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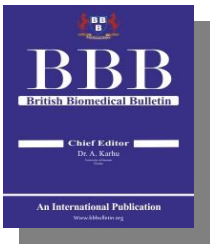
BRITISH BIOMEDICAL BULLETIN



Original

Impact of Hypothyroidism on Serum Malondialdehyde and Lipid Levels in Indian Punjabi Population

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ARTICLE INFO

Received 20 Nov. 2013
Received in revised form 03 Dec. 2013
Accepted 05 Dec. 2013

Keywords:
Hypothyroidism,
Malondialdehyde (MDA),
Oxidative stress (OS),
Lipid Profile and Atherosclerosis.

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ABSTRACT

Background: Oxidative stress has been implicated in the pathophyiology of various diseases like thyroid disorders. Hypothyroidism is widely believed to impair health. The pathological consequences of hypothyroidism point to a high potential for oxidant: antioxidant imbalance. The biochemical factors mediating decline in health, however, are poorly elucidated. Aim: The present study was designed to evaluate the influence of hypothyroidism on the metabolic state and oxidative stress by evaluating malondialdehyde levels along with lipid profile in hypothyroidism patients of north Indian population. Material and Methods: 50 patients with hypothyroidism (study group) and an equal number of age-matched healthy controls (n=50, control group) of both sexes were recruited in the present study. The fasting blood samples were collected from the subjects of both groups for the estimation of malondialdehyde and lipid profile levels. Results: The malondialdehyde levels were significantly higher ($p<0.001$) in hypothyroidism patients with respected to control subjects. Significantly higher values were observed in total cholesterol, triglycerides, low density lipoprotein-cholesterol and very low density lipoprotein-cholesterol levels while a significant decrease was seen in high density lipoprotein-cholesterol levels as compared to the control. Conclusion: These observations suggest that people with hypothyroidism have high risk of free radical damage.

Introduction

Reactive oxygen species (ROS), like superoxide anions, hydrogen peroxide, and hydroxyl radical, as well as organic counterparts such as lipid peroxides are produced as natural consequences of oxidative cell metabolism[1] Under physiological conditions, ROS generation is controlled by a large number of antioxidants systems, which act as protective mechanisms. These systems consist of antioxidant enzymes like superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase as well as non-enzymatic antioxidants, among which the most important are vitamins C and E, carotenoids and glutathione. Disturbance of the prooxidant/antioxidant balance results from the increased production of ROS, inactivation of detoxification systems or excessive consumption of antioxidants. The disturbance is a causative factor in the oxidative damage of cellular structures and molecules such as lipids, proteins, and nucleic acids and other extracellular components like collagen and hyaluronic acid[2,3].

Both hydrogen peroxide and superoxide anion produce highly reactive hydroxyl radicals through the Haber-Weiss reaction. The hydroxyl radical can initiate lipid peroxidation, which is a free radical chain reaction leading to damage of membrane structure and function[4]. Variations in the levels of thyroid hormones can be one of the main physiological modulators of *in vivo* cellular oxidative stress due to their known effects on mitochondrial respiration. In particular, it has been suggested that the increase in reactive oxygen species induced by a deficiency of thyroid hormones can lead to an oxidative stress in various tissues such as liver heart and some skeletal muscles with a consequent lipid peroxidative response[5,6]. The formation of lipid peroxides by the action of the free radicals on unsaturated fatty acids has been implicated in the pathogenesis of atherosclerosis and vascular diseases[7,8].

Thyroid disease affects approximately 5% of the population in various forms. Thyroid hormones are among the most imperative humoral factors involved in setting the basal metabolic rate[5, 9]. Hypothyroidism induced dysfunction of the respiratory chain in the mitochondria leads to accelerated production of free radicals[10,11]. So, in the present work, malondialdehyde levels were determined as indices of lipid peroxidation in hypothyroidism patients of North Indian punjabi population; the overall objective of which is to indirectly assess the role of oxidative tissue damage in the pathogenesis of hypothyroidism.

Materials and Methods

Subjects

The present study was conducted on 50 (6 males, 44 females) hypothyroidism cases and equal number of (10 males, 40 females) healthy controls in the age range of 25-70 years. The patients were screened at Chintpurni Medical College, Pathankot, Punjab, India.

Inclusion and exclusion criteria

The patients with elevated TSH levels (> 5.0µi/ml) and decreased total T_3 with total T_4 levels were recruited in the study group while the patients undergoing treatment with thyroxine/anti thyroid drugs, at end stage renal disease, with post myocardial infarction, with congestive cardiac failure, with type 2 diabetes mellitus, undergoing treatment with anti lipidemic drugs, pregnant woman and women on oral contraceptives were excluded.

Ethical Issues

The study protocol was approved by Research and Publication committee of the

Institute. Written consent was obtained from all cases & controls and confidentiality was also maintained by the researchers.

