ROLE OF OXIDATIVE STRESS MARKERS ON SUBCLINICAL HYPOTHYROIDISM AND ITS ASSOCIATION WITH HS-CRP

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ABSTRACT

Introduction: - Subclinical hypothyroidisms are common clinical entities that encompass mild degrees of thyroid dysfunction. It is an early, mild form of hypothyroidism, where serum level of thyroid-stimulating hormone from the front of the pituitary gland is a little bit above normal. oxidative stress markers can be helpful in assessment of adverse effects of subclinical hypothyroidism, are not very well studied in the past. So, the aim of this study was to investigate the role of oxidative stress markers on subclinical hypothyroidism and its association with hs-CRP in Subclinical hypothyroidism patients

Materials and Methods: The study population consisted of 150 patients with recently diagnosed subclinical hypothyroidism and 150 healthy controls. TSH, FT4 & T3 were estimated by enzyme linked Immunosorbent assay (ELISA) for diagnosis of subclinical hypothyroidism. The SOD activity was estimated by method described by Kono, 1978 by observing the inhibitory rate of NBT (Nitroblue tetraz olium) reduction. Hs-CRP was estimated by enzyme linked Immunosorbent assay (ELISA).

Results: In this study the level of TSH Mean ± SD (9.92±2.42 vs 1.95±1.01) and T3 Mean ± SD (1.01±0.32 vs 1.26±0.34) and T4 Mean ± SD (8.44±0.92 vs 7.67±1.42) were significantly higher (<0.001) in subclinical hypothyroidism. TSH, T4, level was positively correlated with Malondialdehyde and Superoxide Dismutase and hs-CRP in subclinical hypothyroidism.

Conclusion: In conclusion subclinical hypothyroidism patients have raised oxidative stress markers and hs-CRP, this may be due to that future risk and further progression. Level of hs-CRP increases in patients as disease progress if left untreated.

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Introduction

Subclinical hypothyroidism (SCH) is defined as an elevated level of serum thyroid stimulating hormone (TSH) with a normal level of serum free thyroxin (FT4).[1] The overall prevalence of SCH is reported to range from 4% to 10% in large general population screening surveys,[2] although it varies with age, sex, and race.[3]Subclinical hypothyroidism or mild thyroid failure is a common problem, with a prevalence of 3% to 8% in the population without known thyroid disease.[4] The prevalence increases with age and is higher in women.[5] After the sixth decade of life, the prevalence in men approaches that of women, with a combined prevalence of 10%. Anti-thyroid antibodies can be detected in 80% of patients with SCH, and 80% of patients with SCH have a serum TSH of less than 10 mIU/L.

Subclinical hypothyroidism has been associated with a greater prevalence of cardiovascular disease[6]. Thyroid function regulates a wide array of metabolic parameters. Thyroid function significantly affects lipoprotein metabolism as well as some cardiovascular disease (CVD) risk factors, thus influencing overall CDV risk.[7]

There is growing evidence that SCH is associated with lipid abnormalities, increasing cardiovascular risk, particularly in older women. Clinical hypothyroidism is associated with premature atherosclerosis and increased prevalence of coronary disease.

Studies also reported that the induction of acute hyperlipidemia increases the oxidative stress marker.[8] Oxidative stress is the equilibrium between the generation and elimination of reactive oxygen species (ROS). In healthy conditions, cellular antioxidant enzymes are responsible for the regulation of ROS productions.[9] Because of the unique molecular structure, lipids are more vulnerable to oxidation. Oxidative stress occurs when the concentrations of ROS exceed those of antioxidant neutralizing species, such as nicotinamide adenine dinucleotide phosphate (NADPH) and glutathione (GSH). ROS are a heterogeneous population of molecules including free radicals, like hydroxyl (OH), superoxide (O2-), peroxyl (RO2), and hydroperoxyl (HRO2-), and non-radical species, as hydrogen peroxide (H2O2) and hydrochloric acid (HCl).[10,11] Malondialdehyde (MDA) is produced through peroxidation of polyunsaturated fatty acids, and it is atherogenic.[12] Due to presence of altered lipid profile, patients may also have further development of cardiac disease. Atherosclerosis being an inflammatory disorder, associated with accumulated cholesterol concentration might be developed in future in subclinical hypothyroidism.[13] Some of the inflammatory markers are quite effective and well-known future predictors of cardiovascular risk.[14] C-reactive protein CRP is an effective tool for diagnosis of cardiac risk.[15] Therefore, the aim of this study was to is to investigate serum levels of oxidative stress markers (SOD and MDA) and C reactive protein along with lipid profile in subclinical hypothyroidism patients.

