ORIGINAL ARTICLE

Correlation Between Serum Levels of Cholesterol and Homocysteine with Oxidative Stress in Hypothyroid Patients

Rahbani-Nobar M, Bahrami A, Norazarian M, Dolatkhah H.

Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, I.R.Iran

Abnormalities in cholesterol and homocysteine metabolism have been reported in thyroid diseases. Since elevated levels of both parameters are involved in atherogenesis, and thyroid hormones are modulators of oxidative stress. In this study, the correlation between serum levels of cholesterol, and homocysteine, and oxidative stress was assessed in patients with thyroid dysfunction.

Materials and Methods: A total of 60 patients with thyroid dysfunction (30 with hypothyroidism and 30 with hyperthyroidism) were included in this study. Thirty apparently healthy sex and age-matched individuals were selected as control group. The mean age of hypothyroid, hyperthyroid and control groups were 43±7.7, 39±12 and 40±7.9 years, respectively. Serum levels of homocysteine were measured by HPLC and those of thyroid hormones (T_3, T_4, T_3 R. uptake and TSH) by radioimmunoassay techniques. Levels of total antioxidant capacity, lipid profiles and creatinine were determined by standard methods in Cobas Mira Autoanalyzer.

Results: The mean±SD levels of homocysteine in hyperthyroid, hypothyroid and control groups were 7.79±1.44, 17.09±6.93 and 8.08±1.92 µmol/L, respectively. Comparing with control group significant elevation was noted in hypothyroid patients (p=0.0001). Significant correlation between serum levels of creatinine and that of homocysteine was observed (r=0.86, p= 0.0001). Singnificant elevation in the levels of total cholesterol and LDL-C were observed in hypothyroid patients (p<0.05). Significant reduction in the serum antioxidant capacity was found in patients suffering from hypothyroidism (p=0.01). But not in hyperthyroid subjects. Significant inverse correlation was observed between serum levels of antioxidant capacity and those of homocysteine (r=-0.79, p=0.02), total cholesterol (r=-0.93, p= 0.02) and LDL-C (r=-0.83, p=0.001) in hypothyroid patients. This correlations were not significant in the hyperthyroid and control groups (p > 0.05).

Conclusion: The correlation between serum levels of homocysteine, total cholesterol and LDL-C with total antioxidant capacity in hypothyroidism suggests that there is an overproduction of free radicals in these patients. It is concluded that the enhanced production of free radicals might be an important contributing factor in abnormalities seen in homocysteine and cholesterol metabolism.

Key Words: Hyperhomocysteinemia, Oxidative stress, Thyroid dysfunction, Serum lipids, Antioxidant capacity

Correspondence: Amir Bahrami, P.O.Box 51335–1896, Tabriz, I.R.Iran
E-mail: t.u.end.d@tbzmed.ac.ir

Introduction

High plasma homocysteine concentration induces pathologic changes in the arterial wall and thus is strongly associated with an increased risk of atherosclerosis, manifested as cardiovascular, cerebrovascular and pe-

ripheral vascular events.[1] There are consistent reports that patients with hypothyroidism have elevated total homocysteine in plasma and that homocysteine level is reduced following therapy with thyroxine.[2] High serum cholesterol in hypothyroidism and its low concentration in hyperthyroidism are common findings. A significant correlation between serum cholesterol and total homocysteine has been demonstrated.[3] The mechanism behind this correlation has not been clarified, but increases in both cholesterol and total homocysteine levels in hypothyroidism may have an interactive effect, which may contribute to the high prevalence of arterial occlusive disease in hypothyroidism.[4]

Thyroid hormones are physiologic modulators of both tissue oxidative stress and protein degradation.[5] The mechanism linking hypothyroidism with oxidative stress is unknown. Oxidative stress increases the concentration of oxidized LDL, a risk factor for atherosclerosis. Homocysteine is also an inducer of LDL oxidation. A strong covariation between total plasma homocysteine and cholesterol in hypothyroidism may have important medical implications. Determination of TSH in subjects with unexplained hyperhomocysteinemia and high plasma cholesterol has been recommended.[6,7] The aim of the present study is to investigate the correlation between serum levels of cholesterol and homocysteine with that of oxidative stress in patients with thyroid dysfunction.

