

## To Evaluate the Biochemical Changes that Predisposing to CVD in Subclinical Hypothyroidism

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### Abstract

**Background:** Regulation of thyroid hormones is critical to optimum growth, metabolism and organ function. Subclinical thyroidism is a common stable thyroid disorder, mostly found in middle aged and elderly population without any major symptoms. This can be of two types- subclinical hyperthyroidism and subclinical hypothyroidism. Subclinical hypothyroidism as a clinical event where serum thyroid hormones T3 and T4 are at normal respective physiological levels but serum TSH is mildly elevated. Serum TSH has a linear exponential relationship with free thyroid hormones and 2 fold changes in free T4 will lead to 100 fold changes in TSH levels. This SCH is more common in women and normally increases with age. The author further added that the most serious clinical implication of SCH is its gradual progression towards usual hypothyroidism. They added that SCH occurrence happens due to reduced iodide clearance due to impaired glomerular filtration in CKD patients. This results into an elevated plasma iodide concentration that alters thyroid functioning.

**Aim:** The present study was undertaken to identify various biochemical and molecular biomarkers for the early detection of cardiovascular disease (CVD) among subjects with subclinical hypothyroidism (SCH).

**Material and Method:** A case-control study layout was adopted for the present study to identify the various biochemical and molecular biomarkers for the early detection of Cardiovascular Disease among subjects with Subclinical Hypothyroidism. All test and control subjects provided their information on a well-defined proforma regarding their demographic records, family history and disease-related records. The study group included 150 clinically diagnosed individuals with subclinical hypothyroidism and 100 age and sex matched healthy control subjects. Various biochemical parameters, lipid profile, oxidative profile, inflammatory profile, renal profile and emerging biomarkers such as NT-proBNP and PAPP-A were evaluated and compared among study and control subjects.

**Results:** This study provides Biochemical, investigations as well as inflammatory and oxidative risk markers along with emerging biomarker like NT-proBNP and PAPP-A showed a statistically significant

difference between study subjects and control subjects. Variables like PAPP-A, Uric acid, MDA, TC, TG, TSH, Urea, weight and BMI showed a strong positive correlation with NT-proBNP concentration. Moreover, creatinine showed a positive correlation with NT-proBNP. Mean NT-proBNP, uric acid, MDA, TC, LDL, TG, TSH, Urea, creatinine, showed a strong positive correlation with PAPP-A level. HDL-C showed a negative correlation with PAPP-A concentration.

**Conclusion:** Subclinical hypothyroidism is one of the most prevalent autoimmune diseases provoked in genetically susceptible individuals by several triggers, including female sex, advanced age, obesity, abnormal lipid profile and environmental factors. These risk factors seem to contribute DNA damage as indicated by increase in micronuclei frequency. SCH is often linked with higher chance for developing a wider range of adverse health outcomes and that SCH might represent a potentially modifiable risk factor of CVD. Therefore, better understanding of the pathophysiology, prevalence and the risk factors of SCH could help in the prevention of CVD in the population.

**Keywords:** Malondialdehyde, Thyroid Stimulating Hormone, NT-proBNP, PAPP-A, CVD and Subclinical Hypothyroidism

## Introduction

Subclinical hypothyroidism (SCH) is defined as, “a clinical event where serum thyroid hormones T3 and T4 are at normal respective physiological levels but serum TSH is mildly elevated”.<sup>(1,2)</sup> Subclinical or mild hypothyroidism is often associated with adverse cardiovascular risk factors, such as high cholesterol, together with hypertension, endothelial dysfunction and other atherosclerotic cardiovascular risk factors. The ischemic abnormalities are probably related to long-term consequences of a slowly progressing development of hypothyroidism. In recent years, it has become evident that a consensus on the exact limits for cut-off between normal and subclinically hypothyroid individuals is not currently possible. The main reasons for this are differences for measurement of serum thyroid-stimulating hormone (TSH), that reference populations are very different and that a person’s intra-individual variability is much narrower than any population-based interval.<sup>(3)</sup>

Cardiovascular disease (CVD) is a major cause of morbidity and mortality. Newer and improved preventive strategies may reduce the disease burden. According to “the Global Burden of Disease Study, 2015” “ischemic heart disease has topped the list of causes of years of life lost for more than a decade” and Lozano et al in 2012,

highlighted “the shift in the global burden of disease from communicable to chronic disease”.<sup>(4)</sup>