Material & Methods

The present case- control study conducted in Department of Biochemistry Index Medical College, Hospital and Research Centre Indore (M.P.), India. 150 patients with recently diagnosed subclinical hypothyroidism and 150 healthy controls evaluate a study of thyroid function test, lipid profile, oxidative stress and inflammatory markers in subclinical hypothyroidism and controls subjects in the Department of Biochemistry Index Medical College, Hospital and Research Centre Indore (M.P.), India. Recently diagnosed subclinical hypothyroidism patients, 20-45 year of age group both male and female were included for the study population. Male and female healthy individuals without having any history or presence of cardiovascular and thyroid disease were enrol for the study for comparison of results TSH, FT4 & T3 were estimated by enzyme linked Immunosorbent assay (ELISA) for diagnosis of subclinical hypothyroidism. Serum MDA was determined using the thiobarbituric acid (TBA) reaction. The SOD activity was estimated by method described by Kono, 1978 by observing the inhibitory rate of NBT (Nitroblue tetrazolium) reduction. C-reactive protein was estimated by enzyme linked Immunosorbent assay (ELISA).

Inclusion Criteria:

Recently diagnosed subclinical hypothyroidism patients, 20-45 year of age group both male and female were included for the study population

Male and female healthy individuals without having any history or presence of cardiovascular and thyroid disease were enrol for the study for comparison of results

Exclusion Criteria:

- Patients having any previous medical history of disease, family history of thyroid disease or having thyroid medication.
- Patients suffering from cardiovascular risk or having any previous history of heart diseases.
- Diabetes, hypertension, renal diseases and liver disease

- Pregnant women or past history of pregnancy in last two years etc.
- Any inflammatory disease e.g., rheumatoid arthritis, periodontitis etc.
- Any social habits e.g., Smoking, tobacco, alcohol, etc.

Statistical Analysis

Statistical Analysis Was Conducted by Using SPSS Version 20.0(Chicago Us) Unpaired T Test Was Used to Calculate Significance (P Value) In Between The Groups. P <0.05 Was Considered Statistically Significant. Correlation Was Determined by Using Karl's Pearsons Correlation Coefficient.

Observation & Results

The present case-control study was conducted on subclinical hypothyroid patients were selected who attending OPD, Index Medical college, Indore. A total 150 (73 females and 77 males) adult age group 25-45 years of both gender who were diagnosed subclinical hypothyroid patients on the basis of WHO norms were enrolled in this study. 150 (100 male 50 female) cases were similar age and sex normal healthy control. Lipid biomarkers, hs-CRP were compared between subclinical hypothyroid and healthy control and find the association of lipid biomarkers, and hs-CRP with thyroid profile (TSH, T3 and T4).

Table No. 1: Sex group distribution

Sex	Group		P value
	Case (n=150)	Control (n=150)	
Male	77 (51.3%)	100 (66.7%)	**0.007**
Female	73 (48.7%)	50 (33.3%)	

Table No. 2: Thyroid profile level in studied patients

PARAMETERS	GROUP		P value
	Case (n=300)	Control (n=100)	
TSH	9.92±2.42	1.95±1.01	**<0.001**
T3	1.01±0.32	1.26±0.34	**<0.001**
T4	8.44±0.92	7.67±1.42	**<0.001**
Malondialdehyde (MDA) [nmol/ml]	3.73±0.68	1.86±0.72	**<0.001**
Superoxide Dismutase (SOD) [U/ml]	190.02±55.05	150.82±55.03	**<0.001**
Hs-CRP [mg/l]	1.97±0.50	1.40±0.57	**<0.001**

Thyroid profile level (TSH and T4) was significantly higher in the case group in comparison to control group (P<0.05); but T3 level was significantly lower in the case group (P<0.05). Oxidative markers level (Malondialdehyde (MDA) and Superoxide Dismutase (SOD)) were significantly higher in the case group in comparison to control group (P<0.001). Inflammatory markers level (Hs-CR) was significantly higher in the case group in comparison to control group (P<0.001).

