EVALUATION OF MALONDIALDEHYDE AND SUPEROXIDE DISMUTASE ON SUBCLINICAL HYPOTHYROIDISM AND THEIR ASSOCIATION WITH INTERLEUKIN-6

Ajit Kumar Yadav[1], Shreya Nigoskar[2*]

ABSTRACT

Background: - Subclinical hypothyroidism is a common clinical entity that encompasses mild degrees of thyroid dysfunction. It is an early, mild form of hypothyroidism, where the serum level of thyroid-stimulating hormone from the front of the pituitary gland is a little bit above normal. Malondialdehyde and superoxide dismutase, which can be helpful in assessing the adverse effects of subclinical hypothyroidism, have not been very well studied in the past. So, the aim of this study was to investigate the role of malondialdehyde and superoxide dismutase in subclinical hypothyroidism and their association with interleukin 6 in subclinical hypothyroidism patients.

Methodology: 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 the diagnosis of subclinical hypothyroidism. Serum MDA was determined using the thiobarbituric acid (TBA) reaction. The SOD activity was estimated by NBT (nitroblue tetrazolium) reduction. Interleukin 6 was estimated by an enzyme-linked immunosorbent assay (ELISA).

Results: In this study, the levels of TSH mean ± SD (9.92±2.42 vs. 1.95±1.01), 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 and T4 levels were positively correlated with malondialdehyde, superoxide dismutase, and interleukin 6 in subclinical hypothyroidism.

Conclusion: In conclusion, subclinical hypothyroidism patients have raised oxidative stress (MAD and SOD). The level of interleukin 6 increases in patients as disease progresses if left untreated.

Keywords: Subclinical hypothyroidism; Thyroid profile; Oxidative stress; Interleukin-6.

[1]Research scholar, Department of Biochemistry**,** Index Medical College, Hospital and Research Centre Indore (M.P.), India.

[2*]Professor, Department of Biochemistry, Index Medical College, Hospital and Research Centre Indore (M.P.), India.

[*]**Corresponding Author:** Dr. Shreya Nigoskar

[*]Professor, Department of Biochemistry, Index Medical College, Hospital and Research Centre, Indore (M.P.), India, Email id: - shreyanigoskar@gmail.com

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Introduction

Subclinical hypothyroidism (SCH) is an elevated level of serum thyroid stimulating hormone (TSH) with a normal level of serum free thyroxin (FT4).[1] A number of studies have provided that SCH is associated with dyslipidemia, impaired vascular function, atherosclerosis, myocardial dysfunction, and progression to overt hypothyroidism.[2] Because advanced assays enable to measure thyroid hormones more accurately. The incidence of SCH is tend to increase in the last few decades.[3] The overall prevalence of SCH is reported to range from 4% to 10% in large general population screening surveys,[4] although it varies with age, sex, and race.[5] Subclinical hypothyroidism or mild thyroid failure is a common problem, with a prevalence of 3% to 8% in the population without known thyroid disease.[6] The prevalence increases with age and is higher in women.[7] 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. Hypothyroidism results from deficiency of thyroid hormone. Subclinical hypothyroidism is diagnosed when serum TSH is raised and serum free T4 is normal. Subclinical hypothyroidism is found in about 6.1% of women and 3.4% of men.[8] If associated with positive TPO antibody there is about 4% annual risk of developing overt hypothyroidism.[9] Patients with type 2 diabetes mellitus have an increased prevalence of thyroid disorders compared to non-diabetic population and are more common in females than in males. After the sixth decade of life, the prevalence in men approaches that of women, with a combined prevalence of 10.0%. Antithyroid antibodies can be detected in 80% of patients with SCH and 80.0% 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[10]. 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.[11] 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).[12,13] Malondialdehyde (MDA) is produced through peroxidation of polyunsaturated fatty acids, and it is atherogenic.[14] Activity of CRP is induced by a specific cytokine; interleukin-6 (IL-6), important and well-established marker for assessment of inflammation.[15] Studies in the past have created confusion with respect to such inflammatory markers in subclinical hypothyroidism from no risk to definite risk.[16,17] Therefore, the aim of this study was to is to investigate serum levels of oxidative stress markers (SOD and MDA) and Interleukin-6 along thyroid profile in subclinical hypothyroidism patients.

