



Correlation of Osteopontin, Oxidative Stress and Total Antioxidant Capacity in Hypothyroidism Subjects

**Sumesh Prasad Sah ^{a≡}, Jyoti Batra ^{aω*#}, Aarushi Batra ^b, Manisha Arora ^{cω†},
Sonu Sah ^{d‡} and Sudeep Kumar ^{eω‡}**

^a Department of Biochemistry, Santosh Deemed to be University, Ghaziabad, India.

^b LLRM Medical College & SVBP Hospital, Meerut, India.

^c Department of Metabolism and Nutrition, Medical University of Americas, Nevis, Saint Kitts and Nevis.

^d Muzaffarnagar Nursing Institute, Muzaffarnagar, India.

^e Department of Biochemistry, Muzaffarnagar Medical College, Muzaffarnagar, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To find out the correlation between Osteopontin (OPN), oxidative stress and total antioxidant capacity (TAC) in female hypothyroidism subjects.

Study Design: Case Control study

Place and Duration of Study: The present case control study was conducted with the collaboration of the Department of Biochemistry, Santosh Medical college, Ghaziabad and Muzaffarnagar Medical College, Muzaffarnagar from September 2018 to September 2020.

[≡] Ph.D Scholar;

^ω Dr.;

[#] Dean Research & Professor;

[†] Professor;

[‡] Nursing Tutor;

[‡] Associate Professor;

Methodology: The study includes 120 female hypothyroidism subjects and 120 age and sex matched normal healthy individuals as controls. Serum OPN was estimated by commercially available ELISA kit, MDA by method described by Satoh et al. method, TAC by FRAP method and Ceruloplasmin was estimated on Access 2 Beckman Coulter clinical chemistry analyser.

Results: The anthropometric parameters, Body mass index (BMI), waist circumference (WC), hip circumference (HC) and waist hip ratio (WHR) were increased significantly in all the female hypothyroidism subjects as compared to controls. Study showed increased levels of TSH and MDA and decreased levels of serum Osteopontin, T3, T4, TAC and Ceruloplasmin (Cp) in hypothyroid subjects as compared to controls. A Significant positive correlation was found between OPN vs T3, OPN vs T4, OPN vs TAC and OPN vs Cp whereas significant negative correlation was found between OPN vs TSH, and OPN vs MDA.

Conclusion: In the present study, we found decreased levels of Osteopontin in hypothyroidism subject. Pearson's correlation analysis predicts, OPN negatively correlated with MDA and positively correlated with total antioxidant capacity and ceruloplasmin. Hence, Osteopontin, malondialdehyde, total antioxidant capacity and ceruloplasmin should all be considered while assessing the hypothyroidism.

Keywords: Osteopontin; hypothyroidism; ceruloplasmin; oxidative stress; total antioxidant capacity.

1. INTRODUCTION

Hypothyroidism is a clinical syndrome resulting from thyroid hormone deficiency, which causes a general decrease in all metabolic activities [1]. Hypothyroidism is characterised by high levels of thyroid stimulating hormone (TSH) and low levels of thyroid hormones, triiodothyronine (T3) and thyroxine (T4) [2]. The prevalence of hypothyroidism ranges from between 0.2% and 5.3% in Europe and 0.3% and 3.7% in the USA [3,4]. The incidence of hypothyroidism according to Indian population vary in male and female, in male the prevalence is about 6.2% and in female 11.4%. [5]. The overall prevalence of hypothyroidism in western UP is about 8.4% [6].

Osteopontin is a secreted glycoprotein and is high in aspartic acids and it contains highly phosphorylated serine and threonine [7]. OPN plays an important role in different natural physiological processes, including bone remodelling, immune modulation, inflammation and vascularization and pathological conditions like chronic inflammations, including crohn's disease, obesity, severe disease, other autoimmune diseases, many forms of cancer, cardiac fibrosis and atherosclerosis [8]. There exists a direct relationship between OPN and thyroid hormones. A positive correlation was observed between OPN and hypothyroidism and a negative correlation existed in hyperthyroidism.

Increased levels of circulating OPN and/or increased OPN expression by tumour cells are associated with a poor prognosis in various forms of cancer [9]. OPN is expressed in both

benign and malignant thyroid cancers, with increased expression associated with the progression of the cancer [10]. OPN is expressed at higher levels in papillary thyroid carcinoma, and its levels were found to be substantially linked to tumour growth and vascular invasion. In addition to being a prospective target for papillary thyroid cancer therapeutic methods, OPN can be employed as a biomarker in hypothyroidism [11].

