



## Homocysteine Level in Relation to Thyroid Function Tests in Hypothyroid Patients

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**ABSTRACT:** Hypothyroidism is associated with an increased risk for cardiovascular disease, which cannot be fully explained by the atherogenic lipid profile, and other pathogenic factors may be involved. Plasma total homocysteine (tHcy) is an independent risk factor for accelerated atherosclerosis and cardiovascular disease. The aim of this study was to investigate the serum tHcy level and its relationship to thyroid function tests (fT3, fT4 and TSH) in hypothyroid patients compared to control group as well as measurement of serum cholesterol and creatinine in both groups. In this study eighty females were selected of which fifty patients were recently diagnosed with hypothyroidism, and thirty normal volunteers control group. Determination of serum tHcy was assayed by enzyme immunoassay (EIA) technique, fT3, fT4 and TSH by enzyme linked immunosorbent assay (ELISA), whereas total cholesterol by colorimetric method, and serum creatinine by kinetic method. The data was statistically analysed by SPSS-10 and p values less than 0.05 were considered significant. Our results showed that hypothyroid patients revealed a significant increase of tHcy, TSH, T.cholesterol and creatinine levels, and a significant decrease of fT4 and fT3 levels compared to control group. Serum tHcy was higher than in control by 1.7-fold. THcy was significantly positively correlated with TSH, total cholesterol, creatinine, and age, and negatively correlated with free thyroxine (fT4). In conclusion, hypothyroidism was a strongly and inversely related to serum tHcy levels. Hyperhomocysteinemia, which together with hypercholesterolemia, may explain and contribute to an accelerated atherosclerosis seen in these patients. This article was taken from MSc thesis submitted by Mohammed Abdulkader Al-Nuzaily to faculty of medicine and health sciences, Sana'a University.

**Keywords:** Hypothyroidism, Homocysteine, Cardiovascular Disease, Atherosclerosis, Cholesterol, Creatinine, Thyroxine, Triiodothyronine, Thyrotropin.

ORIGINAL ARTICLE  
Received 12 April 2014  
Accepted 25 May 2014

### INTRODUCTION

Hypothyroidism is associated with an increased risk for cardiovascular disease, which is in accordance with autopsy studies showing that the atherosclerotic process is increased in hypothyroidism (Steinberg, 1968) and decreased in hyperthyroidism (Myasnikov et al., 1963). The increased cardiovascular morbidity in hypothyroid patients has been related to elevated cholesterol and lipoprotein levels, which are normalized after thyroid hormones replacement (O'Brien et al., 1997; Martinez-Triquero et al., 1998; Hak et al., 2000). However, lipid abnormalities in hypothyroid patients do not fully account for the accelerated atherosclerosis and cardiovascular disease, and other pathogenic factors may be involved (Mamiya et al., 1989; Masaki et al., 1992).

Total homocysteine (tHcy) is an independent risk factor for cardiovascular disease (Ueland et al., 2000). The plasma level of tHcy is affected by several life-style and physiological factors and is elevated under conditions of impaired folate and cobalamin status and in renal failure (Refsum et al., 1998). In the present study we investigated

the serum tHcy levels and its relationship to thyroid function tests (fT3, fT4 and TSH) in hypothyroid patients compared to control group as well as measurement of serum cholesterol and creatinine in both groups.

### MATERIAL AND METHOD

This study was conducted at Kuwait University Hospital (KUH) in Sana'a, Yemen, from January to April, 2003. It included 80 subjects (females) aged 25 to 64 years. The patient's group consisted of 50 females (mean age  $\pm$ SD, 39.20  $\square$  9.04; median, 38.0; ranged from 25 to 64; 95% CI, 36.63-41.77 years old) selected randomly from those presenting to the out-patients' and in-patients' clinics of medical and general surgery wards of KUH. These patients were newly diagnosed with hypothyroidism by ELISA method, diagnosis were based on decreased levels of serum fT4 (< 0.8 mg/dl), fT3 (< 1.4 pg/ml) and increased TSH (> 2.0 mIU/L), and not yet started therapy. The control group included 30 females (mean age  $\pm$ SD, 31.93  $\square$  7.98; median, 29; ranged from 25 – 55; 95% CI,

28.95-34.91 years old) as normal non-hypothyroid volunteers from the workers and students of KUH. All participants gave their consent to share in this study.