Table No-3: Correlation between Diabetes parameters with lipid profile, oxidative stress makers and inflammatory markers

			Thyroid Profile		
			TSH	**T3**	**T4**
Oxidative stress markers	**SOD**	**Pearson Correlation**	0.369**	-0.129*	0.186**
		P value	**<0.001**	**0.026**	**<0.001**
	MDA	**Pearson Correlation**	0.739**	-0.306**	0.230**
		P value	**<0.001**	**<0.001**	**<0.001**
Inflammatory markers	**Hs-CRP**	**Pearson Correlation**	0.471**	-0.175**	0.246**
		P value	**<0.001**	**0.002**	**<0.001**
**. Correlation is significant at the 0.01 level (2-tailed).					
*. Correlation is significant at the 0.05 level (2-tailed).					

Above table represent the Pearson Correlation of oxidative stress markers and inflammatory markers with the subclinical hypothyroid profile and observed a significant association of oxidative stress markers and inflammatory with TSH, T3 and T4. Negative sign shows the universally proportional correlation.

Table No 4: Correlation between oxidative stress makers and inflammatory markers

Inflammatory markers		Oxidative stress markers	
		SOD	**MDA**
Hs-CRP	**Pearson Correlation**	0.807**	0.516**
	P value	**<0.001**	**<0.001**
**. Correlation is significant at the 0.01 level (2-tailed).			

In this table we noted the positive significant association between oxidative stress and inflammatory markers ($p<0.05$)

Discussion

The role of thyroid in the regulation of the antioxidant systems has been recently reviewed in the context of the reproductive endocrinology.[16] It is well known that thyroid function influences the ovarian activity. ROS play physiological roles in the ovary and hypothyroidism, or a low-T3 syndrome, can induce ovarian dysfunction by interfering with the antioxidant systems. Inflammation and oxidation of lipoproteins are thought to play an important role in the progression and complications of atherosclerosis and CV disease. Low-grade inflammation and oxidative stress may also have a role in the pathogenesis of many complications associated with HT such as impaired endothelial function and atherosclerotic CV disease.[17]

C-reactive protein (CRP), a plasma protein mainly synthesized by the liver, is a sensitive and reliable systemic marker for inflammatory state.[18] CRP has been long recognized as an independent and effective tool for diagnosis of CV risk: CRP concentration is associated with the risk of coronary heart disease, ischemic stroke and vascular mortality.[19,20] Christ-Crain et al. first showed an association between that CRP values and both SHT and OHT.[21] Since then, varying degrees of association between HT and CRP have been noted in different populations. Oxidative stress can result from either an increase in the production of reactive oxygen species, or a reduction in antioxidants. In any case, oxidative stress may result in damage to lipids, proteins, and DNA. Lipids are particularly susceptible to free radical attack.[22] Furthermore, products of lipid peroxidation such as malondialdehyde (MDA) can react with DNA and introduce mutagenic lesions.[22] MDA is one of the most commonly used biomarkers for lipid peroxidation which is often assessed as thiobarbituric acid reactive substances (TBARS). Serum levels of TBARS were found to be strongly predictive of CV events in patients with stable coronary artery disease, independently of traditional risk factors and inflammatory markers.[23] Data concerning MDA levels in HT is variable but until now no meta-analysis has tried to integrate the results of these studies.

This is a case-control, hospital-based study conducted in Department of Biochemistry Index Medical College, Hospital and Research Centre Indore (M.P.), India; and to investigate serum levels of oxidative stress markers (SOD and MDA) and C reactive protein along with lipid profile in subclinical hypothyroidism patients compared with normal healthy adults as control.

