# Total antioxidant status and lipid parameters among patients of hypothyroidism

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**ABSTRACT**

**Objective:** To determine i) the total antioxidant status in hypothyroid patients and ii) its association with hormonal and lipid parameters.

**Materials and Methods:** Total antioxidant status (TAS), lipid and thyroid hormone profile was determined in patients (n= 27) with Hypothyroidism referred for laboratory assessment of thyroid function in GMCH. Normal volunteers (n=24) served as the control group. Data was analyzed on SPSS 19.0 software.

**Results:** The TAS in normal volunteers was found to be 1.65 ± 0.32 mmol/L while in patients with subclinical (N= 12) and overt Hypothyroidism (N= 15) it was found to be 1.25 ± 0.23 and 1.28 ± 0.23 mmol/L respectively. While the difference in TAS between the normal and the overt hypothyroid group was significant ($p < 0.001$), no significant difference could be observed between the subgroups within the hypothyroid state. TAS was found to be significantly lower in patients with low T3 (<3.1nmol/L), however no such association was seen with low T4 levels. Serum TSH level was found to correlate strongly and significantly with total cholesterol and LDL levels. TAS was lower in patients with high serum cholesterol levels ($p<0.001$) but no difference was seen with LDL levels.

**Conclusion:** TAS is significantly lower in patients with both subclinical and overt hypothyroidism compared to normal. TAS levels seem to be associated with low T3 but not T4 levels. While the results appear to show a trend, the sample size is very small and more work is needed. The study is still in progress.

**Key words:** antioxidant status, serum lipid, hypothyroidism

## INTRODUCTION

Hypothyroidism is a common clinical condition, more common among women and prevalence increases with aging[1]. Hypothyroid state manifests itself as a slowing down of mental and physical activity and most of the symptoms are non-specific, often subtle and may be overlooked. Subclinical Hypothyroidism (SCH) is defined as a state where the serum free T4 levels are within the normal range; while the TSH concentration is increased (≥ 4.5mIU/mL) above the normal reference range but not high enough to be classified as overt hypothyroidism. Prevalence of Subclinical Hypothyroidism has been reported to be about 3-8% in a population without known thyroid disease[2].

Thyroid hormones are well known to influence metabolism in tissues. Hyperthyroidism and Hypothyroidism are associated with hyper- and hypo-metabolic states. Metabolic pathways constantly generate reactive oxygen species and produce oxidative stress. Oxidative stress has been implicated in a wide range of biological and pathological conditions including Aging, Alzheimer's disease, Cardiovascular disease and Cancer. Experimental models of Hyper and Hypothyroidism in rats have shown that thyroid hormones play a vital role in generation of oxidative stress[3]. Hypothyroidism was also found to be associated with abnormalities in serum cholesterol and triglyceride levels

and associated with increased risk of cardiovascular disease.Studies on animal models suggest this increased risk could be due to increased oxidative stress due to inefficient antioxidant systems rather than hyperlipidemia[4].The objective of this study was to study the lipid profile and total antioxidant status in hypothyroid (both overt and subclinical) patients and determine the association between them.

## MATERIALS AND METHODS

**Study population:** Patients (18-60 years of age) with suspected or clinically evident Hypothyroidism referred by the Internal Medicine Department of GMCHRC for laboratory assessment of thyroid function. Patients with known cardiovascular, cerebral or peripheral atherosclerotic disease and pregnant women were excluded from the study. Healthy volunteers with no thyroid disease formed the normal group.

**Methodology:** Participants were briefed regarding the study and informed consent was taken. Details of their age, gender, history of other diseases and medication including vitamin and minerals supplements was taken. Fasting blood sample was collected for thyroid function and lipid profile. Serum was stored at -70°C for antioxidant status. Serum TSH level was used to classify the patient as overt hypothyroid (TSH ≥10 ng/mL) or subclinical hypothyroid (TSH ≥ 4.5 but < 10 ng/mL). T3, T4 and TSH and Lipid parameters were estimated in the participants using Cobas analyzer (Roche Diagnostics) by electrochemilumini scence and standard enzymatic colorimetric procedures respectively. Patients were classified as having normal or low values based on our laboratory reference ranges: T3 (1.2-3.1 nmol/L), T4 (66-181 nmol/L), Total Cholesterol (up to 200mg/dL) and LDL Cholesterol (up to 130 mg/dL).