Material & Methods

The present case- control study was conducted in the Department of Biochemistry, Index Medical College, Hospital and Research Centre Indore (M.P.), India after obtaining of ethical approval from institution ethical committee (Approval No: MU/Acm/Ph.D/Med BioChem/2020/014).A total 300 subjects with age group of 20-45 of either gender was enrolled for this study. Out of which 150 subjects were recently diagnosed cases of subclinical hypothyroidism from OPD of Medicine, Index hospital Indore and 150 healthy controls in and around of hospital for the evaluation of thyroid profile, oxidative stress and interleukin-6 in subclinical hypothyroidism and controls subjects. A written and verbal consent was taken from every subjects. Subjects with having any previous medical history or family history of thyroid disease or on having thyroid medication, heart diseases, Diabetes, hypertension, renal diseases, liver disease, Pregnancy (in last two years), rheumatoid arthritis, periodontitis etc. were excluded from this study. Thyroid profile (TSH, T4 & T3) was 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 NBT (Nitroblue tetrazolium) reduction. IL-6 was estimated by enzyme linked Immunosorbent assay (ELISA). Data were presented in the form of Mean & SD. Test of significance (Unpaired t-test [p-value]) and Pearson correlation analysis (r-value) was analysed by using SPSS version 20.0(Chicago US). $p < 0.05$ was considered statistically significant.

Results

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 standard WHO criteria were enrolled in this study. 150 (100 male 50 female) cases were similar age and sex normal healthy control. Oxidative stress and interleukin 6 were compared between subclinical hypothyroid and healthy control and find the association of thyroid profile (TSH, T3 and T4) with oxidative stress and IL-6. 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 stress markers level (Malondialdehyde (MDA) and Superoxide Dismutase (SOD) were significantly higher in the case group in comparison to control group ($P<0.001$). Interleukin-6 was significantly higher in the case group in comparison to control group ($P<0.001$).

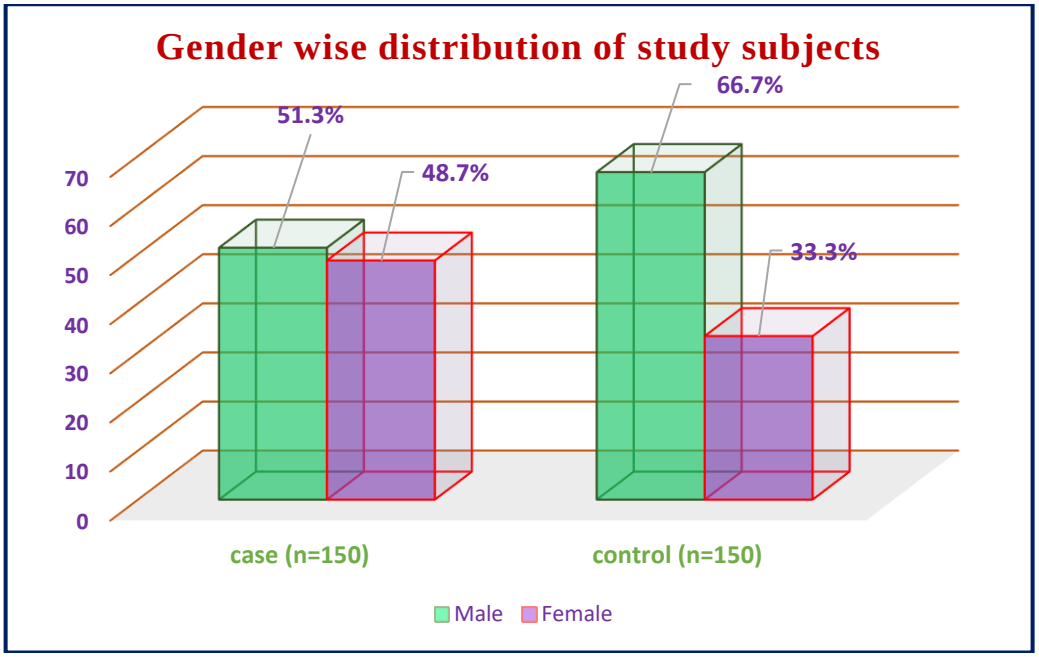

Figure- 1: Gender wise distribution of study subjects

Table-1: Thyroid profile, Oxidative stress markers and IL-6 levels in study subjects

Biochemical parameters	Study Group		P value
	Case (n=150)	Control (n=150)	
TSH (uIU/ml)	9.92±2.42	1.95±1.01	<0.001**
T3 (ng/ml)	1.01±0.32	1.26±0.34	<0.001**
T4 (ug//ml)	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**
IL6 (pg/mL)	31.88±13.99	24.21±14.79	<0.001**

Table-2: Correlation between Malondialdehyde, Superoxide Dismutase and TSH with interleukin

		SOD (U/ml)	MDA (nmol/ml)	TSH (uIU/ml)
IL6 (pg/mL)	***r- value***	0.578**	0.324**	
	P -value	**<0.001**	**<0.001**	
**. Correlation is significant at the 0.01 level (2-tailed).				

