Original Research Paper

Volume-10 | Issue-2 | February - 2020 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Biochemistry

OXIDATIVE STRESS AND GENETIC INSTABILITIES IN FAMILIAL HYPOTHYROIDISM

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ABSTRACT Majority of the hypothyroidism are thought to be sporadic, but <10% of hypothyroid conditions are familial (hereditary). The actual reason behind the familial hypothyroidism and the extent of oxidative stress between familial and sporadic hypothyroidism are to be identified. Hence the present study was under taken to evaluate the extent of oxidative stress and genetic instabilities in familial hypothyroidism and compared with sporadic hypothyroid and healthy control subjects. Thirty eight subjects with varying degrees of familial hypothyroidism, twenty subjects with sporadic hypothyroidism and twenty five healthy control subjects were selected to evaluate the extent of oxidative stress by quantifying the MDA concentration and genetic instabilities by mutagen induced chromosome sensitivity analysis. Laboratory investigations like, RBS, Total Cholesterol, Triglyceride, HDL-C, LDL-C, T3, T4 and TSH were also evaluated and correlated. Subjects with familial hypothyroidism showed statistically significant elevation in MDA concentration and mean b/c value compared to sporadic hypothyroid subjects and control subjects. Moreover, a positive correlation was also observed between these abnormal biochemical concentrations with elevated MDA concentration and mean b/c value. The study frankly observed that, the extent of various biochemical, endocrinological and molecular risk factors are more among subjects with familial hypothyroidism than sporadic hypothyroidism.

KEYWORDS : Familial Hypothyroidism, Oxidative Stress, DNA Repair Mechanism, Reactive oxygen species, Somatic DNA damage

INTRODUCTION:

Hypothyroidism may be defined as the clinical state which results from decreased production of thyroid hormones or very rarely, from their decreased action at a tissue level. Triiodothyronine (T3) deficiency is responsible for the clinical and biochemical manifestations of hypothyroidism (Kostoglou-Athanassiou 2010). The prevalence of hypothyroidism in India is 11%, compared with only 2% in the UK and 4.6% in the USA. The highest prevalence of hypothyroidism (13.1%) is noted in people aged 46–54 years, with people aged 18–35 years being less affected (7.5%) (Bagcchi 2014).

Most of the cases of hypothyroidism are thought to be sporadic, but some are hereditary. Many studies revealed that <10% of hypothyroid conditions are arising from familial cases (Mimuoni M et al 1996). Familial hypothyroidism is the condition in which any of the first or second degree relatives having hypothyroidism. Excess thyroid-stimulating hormone (TSH) might alter oxidative stress processes (Nanda et al 2008). The overall prevalence of hypothyroidism is high among various societies, but the prevalence of familial hypothyroidism is comparatively less.

Thyroid Hormones (TH) are related to oxidative stress not only by their stimulation of metabolism but also by their effects on antioxidant mechanisms. The metabolic effects of TH are directly linked to ROS production and oxidative stress in various ways. TH also promote extra mitochondrial ROS production by modifying the expression of genes coding for enzymes involved in ROS production and elimination (Fernandez et al 1985; Ueta et al 1995).

The actual reason behind the familial hypothyroidism is yet to be identified. Hence the present study was undertaken to evaluate the extent of oxidative stress by quantifying the MDA concentration and genetic instabilities in familial hypothyroidism and comparing with sporadic hypothyroid subjects and healthy control subjects. The oxidative stress was evaluated by quantifying the MDA concentration and the DNA repair efficiency was evaluated by performing mutagen induced chromosome sensitivity analysis. The results were compared and correlated with various other biochemical and endocrinological parameters.

MATERIALS AND METHODS:

Thirty eight subjects suffering from various degrees of hypothyroid with family history of thyroid disorders (familial subjects) and twenty subjects suffering from hypothyroidism without family history of thyroid disorders (sporadic subjects) and twenty five healthy control subjects were selected for the study. Detailed family history and relevant information were recorded. 4 ml of peripheral blood was aseptically collected, 2ml blood transferred to a sodium heparinised vacuutainers for mutagen induced chromosome sensitivity assay and remaining to 2ml blood was allowed to clot. By using the serum investigations like RBS, Total Cholesterol, Triglyceride, LDL-C, HDL-C, T3, T4, TSH and MDA were performed. For chromosome sensitivity analysis the mean number of breaks/cell (b/c) was calculated.