Oxidative stress is defined as disequilibrium between the production of oxidants and antioxidants, leading to accumulation of reactive oxygen species in various cells and tissues and violation of redox signalling pathways and molecular damage [12]. Malondialdehyde (MDA), an end product of arachidonic acid and large PUFAs degradation, is a lipid per-oxidation marker used to measure oxidative stress [13]. Ceruloplasmin (Cp) is considered a defensive antioxidant because of its ability to respond and scavenge free radicals [14]. Oxidative stress or increased susceptibility to oxidative damage may be indicative of low antioxidant ability.

The present study was designed to find out the correlation between Osteopontin, oxidative stress and total antioxidant capacity in female subjects with hypothyroidism.

2. MATERIALS AND METHODS

2.1 Study Design

The present case-control study was conducted in the Department of Biochemistry, Santosh Medical college, Ghaziabad with the

collaboration of Department of Biochemistry, Muzaffarnagar Medical College, Muzaffarnagar from September 2018 to September 2020. Total 240 female subjects were included in the present study. Out of which 120 were female hypothyroidism subjects (Cases) and 120 were female normal healthy subjects (Control) of same age group (30-60 Years).

The hypothyroidism participants were selected from the Department of Medicine those who were already diagnosed by physician on the basis of detailed history and thyroid.

2.2 Inclusion and Exclusion Criteria

All the female hypothyroidism subjects with age between 30-60 years, and patients willing to give informed consent were included in the present study. Subjects with type 2 diabetes, asthma, COPD, cancer, sexually transmitted disease, cardiac disease, renal disease, hepatic disease, gout and arthritis, pregnancy, and subjects taking any thyroid medication, as well as those who refused to give consent were excluded from the study.

2.3 Anthropometric Measurements

Both weight and height were measured in light clothes and without footwear, using the standard apparatus. Waist circumference (WC) was measured using an anthropometric tape at a level on the skin midway between the mean point of iliac peak and the inferior border of the last rib at the level of the umbilicus while in a standing position at the end of gentle expiration. Hip circumference (HC) was measured over the widest part of the gluteal region at the level of pubic tubercle in standing position. Waist-to-hip ratio (WHR) was calculated by dividing the waist circumference (cm) by hip circumference (cm).

Body mass index (BMI) of the participants was calculated as body weight (kg) divided by the square of height (m²) [BMI=Weight (Kg)/[Height (m)]²].

2.4 Biochemical Parameters

Under strict aseptic conditions, 5 mL of venous blood was collected from each participant and the levels of osteopontin, MDA, total antioxidant capacity, and ceruloplasmin were determined. After that, the blood samples were centrifuged for 10 minutes at 3000 rpm. The sample was aliquoted and stored at -20°C until analysis, as per standard protocol. Satoh et al. described a method for estimating malondialdehyde (MDA) in

serum samples using Thiobarbituric acid reacting substance (TBARS) [15]. The ferric reducing antioxidant power (FRAP) method was used to calculate total antioxidant capacity using tripyridyl triazine (TPTZ) [16]. Ceruloplasmin was estimated by Immuno-turbidimetric method by using commercially available kit from Beckman, USA (Catalog Number, ODR3023) on Access 2 Immunoassay clinical chemistry analyser [17]. Serum osteopontin was estimated by Sandwich-ELISA method by using commercially available kit form Elabscience, USA (Catalog Number, E-EL-H1347).

2.5 Statistical Analysis

Descriptive statistics were reported as mean with their standard SD for continuous variables, frequencies (percentage) for categorical variables. Tests of normality namely the Kolmogorov-Smirnov Test was used. Student's t-test was used to compare the results of two groups for all parameters. To assess the possible relationship between studied parameters, the Pearson's correlation analysis was done. A *p* value of less than 0.05 was considered to be statistically significant. Data were statistically evaluated with IBM SPSS Statistics for Mac, Version 25.0., IBM Corp., Chicago, IL.

3. RESULTS

Table 1 represents some of general characteristics of the female hypothyroidism subject. There was not significant difference between age in hypothyroidism subjects as well as control subjects. (40.64 ± 4.84 vs 40.96 ± 4.15). The mean levels of BMI, WC, HC, and WHR were significantly increased in hypothyroidism subjects as compared to control subjects.