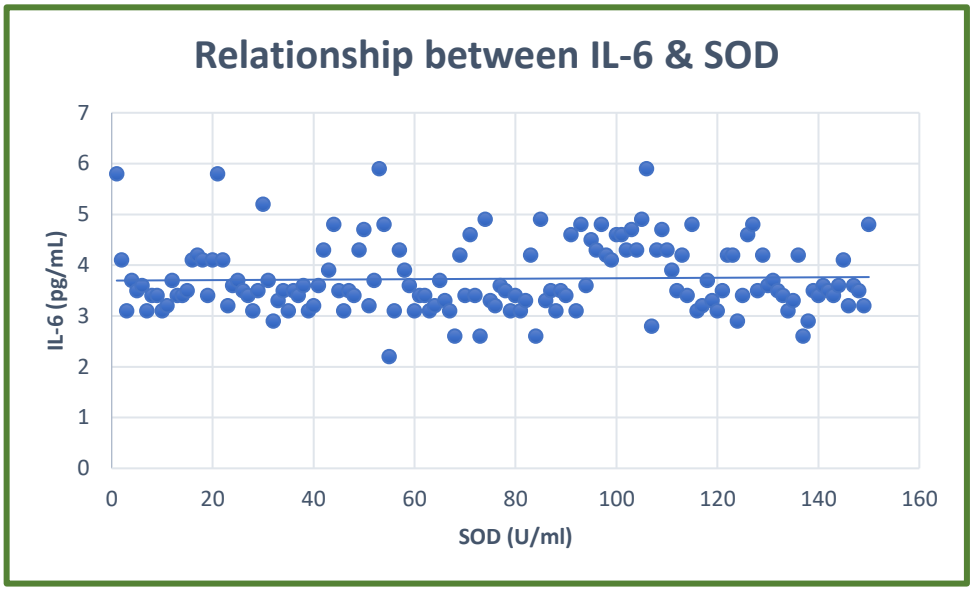

Figure-2: Correlation analysis between IL-6 & MDA in Subclinical hypothyroidism cases.

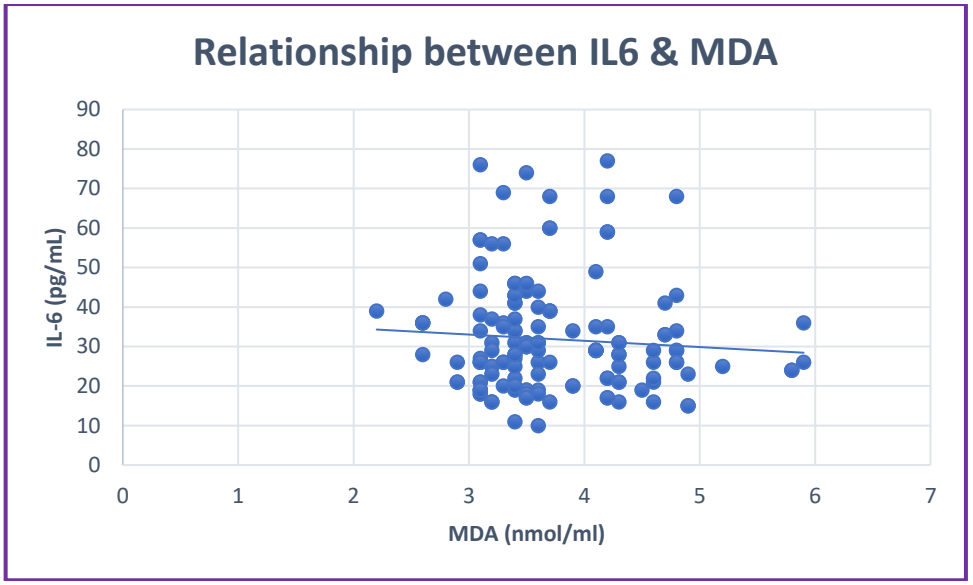

Figure-3: Correlation analysis between IL-6 & MDA in Subclinical hypothyroidism cases.

