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RISK FACTORS IN SUBCLINICAL HYPOTHYROIDISM FOR CARDIOVASCULAR DISEASE

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ABSTRACT

Subclinical hypothyroidism (SCH) is frequent condition affecting millions of people around the world. Subjects with SCH have higher total cholesterol and low density lipoprotein level than normal subjects and also associated with cardiovascular risk factors. The greater prevalence of thyroid abnormalities and dysfunction associated with ageing result with damage to thyroid cells from oxidative stress due to continual exposure to reactive oxygen species and consequent damage to cellular structure, lipid, protein and DNA. The aim of the present study was to evaluate the CVD risk factors in subclinical hypothyroid subjects and detect the extent of somatic DNA damage by Cytokinesis block micronuclei assay. An attempt is also being made to correlate the risk factors and socio demographic characters of subjects. The study was performed in 49 study subjects and 20 healthy control subjects. The mean CBMN frequency and mean MDA value of study subjects was higher than that of the control subjects. The CBMN frequency was found to be significantly associated with age. The biochemical parameters were positively correlated with increased mean CBMN frequency. Lifestyle modifications and treatments will reduce the risk factors and thereby reduce the resulting oxidative DNA damage.

INTRODUCTION

Subclinical hypothyroidism (SCH) is biochemically defined as raised serum thyroid stimulating hormone (TSH) concentrations with simultaneously occurring normal circulating thyroxine (FT4) (Ross, 2001). SCH denotes a declined thyroid activity without clear symptoms, such as fatigue, inability to lose weight, memory impairment, hair loss and depression. The prevalence of subclinical hypothyroidism is approximately 4% in general population; it is more common in females and increases with age (Hollowell et al., 2002).

The clinical and metabolic consequences of SCH include elevated cholesterol level, atherosclerosis, cardiovascular disease and mortality, neuropsychiatric disease, weight gain, neuromuscular disease and poor pregnancy outcomes. The risk of SCH during pregnancy is considerable when women having positive anti thyroid antibodies during first trimester. Thyroid dysfunction also associated with pregnancy complications such as hypertension, preterm birth, low birth weight, placental abruption, and foetal death (Utiger, 1999). With maternal and foetal thyroid insufficiency caused by severe iodine deficiency, the infant has profound neurologic impairment and mental retardation (Xue et al., 1994).

The predisposition of patients with SCH to endothelial dysfunction is an early sign of atherosclerosis. This predisposition may be partially explained by factors which are found in patients with SCH, including changes in lipid profile, low grade chronic inflammation, oxidative stress and insulin resistance. The overproduction of ROS results in increased oxygen consumption by thyroid hormones which disturbs the pro oxidant/antioxidant balance leading to oxidative stress, and consequent damage to cellular structures, lipids, proteins, and DNA (Fernandez et al., 2006). The lipid peroxidation product i.e. MDA levels have been increased significantly in plasma of the patient with coronary artery disease. Oxidative stress increased in SCH due to elevated plasma lipids induced by low thyroid function. Environmental factors and unhealthy lifestyle influence the oxidative stress and leads constituent damage to cellular structure, lipid, protein and DNA. Hence the present study was undertaken to aware people about risk factors in SCH for CVD and monitors the progression of pathology and to prompt the consideration of medical care.

MATERIALS AND METHODS

Forty nine hypothyroid subjects and twenty healthy subjects without any chronic illness were selected for this study. The samples were referred from various centers of Kerala to Genetika, Centre for Advanced Genetic Studies, Thiruvananthapuram, Kerala.

Detailed demographic, clinical and lifestyle characteristics were recorded using proforma. In the present study, Cytokinesis Block Micronuclei (CBMN) assay was performed on each sample by using cytochalasin B for quantitating the extent of somatic DNA damages and MDA test was performed for detecting the oxidative stress.

Collected seven ml of blood sample by venipuncture and transferred 3 ml of blood to sodium heparinized vacutainers for quantifying the extent of somatic DNA damages by cytokinesis-block micronuclei (CBMN) assay. The remaining five ml of blood was transferred into a plain tube. Blood was allowed to clot, serum separated immediately. Blood sugar and lipid profile were estimated using semi-automated clinical chemistry analyzer. The level of the serum lipid peroxide marker, MDA was determined using thiobarbituric acid as main reagent and measuring the values on photoelectric colorimeter at 540nm.

Two ml blood was added to a culture tube containing 10 mL RPMI 1640 medium supplemented with 100 units/mL penicillin, 100µg/mL streptomycin, 15% foetal bovine serum and 10µg/mL phytohemagglutinin. Cytochalasin B was added to the cultures at a final concentration of 4.5µg/mL (Sigma) after 44th hours of initiation of cells with phytohaemagglutinin. Cells were harvested after 72 hr incubation, and they were treated with a hypotonic solution (0.075M KCl) for 10 min and fixed in fresh fixative solution (methanol: acetic acid, 3:1). The cells were dropped onto slides and the slides were air dried and stained with 10% Giemsa. Micronucleated cells were analyzed under light microscopy at 100X magnification. The number of micronuclei is not less than 1000 binucleated cells were scored and the distribution of micronuclei among binucleated cells was recorded.