Materials and Methods

Thirty hypothyroid patients (13 males and 17 females), 30 hyperthyroid subjects (11 males and 19 females) and 30 healthy adults were enrolled in this study. Subjects in hypo- and hyperthyroid groups were sampled from patients who were seen in endocrine clinics. Individuals in the control group were selected from patients with solitary thyroid nodule, who were euthyroid clinically, had normal TFTs, and were not on thyroid hormone preparations. Hyperthyroidism was diagnosed by high free T_4 index and suppressed serum TSH level. Subjects with low free T_4 index and elevated serum TSH were diagnosed as having hypothyroidism.

EDTA-blood samples for plasma homocysteine and total antioxidant capacity were provided after an overnight fasting. Samples were placed on ice, centrifuged within 1 hour, and separated plasma was stored at -70°C before assay. Additional fasting samples were collected for total cholesterol, high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), creatinine, thyroid hormones, T_3R uptake and TSH.

Plasma concentration of homocysteine was measured by high performance liquid chromatography after reduction of plasma disulfides with tris (2-carboxyethyl) phosphine, precipitation of proteins with trichloroacetic acid, derivatization with 7-floro-2, 1, 3-benzoxadiazol-4-sulfonate (SBD-F), and fluorescent detection.[8] Thyroid hormones, TSH and T_3 uptake were measured by standard radioimmunoassay techniques. Serum total cholesterol, HDL-C and LDL-C were measured with standard enzymatic methods in the Cobas Mira Autoanalyzer. The concentrations of creatinine in serum were determined in the same Autoanalyzer using Jaffe reaction. Total antioxidant capacities in plasma samples were assessed using the Randox total antioxidant status kit.

SPSS 11 for Windows computer program was used to perform statistical analysis. Paired student's t-test was employed to determine the significance of differences between the measured parameters of hypothyroid, hyperthyroid and control groups. Com-

parisons between different groups were made using ANOVA and all correlations were evaluated by linear regression. Results are expressed as mean±SD and statistical significance was set at $p<0.05$.

Results

The mean age of the hypothyroid, hyperthyroid, and control groups were 43±7.7, 39± 12, and 40±7.9 years, respectively. Clinical and laboratory characteristics of hypothyroid, hyperthyroid and control groups are shown in Table 1. Significant differences were noticed between the levels of T_3, T_4, TSH and T_3-uptake in the three groups ($p<0.05$). The mean±SD levels of measured parameters, including serum total homocysteine, creatinine, total cholesterol, LDL-C, HDL-C and total antioxidant capacity in hypothyroid and hyperthyroid subjects, were compared with those of control group (Tables 2 and 3, respectively). Significant elevation in the serum levels of total homocysteine, creatinine, total cholesterol and LDL-C and marked reduction in plasma concentrations of total antioxidant capacity were found in the hypothyroid group ($p<0.05$), but the changes in the serum levels of HDL-C were not significant. In the hyperthyroid group, the reductions in the levels of total cholesterol, creatinine and total antioxidant capacity were not significant and no marked changes were noticed in the levels of the other parameters. The correlations between the biochemical factors in hypothyroid, hyperthyroid and control groups are summarized in Table 4. Inverse significant correlation between the levels of total homocysteine and total antioxidant capacity was noticed in the hypothyroid group. The correlation between the levels of creatinine and those of total homocysteine was only marked in the case of the hypothyroid group. An inverse correlation between increased levels of total cholesterol and LDL-C and those of total antioxidant capacity was detected in the hypothyroid group but it was not significant in hyperthyroid and control groups. No meaningful significant correlation was noticed between total antioxidant capacity and HDL-C in subjects of the patients and control groups.

Table 1. Age, sex and thyroid function tests of hypothyroid, hyperthyroid, and control subjects

Parameters	Hypothyroid group (N=30)	Hyperthyroid group (N=30)	Control group (N=30)
Age (years)	43 (7.7)	39 (12)	40 (7.9)
Sex (male/female)	13/17	11/19	11/19
T_4 (μg/dL)	4.1 (2.4)	17.7 (4.5)	6.7 (1.5)
T_3 (ng/dL)	94 (36)	324 (177)	132 (42)
TSH (mIU/mL)	41.0 (35.9)	0.08 (0.07)	1.55 (1.4)
T_3-uptake (%)	22.7 (7.3)	44.6 (5.1)	35.6 (4.0)

Numbers represent mean (SD).