OBSERVATIONS AND RESULTS:

The study was carried out in order to assess the oxidative stress and DNA repair capacity among familial hypothyroid subjects and to compare with sporadic as well as healthy control subjects. Among the 38 familial subjects with hypothyroidism, 32 (84%) were females and 6 (16%) were males. And among the 20 sporadic subjects with hypothyroidism, 19 (95%) were females and 1 (5%) was male. Among the 25 control subjects, 16 (64%) were females and 9 (36%) were males. Regarding the mean b/c value, inter-individual variation was observed. Subjects with familial hypothyroidism showed a mean b/c value of 0.8235±0.048. Whereas the sporadic hypothyroid subjects showed a mean b/c value of 0.7628±0.060 and the healthy control subjects showed a mean b/c value of 0.6906±0.051. Moreover, the MDA concentration was also higher among subjects with familial hypothyroidism (2.82±0.742U/L) than the sporadic hypothyroidism subjects (2.59±0.764U/L) and the control subjects (1.98±0.732U/L). These differences showed a statistical significance (Table 1 and 2). However, the difference in MDA concentration between the sporadic hypothyroid subjects and the control subjects was not statistically significant.

Table 1: Comparison of mean b/c values among study subjects

Category	Mean b/c value	SD	t	p
Familial	0.8255	±0.048	4.66267	< .00001
Sporadic	0.7578	±0.060		
Familial	0.8255	±0.048	9.91575	< .00001
Control	0.6906	±0.051		

Sporadic	0.7578	±0.060	3.80901	.000248
Control	0.6905	±0.051		

Table 2: Comparison of MDA concentration among study subjects

Category	Mean MDA Conc.	SD	t	p
Familial	2.25	±0.742	3.27598	.000905
Sporadic	1.57	±0.764		
Familial	2.25	±0.742	4.14963	.000057
Control	1.4	±0.732		
Sporadic	1.57	±0.764	0.71145	.240577
Control	1.4	±0.732		

The age of the familial and sporadic subjects with hypothyroidism were ranged from 5 to 60 years and 20 to 59 years respectively. The age of the control subjects ranged from 11 to 56 years. The study revealed that mean b/c value was higher among subjects with age ranged from 41 to 60 in both familial and sporadic subjects. Regarding the MDA concentration also subjects with advanced age showed increased concentration of MDA.

Table 3: Distribution of MDA concentration and mean b/c value according to clinical characters

Clinical Characters		MDA			Mean b/c Values		
		Familial Hypothyroidism	Sporadic Hypothyroidism	Control	Familial Hypothyroidism	Sporadic Hypothyroidism	Control
History of Infertility/ Subfertility	Yes	0.8466	0.7621	0.7635	2.21	1.64	2.63
	No	0.8244	0.7460	0.6825	2.25	1.31	1.27
History of Diabetes	Yes	0.8537	0.7608	0.7584	2.48	162	2.16
	No	0.8203	0.7522	0.6831	2.21	1.48	1.32
History of Hypertension	Yes	0.8535	0.7578	0.7543	2.50	1.63	2.38
	No	0.8156	0.7577	0.6794	2.16	1.32	1.23
History of Dyslipidemia	Yes	0.8552	0.8048	0.7512	2.59	2.02	2.33
	No	0.8221	0.7460	0.6799	2.21	1.46	1.24

The MDA concentration and the mean b/c values were evaluated and correlated with various clinical characteristics like H/o Infertility/ Subfertility, H/o Diabetes, H/o Hypertension and H/o Dyslipidemia. The study frankly observed that, subjects with abnormal clinical characteristics showed increased MDA concentration and the mean b/c values, than the subjects without these characteristics (Table. No: 3).

Biochemical evaluations like total cholesterol, LDL-C, HDL-C, Triglyceride and Random Blood Sugar (RBS) were performed and revealed that, there was a significant elevation of these parameters among subjects with familial hypothyroidism than the sporadic subjects, followed by control subjects. Thyroid function test revealed that, a statistically significant decreased concentration of T3 and T4 among familial and sporadic hypothyroid subjects than the control subjects (Table. No: 4). Moreover, a positive correlation was observed between these abnormal biochemical values with MDA concentration and mean b/c value (Table. No: 5).