OBSERVATIONS AND RESULTS

DISTRIBUTION OF MEAN CBMN FREQUENCY AND MDA VALUE ACCORDING TO SUBJECTS

Table 1:

Variables	Number	Mean MDA Value	Mean CBMN Frequency
Control Subjects	20	1.21	10.93
Study Subjects	49	1.74	12.82

DISTRIBUTION OF MEAN CBMN FREQUENCY ACCORDING TO DEMOGRAPHIC CHARACTERS OF SUBJECTS Table 2:

Category	Variables	Number (Percentage)	Mean CBMN frequency
Age (Years)	<30	9 (18.36%)	12.73
	30-40	28 (57.14%)	12.90
	>40	12 (24.48%)	12.96
Birth order	<3	25 (51.02%)	12.66
	≥3	24 (48.97%)	12.98
BMI (kg/m^2)	<25	12 (24.48%)	12.11
	25-30	23 (46.93%)	12.84
	>30	14 (28.57%)	13.4

Age, birth order and BMI of the subjects were categorized into table 1. Advanced age of the subjects was showed highest mean CBMN frequency of 12.96. Subjects with birth order >3 were showed mean CBMN frequency of 12.98. Obese and overweight subjects were showed high mean CBMN frequency.

Clinical characteristics of the subjects were given in table 3. 26 subjects have H/o diabetes and 31 subjects have H/o hypertension with high mean CBMN frequency. Subjects having family H/o thyroid and CAD were showed increased mean CBMN frequency.

DISTRIBUTION OF MEAN CBMN FREQUENCY ACCORDING TO CLINICAL CHARACTERS OF SUBJECTS Table 3:

Category	Variables	Number (Percentage)	Mean CBMN frequency
H/o Diabetes	Yes	26 (53.06%)	13.09
	No	23 (46.93%)	12.51
H/o Hypertension	Yes	31 (63.26%)	12.98
	No	18 (36.73%)	12.54
Family H/o Thyroid disorder	Yes	22 (44.89%)	13.26
	No	27 (55.10%)	12.46
Family H/o Coronary Artery Disease	Yes	9 (18.36%)	13.49
	No	40 (81.63%)	12.67

DISTRIBUTION OF MEAN CBMN FREQUENCY ACCORDING TO BIOCHEMICAL CHARACTERS OF SUBJECTS Table 4:

Category	Variables	Number (Percentage)	Mean CBMN frequency
FBS (mg/dl)	<110	17 (34.69%)	12.42
	≥110	32 (65.30%)	13.03
Total Cholesterol (mg/dl)	<200	18 (36.73%)	12.65
	≥200	31 (63.26%)	12.92
HDL (mg/dl)	< 50	46 (93.87%)	12.95
	≥ 50	3 (6.12%)	12.81
LDL (mg/dl)	< 100	14 (28.57%)	11.2
	≥ 100	35 (71.42%)	13.47
Triglycerides (mg/dl)	< 150	21 (42.85%)	12.77
	≥ 150	28 (57.14%)	12.85

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	25-30	23 (46.93%)	12.84
	>30	14 (28.57%)	13.4

Biochemical characteristics of subjects were given in table 4. High mean CBMN frequency was showed in subjects with abnormal level in lipid profile such as FBS ≥110 mg/dl, total cholesterol ≥200 mg/dl, HDL < 50 mg/dl, LDL ≥ 100 mg/dl and triglyceride ≥ 150 mg/dl.

DISCUSSION

Surks and Hollowell, (2007) reported that the prevalence of SCH seemingly increases with age. Roger et al., (2013) also reported the incidence of CVD rises with advanced age of SCH subjects. In the present study, the CBMN frequency was also increased with advancing age.

Various studies have reported the positive correlation between increased TSH level and body mass index (BMI) (Bastemir et al., 2007). The present study also showed that the mean CBMN frequency increased with increasing BMI.

The diastolic blood pressure was higher in women with SCH than in the euthyroid controls (Luboshitzky et al., 2002). In addition to this, the present study clearly demonstrated that the frequency of micronuclei was increased in subjects with hypertension.

Bauer et al., (1998) found that subjects with subclinical hypothyroidism have higher total cholesterol and low density lipoprotein cholesterol levels than euthyroid subjects. In the present study elevated LDL cholesterol and triglycerides and decreased HDL cholesterol was observed in hypothyroid patients and showed increased mean CBMN frequency in subjects.

Prospective studies indicate that patients with SCH have increased risk for all cause and cardiovascular mortality (Tseng et al., 2012), and thus the presence of oxidative stress in these patients could further enhance risks. Santi et al., (2012) reported that marked increase in MDA in SCH compared with euthyroid controls (indicates increased oxidative stress), the present study also showed mean MDA value was increased in study subjects than the control subjects.

CONCLUSION

Subclinical hypothyroidism is one of the most prevalent autoimmune diseases provoked in genetically susceptible individuals by several triggers, including female sex, advanced age, obesity, abnormal lipid profile and environmental factors. These risk factors seem to contribute DNA damage as indicated by increase in micronuclei frequency. SCH is associated with an increased risk of developing a wide range of adverse health outcomes and that SCH might represent a potentially modifiable risk factor of CVD and mortality. Therefore, understanding the prevalence and risk factors of SCH could aid in the prevention of CVD in this population. Careful attention to risk factors modification is essential to prevent primary and recurrent events. Understanding the symptoms of hypothyroidism and getting regular screening to ensure an early diagnosis will prevent the onset of the complications. Lifestyle determinants of cardiovascular risk like diet and physical activity may be useful in preventive medicine.

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