Table 2. Comparison of the mean (SD) levels of the measured parameters in hypothyroid and control subjects

Parameters	Hypothyroid (N=30)	Control (N=30)	P Value
Total Homocysteine (œmol/L)	17. 9	8.08 (1.39)	0.0001
Creatinine (mg/dL)	1.52 (0.92)	0.90 (0.22)	0.007
Total Cholesterol (mg/dL)	252 (27)	166 (48)	0.004
HDL-C (mg/dL)	46.2 (16.6)	45.3 (18.2)	0.48
LDL-C (mg/dL)	137 (18)	90 (51)	0.0001
Total Antioxidant capacity (mmol/L)	0.94 (0.39)	1.40 (0.18)	0.04

Numbers represent mean (SD).

Table 3. Comparison of the mean (SD) levels of the measured parameters in hyperthyroid and control subjects

Parameters	Hyperthyroid (N=30)	Control (N=30)	P Value
Total Homocysteine (œmol/L)	7.79 (1.44)	8.08 (1.39)	0.079
Creatinine (mg/dL)	0.78 (0.32)	0.90 (0.22)	0.54
Total Cholesterol (mg/dL)	155 (98)	166 (48)	0.09
HDL-C (mg/dL)	43.8 (16.4)	45.3 (18.2)	0.51
LDL-C (mg/dL)	84 (48)	90 (51)	0.48
Total Antioxidant capacity (mmol/L)	1.3 (0.19)	1.40 (0.18)	0.081

Numbers represent mean (SD).

Table 4. Correlation between the measured parameters in hypothyroid, hyperthyroid and control subjects

Correlation Between	Hypothyroid group		Hyperthyroid group		Control group	
	r	p	r	p	r	p
Homocysteine and total antioxidant capacity	-0.79	0.02	0.63	0.06	0.03	0.71
Creatinine and homocysteine	0.86	0.0001	0.69	0.057	0.55	0.06
Cholesterol and total antioxidant capacity	0.93	0.021	-0.21	0.059	0.11	0.49
LDL-C and total antioxidant capacity	.083	0.001	0.59	0.53	0.44	0.67
HDL-C and total antioxidant capacity	0.53	0.08	-0.02	0.71	-0.03	0.71

Discussion

Elevated plasma levels of the amino acid homocysteine has been identified as an independent risk factor for atherosclerosis.[9] The mechanisms by which hyperhomocysteinemia causes atherosclerosis are not completely understood. Animal models of hyperhomocysteinemia have shown altered vascular function, including the promotion of smooth muscle cell growth and the development of atherosclerosis. Diffuse arterial damage and an increased propensity to thrombus formation are commonly noted in people with homocysteinuria. Concomitant lipoprotein abnormalities, including increased oxidation and binding, may also be mechanisms by

normalities, including increased oxidation and binding, may also be mechanisms by which hyperhomocysteinemia promotes atherosclerotic process.[10]

There are consistent reports that patients with hypothyroidism have elevated total homocysteine in plasma and that total homocysteine is reduced following therapy with thyroid hormones.[11,12] Hypothyroidism is associated with increased cardiovascular morbidity, which cannot be fully explained by the atherogenic lipid profile observed. Other abnormalities have also been suggested to be responsible for the increased cardiovascular morbidity in hypothyroid patients.[13,14] Homocysteine causes LDL oxidation probably through superoxide anion formation in the auto-oxidation process of the amino acid.[15] Homocysteine thiolactone also acetylates extracellular proteins such as apoB and LDL.[16] Since substantial evidence indicates that oxidized low-density lipoprotein contributes to atherogenesis through a number of mechanisms,[17] the relationship between oxidative stress and the levels of cholesterol and homocysteine was evaluated in this study.

In the present work, we observed significantly higher levels of homocysteine in hypothyroid patients in comparison with thyrotoxic individuals which is in agreement with previously reported data.[18] Another important finding was the association of changes in serum levels of creatinine and cholesterol with concentrations of homocysteine in hypothyroidism. Renal function is a well-known determinant of plasma homocysteine level. Altered thyroid function not only leads to changes in glomerular filtration rate but also to changes in body weight and body composition. An alterative explanation for the concurrent elevation of plasma homocysteine and serum creatinine in hypothyroidism is the formation of homocysteine in conjunction with creatine synthesis, which is related with creatine synthesis, which is related to muscle mass.[19]

