CORRELATION OF OSTEOPONTIN, OXIDATIVE STRESS AND TOTAL ANTIOXIDANT CAPACITY IN HYPOTHYROIDISM SUBJECTS

Abstract

Background and Objective: Hypothyroidism is a failure of the thyroid gland to produce sufficient thyroid hormone to meet the metabolic demands of the body. Osteopontin is a plasma glycoprotein and it may be downregulated and oxidative stress disturbed in hypothyroidism. The present study was conducted with the objective to find out the correlation of osteopontin (OPN), oxidative stress and total antioxidant capacity (TAC) in hypothyroidism subjects. **Methods:** The study includes 120 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 Beckman Coulter AU 480 clinical chemistry analyzer. **Results:** The anthropometric parameters, Body mass index (BMI), waist circumference (WC), hip circumference (HC) and waist hip ratio (WHR) were increased significantly in 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 were found between OPN vs T3, OPN vs T4, OPN vs TAC and OPN vs Cp whereas significant negative correlation were 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, OPN can be done in the hypothyroidism patients.

Keywords: Osteopontin, Hypothyroidism, Ceruloplasmin, Oxidative stress, Total antioxidant capacity

Introduction

Hypothyroidism is characterized as the failing to produce enough thyroid hormone delivering metabolic demands of the body either due to primary or secondary or tertiary thyroid disorder. [1] Osteopontin is a secreted glycoprotein and is high in aspartic acids and it contains highly phosphorylated serine and threonine. [2] 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. Hence, OPN was considered as the biomarker for thyroid disorders. [3] 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 signaling pathways and molecular damage. [4] Malondialdehyde (MDA), an end product of arachidonic acid and large PUFAs degradation, is a lipid per-oxidation marker used to measure oxidative stress. [5] Ceruloplasmin (Cp) is considered a defensive antioxidant because of its ability to respond and scavenge free radicals. [6] Oxidative stress or increased susceptibility to oxidative damage may be indicative of low antioxidant ability. Little information is available about antioxidant potential in hypothyroidism patients of different races. The study regarding the role of osteopontin and oxidative stress in hypothyroidism patients is very limited. Keeping this in mind, the present study has been planned to find out the correlation of osteopontin, oxidative stress and total antioxidant capacity in subjects with hypothyroidism subjects.

MATERIAL AND METHODS

Study Design:

A type of case control study was carried out with the collaboration of the Department of Biochemistry, Santosh Medical college, Ghaziabad and Muzaffarnagar Medical College, Muzaffarnagar from September 2017 to September 2019. The study was approved from Institutional ethical committee, Santosh Medical College & Hospital [F.No SU/2018/528(33)] and Muzaffarnagar Medical College [MMC/IEC/2019/225]. Verbal and written consent was taken from all the participants.

The study included 240 subjects of age group 30-70 years. Out of them, 120 were normal healthy individuals as controls and 120 of them were hypothyroid who had already been diagnosed due to thyroid status and were willing to give written informed consent were included. 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.

Anthropometric Measurements: The standard apparatus was used to measure and calculate both body mass index (BMI) and waist-to-hip ratio (WHR) (cm) were calculated.

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) [7]. The ferric reducing antioxidant power (FRAP) method was used to calculate total antioxidant capacity using tripyridyl triazine (TPTZ) [8]. Ceruloplasmin was measured in a serum sample using a commercially available kit on a Beckman Coulter AU 480 clinical chemistry analyzer. Serum osteopontin was measured by ELISA method.

Statistical Analysis: Descriptive statistics were reported as mean (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.

RESULTS

A total of 240 study participants were present in our study. Significant value of the Kolmogorov-Smirnov Test was greater than 0.05, hence our data is normally distributed. Table 1 represents the studied subjects' general characteristics. Because there was no difference in age or gender between hypothyroidism and control subjects, the subjects in both groups were age and gender matched. In hypothyroidism subjects, mean levels of BMI, WC, HC, and WHR were significantly higher than in control subjects. The biochemical parameters, oxidative stress markers, and total antioxidant capacity of the subjects studied were all depicted in Table 2. 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. In hypothyroidism patients, the researchers discovered a link between OPN and other biochemical parameters, oxidative stress markers, and total antioxidant capacity. OPN and T3, T4, TAC, and Cp (had a significant positive correlation, while OPN and TSH, MDA had a significant negative correlation (Figure 1,2; Table 3).

Table 1: General characteristics of the studied subjects (N=240)

Variable	Hypothyroidism Subjects (n=120)	Control Subjects (n=120)	95% CI	t	p value
Age (Years)	40.64 ± 4.84	40.96 ± 4.15	[-0.83,1.46]	0.549	0.58 **NS**
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 (N=240)

Variables	Hypothyroidism Subjects (n=120)	Control Group (n=120)	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 (n=120)

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

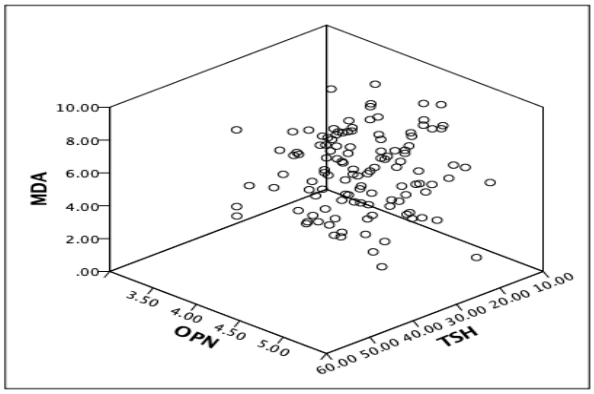

Figure 1: Bubble plot correlation of OPN with TSH and MDA (n=120)