Table 4: Distribution of Laboratory Investigations among study subjects

Variable	Familial		Sporadic		Control	
	Mean	±SD	Mean	±SD	Mean	±SD
Total Cholesterol	206.10	63.382	244.75	54.315	146.45	25.656
HDL-C	36.47	9.468	32.7	10.157	47.9	5.035
LDL-C	115.84	35.251	143.6	32.679	94.85	18.635
Triglyceride	155.39	37.862	178.55	40.855	127	24.449
RBS	144.05	78.853	164.05	106.402	92.8	11.241
T3	70.76	11.146	74.4	8.002	102.5	27.668
T4	3.72	1.606	3.54	1.199	7.14	1.573
TSH	7.88	2.6152	7.82	2.359	3.54	1.550

Table 5: Distribution of MDA concentration and mean b/c value according to Laboratory Investigations

Laboratory Investigations		MDA			MEAN b/c VALUES		
		Familial Hypothyroidism	Sporadic Hypothyroidism	Control	Familial Hypothyroidism	Sporadic Hypothyroidism	Control
Total Cholesterol	≤200	2.20	1.22	1.43	0.8218	0.7698	0.6827
	>200	2.30	1.66	0.78	0.8297	0.7098	0.6910
LDL-C	≤120	2.07	1.22	1.21	0.8246	0.7098	0.6778
	>120	2.47	1.66	2.46	0.8267	0.7698	0.7630
HDL-C	≤40	2.34	1.66	1.53	0.8288	0.7698	0.6912
	>40	2.06	1.22	1.39	0.8193	0.7098	0.6855
Triglyceride	≤150	2.09	0.99	1.24	0.8217	0.7012	0.6795
	>150	2.39	1.67	2.42	0.8290	0.7678	0.7563
RBS	≤110	2.18	1.54	1.43	0.8238	0.7505	0.6874
	>110	2.31	1.60	0.88	0.8271	0.7650	0.6908
T3	≤75	2.10	1.29	1.21	0.8234	0.7320	0.6179
	>75	2.30	1.85	1.45	0.8304	0.7835	0.7088
T4	≤6	2.22	1.48	1.34	0.8239	0.7511	0.6623
	>6	2.50	2.41	1.64	0.8396	0.818	0.6977
TSH	≤5	2.03	1.24	1.40	0.8229	0.6696	0.6866
	>5	2.27	1.63	1.42	0.8484	0.7733	0.7132

DISCUSSION:

Variation in thyroid hormones (TH) is the most important factors involved in the regulation of the basal metabolic condition as well as in the oxidative metabolism. TH stimulates the production of free radicals and their disorders have pathogenic impact on human tissues. Depression of metabolism due to hypothyroidism and hyperthyroidism has been reported to decrease oxidant production and thus protects tissues against oxidant damage. Oxidative stress, characterized by an elevation in the steady state concentration of reactive oxygen species (ROS), has been involved in a wide range of biological and pathological conditions (Dursun et al 2008). However, data on the oxidative status of hypothyroidism and hyperthyroidism are limited and controversial (Isman et al 2003; Sarandol et al 2006). Most cases of hypothyroidism are sporadic, when inherited the condition usually has an autosomal recessive inheritance pattern, which means both copies of the gene in each cell have mutations. The actual reason behind the familial hypothyroidism is yet to be identified. Hence the present study was undertaken to evaluate the extent of oxidative stress by quantifying the MDA concentration and genetic instabilities in familial hypothyroidism and comparing with sporadic hypothyroid subjects and healthy control subjects.

The present study identifies hypothyroidism as familial and sporadic. The hereditary pattern of hypothyroidism might be associated with DNA repairing efficiency of the subjects. Helfend and Redfern (1998) reported that thyroid diseases are very common in middle aged and older adults. According to Vanderpump et al (1996) reported that thyroid disease is much more prevalent in women than men. Usha et al (2009) reported that approximately 1 out of every 7 women develops thyroid disease and its prevalence increases with age. These findings suggest that hypothyroidism is much more prevalent in the female population and it increases with increasing age. In the present study, both familial and sporadic hypothyroid subjects were belonged to the age range 41-60 years. Among the study subjects majority are females also. Thus the increase in hypothyroidism with progression of age within the female community observed in the present study is in agreement with that of previous study done by Unnikrishnan et al in 2011.

