Original Research Paper

Volume - 11 | Issue - 06 | June - 2021 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Healthcare

SOMATIC DNA DAMAGES AND OXIDATIVE STRESS AMONG SUBJECTS WITH SUBCLINICAL HYPOTHYROIDISM

Anoop A	Department of Zoology, Mar Ivanios College, Thiruvananthapuram, Kerala.
Lija L Raju	Department of Zoology, Mar Ivanios College, Thiruvananthapuram, Kerala.
Leenamma Joseph	Department of Zoology, Mar Ivanios College, Thiruvananthapuram, Kerala.
Aswathy Sundaresh	Genetika, Centre for Advanced Genetic Studies, Pettah P O, Thiruvananthapuram, Kerala- 695024.
Dinesh Roy D*	Genetika, Centre for Advanced Genetic Studies, Pettah P O, Thiruvananthapuram, Kerala- 695024. *Corresponding Author

ABSTRACT Subclinical hypothyroidism (SCH), also called mild thyroid failure, is defined as a serum thyroid-stimulating hormone (TSH) level above the upper limit of normal despite normal levels of serum free thyroxine. The overproduction of ROS results in increased oxygen consumption by thyroid hormones which disturb the pro-oxidant/antioxidant balance leading to oxidative stress and consequent damage to cellular structures, lipids, proteins and DNA. 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. The aim of the present study was to quantify the extent of somatic DNA damage (by using Cytokinesis block micronuclei assay) and Oxidative stress among subjects with subclinical hypothyroidism. The study was performed in 30 study subjects with SCH and 15 healthy control subjects. The mean CBMN frequency and mean MDA value of study subjects was higher than that of the control subjects. The biochemical parameters were also positively correlated with increased mean CBMN frequency. Lifestyle modifications and proper clinical management will reduce the risk factors and thereby reduce the resulting oxidative DNA damage and subsequent cardiovascular disease risks.

KEYWORDS : Subclinical hypothyroidism, Oxidative stress, DNA Damage, Cardiovascular disease

INTRODUCTION

Subclinical hypothyroidism (SCH), also called mild thyroid failure, is defined as a serum thyroid-stimulating hormone (TSH) level above the upper limit of normal despite normal levels of serum free thyroxine (Cooper 2001). Kim and Park (2014) estimated that, "Subclinical hypothyroidism showed a higher prevalence in women (6% to 10%) than in men (2% to 4%) in all previous studies". In 2019, Gosi and Garla estimated that, "the prevalence of subclinical hypothyroidism varies from 3 to 15% based on the study population. According to, Somwaru et al (2012), "the risk of subclinical hypothyroidism progression to overt hypothyroidism is 2 to 6% per year". In 2015, Chaker et al suggested that, "Subclinical hypothyroidism correlates with an increased risk of fatal and non-fatal coronary artery disease (CAD) events, congestive heart failure and fatal stroke".

According to Rizos et al (2011), "Thyroid hormones regulate a wide array of metabolic parameters including lipoprotein metabolism and cardiovascular disease (CVD) risk factors, thus influencing overall CVD risk". Rodondi et al (2005) also observed, "an increased risk of congestive heart failure among older patients with SH that had a TSH level >7.0mU/l, but no significant correlation between SH and stroke, peripheral arterial disease, cardiovascular-associated or total mortality was revealed". Another cross-sectional analysis further revealed SH to be an independent risk factor for coronary heart disease, in parallel to hypercholesterolemia, hypertension, smoking and diabetes (Walsh et al 2005).

Thyroid hormones have a considerable impact on oxidative stress (Tejovathi et al 2013), ascribed to their role in cellular metabolism and oxygen consumption. Fernandez et al in 2006 explained that, "the overproduction of ROS results in increased oxygen consumption by thyroid hormones which disturb the pro-oxidant/antioxidant balance leading to oxidative stress and consequent damage to cellular structures, lipids, proteins and DNA". 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 quantify the extent of somatic DNA damage and Oxidative stress among subjects with subclinical hypothyroidism.

