, subclinical and hypothyroidism

Table 1
Median IQR of selenium among the study groups

Study Group		Selenium
Control (n=20)	Median	200
	Minimum	50
	Maximum	260
Subclinical hypothyroidism(n=20)	Median	70
	Minimum	30
	Maximum	150
Hypothyroidism(n=20)	Median	110
	Minimum	48
	Maximum	260

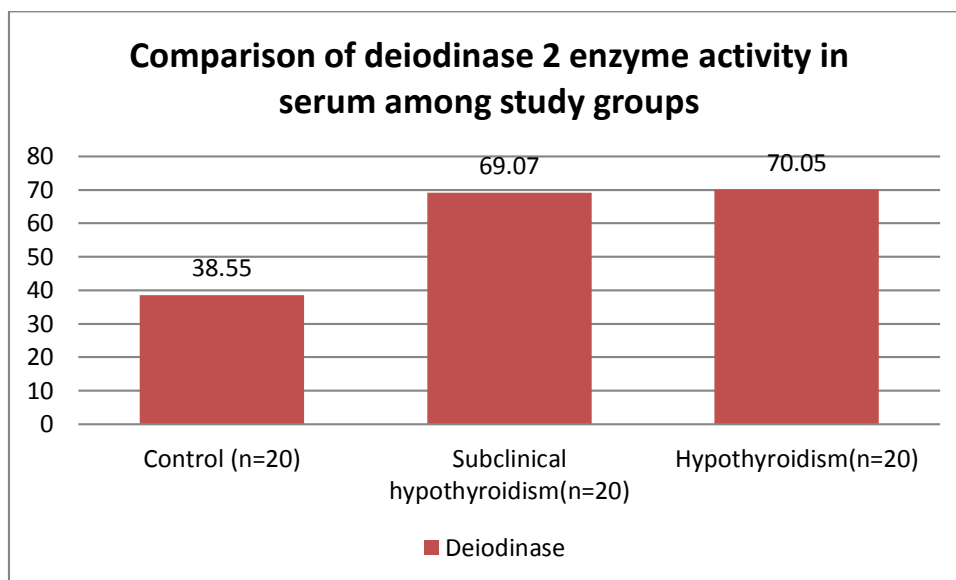


Figure 2: Comparison of deiodinase 2 enzyme activity in serum among study groups

Table 2
Correlation of selenium with Deiodinase 2 enzyme and T3

		Pearson Correlation	p-value
Control	Se and DIO 2	-0.178	0.452
	T3 and Se	-0.428	0.060
Subclinical Hypothyroidism	Se and DIO 2	0.178	0.453
	T3 and Se	0.374	0.104
Hypothyroidism	Se and DIO 2	0.449	0.047
	T3 and Se	0.180	0.447

Discussion

Hypothyroidism is characterised by low T3 and T4 with increased level of TSH (fig :1). The normal range of T3 observed in Subclinical hypothyroidism may be due to the fact that the production of thyroid hormone is regulated by a endocrine feedback loop. This may be due to the fact low level of T3 and T4 stimulate the TRH which in turn stimulates the TSH secretion and regulates thyroid hormone levels.(12) It has been shown selenoproteins are involved in thyroid hormone metabolism and recent reports suggest there is increasing prevalence of subclinical hypothyroidism when compared to hypothyroidism(6)(9). Table 1 shows the selenium levels in plasma among the study groups. In the present study the median IQR selenium concentration in control, subclinical hypothyroidism and hypothyroidism are (200(50-260), 70(30-150),110(48-260) ug/L respectively and the levels differed significantly(p=0.001). The normal level of selenium in the blood ranges between 60-120ug/L(13).Several studies have shown that low selenium status is associated with increased risk of thyroid