#### Sample collection:

Non-fasting blood samples (5ml) of venous blood were collected from each of patients and controls and centrifuged within 30 minutes at 4000 x g for 5 minutes; the separated serum was divided into several aliquots. Determination of serum fT3, fT4, and TSH were carried out immediately. The remaining serum samples were stored at  $-70^{\circ}\text{C}$  for later analysis for the estimation of total homocysteine, total cholesterol, and creatinine levels.

#### Determination of total homocysteine (tHcy):

Serum tHcy concentrations were determined by a commercially available Axis @ Homocysteine enzyme immunoassay (EIA) reagent kit supplied by (Axis-Shield, Axis Biochemicals ASA, Distributed by IBL, Hamburg, Germany) and run on Multiscan EX from Labsystem, Finland. The reference values for adult male and female between 5 and 15  $\mu\text{mol/L}$  and among elderly ( $>60$  years) was 5–20  $\mu\text{mol/L}$ .

#### Determination of thyroid hormones:

Serum fT3, fT4 and TSH concentrations were determined by ELISA technique reagent kits supplied by Human GB and Dmbh, Wiesbaden, Germany. The ELISA kit was run on Multiscan EX from Labsystem, Finland. The reference range for serum fT3 (1.4-5.0 pg/mL), fT4 (0.80-1.6 ng/dL) and TSH (0.50-2.0 mIU/L).

#### Determination of total cholesterol and creatinine

Serum total cholesterol and creatinine concentrations were estimated using kits (colorimetric method for cholesterol and kinetic method for creatinine) supplied by Randox Lab. Ltd. (United Kingdom) and run on RA-50 Chemistry Analyser from Bayer, Ireland. The reference range for serum total cholesterol (up to 5.18 mmol/L), creatinine (female; 44.2-79.5  $\mu\text{mol/L}$ ).

#### Statistical analyses:

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS software version 10, Chicago, Illinois) to indicate the degree of significant between the mean values of the patient groups and the mean values of the corresponding controls. Descriptive data were given as mean  $\pm$  standard deviation (SD). All tests were two-tailed, and p values less than 0.05 were considered significant. Correlation between tHcy and other variables were performed by Pearson correlation.

## RESULTS

There was a significant increase in tHcy in hypothyroid patients than in control groups by 1.7-fold (Mean  $\pm$  SD, 27.28  $\pm$  6.53  $\mu\text{mol/l}$ ; 95% confidence interval (CI), 25.42-29.14 vs 10.04  $\pm$  2.86  $\mu\text{mol/l}$ ; 95% confidence interval (CI), 8.97-11.11, respectively;  $p=0.001$ ) (Table1). The median tHcy of hypothyroid

patients was 17.6  $\mu\text{mol/l}$  higher than that of control subjects. In hypothyroid patients, tHcy was ranged from 10.80 to 44.88 compared to control 4.75 to 16.50  $\mu\text{mol/l}$ . 43 of 50 (86%) hypothyroid patients had tHcy levels  $>16.5$   $\mu\text{mol/L}$ , the upper limit of the control. Table (2) showed that there were a significant positive correlation in hypothyroid patients between tHcy with TSH ( $p=0.043$ ), total cholesterol ( $p=0.029$ ), creatinine levels ( $p=0.006$ ), and age ( $p=0.001$ ). In contrast, tHcy was negatively correlated with fT4 ( $p=0.001$ ), and non-significantly correlated with fT3 (Figures 1, 2, 3 and 4).