MATERIALS AND METHODS

Thirty subjects with clinically proven subclinical hypothyroidism and fifteen healthy subjects without any chronic illness were selected for this study. The samples were recruited from Hridayalaya, Institute for Preventive Cardiology, Thiruvananthapuram, Kerala to Genetika, Centre for Advanced Genetic Studies, Thiruvananthapuram, Kerala for genetic studies. 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 as described by Michael Fenech in 1993 and MDA concentration was quantified for evaluating the oxidative stress. Five ml of blood sample was collected by venipuncture, transferred 2 ml of blood to sodium heparinized vacutainer for quantifying the extent of somatic DNA damages. The number of micronuclei is not less than 1000 binucleated cells were scored and the distribution of micronuclei among binucleated cells was recorded. The remaining three ml of blood was transferred into a plain tube and allowed to clot, serum separated immediately. Blood sugar and lipid profile, T3, T4, TSH, fT3 and fT4 were estimated. The level of the serum lipid peroxide marker, MDA was determined using thiobarbituric acid (described by Sato et al 1979) as main reagent and measuring the values on photoelectric colorimeter at 540nm.

OBSERVATIONS AND RESULTS

The demographic, anthropometric, physiological, clinical, biochemical and endocrinology characteristics were recorded and CBMN assay was done to evaluate the extent of somatic DNA damages in each subjects. Moreover, Malondialdehyde (MDA) level was also measured, for identifying the level of Oxidative stress. The observed mean CBMN frequency of study subjects was 12.64 and for control it was 10.39 ($t=6.894$; $p<0.05$). An increased mean CBMN frequency was noted among study subjects with SCH when compared to the control group. Similarly, an elevated MDA concentration was also observed among study subjects (2.82µmol/L) than the control subjects (1.55µmol/L) ($t=6.179$; $p<0.05$). Fasting Blood Sugar (FBS), Total Cholesterol (TC), Triglyceride (TG) and Thyroid stimulating hormone (TSH) showed a statistically significant difference between study and control subjects with p-value less than 0.05. However, levels of T3, T4, fT3 and fT4 did not show any statistically significant difference between study and control subjects.

The study subjects with advanced age showed an increased mean CBMN frequency (13.31) and MDA concentration (2.99µmol/L). Among the 30 study subjects, 16 were male and the remaining were

female subjects. The mean CBMN frequency of female subjects was 12.7 and that of male subjects it was 12.59. The observed MDA concentration was also higher in females (2.9µmol/L) as compared to that of males (2.74µmol/L). According to residence, 30 study subjects were categorized into three group such as, urban (n=20), rural (n=6) and coastal (n=4). The distribution of mean CBMN frequency was noted to be higher among subjects who reside in urban area, which was followed by coastal and rural areas. The level of MDA was higher among subjects who reside in coastal areas (3.01µmol/L) and when compared to the rest. Subjects who had sedentary type of occupation showed an increased mean CBMN frequency (13.11) and MDA concentration (3.05µmol/L) when compared to subjects with non-sedentary type of occupation.