Blood Collection and Sample Preparation

10ml of blood each was withdrawn from patients of hypothyroidism and healthy controls after overnight fasting with dry disposable syringe and needle, under aseptic conditions. Venipuncture of thee antecubital vein was performed and the blood collected into sterile, dry and acid washed vial. The blood samples were incubated at 37^0C temperature for 25-30 minutes for proper clot formation and these blood samples then centrifuged at 3000rpm for 10 minutes for serum separation. This serum sample was used for various biochemical assays.

Biochemical Assays

1. **Total cholesterol:** Serum cholesterol level was assayed as per the method given by Allain *et al.*, 1974[12].
2. **Triglycerides:** Serum triglyceride level was estimated by using enzymatic GPO-PAP method given by Mcgowan *et al.*, 1983[13].
3. **HDL Cholesterol:** HDL-C was determined by the method given by Burstein *et al*, 1970[14].
4. **LDL Cholesterol:** LDL-Cholesterol was analyzed by applying the method of Bates and Warren, 1989[16].
5. **VLDL- Cholesterol:** VLDL-C was estimated by using the method of Lowenstein and Varrier, 1984[17].
6. **T_3, T_4 and TSH Estimations*:*** A fully automated Immunoflorescence immunoassay analyzer (Tosoh, AIA -360) was used for the estimation of T3, T4 and TSH.
7. **Malondialdehyde (MDA):** MDA levels were estimated by measurement the pink coloured chromophore formed by the reaction of thiobarbituric acid reactive substances in serum according to the method of Satoh, 1978[18].

Statistical Analysis

Numerical data was presented as mean ± S.D. The statistical significance was evaluated by Student's t-test using SPSS version 10.0. The difference from normal healthy control subjects was considered significant at $p<0.05$.

Results and Discussion

Table 1 compares the demographic and lipid parameters between the hypothyroid patients and the controls. Although the two comparison groups did not differ significantly in age and sex distribution (NS for each). The hypothyroid patients, compared with healthy controls, were characterized by significantly higher levels of total cholesterol ($p<0.05$), triglycerides ($p<0.05$), LDL ($p<0.05$), VLDL ($p<0.05$) and malondialdehyde ($p<0.001$). However, the HDL levels were found to be lower among the hypothyroidism patients relative to the controls ($p<0.05$)(Table-1). That, there is a relationship between hypothyroidism and serum lipid and lipoprotein abnormalities is well established[18,19]. The United State Lipid Research Clinical trial has shown that cholesterol makes most significant individual contribution to risk of coronary heart disease (CHD)[20-21]. The significantly higher serum total cholesterol, LDL-Cholesterol and triglyceride observed among the hypothyroidism patients in the current study compared with healthy controls are in agreement with the literature reports that hypothyroidism is one the risk factor for the onset of CHD[2,4,10].

The significantly higher MDA levels found among our patients, relative to the controls, appears to point towards increased lipid peroxidation as one possible patho-mechnism for the increased cardiovascular risk in hypothyroid patients. An increase in

MDA levels is said to be indicative of the involvement of free oxygen radicals in tissue damage[22-23]. Free oxygen radicals might cause the lipid peroxidation of biological membranes through a chain reaction. The first step is the initiation reaction, which begins by the removal of hydrogen atom from polyunsaturated fatty acid (PUFA) by free oxygen radicals. The second is the propagation, which culminates in the final step of termination[24,25]. The extent of LPO has often been determined by the thiobarbituric acid (TBA) test, which has also been considered for the detection of MDA[26].

In conclusion, apart from hyperlipidaemia increased lipid peroxidation may be an additional factor contributing towards high cardiovascular risk in patients with hypothyroidism. This however, is subject to confirmation.