Table 2 represents the biochemical parameters, oxidative stress marker and total antioxidant capacity of the hypothyroidism subjects and control subjects. When compared to controls, hypothyroidism patients had significantly higher levels of TSH and MDA and significantly lower levels of T3, T4, TAC, ceruloplasmin, and OPN.

Table 3 shows the correlation between Osteopontin with other biochemical parameters, oxidative stress markers and total antioxidant capacity in hypothyroidism subjects. OPN was positively and significantly correlated with T3, T4, TAC, and Cp and negatively & significantly correlated with TSH, MDA.

Table 1. Some general characteristics of the studied subjects

Variable	Hypothyroidism Subjects	Control Subjects	95% CI	T	p value
BMI (Kg/m²)	32.19 ± 7.16	22.61 ± 4.15	[8.08,11.06]	12.673	< 0.001 S
WC (cm)	106.32±8.26	84.06 ± 9.76	[19.96,24.55]	19.077	< 0.001 S
HC (cm)	115.44 ± 8.03	99.35 ± 8.47	[13.99,18.19]	15.112	< 0.001 S
WHR	0.92 ± 0.09	0.85 ± 0.12	[0.04,0.100]	5.139	< 0.001 S

BMI: Body mass index; WC: Waist circumference; HC: Hip circumference; WHR: Waist to Hip ratio; p value < 0.05 considered as statistically significant. NS Stands for statistically not significant and S stands for statistically Significant.

Table 2. Biochemical parameters, oxidative stress markers and total antioxidant capacity in control and hypothyroidism subjects

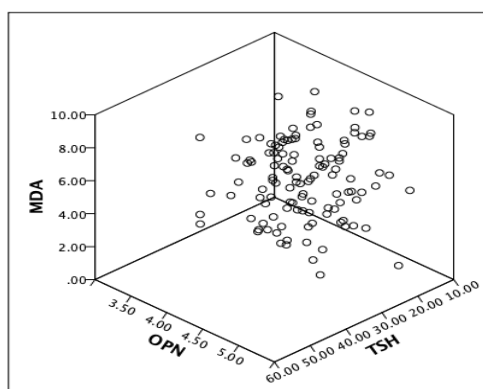
Variables	Hypothyroidism Subjects	Control Group	95% CI	t	p value
TSH (μIU/ml)	30.75± 8.09	2.05± 0.80	[27.24,30.16]	38.66	< 0.001
T3 (ng/ml)	1.35± 0.27	1.44± 0.30	[-0.17,-0.29]	-2.78	< 0.001
T4 (μg/dl)	4.57± 1.78	8.80± 2.18	[-4.74,-3.72]	-16.44	< 0.001
Malondialdehyde (μm)	6.05± 2.05	4.09 ± 1.92	[1.45,2.47]	7.62	< 0.001
Total antioxidant capacity (mmol/L)	1.03± 0.26	1.70 ± 0.35	[-0.75,-0.59]	-16.97	< 0.001
Ceruloplasmin (mg/dl)	18.83± 5.82	27.39± 7.59	[-10.28,-6.84]	-9.81	< 0.001
Osteopontin(ng/ml)	4.45± 0.39	7.22 ± 1.53	[-3.06,-2.49]	-19.26	< 0.001

TSH: Thyroid stimulating hormone; T3: triiodothyronine; T4: thyroxine; MDA: Malondialdehyde; TAC: Total antioxidant capacity; CP: Ceruloplasmin; OPN: Osteopontin; p- value < 0.05 considered as statistically significant.

Table 3. Correlation of osteopontin with other biochemical parameters, oxidative stress markers and total antioxidant capacity in hypothyroidism subjects

Parameters	Osteopontin	
	R	p-value
TSH	-0.466	< 0.001
T3	0.383	< 0.001
T4	0.463	< 0.001
MDA	-0.359	< 0.001
TAC	0.435	< 0.001
CP	0.493	< 0.001

TSH: Thyroid stimulating hormone; T3: triiodothyronine; T4: thyroxine; MDA: Malondialdehyde; TAC: Total antioxidant capacity; CP: Ceruloplasmin; OPN: Osteopontin; p- value < 0.05 considered as statistically significant