History of (H/o) Diabetes was reported among 23.33% of the study subjects. Moreover, an increased mean CBMN frequency (13.39) and MDA concentration (2.99µmol/L) was observed among the study subjects reported with H/o Diabetes when compared to the subjects without H/o Diabetes. Out of 30 study subjects, 10 of them reported with H/o Hypertension, showed higher mean CBMN frequency of 13.44 and MDA level of 3.04µmol/L. The observed mean CBMN frequency and MDA level of study subjects with H/o Dyslipidemia was 13.56 and of 2.94µmol/L respectively. An increased oxidative stress and DNA damage was observed among study subjects reported with H/o Dyslipidemia. H/o CAD was reported in 20% (n=6) of the study subjects and these subjects revealed increased, MDA level (3.07µmol/L) and mean CBMN frequency (13.56). Study subjects with H/o thyroid disorder showed an increased mean CBMN frequency of 13.43 and along with an elevated MDA concentration of 3.18µmol/L as compared to that of subjects without H/o thyroid disorder (they showed a mean CBMN frequency of 12.3 and MDA level of 2.66µmol/L respectively). Out of 30 study subjects, 10 were reported with H/o Chest pain. The observed mean CBMN frequency of subjects reported with H/o Chest pain was 12.9 and their observed MDA concentration was 2.92µmol/L. Subjects with obesity, habit of smoking, chewing and alcohol consumption, irregular exercise, etc showed an increased mean CBMN frequency along with an elevated level of MDA concentration.

Study subjects with an increased concentration of FBS (>110mg/dL), Total Cholesterol (>200mg/dL) and Triglyceride level (>150mg/dL) showed a mean CBMN frequency of 13.12, 12.98 and 13.15 respectively and MDA concentration of 3.03µmol/L, 3.03µmol/L and 2.98µmol/L respectively. Furthermore, study subjects with TSH level >5.5 mIU/L showed an increased mean CBMN frequency of 12.01 and MDA concentration of 2.46µmol/L. Remaining subjects with TSH level ≤5.5mIU/L showed a decreased mean CBMN frequency and MDA concentration.

DISCUSSION

Canaris et al (2000) reported that, "the incidence of subclinical hypothyroidism varies among populations and ranges from 3 to 15% and a higher incidence associated with increasing age, female gender and a suboptimal iodine status". Study done by Thakkar and Jain (2010) observed that, "increase in cell damage was also observed in hypothyroid and Diabetes Mellitus (DM) subjects ($p<0.05$). Hypothyroidism affects the metabolism rate and thereby mitochondrial function". In the current study, study subjects with SCH showed an elevated level of oxidative stress marker (MDA) and increased mean CBMN frequency.

According to Kanaya et al (2002), "the overall prevalence of SCH was 3.4% in men and 6.3% in women and increased with age, to 14.6% in 70-year-old women and 10.1% in 70-year-old men". In 2012, Tseng et al mentioned that, "the prevalence of SCH increases with age and is higher in women". In the present study also subjects with advanced age were observed with an increased mean CBMN frequency and MDA concentration.

In a study done by Sharma et al (2020) it was noted that, "Type 2 diabetes mellitus (T2DM) has been associated with subclinical hypothyroidism (SCH)". Palma et al in 2013 also reported that, "the prevalence of SCH was more in patients with T2DM (2.2% to 17%) than the general population (4 to 10%)". In the current study, 7 subjects were reported with H/o Diabetes and also their observed mean CBMN frequency and MDA level was higher when compared to the rest.

In 2012, Pearce estimated that, "1 to 11% of all patients with dyslipidemia have subclinical hypothyroidism". According to Hak et al (2000), "SCH is a strong indicator of the risk for atherosclerosis and myocardial infarction in elderly women". According to Hussain et al (2019), "SCH is associated not only with elevated low-density lipoprotein-cholesterol (LDL-C) levels and low high-density lipoprotein-cholesterol (HDL-C) levels but also with elevated lipoprotein(a). This may further increase the risk of the development of atherosclerosis". In the present study, 5 subjects were reported with H/o Dyslipidemia and these subjects reported an increased mean CBMN frequency and MDA concentration.

Rajendra et al in 2015 observed, "a significantly higher level of diastolic BP, TC and LDL-C among SCH subjects when compared to healthy control. Moreover, High diastolic BP, hypercholesterolemia, low HDL-C, undesirable LDL-C and high hs-CRP were much common in SCH subjects compared to control". Sharma et al (2011) also observed that, "significant positive correlation between TSH and hs-CRP, LDL-C and TC among subjects with subclinical hypothyroidism". In the current study it was observed that, SCH subjects with high concentration of FBS, TC, TG and TSH showed an elevated mean CBMN frequency and MDA concentration. Cheserek et al (2015) suggested that, "Plasma MDA is an important biomarker of oxidative damage to lipids. A marked increase in MDA in SCH compared with euthyroid controls which indicate increased oxidative stress".