The present **c**ase- control study conducted evaluate a study of thyroid function test, lipid profile, oxidative stress and inflammatory markers in subclinical hypothyroidism and controls subjects in the Department of Biochemistry Index Medical College, Hospital and Research Centre Indore (M.P.), India. Recently diagnosed subclinical hypothyroidism patients, 20-45 year of age group both male and female were included for the study population. We investigate serum levels of oxidative stress markers (SOD and MDA) and C reactive protein along with lipid profile in subclinical hypothyroidism patients compared with normal healthy adults as control.

This study noted that the thyroid profile level (TSH and T4) was significantly higher in the case group in comparison to control group ($P<0.05$); but T3 level was significantly lower in the case group ($P<0.05$). These findings were supported by previous studies**. Arikan S et al**[24] and **Ujwal Upadya B et al**[25] studies done in the past, have produced varied results regarding the subclinical hypothyroidism and disturbed lipid parameters, which are still not clearly defined. Higher concentration of total cholesterol, triglycerides and LDL cholesterol in SCH patients in this study were also observed by **Sridevi A et al**[26] and **Kvetny J et al**[27]. **Erdem TY et al**[28] supported this study by observing the decreased concentration of HDL cholesterol. **Ford ES**[29] reported that the C-reactive protein an acute phase reactant is known to influenced by several factors e.g. body mass index and cholesterol concentration stated by **Ridkar PM et al**[30], etc. which may be associated with subclinical hypothyroidism reported by **Roy S et al**[31]. **Gupta G et al**[32] study suggests that level of inflammatory markers was relatively higher in SCH patients than control group and they were positively correlated with TSH level in SCH

group. **Karoli R et al[33]** and **Mahto M et al[34]** studies have described an elevated concentration of C reactive protein in SCH patients which was positively correlated with TSH concentration reported by **Sharma R et al[35]**.Our study noted that the oxidative markers level (Malondialdehyde (MDA) and Superoxide Dismutase (SOD)) were significantly higher in the case group in comparison to control group (P<0.001). **Cheserek MJ et al[36] reported the** oxidative stress was increased in subclinical hypothyroidism as evidenced by the elevated lipid peroxidation product, malondialdehyde, while protein oxidation was absent. Thus, reduction of oxidative stress may be beneficial in patients with subclinical hypothyroidism. **Chakrabarti SK et al[37] reported the** hypothyroidism is a state of increased oxidative stress. **Kalaivanam KN et al[38]** concluded that the increased MDA, carbonyl protein concentrations and decreased concentration of total antioxidant capacity, are evident for SCH patients' at asymptomatic stage itself. **Kaushik GG et al[39] reported the** mean hs-CRP level of SCH subjects is found higher than Euthyroid controls (p<0.0001). **A**pplying the Pearson Correlation of lipid profile, oxidative stress markers and inflammatory markers with the subclinical hypothyroid profile this study noted a significant association of oxidative stress (MAD & SOD) and inflammatory markers (hs-CR) with TSH, T3 and T4. Our study also noted the positive significant association between oxidative stress and inflammatory markers (p<0.05). **Vyakaranam S et al[40]** reported that the multivariate linear regression suggested that hs-CRP is significantly and positively associated with SCH after adjusting for age and body mass index (BMI) in their study. **Duntas et al[41]** suggested that SCH has been strongly associated with dyslipidemia and cardiovascular risk along with abnormal C-reactive protein level. **Vaya et al.,**[42] supported this study by reporting that CRP level was significantly higher in SCH. Further this study was noted that the TSH, T3 and MAD level was significantly higher in male in compare to female p<0.05). but T4, lipid profile (total cholesterol, triglycerides, HDL, LDL, & VLDL), oxidative stress (SOD) and inflammatory markers hs-CRP & was insignificantly distributed in among groups.

Conclusion:

On the basis of results of this study, it can be suggested that SCH profile (TSH, T3 and T4) patients are associated with oxidative stress (MAD and SOD) and & hs-CRP. By the outcomes of this study, we can give torch to the clinicians working in their general practice that subclinical hypothyroidism should be treated in early detection to avoid the risk of various clinical syndrome. Our findings contribute to the growing evidence about the adversity created due to impact of subclinical Hypothyroidism. Though, our findings require confirmation in additional cohorts.

Conflict of Interest: The authors have declared that there is no conflict of interest

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