Original Article

Subclinical Hypothyroidism Association with Lipid Metabolism Disturbance and Oxidative Stress

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ABSTRACT

OBJECTIVE: To evaluate the lipid disturbance and oxidative stress in females with subclinical hypothyroidism.

METHODOLOGY: This case-control study was conducted at the Department of the Physiology University of Sindh, Jamshoro from April – September 2018. A total of 80 females were selected by random technique and were divided into two groups. Subjects with a history of episodic higher TSH discordant to FT4 levels and current levels of TSH ranging between 4.5ml U/L-<10ml U/L were selected for the study, subjects with hormone replacement therapy, chronic diseases or medication, pregnancy, or smoking habits were excluded. Thyroid profile (TSH, fT4), lipid profile (LDL, HDL, triglyceride, and total cholesterol), oxidative stress markers (MDA, AOPP, ABTS assay, CAT, SOD, GSH, and NO), and C reactive proteins were investigated and compared in subclinically hypo thyroid's females (SCH) with controls (Cont) females reside in Hyderabad Sindh, Pakistan. SPSS (statistical package for the social sciences) version 20.0 was used for statistical analysis.

RESULTS: We observed SCH group subjects with elevated TSH (7.41±0.23) having a slight increase in their LDL (116.39±2.52) and triglyceride levels (122.75±4.74), and also showing elevated MDA (2.49±0.17), AOPP (91.25±1.98) level, but had decreased level of GSH (119.33±2.19). However, no significant difference has been found in CAT, SOD, NO, and C - reactive protein between the two groups.

CONCLUSION: Observations of present studies suggestive of disturbed lipid metabolism in subclinically hypothyroid's females and a burdened antioxidants status.

KEYWORDS: Lipid Profile, Thyroid profile, Oxidative Stress markers, Subclinical hypothyroidism, C reactive protein.

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INTRODUCTION

Thyroid hormones mainly regulate the basal and oxidative metabolism of the body. Thyroid hormones have several protective effects on every system of the body and are associated with the oxidative and anti-oxidative status of the organism[1].

Subclinical hypothyroidism is associated with a disturbance of thyroid functions and is characterized by a persistently elevated level of Thyroid-stimulating hormone (TSH) while circulating free triiodothyronine (f T3) and free thyroxin (f T4) levels are within normal range[2]. The prevalence of subclinical hypothyroidism is 15% in general population/population-based studies[3] with persistent effects on almost each organ system and is more common in elderly females[4].

Several studies reported that sub-clinical hypothyroid patients have elevated serum levels of total cholesterol (TC) and low-density lipoprotein (LDL) than normal subjects, and that has been associated with a high risk of cardiovascular diseases, atherosclerosis, renal impairment, and diabetes mellitus[5-7]. Studies suggestive of oxidative stress in hypothyroid as well as subclinically hypothyroid patients are also available.

Oxidative stress is characterized by the imbalance production 0f antioxidants and reactive oxygen species (ROS), which play a vital role in biological processes and the development of pathological conditions[8]. Mostly ROS which is released in oxidative stress is hydrogen peroxide (H_2O_2), nitric oxide (NO), superoxide anion (O^{2-}), hydroxyl radical (HO^{-}), peroxyl (ROO^{-}), and reactive aldehyde (ROCH)[1,8], and these ROS are deal by the body in several ways, such as antioxidant enzymes usage. The biological oxidative impacts of ROS on lipids, proteins, and DNA are mainly controlled by a wide range of antioxidants. Many antioxidant enzymes protect against ROS and the oxidative products of lipids, DNA and protein[9]. Superoxide dismutase, SOD, is an antioxidant enzyme that dumps the superoxide radicals into hydrogen peroxide, which is later converted to water by the action of CAT, catalase, glutathione

peroxidase, and GPX[10]. Lipid peroxidation and cellular dysfunction malfunction are caused by the increase in NO, synthesis of peroxynitrite, and disturbed antioxidant[1]. It is elicited by hydroxyl radical and is associated with the breakdown of membrane[9, 11].

Disturb lipid metabolism in subclinical hypothyroid patients leads to overproduction of oxidants, and stresses the antioxidant protection system[12]. The oxidative products and the imbalance thus created leads to damage to various cell types which may lead to the development of an array of pathological conditions[13].