#### Thyroid hormones Parameters:

Serum TSH levels in all 50 (100%) hypothyroid patients were significantly higher than the control group by 26-folds (Mean $\pm$ SD, 33.44 $\pm$ 10.95 mIU/L; median, 34.81; ranged from 5.92 to 51.60 vs 1.23 $\pm$ 0.28 mIU/L, median, 1.30; ranged from 0.60 to 1.70, respectively;  $p=0.001$ ) (Table 1). Serum TSH level was significantly positively correlated with tHcy and non-significantly with other variables. On the other hand, both fT4 and fT3 were observed to be significantly lower in the hypothyroid patients by 66% and 60%, respectively, compared to the control group (Table 1). For fT4, the mean $\pm$ SD was 0.41 $\pm$ 0.22 ng/dl; median was 0.36; and ranged from 0.10 to 0.90 vs 1.23 $\pm$ 0.11 ng/dl, median, 1.23; ranged from 0.96 to 1.45, respectively;  $p=0.001$ . For fT3, the mean $\pm$ SD was 1.13 $\pm$ 0.69 pg/ml; median was 0.99; and ranged from 0.14 to 3.10 vs 2.84 $\pm$ 0.86 pg/ml, median, 2.55; ranged from 1.66 to 4.60, respectively;  $p=0.001$ ). 44 of 50 (88%) hypothyroid patients had fT4 levels  $<0.8$  ng/dL, the lower limit of assay, whereas 35 of 50 (70%) had fT3 levels  $<1.4$  pg/ml, the lower limit of assay.

#### T. cholesterol and creatinine:

Both serum total cholesterol and creatinine levels were significantly higher by 54% and 46%, respectively, compared with the control group (Table 1). For T.cholesterol, the mean $\pm$ SD was 6.38 $\pm$ 1.85 mmol/l; median was 6.05; ranged from 3.18 to 12.0 vs 4.13 $\pm$ 0.17 mmol/l; median, 4.17; ranged from 3.73 to 4.50, respectively;  $p=0.001$ ). For creatinine, the mean $\pm$ SD was 91.88 $\pm$ 24.93  $\mu\text{mol/l}$ ; median was 95.0; ranged from 41.9 to 145.0  $\mu\text{mol/l}$  vs 62.97 $\pm$ 7.46  $\mu\text{mol/l}$ ; median, 63.6; ranged from 45.0 to 78.0, respectively;  $p=0.001$ ). 40 of 50 (80%) of hypothyroid patients were hypercholesterolemic, and 86% of hyperhomocysteinemic hypothyroid patients were hypercholesterolemic, whereas 37 of 50 (74%) of hypothyroid patients had elevated serum creatinine levels.

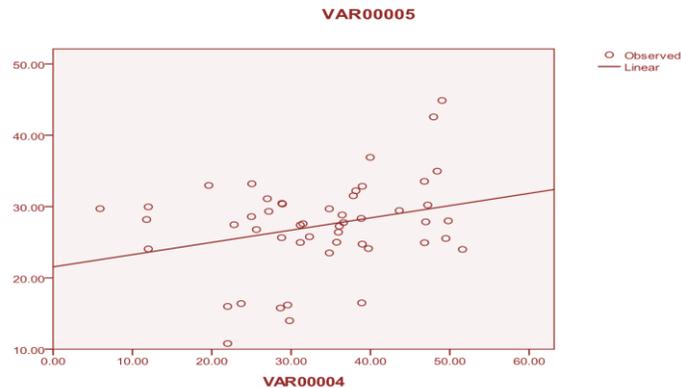
**Table 1.** Comparison between serum levels of tHcy, FT3, FT4, TSH, T.cholesterol and creatinine in hypothyroid patients and control subjects

Groups Variables	Hypothyroid patients (n=50) (Mean ± SD)	Control Subjects (n=30) (Mean ± SD)	P value
Total homocysteine (tHcy) (µmol/L)	27.28 ± 6.53	10.04 ± 2.86	0.001
Free triiodothyronine (FT3) (pg/ml)	1.13 ± 0.69	2.81 ± 0.79	0.001
Free thyroxine (FT4) (ng/dL)	0.41 ± 0.22	1.23 ± 0.11	0.001
Thyroid-stimulating hormone (TSH) (mIU/L)	33.44 ± 10.95	1.23 ± 0.28	0.001
Total cholesterol (mmol/L)	6.38 ± 1.85	4.13 ± 0.17	0.001
Creatinine (µmol/L)	91.88 ± 24.93	62.97 ± 7.46	0.001
Age (Year)	39.20±9.04	31.93±7.98	0.001

**Table 2.** Correlations between serum tHcy and other variables in hypothyroid patients

Variable	Hypothyroidism (n=50)	
	r	P†
FT3	0.009	0.951
FT4	-0.583	0.001
TSH	0.288	0.043
T. cholesterol	0.308	0.029
Creatinine	0.385	0.006
Age (Year)	0.775	0.001

†Pearson correlation, Correlation is significant at the 0.05 level (2-tailed)