We observed high serum cholesterol and LDL-C in hypothyroid and low concentration in hyperthyroid patients, which is in agreement with other reports.[20,21] Significant correlation was noticed between the levels of homocysteine and those of cholesterol and LDL-C in the hypothyroid group. A marked correlation between serum cholesterol and homocysteine has also been demonstrated in some epidemiological studies.[22] Although the mechanism behind this covariation has not been clarified,[23] increases in both cholesterol and homocysteine in hypothyroid subjects may have an interactive effect,[24] which may contribute to the high prevalence of arterial occlusive diseases in hypothyroid patients.[25] On the other hand, low levels of both factors in hyperthyroid patients may be protective.[26]

Oxidative stress induced by homocysteine is reflected by an increase in malondialdehyde (MDA), a measure of membrane lipid peroxidation, and a decrease in plasma antioxidant capacity eight hours after methionine loading in healthy subjects.[27] Homocysteine may induce intracellular inactivation of the glutathione (GSH) antioxidant defense system, and possibly reduces homocysteine derived GSH synthesis.[28] Excess or deficiency of the thyroid hormones cause alteration in MDA levels and glutathione peroxidase activities of tissues.[29] In the present study, the level of total antioxidant capacity and correlation with the levels of cholesterol and the other measured parameters were evaluated in thyroid dysfunction. A reverse and significant correlation was observed between the level of total antioxidant capacity and the concentration of homocysteine in the hypothyroid group but the correlation was not significant in the case of the hyperthyroid group.

Similar results have been reported in other studies.[30]

In conclusion, homocysteine levels were increased and plasma levels of total antioxidant capacity were decreased significantly in hypothyroidism. A strong covariation between homocysteine and cholesterol and their correlation with the levels of total antioxidant capacity in hypothyroidism may increase cardiovascular risk. Determination of serum levels of thyroid hormones is recommended in subjects with unexplained hyperhomocysteinemia and hypercholesterolemia.

References

1. Robinson K, Mayer E, Jacobsen DW. Homocysteine and coronary artery disease. Cleve Clin J Med. 1994 Nov-Dec;61(6):438-50.
2. Hussein WI, Green R, Jacobsen DW, Faiman C. Normalization of hyperhomocysteinemia with L-thyroxine in hypothyroidism. Ann Intern Med. 1999 Sep 7;131(5):348-51.
3. Lien EA, Nedrebo BG, Varhaug JE, Nygard O, Aakvaag A, Ueland PM. Plasma total homocysteine levels during short-term iatrogenic hypothyroidism. J Clin Endocrinol Metab. 2000 Mar;85(3):1049-53.
4. Steinberg AD. Myxedema and coronary artery disease--a comparative autopsy study. Ann Intern Med. 1968 Feb;68(2):338-44.
5. Pamplona R, Portero-Otin M, Ruiz C, Bellmunt MJ, Requena JR, Thorpe SR, et al. Thyroid status modulates glycoxidative and lipoxidative modification of tissue proteins. Free Radic Biol Med. 1999 Oct;27(7-8):901-10.
6. Arnesen E, Refsum H, Bonaa KH, Ueland PM, Forde OH, Nordrehaug JE. Serum total homocysteine and coronary heart disease. Int J Epidemiol. 1995 Aug;24(4):704-9.
7. Nedrebo BG, Nygard O, Ueland PM, Lien EA. Plasma total homocysteine in hyper- and hypothyroid patients before and during 12 months of treatment. Clin Chem. 2001 Sep;47(9):1738-41.
8. Gilfix BM, Blank DW, Rosenblatt DS. Novel reductant for determination of total plasma homocysteine. Clin Chem. 1997 Apr;43(4):687-8.
9. den Heijer M, Koster T, Blom HJ, Bos GM, Briet E, Reitsma PH, et al. Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. N Engl J Med. 1996 Mar 21;334(12):759-62.
10. Fallest-Strobl PC, Koch DD, Stein JH, McBride PE. Homocysteine: a new risk factor for atherosclerosis. Am Fam Physician. 1997 Oct 15;56(6):1607-12, 1615-6.
11. Catargi B, Parrot-Roulaud F, Cochet C, Ducassou D, Roger P, Tabarin A. Homocysteine, hypothyroidism, and effect of thyroid hormone replacement. Thyroid. 1999 Dec;9(12):1163-6.
12. Lien EA, Nedrebo BG, Varhaug JE, Nygard O, Aakvaag A, Ueland PM. Plasma total homocysteine levels during short-term iatrogenic hypothyroidism. J Clin Endocrinol Metab. 2000 Mar;85(3):1049-53.
13. O'Brien T, Katz K, Hodge D, Nguyen TT, Kottke BA, Hay ID. The effect of the treatment of hypothyroidism and hyperthyroidism on plasma lipids and apolipoproteins AI, AII and E. Clin Endocrinol (Oxf). 1997 Jan;46(1):17-20.
14. Ishikawa T, Chijiwa T, Hagiwara M, Mamiya S, Hidaka H. Thyroid hormones directly interact with vascular smooth muscle strips. Mol Pharmacol. 1989 Jun;35(6):760-5.
15. Jones BG, Rose FA, Tudball N. Lipid peroxidation and homocysteine induced toxicity. Atherosclerosis. 1994 Feb;105(2):165-70.
16. Jakubowsk H. Homocysteine thiolactone: Metabolic origin and protein acetylation in humans. J Nutr. 2000; 130: S371-81.
17. Chisolm GM, Penn MS. Oxidized lipoproteins and atherosclerosis. In: Fuster V, Ross R and Topol EJ, atherosclerosis and coronary artery disease. editors, Philadelphia, Lippincott-Raven Publisher,. 2000: p. 129-149.
18. Catargi B, Parrot-Roulaud F, Cochet C, Ducassou D, Roger P, Tabarin A. Homocysteine, hypothyroidism, and effect of thyroid hormone replacement. Thyroid. 1999 Dec;9(12):1163-6.
19. Lafayette RA, Costa M and King A. Increased serum creatinine in the absence of renal failure in profound hypothyroidism. Am. J. Med; 43(3): 786-790, 1998.
20. Mason RL, Hunt HM, Hurxthal L. Blood cholesterol values in hyperthyroidism and hypothyroidism. Their significance. N Engl J Med. 1980; 203: 1273-8.
21. Hoch FL. Lipids and thyroid hormones. Prog Lipid Res. 1988;27(3):199-270.
22. Nygard O, Vollset SE, Refsum H, Stensvold I, Tverdal A, Nordrehaug JE, et al. Total plasma homocysteine and cardiovascular risk profile. The Hordaland Homocysteine Study. JAMA. 1995 Nov 15;274(19):1526-33.