With the consideration of the high prevalence of subclinical hypothyroidism and its association with the complications of dyslipidemia and oxidative stress. The objective of our study was to understand the relationship of lipid metabolism disturbance due to abnormal levels of TSH and the following oxidative stress development that a need for replacement therapy or preventive measure in subclinically hypothyroid patients can be assessed.

METHODOLOGY

This case-control study was conducted at the department of physiology university of Sindh from April -September 2018, after approval by Institutional Review Board (Physiol:/IRB/125). A randomly selected 80 adult females between 20 to 40 years were recruited from a different area of Hyderabad, Sindh, Pakistan for the present study. Before the study the written informed consent was taken from each participant and experimentation was carried out according to the guiding principle of the Declaration of Helsinki 1964. The participants were divided into two groups: Control group (40 female) having normal serum-free T4 (FT4) and TSH level, and SCH (subclinically hypothyroid) group with TSH level (above than normal limit of 4.5mIU/L and less than 10mIU/L) while having FT4 level within the reference range. The subjects with a history of high-level TSH discordant to FT4 level and not on replacement therapy currently were selected for this study. Exclusion criteria were diabetes, cardiovascular disorder, high iodine therapy, malignant diseases, pregnant, smokers, alcoholic, and taken antihypertensive, antihistamines, lipid controlling drugs, vitamins supplement.

Biochemical assay

Blood samples were withdrawn from both groups after 12 overnight fastings, in EDTA-k2 tubes(BD Diagnostics, Plymouth, UK) for collection of whole blood and obtaining plasma, a gel containing (yellow cap) vacutainers (BD Diagnostics, Plymouth, UK) for serum separation. Plasma and serum were separated through centrifuged and were kept at -20 ^{0}C till investigation.

Thyroid profiles (TSH and FT4) and C reactive proteins (CRP) determination were performed using a Human diagnostic worldwide commercial ELISA kit.

The serum concentration of total cholesterol (TC), high-density lipoprotein (HDL), triglyceride (TG), and low-density lipoprotein (LDL) were measured using micro lab with kits from Human diagnostic worldwide commercial.

Antioxidant assay

Catalase activity in serum was estimated by following Aebi's methods[14]. Serum MDA was analyzed by following the methods of Ohkawa H 1979[15] and Chole RH 2010[16]. SOD activity was evaluated by Kakkar P 1984[17], reduced glutathione (GSH) estimation was carried out giving to the method of Jollow DJ 1974[18] method AOPP in serum were measured by the Kalousva 2002[19] method. Nitric oxide (NO) level in serum was determined according to the Taddei S et al[20] method.

Descriptive statistics were performed to obtain mean ± standard error (SE) and Q25% and Q75%. One way ANOVA test using Bonferroni post hoc test was applied to compare means between Cont (control) group and SCH (subclinical hypothyroidism group) group. The data were analyzed using the SPSS v.20. The significance level was set at $p < 0.05$.

RESULTS

In the present research, out of 80 females participants aged between 20-40 years from the urban region of Hyderabad, Sindh, Pakistan were selected which further divided into two groups; control (40 females) and Subclinical hypothyroid patients (40 females).

Patients with subclinical hypothyroidism had a significantly higher level of TSH (7.41±0.23) but FT4 level within the normal range, as shown in Table I. A significant increase ($p <0.05$) in total cholesterol (184.08±3.03) and LDL (116.39±2.52) have been observed in the SCH group, and also significant increase ($p < 0.05$) of triglycerides (122.75±4.74) have been observed in the SCH group as compared to control group, as shown in Table I. No difference was evident for the values of C-reactive proteins in both groups.

A significant increase ($p < 0.05$) has also been observed in MDA (2.49±0.17) and AOPP (91.25±1.98) levels in the SCH group as compared to the control group, as shown in **Table II**.

In **Table III**, a significant increase ($p < 0.05$) in GSH level was observed in the SCH group while a small non-significant ($p > 0.05$) decrease in CAT and SOD activity and similarly increased NO activity in the SCH group in comparison to the control group.