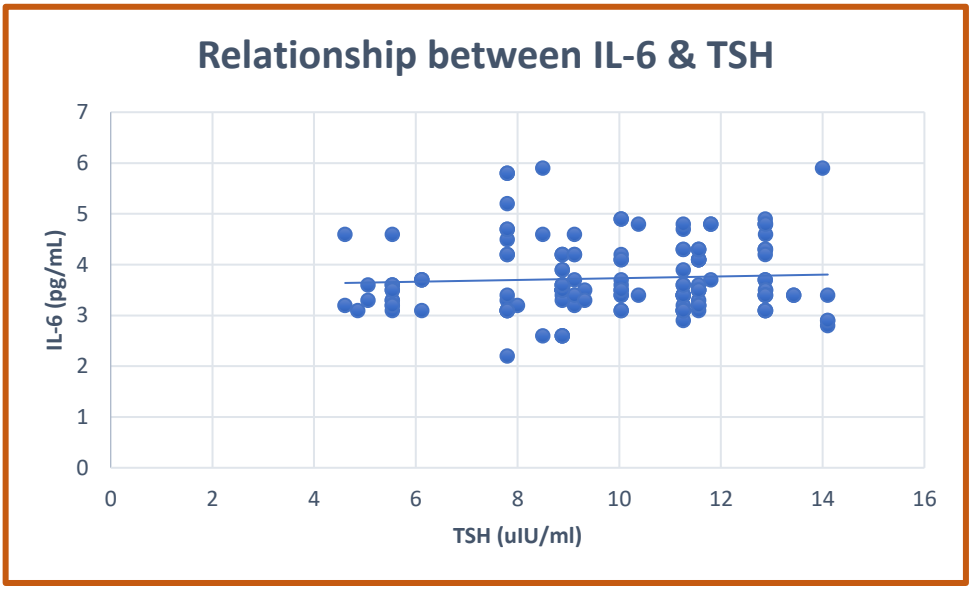

Figure-4: Correlation analysis between IL-6 & MDA in Subclinical hypothyroidism cases.

Discussion

The role of thyroid in the regulation of the antioxidant systems has been recently reviewed in the context of the reproductive endocrinology.[18] It is well known that thyroid function influences the ovarian activity. Reactive oxygen species play physiological roles in the ovary and hypothyroidism, or a low-T3 syndrome, which 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 cardiovascular 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.[19] 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. Products of lipid peroxidation such as malondialdehyde (MDA) can react with DNA and introduce mutagenic lesions.[13] 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.[20] Data concerning MDA levels in HT is variable but until now no meta-analysis has tried to integrate the results of these studies. A case-control, hospital-based study conducted in the Department of Biochemistry Index Medical College, Hospital and Research Centre Indore (M.P.), India; and to investigate serum levels of thyroid profile, oxidative stress markers (SOD and MDA) and Interleukin-6 in subclinical hypothyroidism patients compared with normal healthy adults as control. Chakrabarti SK et al[21] has made a case-control study of the oxidative stress in hypothyroid patients and the role of antioxidant supplementation and concluded that the oxidative stress compounds hypothyroidism. Hypothyroidism is a state of increased oxidative stress. Cheserek MJ et al[22] conducted a case-control study of the association between thyroid hormones, lipids and oxidative stress markers in subclinical hypothyroidism and concluded that 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. Torun AN et al[23] has made a case-control study of the serum total antioxidant status and lipid peroxidation marker malondialdehyde levels in overt and subclinical hypothyroidism and concluded that an increased oxidative stress in both hypothyroid and subclinical hypothyroidism states, which can be explained by both the insufficient increase in the antioxidant status and the altered lipid metabolism in these cases.

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[21] 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[22] reported the hypothyroidism is a state of increased oxidative stress. Kalaivanam KN et al[23] concluded that the increased MDA, carbonyl protein concentrations and decreased concentration of total antioxidant capacity, are evident for SCH patient at asymptomatic stage itself. Present study noted that the IL6 was significantly higher in the case group in comparison to control group ($P<0.001$). Gupta G et al[24] study reported the IL-6 were significantly higher in SCH group when compared with control group. Torun AN et al[23] reported that an increased oxidative stress in both hypothyroid and subclinical hypothyroidism states, which can be explained by both the insufficient increase in the antioxidant status and the altered lipid metabolism in these cases. On applying the Pearson Correlation on oxidative stress markers and IL-6, the subclinical hypothyroid profile this study noted the positive significant association between oxidative stress and inflammatory markers ($p<0.05$). Goyal S. et al[25] reported that a positive correlation between serum levels of IL-6 (r- value=0.5778, p-value=0.0008) and TNF-α (r- value=0.521, p-value=0.003) with serum TSH levels.

Conclusion:

- On the basis of results of this study, it can be suggested that SCH patients have a role on oxidative stress (MAD and SOD) and & Interleukin 6.
- Subclinical hypothyroidism patients have raised oxidative stress (MAD and SOD). Elevated levels of interleukin 6 afflict the patients as disease in progress if it is untreated.

- 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.

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

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