chim Biophys Acta. 1998 Aug 28;1393(2-3):317-24.
24. Graham IM, Daly LE, Refsum HM, Robinson K, Brattstrom LE, Ueland PM, et al. Plasma homocysteine as a risk factor for vascular disease. The European Concerted Action Project. JAMA. 1997 Jun 11;277(22):1775-81.
25. Fowler PB, Ikram H, Banim SO. Serum-cholesterol, thyroid failure, and coronary-artery disease. Lancet. 1972 Mar 25;1(7752):685.
26. Jeffers WA, Littman DS, Rose E. The infrequency of myocardial infarction in patients with thyrotoxicosis. Am J Med Sci. 1957 Jan;233(1):10-5.
27. Cavalca V, Cighetti G, Bamonti F, Loaldi A, Bortone L, Novembrino C, et al. Oxidative stress and homocysteine in coronary artery disease. Clin Chem. 2001 May;47(5):887-92.
28. Mosharov E, Cranford MR, Banerjee R. The quantitatively important relationship between homocysteine metabolism and glutathione synthesis by the transsulfuration pathway and its regulation by redox changes. Biochemistry. 2000 Oct 4;39(42):13005-11.
29. Bilgihan K, Bilgihan A, Diker S, Ataoglu O, Dolapci M, Akata F, et al. Effects of hyper- and hypo- thyroidism on oxidative stress of the eye in experimental acute anterior uveitis. Acta Ophthalmol Scand. 1996 Feb;74(1):41-3.
30. Ohidar Rahaman SK, Ghosh S, Mohanakumar KP, Das S, Kumar Sarkar P. Hypothyroidism in developing rat brain is associated with marked oxidative stress and aberrant intraneuronal accumulation of neurofilaments. Neuroscience Research. 2001; 40: 273-9.

Correction:

Amani R, Zand-Moghaddam A, Jalali MT, Hatamizadeh MA. Effects of Soy Protein Isoflavones on Lipid Profile and Serum Hormones in Hypercholesterolemic men. Int J Endocinol Metab 2004;2:29-34.

In table 2 (page 32), comparison of SPI and placebo groups for total cholesterol in the column "Post" has a $p<0.055$. In Table 3, p value for FSH should read 0.2 instead of 0.02, and in the footnote "† is SPI group" should be added.