**Fig. 1. Bubble plot correlation of OPN with TSH and MDA (n=120)**

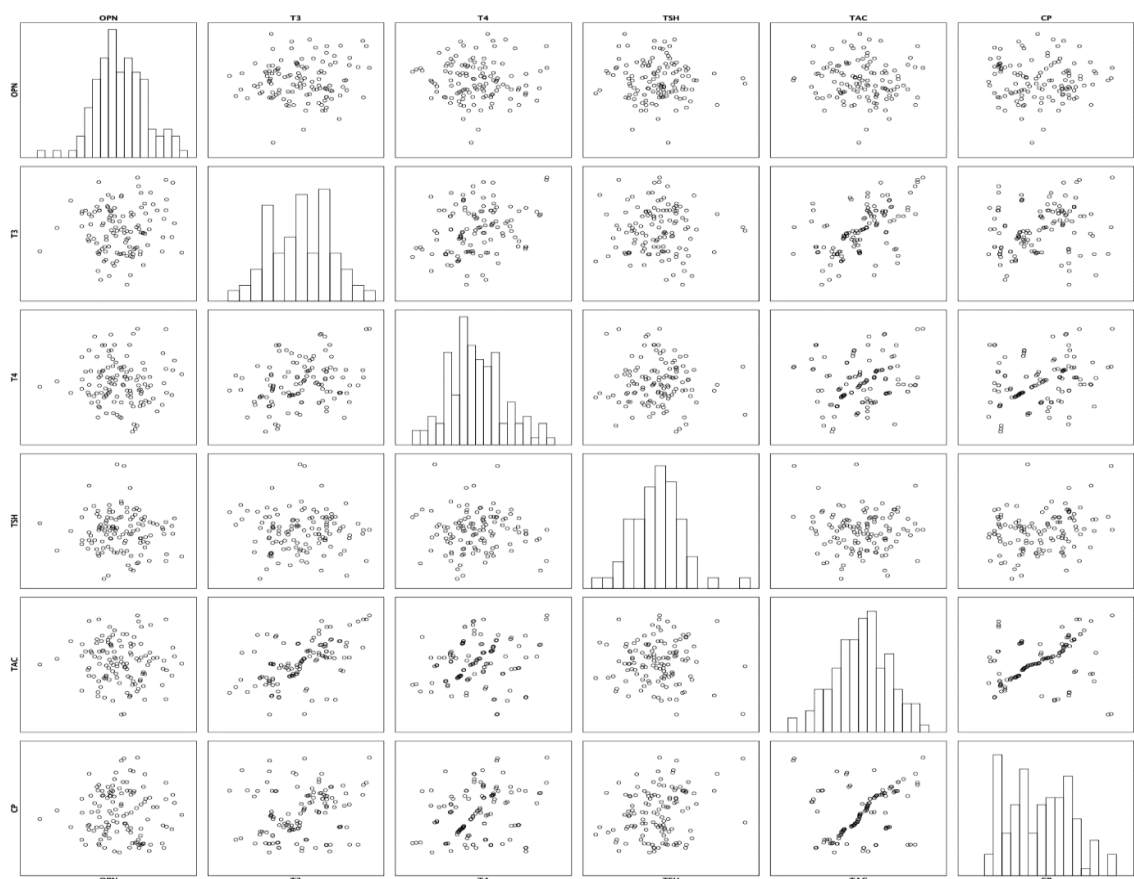


Fig. 2. Matrix plot correlation of OPN with T3, T4, TAC, and Cp (n=120)

4. DISCUSSION

Thyroid disorders are the most common endocrine diseases in the world. In India, among all endocrine disorders, thyroid disorders are more prevalent and hypothyroidism is more prevalent than other thyroid disorders. In this case-control study, we have evaluated the correlation between osteopontin, oxidative stress markers and total antioxidant capacity in hypothyroidism subjects and found significant differences between in the levels of osteopontin, oxidative stress markers and total antioxidant capacity in hypothyroidism subjects as compared to control. In addition, we found strong correlation between osteopontin, oxidative stress markers and total antioxidant capacity in hypothyroidism subjects.

In the present study, the anthropometric parameters namely BMI, WC, HC and WHR were significantly increased in hypothyroidism subjects as compared to controls which in concordance with Savita et al in-hypothyroidism individuals [18]. It may be attributed to weight

gain due to the accumulation of glycosaminoglycans in the skin and muscle in intracellular spaces and the hypo-catabolic condition of hypothyroidism. Hypothyroidism subjects have higher rate of obesity which may increase to increased risk of CVD.