CONCLUSION

Subclinical hypothyroidism is one of the most common autoimmune diseases, triggered by wide range of factors such as advanced age, obesity, an irregular lipid profile and environmental factors. Moreover, the current study also demonstrated that oxidative stress was increased in subclinical hypothyroidism as indicated by the elevated lipid peroxidation product, malondialdehyde. This study clearly demonstrated that, there was an increased DNA damage and oxidative stress among study subjects with SCH. This is mainly due to the lifestyle and dietary habits and sometimes due to various hereditary factors. The subclinical thyroid dysfunction is common and highlights the usefulness of screening to allow early detection and therefore, preventing associated adverse health outcomes. Thus it can be concluded that certain dietary modification, changes in life style parameters and proper medication can reduce the risk of SCH and subsequent development of CVD risks.

REFERENCES

[1] Canaris, G.J., Manowitz, N.R., Mayor, G. and Ridgway, E.C., 2000. The Colorado thyroid disease prevalence study Arch Intern Med 160: 526–534. Find this article online.

[2] Chaker, L., Baumgartner, C., Den Elzen, W.P., Ikram, M.A., Blum, M.R., Collet, T.H., Bakker, S.J., Dehghan, A., Drechsler, C., Luben, R.N. and Hofman, A., 2015. Subclinical hypothyroidism and the risk of stroke events and fatal stroke: an individual participant data analysis. The Journal of Clinical Endocrinology & Metabolism, 100(6), pp.2181-2191.

[3] Cheserek, M.J., Wu, G.R., Ntazinda, A., Shi, Y.H., Shen, L.Y. and Le, G.W., 2015. Association Between Thyroid Hormones, Lipids and Oxidative Stress Markers in Subclinical Hypothyroidism/Povezanost Izme\U Tireoidnih Hormona, Lipida I Markera Oksidativnog Stresa U SubkliničKoj Hipotireozi. Journal of medical biochemistry, 34(3), pp.323-331.

[4] Cooper, D.S., 2001. Subclinical hypothyroidism. New England Journal of Medicine, 345(4), pp.260-265.

[5] Fernandez, V., Tapia, G., Varela, P., Romanque, P., Cartier-Ugarte, D. and Videla, L.A., 2006. Thyroid hormone-induced oxidative stress in rodents and humans: a comparative view and relation to redox regulation of gene expression. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 142(3-4), pp.231-239.

[6] Gosi, S.K.Y. and Garla, V.V., 2019. Subclinical hypothyroidism. StatPearls [Inte13 Canaris GJ, Manowitz NR, Mayor G, Ridgway EC. The Colorado Thyroid Disease Prevalence Study. Arch Intern Med 2000;160:526-34.

[7] Hak, A.E., Pols, H.A., Visser, T.J., Drexhage, H.A., Hofman, A. and Witteman, J.C., 2000. Subclinical hypothyroidism is an independent risk factor for atherosclerosis and myocardial infarction in elderly women: the Rotterdam Study. Annals of internal medicine, 132(4), pp.270-278.

[8] Hussain, A., Elmahdawi, A.M., Elzeraidi, N., Nouh, F. and Algathafi, K., 2019. The Effects of Dyslipidemia in Subclinical Hypothyroidism. Cureus, 11(11).

[9] Kanaya, A.M., Grady, D. and Barrett-Connor, E., 2002. Explaining the sex difference in coronary heart disease mortality among patients with type 2 diabetes mellitus: a meta-analysis. Archives of internal medicine, 162(15), pp.1737-1745.