Total antioxidant status (TAS) was measured in the serum of participants by the Myoglobin-ABTS assay kit (Sigma, USA). The principle of this assay is formation of a ferryl myoglobin radical from metmyoglobin and hydrogen peroxide, which oxidizes the ABTS(azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) to produce a radical cation, ABTS· and a soluble chromogen that is green in color and can be determined spectrophotometrically at 405 nm. Antioxidants prevent formation of this radical. Values were interpolated from a standard curve using Trolox (Vitamin E) as a standard and expressed as mmol/L of Trolox equivalents. Data obtained was analyzed using SPSS 19. Total antioxidant status was analyzed between and within the groups using ANOVA and significance tested using Duncan multiple comparison test. Pearsons test was used to study the correlation between the total antioxidant status and the various hormonal and lipid parameters.

## RESULTS

On the basis of their TSH values, 27 patients (13 female and 14 male) were included in the study. Of them, 15 patients were Overt hypothyroid and 12 patients were classified as having subclinical hypothyroidism. 24 healthy volunteers (15 female and 9 male) served as the normal group. The average age in the normal group was 37 years (ranged from 23-57 years) while that in the patients of hypothyroidism was 35 years (ranged from 21-48 years).

Table 1. Total antioxidant (TAS) and Total cholesterol values in normal, subclinical hypothyroid and overt hypothyroid groups. Values expressed are Mean ± Std. Deviation

| Groups | N | TAS (mmol/L) | Total Cholesterol (mg/dL) |
|---|---|---|---|
| Normal | 24 | 1.65 ± 0.32 | 200.12 ± 22.2 |
| Subclinical Hypothyroid | 12 | 1.25± 0.23* | 212.92 ± 16.3 |
| Overt hypothyroid | 15 | 1.28 ± 0.23* | 252.20 ± 30.8** |

*Significantly ($p < 0.01$) different from the Normal group,
** Significantly different from the normal and the subclinical hypothyroid group.

As shown in Table 1, the serum total cholesterol was 200.12 ± 22.2 mg/dL in the normal group while it was 212.9 ± 16.3 in the subclinical hypothyroid group and 252.2 ± 30.8 mg/dL in the Overt hypothyroid group. Significant difference was observed in the total cholesterol values between the normal and the overt hypothyroid groups. However there was no difference in total cholesterol between the normal and the subclinical hypothyroid groups. Mean serum LDL level in the normal group was 108.5 mg/dL, in the subclinical hypothyroid group was 118.3 mg/dL and in the Overt hypothyroid group was 141.73 mg/dL. Serum TSH level was found to correlate strongly and significantly with total cholesterol (r= 0.80) and LDL (r= 0.66) levels. Both the correlations were also found to be significant (p< 0.01).

TAS in the normal group was found to be 1.65±0.32 mmol/L. In patients with subclinical and overt Hypothyroidism, it was found to be 1.25±0.23 and 1.28±0.23 mmol/L respectively. While the difference in TAS between the normal and the overt hypothyroid group was significant (p < 0.001), no significant difference could be observed between the subclinical and the overt hypothyroid groups.

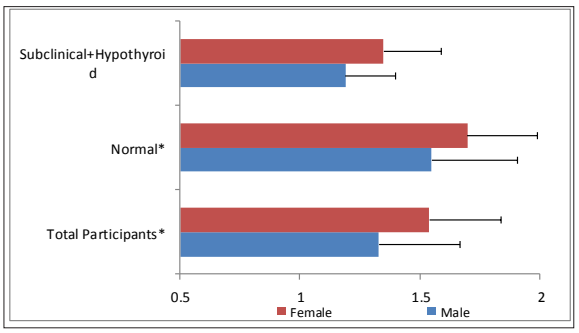


Figure 1: Total antioxidant status (Trolox equivalents) in mmol/L (Mean ± SD) in males and females in the various groups. *Significant difference (P<0.05) between the genders

When TAS was compared between the genders (Figure 1) taking all participants together, females had higher TAS compared to males. However, when further grouped according to the thyroid status, this difference was seen only within the normal group and no significant difference could be seen between the males and the females within the subclinical hypothyroid and the overt hypothyroid groups.