Prospective studies indicate that patients with Subclinical Hypothyroidism (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 among study subjects with hypothyroidism than the control subjects. Surks and Hollowell (2007) reported that the prevalence of SCH seemingly increases with age. Roger et al (2014) also reported the incidence of CVD rises with advanced age of SCH subjects. In the present study, the MDA concentration frequency was also increased with advancing age.

The present study identifies hypothyroidism and dyslipidemia as a common disorder and is leading cause for coronary heart diseases. Hypothyroid patients have increased levels of TC and LDL-C. Indeed, hypothyroidism is a common cause of secondary dyslipidemia (Canaris et al 2000). In the present study, subjects with total cholesterol greater than 200 mg/dl were observed with increased mean b/c value and MDA concentration. Oxidized low-density lipoprotein (LDL) is a known risk factor for atherosclerosis. Diekman et al (1998) showed that LDL-C from hypothyroid patients is more vulnerable for oxidation, indicating oxidative stress (OS). Olinescu et al (1998)

showed an increase in malondialdehyde (MDA) level in obese hypothyroid women.

A report by Dardano et al in 2006 suggested that increased TSH itself may lead to low-grade inflammation and OS. This was an interesting study because raised TSH is a hallmark of primary hypothyroidism. Before entering into a phase of overt hypothyroidism, the body experiences a gradual buildup of TSH and the time period varies among patient to patient depending on genetic-endocrinal-immunological differences. According to Nanda et al (2008) it was observed that TSH level correlates with the degree of lipid peroxidation and marker of inflammation such as ultrasensitive C-reactive protein, indicating a proportionate increase in cardiovascular risk factors. An association of increased TSH beyond its physiological limit with increased inflammation and OS was observed (Nanda et al 2011). In the present study also a positive correlation observed between increased TSH level and MDA concentration.

SUMMARY AND CONCLUSIONS

Majority of the hypothyroidism are thought to be sporadic, but <10% of hypothyroid conditions are familial (hereditary). Hypothyroidism-associated ROS is the consequence of both increased production of free radicles and reduced capacity of the anti-oxidative defense. Excess TSH might alter oxidative stress. The actual reason behind the familial hypothyroidism and the extent of oxidative stress between familial and sporadic hypothyroidism are to be identified. Hence the present study was under taken to evaluate the extent of oxidative stress and genetic instabilities in familial hypothyroidism and compared with sporadic hypothyroid and healthy control subjects. Mutagen induced chromosome sensitivity assay was performed as described by Hsu et al, 1987. The mean b/c value observed among familial hypothyroid subjects, sporadic and control subjects were 0.8255, 0.7578 and 0.6906 respectively. Similarly, the MDA concentration of among familial hypothyroid subjects, sporadic and control subjects were 2.25, 1.57 and 1.4 respectively. Laboratory investigations like, RBS, Total Cholesterol, Triglyceride, HDL-C, LDL-C, T3, T4 and TSH were also evaluated and correlated. Subjects with familial hypothyroidism showed statistically significant elevation in MDA concentration and mean b/c value compared to sporadic hypothyroid subjects and control subjects. Moreover, a positive correlation was also observed between these abnormal biochemical concentrations with elevated MDA concentration and mean b/c value. The study frankly observed that, the extent of various biochemical, endocrinological and molecular risk factors are more among subjects with familial hypothyroidism than sporadic hypothyroidism. Subjects with sedentary type of lifestyle showed increased MDA concentration and mean b/c value than subjects with non-sedentary type of lifestyle. Oxidative stress and subsequent genetic instabilities in hypothyroidism is a multifactorial condition, if not properly addressed, it may become the linking factor for various lifestyle associated diseases like cardiovascular diseases. In view of extensive OS and genetic instabilities in hypothyroidism along with the presence of atherogenic biochemical profile, more interventions are required to reduce the cardiovascular morbidities.

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