**Figure 1.** Curve estimation between tHcy (var5) and TSH (var4): There was a positive relation

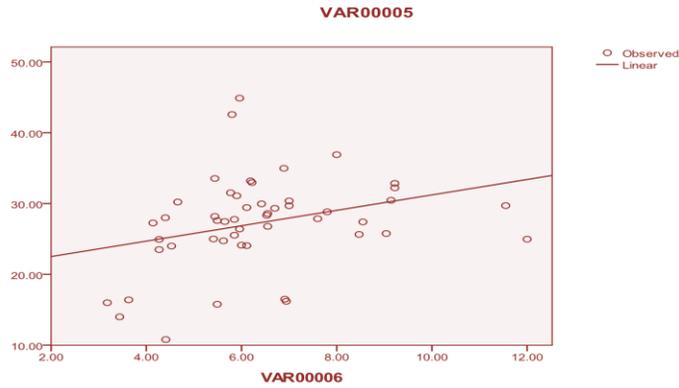


Figure 2. Curve estimation between tHcy (var5) and T.cholesterol (var6): There was a positive relation

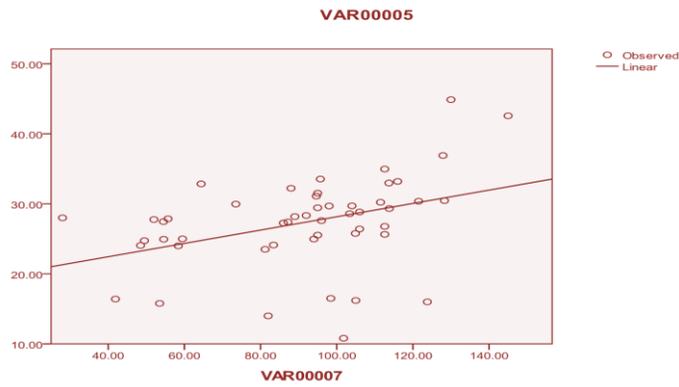


Figure 3. Curve estimation between tHcy (var5) and creatinine (var7): There was a positive relation

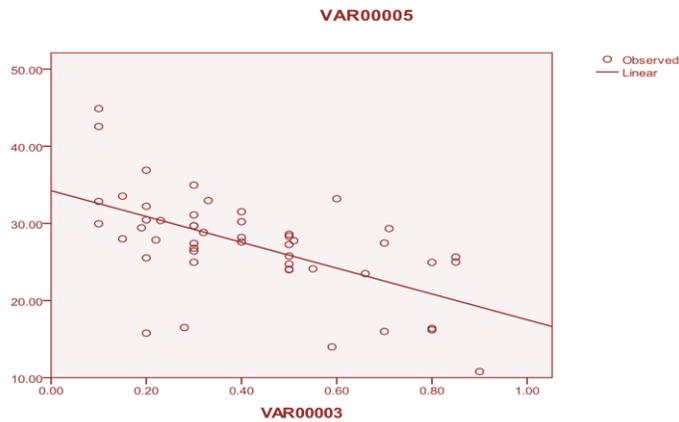


Figure 4. Curve estimation between tHcy (var5) and fT4 (var3): There was a negative relation

### DISCUSSION

In the present study, elevated serum total homocysteine (tHcy) was found to be strongly associated with hypothyroidism. As shown in Table (1), serum levels of tHcy were significantly higher in patients with hypothyroidism by 1.7-fold ( $p < 0.001$ ), and it was

significantly correlated positively with TSH, cholesterol, creatinine, and age, and negatively correlated to free thyroxine (fT4), with no significant correlation with fT3. The increased observed in tHcy in these patients may explain and contribute to a higher cardiovascular risk, since an earlier study indicates that an increase in plasma

Hcy level of 4 mmol/L, confers a 40% increase in relative risk for coronary heart disease compared with healthy controls (Boushey et al., 1995). The apparent close relation between the serum tHcy and thyroid hormone levels indicates a hormone effect on homocysteine metabolism, distribution, or clearance.