disease.(14) Though normal, there was a significant decrease in selenium levels in patients with subclinical hypothyroidism when compared to hypothyroidism and controls. Selenium levels in blood mostly reflect the selenium intake through diet. A recent cross-sectional observational study involving 6152 participants conducted in two counties of Shaanxi province, China suggests that the prevalence of thyroid disorders was lower in the adequate selenium county than in the low selenium county. The author suggests increased selenium intake may reduce the risk of thyroid function in areas of low selenium intake which exist not only in China, but in many other parts of the world(15). Our earlier report in the same study area revealed a low selenium and GPX activity among the diabetics (16). A recent study has shown intake of kabasura kudineer significantly increased the antioxidant enzymes in asymptomatic covid 19 patients and also reduced oxidative stress and inflammation (17). The normal selenium levels observed in the thyroid patients and a high level observed in controls may be due to the additional or hidden supplements consumed by the participants in the current pandemic to prevent themselves from corona virus. Literature also suggests that selenium supplementation helped in increasing TPO antibodies in autoimmune thyroiditis(18)(19). A retrospective study conducted in Jordan in 2020, by following up subclinical hypothyroid patients after 2 years revealed that thyroid positive TPO antibodies was associated with high TSH and hypoechogenicity was observed in TPO positive patients. The results suggested that thyroid ultrasonography and TPO antibodies should be tested at an early stage in evaluating and treating patients with subclinical hypothyroidism (20). The limitation in the present study is TPO antibodies were not measured. Hence further study should focus on evaluating the TPO antibodies among the study population to assess the relation of high TSH and the occurrence of subclinical hypothyroidism.

Figure 2 shows the serum deiodinase 2 enzyme activity among the study groups. In the present study the mean deiodinase levels were 58.55ng/dl $\pm$ 13.6, 69.07 ng/dl $\pm$ 15.15 and 70.05 ng/dl $\pm$ 15.04 in control, subclinical hypothyroidism and hypothyroidism respectively and were statistically significant ($p=0.021$). Deiodinase 1 and 2 are responsible for the local activation of thyroid hormones and selenium content also directly affects the activity and indirectly the T3 synthesis(21). Plasma levels of deiodinases are less studied. Mostly the tissue level or the expression of deiodinase enzyme are studied in tissues to quantify the thyroid status(22)(23)(24). In a recent study, serum DIO2 was measured in COPD patients and it was shown that DIO2 was increased when compared to controls which also marks inflammation and the author suggests further investigations are needed if they are intended to be considered markers of processes involved in COPD mechanisms(25). The reports of our study revealed the deiodinase 2 activity was found to be increased in subclinical hypothyroid and hypothyroid patients when compared to controls. Literature suggests in hypothyroidism when T4 production is reduced, D2 activity is increased due to the reduced ubiquitination and proteosomal degradation induced by T4. Since T4 to T3 conversion by deiodinase 2 is double to that of deiodinase 1, it increases the activation of residual T4 and facilitates the T3 homeostasis in serum and peripheral tissues(26). Hence the increased level of deiodinase 2 observed in the present study may be due to the above said mechanism. The correlation analysis showed no significant association between selenium and Deiodinase 2 and T3 in

the study group (Table 2). According to a review by Annie Drutel, currently available clinical data does not demonstrate a clear relation between selenium and deiodinase status among thyroid dysfunction patients after selenium intake and the observations reveal even small selenium concentrations are sufficient for deiodinase activity and thyroid function (13). Future studies should be focussed with large sample size to determine the levels of serum deiodinases and study the association of selenium with the types of deiodinases among thyroid dysfunction.

Conclusion

The sample size is a limitation of this study. Another limitation for the high normal selenium observed in controls and normal level of selenium in both the study group may be due to the current pandemic where the people were indirectly on many home remedies rich in antioxidants as a preventive measure from coronavirus. In spite of these factors our results revealed a significant difference in selenium levels and deiodinase 2 activity which suggests the selenium deficiency affects the deiodinase enzyme activity which in turn affects the thyroid hormone levels and ultimately impairment in metabolism in subclinical hypothyroidism and hypothyroid subjects. The deficiency observed may be due to dietary deficiency or the low level of selenium in soil. Hence a large prospective study should be carried out along with TPO antibodies at an early stage to evaluate whether selenium supplementation will be effective in treating subclinical hypothyroidism.

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