In our study, we found the increased level of TSH and decreased levels of T3 and T4 in hypothyroidism as compared to normal healthy individual which are in accordance with the many researchers [19,20]. The increase in TSH and decrease in T3 & T4 may be due to the insufficient thyroid hormone production by the body sometimes associated to deficiency of iodine in body.

The imbalance between the production of free radicals derived from oxygen and their elimination by antioxidants is oxidative stress. Malondialdehyde (MDA) is the most widely used marker for examining the involvement of oxidative stress in biological systems [21]. While comparing with other studies, in our study we found a significant increase in the level of MDA in

hypothyroidism subjects [22,23]. Hypothyroidism may be associated with increased oxidative stress and lipid peroxidation, and supposed that this might be lead to the development and progression of atherosclerosis.

In our present study, serum ceruloplasmin level was significantly lower in hypothyroid subjects as compared to controls. The results of our study are inconsistent with study done by Bhattacharya et al. [14]. In our present study, the serum TAC level was significantly lower in hypothyroid patients as compared to control subjects. Our results consistent with previous studies [24,25]. The reduction of TAC in hypothyroid patients reflects increase oxidative stress in hypothyroidism. The increase of free radicals is due to a decrease of Ceruloplasmin antioxidants. Erdamar et al. discussed the correlation between hypothyroidism by reducing the function of components of the antioxidant system which indicate the impact of thyroid hormones on oxidative stress and antioxidant systems is powerful [26].

In our present study, we studied osteopontin in patients of age group 30-60 years with hypothyroidism subjects and we found lower levels of osteopontin in hypothyroidism subjects as compared to controls subjects. The decrease in OPN may be due to the various cell processes going in the thyroid gland under the influence of osteopontin. Our results are in accordance with study done by Liou YM et al [27]. Reza et al studied on thyroid disorder patients and observed a significant difference in serum osteopontin levels between hypothyroid and hyperthyroid patients and this may due to the fact that OPN promotes pathogenesis of autoimmune diseases by inducing immune cell activation and migration and inflammatory cytokine production [28]. Osteopontin is a plasma glycoprotein and it may be downregulated in hypothyroidism and it may be due to increased oxidative stress and reduction of antioxidant defenses reflect increased free radical production in electron transport chain in mitochondrial inner membrane. Ambrosi et al studied in patients with Grave's disease in Chinese population and observed increased serum OPN level which coincided with an increase in OPN receptor coexpression and enhancement in proinflammatory cytokine and chemokine production [29].

When we correlate the biochemical parameters in hypothyroidism subjects, we found a positive correlation of OPN with T3 & T4 and it was

statistically significant whereas OPN significantly and negatively correlated with TSH. Reza et al. found that OPN was upregulated in most of the patients of hyperthyroidism and downregulated in hypothyroid patients. While OPN was positively correlated with fT3 and fT4 and it was negatively correlated with TSH [28]. Alwakeel et al studied on hypothyroid and hyperthyroid subjects both and obtained the same results. [30]. Furthermore, TAC & CP were positively correlated with OPN and negatively correlated with MDA. Our results are in accordance with study done by Xu et al [31].

5. CONCLUSION

The findings of the current study suggested that oxidative stress and total antioxidant capacity is correlated with hypothyroidism in which osteopontin is negatively correlated with Malondialdehyde and positively correlated with total antioxidant capacity and ceruloplasmin. Therefore, Osteopontin, malondialdehyde, total antioxidant capacity and ceruloplasmin should all be considered while assessing the hypothyroidism.

6. LIMITATION OF THE STUDY

In this investigation, the patient population was small and selected all the female patient for the present study. In order to determine the role of Osteopontin, malondialdehyde, total antioxidant capacity, and ceruloplasmin in hypothyroidism, Authors proposed conducting more prospective population-based studies in this manner.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL AND CONSENT

The study was reviewed and approved by Ethics Committee of Santosh Medical College, Ghaziabad [F.No SU/2018/528(33), dated 25.05.2018] and Muzaffarnagar Medical College

[MMC/IEC/2019/225 dated 27.03.2019]. Verbal and written consent was taken from all the participants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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