Increased tHcy levels might be the result of either a decrease in the levels of vitamins (folic acid, vit B12, and /or B6) from dietary sources (Selhub et al., 1993) or a direct effect of hypothyroidism on the tHcy metabolism and clearance. The former may be explained as a consequence of malnutrition that is often associated with hypothyroidism, partly because hypothyroidism induces anorexia, as well as, it is associated with weakness and fatigue and overweight (Yazbeck et al., 2001) resulting from decreased metabolism rate. Alternatively, it may be explained as a direct effect of thyroid hormones on the Hcy metabolism in the liver. Thyroid hormone deficiency decreases hepatic levels of enzymes involved in the remethylation pathway of Hcy (Cataragi et al., 1999). Experimental studies have also indicated that thyroid hormones affect folate metabolism. The observations that methylenetetrahydrofolate reductase (MTHFR) is increased in hyperthyroidism and decreased in hypothyroidism may be relevant for the relation between the Hcy level and thyroid status (Nair et al., 1994). This enzyme is responsible for the formation of 5-methyl-tetrahydrofolate, which functions as methyl donor during remethylation of Hcy to methionine (Finkelstein, 1990).

In line with previous studies (Nakahama et al., 2001), we found that serum creatinine was significantly ( $p < 0.001$ ) higher in hypothyroid patients when compared with controls. Serum creatinine levels were reported to decrease in hyperthyroidism and increase in hypothyroidism, and elevated levels can be reduced by thyroid hormone replacement (Diekman et al., 2001). There was a positive relation between tHcy and serum creatinine level in hypothyroidism. This may partly be explained by direct effect of thyroid hormones on renal function and/or tHcy metabolism and clearance in kidney (Nakahama et al., 2001). The former may be explained by the hypodynamic circulation in hypothyroidism (Polikar et al., 1993). Thyroid hormones are cardiostimulatory agents, which increase cardiac output while lowering systemic vascular resistance (Klemperer et al., 1995), resulting in increased renal blood flow (Polikar et al., 1993). This, in turn, may increase the glomerular filtration rate (GFR), which is related to serum creatinine, but also closely associated with plasma tHcy (Bostom et al., 1999; Wollesen et al., 1999).

The later mechanism may be explained as a result of impaired renal tHcy clearance. tHcy shows a negative relation to the GFR, and patients with renal failure have hyperhomocysteinemia attributed to low tHcy clearance, possibly due to impaired renal tHcy metabolism

(Guttormsen et al., 1997). Thus, thyroid hormones may influence the tHcy serum levels both through effects on Hcy formation and its elimination from plasma.

We observed that serum total cholesterol was significantly ( $p < 0.001$ ) higher in patients with hypothyroidism, by 54%, when compared with controls. These results were consistent with (Yazbeck et al., 2001), who stated that T.cholesterol levels were higher in patients with hypothyroidism. Hypercholesterolemia was seen in 80% of hypothyroid patients, and in 86% of hyperhomocysteinemic hypothyroid patients. There was a positive relation between tHcy and T.cholesterol. The increase of serum cholesterol might be attributed to the influence of thyroid hormones on the cholesterol metabolism or disposition (Ness and Lopez, 1995), and this may contribute to the association between hypothyroidism and hypercholesterolemia. To date, there is only one study on tHcy effects on cholesterol production and secretion showing tHcy to stimulate the production and secretion of cholesterol in hepatic cells (Karmin et al., 1998), and this may contribute to the association between cholesterol and homocysteine observed in the present study as in some epidemiological studies (Arnesen et al., 1995; Nygard et al., 1995). Since hypercholesterolemia may partly be responsible for increased cardiovascular morbidity, but cannot fully explain the accelerated atherosclerosis, therefore, an increased in both serum tHcy and cholesterol may confer increased cardiovascular risk factor observed in these hypothyroid patients.

## CONCLUSION

In conclusion, we observed that hypothyroidism was a strongly and inversely related to serum tHcy levels. Hyperhomocysteinemia, which together with hypercholesterolemia, may explain and contribute to an accelerated atherosclerosis seen in these patients. We recommended tHcy screening of hypothyroid patients, as an independent risk factor for accelerated atherosclerosis and cardiovascular disease. In addition, we recommended that hypothyroid patients should be taken folic acid and vitamin B complex supplements as well as thyroid hormone therapy to reduce cardiovascular complications caused by elevated tHcy levels.

## Competing interest

The authors declare no competing interests to disclose.

## Acknowledgment

The authors thank doctors of Lab., technician and the health workers in KUH for their help and cooperation during this study.

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