TABLE I:
BIOCHEMICAL PARAMETERS IN CONTROL AND SUBCLINICAL HYPOTHYROID PATIENTS GROUP

		N	Min	Mean±S.E	Max	25%	75%	ANOVA
TSH	Cont.	40	40	2.5±0.11	0.11	1.94	3.02	
	SCH	40	40	7.41±0.23	0.23	6.15	8.37	0.001
	All	80	80	4.95±0.31	0.31	2.34	7.5	
FT4 ng/dl	Cont.	40	40	1.4±0.03	0.03	1.27	1.51	
	SCH	40	40	1.31±0.04	0.04	1.17	1.47	0.032
	All	80	80	1.36±0.02	0.02	1.23	1.5	
LDL mg/dl	Cont.	40	88.82	104.46±1.46	118.53	96.02	113.17	
	SCH	40	83.06	116.39±2.52	155.67	104.64	125.31	0.001
	All	80	83.06	110.43±1.6	155.67	98.86	118.4	
HDL mg/dl	Cont.	40	36.52	44.12±0.53	51.4	41.86	46.7	
	SCH	40	39.5	43.15±0.37	47.63	40.89	45.04	0.133
	All	80	36.52	43.63±0.33	51.4	41.58	45.71	
Triglyceride mg/dl	Cont.	40	72.03	100.34±2.93	130.78	84.96	118.15	
	SCH	40	76.57	122.75±4.74	172.63	97.48	154.05	0.001
	All	80	72.03	111.54±3.05	172.63	89.35	127.41	
Total Cholesterol mg/dl	Cont.	40	144.93	168.64±2.23	190.93	158.71	180.48	
	SCH	40	141.49	184.08±3.03	220.49	172.32	198.57	0.001
	All	80	141.49	176.36±2.06	220.49	161.21	187.98	
C reactive proteins	Cont.	40	0.0005	0.1527±0.01781	0.3376	0.03	0.24	
	SCH	40	0.0059	0.1576±0.0176	0.3657	0.05	0.27	0.848
	All	80	0.0005	0.1552±0.0125	0.3657	0.04	0.25	

Min = minimum, Max = maximum, S.E = standard error, Q25% = 25% quartile and Q75% = quartile 50%

TABLE II:
PROTEIN AND LIPIDS OXIDATIVE PRODUCTS IN CONTROL AND SUBCLINICAL HYPOTHYROID PATIENTS GROUP

		No	Min	Mean±S.E	Max	25%	75%	ANOVA
MDA	Cont.	40	0.5	1.57±0.09	3.17	1.24	1.97	
	SCH	40	0.96	2.49±0.17	4.91	1.65	3.21	0.001
	All	80	0.5	2.03±0.11	4.91	1.37	2.4	
AOPP	Cont.	40	47.04	75.11±2.37	105.3	61.75	86.19	
	SCH	40	66.98	91.25±1.98	124.39	83.36	100.24	0.001
	All	80	47.04	83.18±1.79	124.39	72.43	93.25	

Min = minimum, Max = maximum, S.E = standard error, Q25% = 25% quartile and Q75% = quartile 50%

TABLE III:
OXIDATIVE STRESS MARKERS IN CONTROL AND SUBCLINICAL HYPOTHYROID PATIENTS GROUP

		N	Min	Mean±S.E	Max	25%	75%	ANOVA
GSH	Cont.	40	100.64	137.86±2.51	161.03	126.15	151.68	
	SCH	40	92.01	119.33±2.19	144.23	109.85	130.83	0.001
	All	80	92.01	128.6±1.96	161.03	114.57	141.1	
CAT	Cont.	40	40.29	68.44±1.68	85.42	59.91	76.81	
	SCH	40	43.66	65.84±1.6	88.49	58.73	74.07	0.266
	All	80	40.29	67.14±1.16	88.49	58.96	75.45	
SOD	Cont.	40	1.07	4.45±0.21	8.153	3.6	5.21	
	SCH	40	1.47	3.93±0.24	8.025	2.63	5.07	0.107
	All	80	1.07	4.19±0.16	8.153	3.03	5.14	
Nitric oxide	Cont.	40	23.75	31.89±0.83	42.31	27.52	36.05	
	SCH	40	18.58	34.44±1.21	51.39	27.56	40.33	0.086
	All	80	18.58	33.16±0.75	51.39	27.56	38.5	

Min = minimum, Max = maximum, S.E = standard error, Q25% = 25% quartile and Q75% = quartile 50%.