Participants were classified into groups based on T3 and T4 status (normal or low); and Total cholesterol and LDL cholesterol status (normal or high), irrespective of their TSH status. As shown in Table 2, participants in the low T3 and high cholesterol groups both had significantly lower TAS compared to the normal group. LDL cholesterol and T4 levels showed no such relationship with TAS.

## DISCUSSION

Reactive oxygen species are generated constantly during the oxidation of cellular

Table 2. Total antioxidant status in patients with normal or low T3, T4 and normal or high Total Cholesterol and LDL levels.

| Status | | N | TASmmol/L (Mean ±Deviation) | Significance |
|---|---|---|---|---|
| T3 | Normal | 39 | 1.51 ±0.34 | P<0.01 |
| | Low | 12 | 1.23 ±0.2 | |
| T4 | Normal | 45 | 1.46 ±0.34 | NS |
| | Low | 6 | 1.35 ±0.32 | |
| Total Cholesterol | Normal | 23 | 1.65 ±0.32 | P<0.001 |
| | High | 28 | 1.28 ±0.24 | |
| LDL | Normal | 40 | 1.49 ±0.34 | NS |
| | High | 11 | 1.29 ±0.26 | |

macromolecules such as carbohydrates, lipids and proteins. Oxidative stress can result from either overproduction of these species or from failure of the antioxidant defense systems. Under normal conditions, the antioxidant defense of the body consists of enzymes such as Superoxide Dismutase, Catalase, Glutathione Peroxidase and Glutathione Reductase along with non-enzymatic antioxidants, vitamins C and E, Carotenoids, and Glutathione.

The total antioxidant status in our normal group corresponds well with the values reported from other studies[5]. Within the normal group, females were found to have significantly higher antioxidant status in our study. This is not surprising since oxidative stress is known to be different between men and women. Oxidative stress has been reported to be higher in males than females and estrogens have been implicated to have a protective role[6].

TAS was significantly lower in subclinical and the over thypothyroid group compared to normal but no significant difference could be observed between the subclinical and the overt hypothyroid groups. Increased generation of reactive oxygen species and impairment of the antioxidant system has been reported in patients with both hyperthyroidism and hypothyroidism[7-8]. Serum oxidative stress markers have also been reported elevated in women with hypothyroidism[9]. Serum levels of oxidant-antioxidant system and thyroid hormone status in hypothyroidism pre- and post-treatment showed malondialdehyde levels to be higher in patients with hypothyroidism before treatment and were lowered following treatment but still remained higher than the controls[10].

Hypothyroidism has been known to be associated with increased risk of atherosclerotic cardiovascular diseases while evidence for subclinical hypothyroidism is also emerging[11-12]. Our earlier results had also shown significantly higher serum total cholesterol and triglyceride levels in hypothyroid patients compared to normal or subclinical hypothyroid[13]. In this study too, total cholesterol in the hypothyroid group was significantly elevated while in the subclinical hypothyroid group it was not significantly different from normal. The association between hypothyroidism and serum Total Cholesterol and LDL-Cholesterol has been investigated in several large population-based studies with conflicting reports. In the NHANES III study, mean total cholesterol levels were higher in subclinical hypothyroidism than in euthyroid controls while there were no differences in LDL or HDL levels. However, when adjusted for age, race and sex, subclinical hypothyroidism was not found related to increased cholesterol levels[1]. Total Cholesterol and Low-density lipoprotein (LDL) cholesterol levels have been reported to be significantly higher in both hypothyroid and subclinical hypothyroid patients while triglyceride levels were high only in hypothyroid patients[4]. In recent studies, overt hypothyroidism has been shown to be associated with an increase in oxidative stress. Total and LDL cholesterol, malondialdehyde as well as superoxide dismutase, catalase and vitamin E were significantly higher in the overt hypothyroid patients. Significant correlation was observed for TSH and superoxide dismutase, catalase, vitamin E and thiobarbituric acid reactive substances. Correlation between T3 and catalase remained significant after controlling for total cholesterol concentrations[14]. In our study too, total antioxidant status of participants with low T3 was significantly lower than those with normal T3 levels. Similarly, the total antioxidant status of participants with high cholesterol was lower than those with normal cholesterol. Serum TSH levels correlated strongly with the total cholesterol and the LDL levels and the correlation was significant.