Earlier in 1979, Sawin et al observed that “Subclinical hypothyroidism has been found to be associated with type 1 diabetes mellitus and possibly with autoimmune diseases”.<sup>(5)</sup> Moreover, according to Klein et al (1991)<sup>(6)</sup> “two percent of pregnant women also have subclinical hypothyroidism”. In two population-based studies conducted by Spencer et al in 1990 and Al Eidan et al in 2018, reported “the prevalence of subclinical hypothyroidism ranged between 7.5–8.5% in women and 4.4% in men”.<sup>(7,8)</sup>

SCH is positively correlated with increased prevalence of some CVD risk factors. However, it is imperative to investigate the available data on the incidence of CVD morbidity and mortality in SCH subjects. There are several conventional markers which were used as CVD markers, but emerging risk markers like Pregnancy-associated plasma protein A (PAPP-A) and NT-proBNP may provide specific value when compared to traditional markers.

Oxidative stress is known to be associated with both hyperthyroidism and hypothyroidism. In most cases increased reactive oxygen species (ROS) is associated with hyperthyroidism and

lower production of anti-oxidants in hypothyroidism. According to Villanueva et al (2013), —the physiological function of thyroid hormones can be divided into two broad categories- (a) role in growth and development and (b) role in metabolism.<sup>(9)</sup> This regulation of metabolism by thyroid hormones corresponds to oxidative stress and ROS production. Under such circumstances, ROS production increases within cells.

Individuals with subclinical hypothyroidism (SCH) have high risk of organ damage and cardiovascular morbidity and mortality. Risk factors may alter the DNA repair mechanism which predisposes to DNA damage among SCH subjects and it may lead to cardiovascular diseases. The relationship between subclinical hypothyroidism (SCH) and cardiovascular disease has been one of the most popular topics in recent times. The pathophysiological relation between SCH and CVD is still unclear. The clinical importance of subclinical hypothyroidism in cardiovascular disease and mortality remains controversial, with most studies providing conflicting results. Systematic approaches on demographic, anthropometric, biochemical and genetic studies are necessary to find out the link between SCH and CVD. If there is any correlation observed between SCH and CVD, then all the subjects are screened for CVD using advanced biomarkers which will help in the early prediction of CVD.

### Material and Methods

A case-control study layout was adopted for the present study to identify the various biochemical and molecular biomarkers for the early detection of Cardiovascular Disease among subjects with Subclinical Hypothyroidism. All test and control subjects provided their information on a well-defined proforma regarding their demographic records, family history and disease-related records. The clinically diagnosed individuals with subclinical hypothyroidism subjects were selected from the OPD and IPD.

### Sample

In order to identify the risk factors for cardiovascular disease in subclinical hypothyroidism subjects, a case-control study was implemented. The case/study group included 150 clinically diagnosed individuals with subclinical hypothyroidism and 100 age and sex matched healthy subjects were selected as the control group.

### Inclusion Criteria

- The criteria for SCH: normal T3 (3.5-7.8 pmol/L), T4 (10-25 pmol/L) and elevated TSH ( $\geq 5$   $\mu$ IU/ml).
- Criteria for inclusion were being 18 to 55 years.

### Exclusion Criteria

- Age  $>55$  years, Hyper and Hypothyroidism, Ovarian dysfunctions, Renal dysfunctions, Cardiac dysfunctions, Acute and Chronic illness, Previous use of any thyroid agents and those undergone lipid lowering therapy in last 6 months.
- The same exclusion criteria are applied for the control subjects also.

### Blood Sample Collection

After 10 to 12 hours of fasting, eight mL (8 mL) of venous blood was collected and 2 mL of blood was transferred aseptically to a sodium heparinsed vacutainer for evaluating the DNA repair proficiency and to quantify the extent of somatic DNA damages. The rest of blood was transferred to a plain tube, serum separated after clot formation and investigations were done for biochemical and ELISA test.

### Methods:

- Fasting Blood Sugar (GOD-POD Method)<sup>(10)</sup>
- Serum Triglyceride (Enzymatic- GPO Method)<sup>(11)</sup>
- Serum Total Cholesterol (CHOD-PAP Method)<sup>(12)</sup>
- Serum HDL Cholesterol<sup>(13)</sup>

- Serum LDL Cholesterol <sup>(14)</sup>
- High sensitive C Reactive Proteins (hsCRP) <sup>(15)</sup>
- Thyroid stimulating hormone (TSH) <sup>(16)</sup>
- Urea <sup>(17)</sup>
- Enzymatic Creatinine Assay <sup>(18)</sup>
- Malondialdehyde(Thiobarbituric acid Assay) <sup>(19)</sup>
- Uric acid (Uricase Method) <sup>(20)</sup>
- PAPP-A and NT-proBNP (ELISA) <sup>(21,22)</sup>

### Statistical Analysis:

Statistical evaluation was carried out using SPSS (Version 14.0) Data obtained from the study groups were compared by the parametric student's t test; correlation analysis between variables were made by Pearson test. All the results were expressed as means with their

standard deviation. Statistical analysis was also performed by using standard deviation and ANOVA.