[10] Kim, Y.A. and Park, Y.J., 2014. Prevalence and risk factors of subclinical thyroid disease. Endocrinology and Metabolism, 29(1), p.20.

[11] Michael Fenech., 1993. Cytokinesis block micronucleus method in human lymphocytes. A detailed description of the method and its application to genotoxicity studies in human population research, 285, 35-44.

[12] Palma CC, Pavesi M, Nogueira VG, Clemente EL, Pereira MD, Pereira LC, Pacheco FF, Braga TG, de Faria Bello L, Soares JO, Dos Santos SC., 2013. Prevalence of thyroid dysfunction in patients with diabetes mellitus. Diabetology & metabolic syndrome. 2013 Dec;5(1):1-5.

[13] Pearce, E.N., 2012. Update in lipid alterations in subclinical hypothyroidism. The Journal of Clinical Endocrinology & Metabolism, 97(2), pp.326-333.

[14] Rajendra. Kc,, Khatiwada, S., Deo Mehta, K., Pandey, P., Lamsal, M. and Majhi, S., 2015. Cardiovascular risk factors in subclinical hypothyroidism: a case control study in Nepalese population. Journal of thyroid research, 2015.

[15] Rizos, C.V., Elisaf, M.S. and Liberopoulos, E.N., 2011. Effects of thyroid dysfunction on lipid profile. The open cardiovascular medicine journal, 5, p.76.

[16] Rodondi, N., Newman, A.B., Vittinghoff, E., de Rekeneire, N., Satterfield, S., Harris,

T.B. and Bauer, D.C., 2005. Subclinical hypothyroidism and the risk of heart failure, other cardiovascular events, and death. Archives of internal medicine, 165(21), pp.2460-2466.

[17] Sato, Y., Hotta, N., Sakamoto, N., Matsuoka, S., Ohishi, N. and Yagi, K., 1979. Lipid peroxide level in plasma of diabetic patients. Biochemical medicine, 21(1), pp.104-107.

[18] Sharma, R., Sharma, T.K., Kaushik, G.G., Sharma, S., Vardey, S.K. and Sinha, M., 2011. Subclinical hypothyroidism and its association with cardiovascular risk factors. Clinical laboratory, 57(9-10), pp.719-724.

[19] Sharma, S., Ray, A. and Sadasivam, B., 2020. Metformin in COVID-19: a possible role beyond diabetes. Diabetes research and clinical practice, 164, p.108183.

[20] Somwaru, L.L., Rariy, C.M., Arnold, A.M. and Cappola, A.R., 2012. The natural history of subclinical hypothyroidism in the elderly: the cardiovascular health study. The Journal of Clinical Endocrinology & Metabolism, 97(6), pp.1962-1969.

[21] Tejovathi, B., Suchitra, M.M., Suresh, V., Reddy, V.S., Sachan, A., Rao, P.S. and Bitla, A.R., 2013. Association of lipid peroxidation with endothelial dysfunction in patients with overt hypothyroidism. Experimental and Clinical Endocrinology & Diabetes, 121(05), pp.306-309.

[22] Thakkar, N.V. and Jain, S.M., 2010. A comparative study of DNA damage in patients suffering from diabetes and thyroid dysfunction and complications. Clinical pharmacology: advances and applications, 2, p.199.

[23] Tseng, F. Y., Lin, W. Y., Lin, C. C., Lee, L. T., Li, T. C., Sung, P. K., & Huang, K. C. (2012). Subclinical hypothyroidism is associated with increased risk for all-cause and cardiovascular mortality in adults. Journal of the American College of Cardiology, 60(8), 730-737.

[24] Walsh, J.P., Bremner, A.P., Bulsara, M.K., O'Leary, P., Leedman, P.J., Feddema, P. and Michelangeli, V., 2005. Subclinical thyroid dysfunction as a risk factor for cardiovascular disease. Archives of internal medicine, 165(21), pp.2467-2472.