## CONCLUSION

TAS is significantly lower in patients with both subclinical and overt hypothyroidism compared to those with a normal thyroid status. TAS levels seem to be associated

with low T3 and high total cholesterol but not with lower T4 levels. While the results appear to show a trend, more work is needed with a larger number of participants. The study is still in progress.

**REFERENCES**


1. Aoki Y BR, Clickner R, Jeffries R, et al. Serum TSH and total T4 in the United States population and their association with participant characteristics: National Health and Nutrition Examination Survey (NHANES 1999-2002). Thyroid 2007;17(12):1211-23.
2. Karmisholt JAS and Laurberg P. Variation in thyroid function tests in patients with stable untreated subclinical hypothyroidism. Thyroid 2008;18(3):303-8.
3. Kale MK, Bhusari KP, Umathe SN. Role Of Thyroid Hormones In The Generation Of Widespread Oxidative Stress. Journal of Cell and Tissue Research 2007;7:871-6
4. Peppa MB and Dimitriadis G. Lipid abnormalities and cardiometabolic risk in patients with overt and subclinical thyroid disease. Journal of lipids 2011;2011:575840. Epub 2011/07/18. doi: 10.1155/2011/575840
5. Lamont J, Campbell J and FitzGerald P. Measurement of Individual vs Total Antioxidants. Clinical Chemistry 1997;43(5):852-4.
6. Vina J GJ, Lopez-Grueso R, Abdelaziz KM, et al. Females live longer than males: role of oxidative stress. Curr Pharm Des 2011;17(36):3959-65.
7. Erdamar HDH, Yaman H, Erbil MK, et al. The effect of hypothyroidism, hyperthyroidism, and their treatment on parameters of oxidative stress and antioxidant status. Clin Chem Lab Med 2008;46(7):1004-10.
8. Kandasamy IAJ, Jeganathan P. Effect of Abnormal thyroid hormone changes in lipid peroxidation and Antioxidant imbalance in Hypothyroid and Hyperthyroid patients. Int J Biol Med Res 2011;2(4):1122-6.
9. Coria MJP, Gimenez MS. Serum oxidative stress parameters of women with hypothyroidism. . Acta Biomed 2009;80:135-9.
10. Baskol GAH, Tanrıverdi F, Baskol M, et al. Oxidative Stress and Enzymatic Antioxidant Status in Patients with Hypothyroidism before and after Treatment. Exp Clin Endocrinol Diabetes 2007;115(8):522-6.
11. Duntas WL. Cardiovascular Risk and Subclinical Hypothyroidism : Focus on Lipids and New Emerging Risk Factors. What Is the Evidence? . Thyroid 2007;17(11):1075-84.
12. Turhan SS, Sezer S, Erden G, et al. Plasma homocysteine concentrations and serum lipid profile as atherosclerotic risk factors in subclinical hypothyroidism. Ann Saudi Med 2008;28:96-101.
13. IA Shaafie IG, Gomathi KG, SA Basha, et al. Subclinical Hypothyroidism in patients attending GMC Hospital and its association with lipid disorders- A Preliminary Study. Proceedings of the 3rd Annual Scientific Meeting of Gulf Medical University; 2011 November 29-30:104-8.
14. Santi ADM, Moresco RN, Menezes C, et al. Association between thyroid hormones, lipids and oxidative stress biomarkers inovert hypothyroidism. Clin Chem Lab Med 2010;48(11):1635-9. Epub 2010 Aug 13.