DISCUSSION

Every aspect of lipid metabolism is influenced by thyroid hormones. LDL clearance by the liver depends on stimulation by thyroid hormones. With lower level availability of thyroid hormones the delayed LDL clearance results in a higher level of LDL as well as of cholesterol[21]. It has been observed that TSH concentration increase within normal ranges can result in a linearly raised concentration of LDL, Triglycerides, Total Cholesterol, and vice versa a lowered HDL concentration[22]. In addition to the effects of thyroid hormones on adipose tissue uptake and utilization of lipids, the associated effects of thyroid hormones with the development of insulin resistance which results in increased production of LDL-c by the liver further exacerbate the conditions[23].

This results in the clustering of conditions supporting the accumulation of lipid metabolites, their oxidation and supporting the conditions like atherosclerosis, cardiovascular disorders, renal disorders, and diabetes mellitus.[24] TSH has also been positively correlated with oxidative stress, however with controversial outcomes and scanty[23-25]. Several studies reported that depressed metabolism in hypothyroidism may lead to lower production of oxidative products and negatively correlate with oxidative stress. Our findings show that in subjects with subclinical hypothyroidism, a raised level of TSH even with a normal level of FT4 can affect the lipid metabolism negatively. A small but significantly increased level of LDL, triglycerides and total cholesterol have been observed in sub-clinical hypothyroid subjects in comparison to subjects in the control group, a consistent finding in association to subclinical hypothyroidism. The change is the possible effects of a raised level of TSH on liver steatosis and dyslipidemia via expression of SREBP-2 expression in the liver[26].

This TSH level associated with dyslipidemia and oxidative stress has been correlated with the cardiovascular disorder by many studies. Considerable data is available suggesting that oxidative stress may result in endothelial damage and cardiovascular diseases. Persistence high level of circulating LDL made them available as a substrate for oxidation and production of ox-LDL results in dysfunctional endothelial cells[27]. The same increase level of LDL may be an explanation for the finding of a significant increase in MDA level in subclinical hypothyroid subjects in comparison to normal subjects. MDA is one of the many aldehydes formed during lipid peroxidation and used as a reliable marker of lipid peroxidation[28]. This persistent oxidative environment may burden the antioxidant system which is evident by a lower level of GSH in subclinical hypothyroid subjects. GSH is considered to play its neutralizing role where a low level of H2H2 is present, whereas it is the catalase that comes into action in the presence of high production of H2O2 to neutralize[21].

The results of the present study are suggestive of a mild oxidative environment in the subclinical hypothyroid group as the significant decrease in GSH level is not accompanied by increased activity of catalase. There is no significant difference in the two groups for catalase activity and no correlation has been found in TSH level and CAT activity. These are not only the lipids that are prone to alteration in the oxidative environment, proteins are also get modified

and get damaged by the excess of free radicals. Currently, the available data suggestive of protein damage in subclinical hypothyroidism is scarce[29]. However, in the present study, a significant increase in the AOPP had been observed. AOPP has been found to play a role in the induction of renal fibrosis via redox-sensitive inflammatory pathway[30] and in the development of cardiovascular diseases and diabetes via AGEs receptor by resulting in damage to endothelial cells damage in blood vessels and pancreas respectively.

CONCLUSION

In conclusion, the present study suggests a mild oxidative stress environment in subjects suffering subclinical hypothyroidism, where dyslipidemia due to the raised level of TSH results increased and persistence availability of lipids for oxidation. This not only can lead to the development of damage-induced diseases like atherosclerosis but also can exacerbate the thyroid function itself by inducing damage to the follicular cells.

RECOMMENDATION

It is recommended that oxidative stress may further be studied at a large scale that the need for oxidant stress testing may be fully evaluated and preventive measures or replacement therapy measures can be designed.

Conflict of Interest: There is *no* conflict of *interest among the authors.*

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DATA SHARING STATEMENT: The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions

AUTHOR CONTRIBUTIONS

Mughal ZN: Conceived the study, designed the study, wrote the initial and final drafts of the article, collected samples, conducted the research, and provided research materials, and also organized, analyzed, and interpreted the data.
Zai JA: Designed the study, collected samples, conducted the research, and provided research materials and also organized, analyzed, and interpreted the data, provided logistic support
Gill NP: Performed Statistical analysis
Chugtai LA: Collected samples, conducted the research, and provided research materials, and also organized, analyzed, and interpreted the data
Khand AA: Collected samples, conducted the research, and provided research materials, and also organized, analyzed, and interpreted the data.
Soomro A: Collected samples, conducted the research, and provided research materials, and also organized, analyzed, and interpreted the data.
All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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