### Result:

In the present study, 150 clinically proven subjects with subclinical hypothyroidism were selected as the test subjects. These test subjects were referred from Datta Meghe Medical college and Shalinitai Hospital and research center Nagpur. Detailed demographic, physiological and lifestyle characteristics were recorded using proforma. Eight-two age and sex matched healthy subjects were selected as controls. All subjects were taken within the age group of 18 to 55 years.

**Table 1: Comparison of Biochemical Parameters Among Study and Control Subjects.**

Biochemical parameters		Study Subjects			Control Subjects		
		MDA U/L	PAPP-A mIU/L	NT-proBNP pg/ml	MDA U/L	PAPP-A mIU/L	NT-proBNP pg/ml
FBS mg/dL	≤110	4.10	43.74	135.10	4.46	47.87	138.55
	>110						
Triglyceride mg/dL	≤150	3.98	39.64	130.41	4.44	48.88	140.33
	>150						
Total Cholesterol mg/dL	≤200	4.00	40.26	130.91	4.68	48.75	139.84
	>200						
HDL mg/dL	≤40	4.35	45.51	137.47	4.13	42.28	135.33
	>40						
LDL mg/dL	≤100	4.14	41.22	134.26	4.32	46.25	138.29
	>100						
hsCRP mg/L	≤2	2.98	43.39	131.04	3.61	46.82	143.44
	>2						
TSHμIU/ml	≤10	3.49	43.66	135.11	3.69	49.58	142.16
	>10						
Urea mg/dL	≤20	3.82	42.52	133.28	4.93	46.78	139.34
	>20						
Creatinine mg/dL	≤1.2	3.55	42.22	133.73	3.95	45.77	137.64
	>1.2						
Uric Acid mg/dL	≤6.2	4.23	43.21	132.69	4.58	44.88	133.72
	>6.2						

It was observed that, study subjects with increased FBS level showed higher MDA

concentration. An increased level of PAPP-A level was observed among study subjects with

high concentration of fasting blood sugar level. Regarding to the NT-proBNP concentration, FBS level was compared among study subjects and it was observed that, study subjects have high level of FBS showed an increased level of NT-proBNP. It was observed that, subjects with higher cholesterol level showed an increased concentration of PAPP-A. An increased level of NT-proBNP was reported among study subjects with total cholesterol  $>200$  mg/dL and subjects with total cholesterol  $\leq 200$  mg/dL. Study subjects with lower HDL-C level showed higher concentration of MDA (3.32 mg/dL) than subjects with increased HDL-C level. It is evident that, subjects with lower HDL-C concentration showed an increased concentration of PAPP-A. A higher MDA concentration was observed among study subjects with increased LDL-C level.

Study subjects with LDL-C level  $>100$  mg/dL showed an increased level of PAPP-A when compared to subjects with HDL-C level  $\leq 100$  mg/dL. It was observed that, subjects with LDL-C level  $>100$  mg/dL showed an increased NT-proBNP and subjects with LDL-C level  $\leq 100$  mg/dL showed a lower level of NT-proBNP. It was observed that, subjects with higher triglyceride showed an increased MDA concentration. An increased level of PAPP-A was observed among study subjects with triglyceride level  $>150$  mg/dL. It was observed that, subjects with increased TSH concentration showed an increased PAPP-A level. According to NT pro-BNP concentration, TSH value was

compared among study and control subjects. Subjects with TSH  $>10$   $\mu$ IU/ml showed an increased NT pro-BNP level than the subjects with TSH level  $\leq 10$   $\mu$ IU/ml. It was observed subjects with increased level of urea showed a high level of MDA concentration. Study subjects with increased urea level observed an increased PAPP-A level in study subjects.

According to creatinine level, MDA concentration was compared among the study subjects and it was observed that subjects increased creatinine level showed a higher MDA concentration. It was observed subjects with increased creatinine concentration reported high level of NT-proBNP

An increased MDA concentration was observed among study subjects with increased hsCRP level. Study subjects with increased level of hsCRP ( $>2$  mg/L) showed higher PAPP-A level, when compared to subjects. An increased PAPP-A level was observed among study subjects with higher level of uric acid. Parameters like, NT-proBNP, uric acid, MDA, TC, LDL, TG, TSH, Urea, creatinine, showed a strong positive correlation with PAPP-A level. HDL-C showed a negative correlation with PAPP-A concentration.