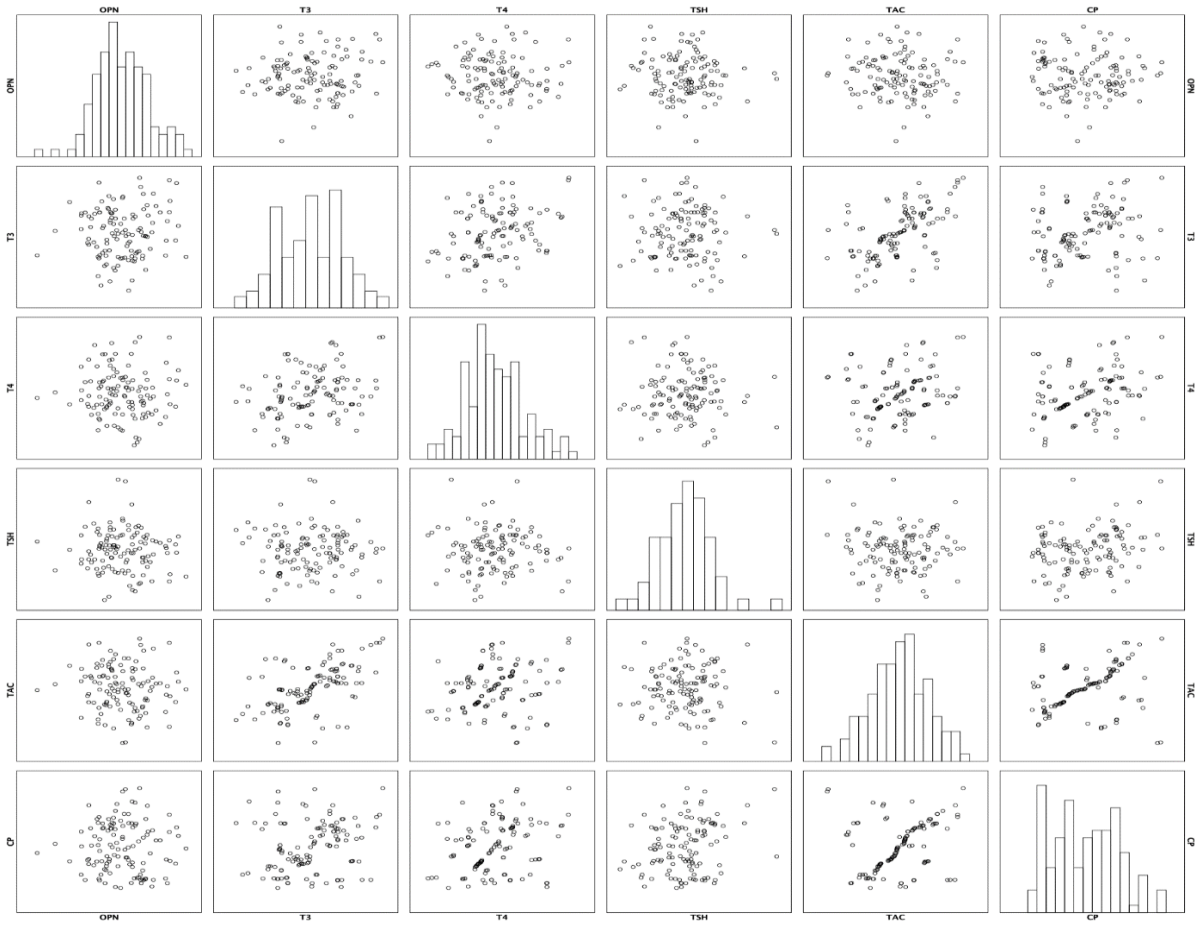

Figure 2: Matrix plot correlation of OPN with T3, T4, TAC, and Cp (n=120)

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 cross-sectional 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 [9]. 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 [10,11]. The increase in TSH and decrease in T3 & T4 may be due to the insufficient thyroid hormone production by the body and also due to the deficiency of iodine in our 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 [12]. While comparing with other studies, in our study we found a significant increase in the level of MDA in hypothyroidism subjects [13,14]. 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 Bhattarcharya et al [15]. In our present study, the serum TAC level was significantly lower in hypothyroid patients as compared to control subjects. Our results consistent with study done by K.N. et al, Suresh et.al [15,16]. 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 [17].

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, and Reza et al [18,19].

When we correlate the biochemical parameters in hypothyroidism subjects, we found a significant and positive correlation of OPN with T3, T4, TAC & CP whereas OPN significantly and negatively correlated with TSH and MDA. Our results are in accordance with study done by lingyan et al. [20], found osteopontin is positively correlated with T3 and T4 & negatively correlated with TSH.

Osteopontin is a plasma glycoprotein and it may be downregulated in hypothyroid patients 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 so it could be a new biochemical marker in the diagnosis of hypothyroidism subjects.

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, in hypothyroidism, these parameters should be done. However, it should be considered that there is little evidence is still required about the correlation between osteopontin and hypothyroidism.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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.

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