References

1. Komosinska VK, Olczyk K, Kucharz EJ, Marcisz C, Winsz SK, Kotulska A. Free radical activity and antioxidant defense mechanisms in patients with hyperthyroidism due to Graves'disease during therapy. *Clinica Chimica Acta,* 300: 107-17 (2000).
2. Kehrer JP. Free radicals as mediators of tissue injury and disease. *Crit Rev Toxicol,* 23: 21-48(1993)
3. Halliwell B: Free radicals and antioxidants: updating a personal view. *Nutr Rev,* 70: 257–65 (2012).
4. Halliwell B, Gutteridge JMC. Role of free radicals and catalytic metal ions in human disease: an overview. *Methods Enzymol,*186: 1-85 (1990).
5. Małgorzata KL, Agnieszka KB. Oxidative damage to macromolecules in the thyroid - experimental evidence. *Thyroid Res,* 5: 25-30 (2012).
6. Guerrero A, Pamplona R, Postero OM, Barja G, Lopez TM. Effect of thyroid status on lipid composition and peroxidation in the mouse liver. *Free Rad Biol Med,* 26: 73-80 (1990).
7. Frei B. Reactive oxygen species and antioxidant vitamins: mechanisms of action. *Am J Med,* 97: S5-S13 (1994).
8. Donald DH. Oxidative stress and vascular disease. *Arterioscler Thromb Vasc Biol,* 26: 689-95 (2005).
9. Bhawna B, Agrawal BK, Sharma VK, Sarika SC. Oxidative stress status in hypothyroid patients. *Biomed Res,* 23 (2): 286-88 (2012).
10. Yilmaz S, Ozan S, Benzer F, Canatan H. Oxidative damage and antioxidant enzyme activities in experimental hypothyroidism. *Cell Biochem Funct,* 21(4): 325-30 (2003).
11. Venditti P, Balestrieri M, Di MS, De L T. Effect of thyroid state on lipid peroxidation, antioxidant defenses, and susceptibility to oxidative stressin rat tissues. *J Endocrin,* 155(1): 151-57 (1997).
12. Allain CC, Poon S, Chan CSG, Richmond S, Fu PC. An enzymatic method for estimating serum cholesterol. *Clin Chem,* 20: 470-74 (1974).
13. Mcgowan MW, Artiss JD, Standbergh DR, Zark B. A peroxidase coupled method for the colorimetric determination of serum triglycerides. *Clin Chem,* 29: 538-42 (1983).
14. Brustein M, Scolnick K, Morfin R. Estimation of HDL-C. *J Lipid Res,* 19: 583-93 (1970).
15. Bates CM, Warren GSC. Estimation of low density lipoprotein cholesterol. *South Med J,* 582; 570-74 (1989).
16. Lowenstein S, Varrier AG. Very low density lipoprotein cholesterol (VLDL-C). *J Am Med Assoc,* 276: 285-92 (1984).
17. Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clin Chem Acta,* 90: 36-43 (1978).
18. Casrelli WP. Lipid risk factors and ischemic heart disease. *Atherosclerosis,* 124: S1-S9 (1996).
19. Yeolekar ME. Coronary artery disease in Asian Indians. *JPGM,* 44: 26-28 (1998).
20. Wilson PW. High density lipoprotein, low density lipoprotein and coronary artery disease. *Am J Cardiol* 66: 7A-10A (1991).
21. Elisaf MS, Siamopoulos KC, Tselegarides TJ. Lipid abnormalities in Greek patients with coronary artery disease. *Inter J Cardiol,* 59(2): 177-84 (1997).

22. Sen S, Chakraborty R, Sridhar C, Reddy YSR, Biplap D. Free radicals, antioxidants, disease and phytomedicines: Current status and future prospects. *Intr. J Pharma Sci*, 1: 91-100 (2010).
23. Simon RJ, Maxwell L, Gregory YP. Free radicals and antioxidants in cardiovascular disease. *Br J Clin Pharma*, 44: 307-17 (1997).
24. Moamen AA, Badry EI. Serum malondialdehyde levels as a biomarker of cellular injury in human fascioliasis. *JTU Med Sci* 1: 57-64 (2006).
25. Khan HA Alhomida AS, Sobki SH et al. Serum markers of tissue damage and oxidative stress in patients with acute myocardial infarction. *Biomedical Res*, 24: 15-20 (2013)
26. Traverso N, Menini S, Maineri EP. Malondialdehyde, a lipoperoxidation-derived aldehyde, can bring about secondary oxidative damage to proteins. *J Gerontol*, 59(9):890-95(2004).

Table 1. A comparison of the demographic and lipid parameters between the hypothyroid patients and the controls

S/No.	Parameter	Controls (n=50) Mean ± SD or n	Hypothyroidism Patients (n=50) Mean ± SD or (%)	P-Value
1	Age (years)	45 ±10	42±6	NS
2	Males	10	6	-
3	Females	40	44	-
4	Total Cholesterol (mg/dl)	151.83 ± 31.92	188.58 ± 35.45 (+24.20)	<0.05
5	Triglycerides (mg/dl)	126.10 ± 23.67	180.68 ± 26.92 (+43.28)	<0.05
6	LDL (mg/dl)	78.38 ± 16.54	112.37 ± 21.56 (+54.84)	<0.05
7	HDL (mg/dl)	48.21 ± 11.64	40.08 ± 17.48 (-12.71)	<0.05
8	VLDL (mg/dl)	25.24 ± 8.43	36.13 ± 10.32 (+26.42)	<0.05
9	Malondialdehyde (nmol/ml)	3.16± 0.51	8.99 ± 1.25 (+184.49)	<0.001

n- Number of subjects.
NS= Not Significant
%= Percent
LDL= Low Density Lipoprotein (Reference Range = 60-170mg/dL)
HDL= Density Lipoprotein (Reference Range = 30-70mg/dL)
VLDL= Density Lipoprotein (Reference Range = 20-35mg/dL)
Total Cholesterol Reference Range=150-200mg/dL
Triglyceride Reference Range=50-150mg/dL
Malondialdehyde (MDA) Reference Range=3.00- 4.45nmol/ml