## Discussion

The present study was performed using 150 subclinical hypothyroidism subjects and 100 healthy control subjects in order to evaluate the various biochemical changes associated with subclinical hypothyroidism and its risk for cardiovascular disease.

**Cooper in 2001**<sup>(23)</sup> reported that, "Subclinical hypothyroidism (SCH) is a clinical condition in which the concentration of serum TSH is above the upper limit of normal, despite of normal levels of serum free thyroxine". Later **Cooper et al 2012**<sup>(24)</sup> emphasized that, "subclinical hypothyroidism (SCH), is usually associated with isolated elevation of thyroid stimulating hormone (TSH) levels with a normal free thyroxine (fT4) level and often leads to early or mild thyroid failure". Most recently, a study done by **Xu et al (2012)**<sup>(25)</sup> reported that, "the relationship between TSH levels and lipid status after adjustments for the thyroid hormones and/or other potentially confounding factors in patients with CHD". **Klein et al (2007)**<sup>(26)</sup> reported that, "an increased risk of CHD events associated with higher TSH levels might be related to the known effects of

thyroid hormone on the heart and metabolism; consistent with the concept that subclinical hypothyroidism is a milder form of overt hypothyroidism”.

**Gullberg et al (2002)**<sup>(27)</sup> observed that, “an increased CRP levels are not associated with SCH”. **Toruner et al (2008)**<sup>(28)</sup> reported that, “serum CRP levels were not higher, or lower, in the SCH group compared with controls. There are conflicting reports about the association of SCH and CRP”. **Toruner et al in 2008**<sup>(28)</sup> showed that, “all lipid parameters, except HDL-C, were higher in patients with SCH. The association between SCH and dyslipidemia is unclear in the literature”. **Badyal et al (2019)**<sup>(29)</sup> also reported that, “the CVD incidence was increased with increasing the concentration of NT-proBNP. Moreover, **Badyal et al (2019)**<sup>(29)</sup> observed a positive correlation between the severity of CVD and the concentration of NT-proBNP.

**Cosin-Sales et al 2005**<sup>(30)</sup> reported that, “pregnancy-associated plasma protein-A (PAPP-A) appears to offer an interesting profile for predicting the risk of cardiovascular profile. Moreover, an increased plasma PAPP-A levels correlate with the presence of vulnerable coronary artery stenosis and the extent of angiographic CAD”. **Santi et al in 2012**<sup>(31)</sup> reported “a positive correlation between total cholesterol and LDL with TSH, as well as thyroid hormones (T3 and FT4) showing correlation with triglyceride levels”.

**Haribabu et al (2013)**<sup>(32)</sup> demonstrated that, “unlike lipid peroxidation, studies reporting protein oxidation in SCH is currently limited. Recently, reported that patients with SCH have increased MDA and protein carbonyls”. In the present study a statistically increased MDA concentration was observed among subjects with SCH than the control subjects

**Danesh et al (2006)**<sup>(33)</sup> reported that, “moderate elevations of CRP (below those of most routine CRP assays) correlate with future cardiovascular

events validate the use of this test to assess cardiovascular risk”. Kushner and **Sahay in 2002**<sup>(34)</sup> recommended that, “hsCRP is an effective screening test for cardiovascular risk”. **Jin et al 2013**<sup>(35)</sup> reported that, “elevated serum uric acid contributes to CVD development, even below the clinical threshold for hyperuricemia by increasing oxidative stress, promoting endothelial dysfunction and enhancing inflammation”. **Badyal et al (2019)**<sup>(29)</sup> reported that, “a significant relation between the levels of urea and creatinine and hypothyroidism. There is a positive relation between TSH levels and overt hypothyroidism. It was further seen that primary hypothyroidism is associated with a reversible elevation of serum creatinine in adults as well as children.

### Conclusion:

Subclinical hypothyroidism is one of the most prevalent autoimmune diseases provoked in genetically susceptible individuals by several triggers, including female sex, advanced age, obesity, abnormal lipid profile and environmental factors. Despite the fact, an earlier assessment of emerging risk markers such as NT-proBNP and PAPP-A is necessary for the earlier prevention of CVD events. The subclinical thyroid dysfunction is common and highlights the usefulness of screening to allow early detection and therefore, prevent associated adverse health outcomes. Thus it can be concluded that certain dietary modification, changes in life style characters and proper medication can reduce the risk of CVD.

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