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Role of Osteopontin in Hypothyroid Anemic Woman and Their Association with Oxidative Stress

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Abstract

Background: Hypothyroidism is frequently associated with anemia and oxidative stress, necessitating exploration of biomarkers like Osteopontin to better understand disease mechanisms.

Objective: To explore the association between Osteopontin, oxidative stress, and antioxidant capacity in hypothyroid woman with or without anemia.

Methods: The study was conducted at the Santosh Medical College Department of Biochemistry in Ghaziabad, India, from September 2019 to October 2021. This study was cross-sectional and recruited 360 female subjects, divided into three groups: hypothyroidism with anemia, hypothyroidism without anemia, and normal healthy controls. Osteopontin, thyroid profile, malondialdehyde (MDA) and total antioxidant capacity were measured.

Results: Osteopontin, T3, T4, and total antioxidant capacity levels were significantly lower ($p<0.001$), and the TSH and MDA levels significantly increased ($p<0.001$) in female subjects experiencing hypothyroidism, with or without anemia, as compared to normal healthy female control groups. Osteopontin demonstrated significant negative relationship with TSH and MDA, while showing a significant positive relationship with T3, T4, and total antioxidant status in both studied groups.

Conclusion: Osteopontin and oxidative stress/antioxidant status significantly correlate in female subjects experiencing hypothyroidism, with or without anemia. In these patients, Osteopontin might be a useful biomarker for evaluating antioxidant levels and oxidative stress.

Keywords: Antioxidant, hypothyroidism, osteopontin, oxidative stress

Introduction

Hypothyroidism is a clinical condition marked by elevated levels of thyroid stimulating hormone (TSH) and lower levels of triiodothyronine (T3) and thyroxine (T4).[1] Hypothyroidism and subclinical hypothyroidism were more prevalent in women than in men, with prevalence's of 4.1% and 5.4% respectively. In India, 200 million individuals are susceptible to iodine deficiency disorders, while 42 million are affected by thyroid conditions.[2]

Osteopontin (OPN), a glycoprotein was first discovered in 1986 in osteoblasts and is made up of 300 amino acids. It is downregulated in people with hypothyroidism and is found on the long arm of chromosome 4 region 13 (4q13). Osteopontin overexpression in thyroid cancers links to its role in immune cell

movement and promoting malignant tumor development.[3] Osteopontin has significant role in both normal physiological & pathological processes and Numerous diseases including obesity, atherosclerosis, cardiac fibrosis, immune system disorders like Graves' disease, chronic inflammation, and several forms of cancer.[4]

Anemia is a clinical condition, occurs when the blood's hemoglobin or red blood cell count is below normal and has been prevalent in patients with hypothyroidism by 20 to 60%. Hypothyroidism is related to development and severity of anemia.[5] Results from previous studies indicated that iron deficiency anemia causes minor abnormalities without any significant changes in thyroid function. However, studies in animals have shown iron deficiency anemia to be associated with thyroid metabolism and Iron deficiency are more serious for women.[6]

Oxidative stress, defined as an imbalance between pro-oxidants and antioxidants, leads to molecular damage, including oxidative DNA damage and lipid peroxidation.[7] Free radical production decreases due to lowered thyroid hormone levels and metabolic suppression in hypothyroidism.[8] Malondialdehyde (MDA), a byproduct of lipid peroxidation, serves as a key biomarker of oxidative stress, particularly in the context of polyunsaturated fatty acid oxidation, such as linoleic and linolenic acids.[9] A reduced total antioxidant capacity may suggest the presence of oxidative stress or a heightened vulnerability to oxidative damage.[10]

This present study aimed to assess OPN, MDA, and total antioxidant capacity levels and to investigate the correlation between OPN, MDA, and total antioxidant capacity in subjects with hypothyroidism, with or without anemia subjects.

Methods

This case-control study was conducted from September 2019 to 2021 in the Department of Biochemistry at Santosh Medical College & Hospital, Ghaziabad, Uttar Pradesh, India. The study was approved by the Institutional Ethical Committee (F. No. SU/2018/528(33), dated May 25, 2018), and all patients provided informed permission prior to the study. A total of 360 subjects aged 30 to 60 years were enrolled in the study. The participants were divided into three groups: 120 hypothyroid subjects with anemia, 120 hypothyroid subjects without anemia, and 120 healthy controls within the same age range. The hypothyroid and anemic subjects were taken from the Department of Medicine and had previously been diagnosed by a physician based on a complete history, thyroid profile testing, and hemoglobin test.

The current study included all hypothyroid subject aged between 30–60 years who were willing to provide informed consent. The study excluded patients with type 2 diabetes mellitus, asthma, COPD, cancer, sexually transmitted disease, cardiac disease, renal disease, hepatic disease, gout and arthritis, pregnancy, on thyroid medication, and refuse to provide prior permission.

A 5 mL of venous blood sample were collected from their medial cubital vein into a plain vial. The serum was tested after 3 minutes of centrifugation at 1500 rpm. All parameters were determined using the enzymatic technique and an automated analyzer (Beckman Coulter-AU-480). Satoh *et al*. established a method for determining malondialdehyde (MDA) in serum samples that employs thiobarbituric acid reactive material. The ferric reducing antioxidant power (FRAP) method was used to calculate total antioxidant capacity using tripyridyl triazine (TPTZ). Serum Osteopontin was measured using the Sandwich-ELISA method with a commercially available kit from Elabscience, USA (Catalogue Number: E-EL-H1347).

Descriptive data were presented as the mean with its standard deviation. Normality test, specifically the Kolmogorov-Smirnov test, was used. The student's t-test was used to compare the outcomes of two groups across all parameters. Pearson's correlation analysis was used to determine any potential relationships between the analyzed parameters. A p-value of less than 0.05 was considered statistically significant. Statistical analysis was carried out using IBM SPSS Statistics for Mac, Version 25.0 (IBM Corp., Chicago, IL, USA).

Results

Table 1 presents the biochemical parameters of the studied subjects. Serum TSH and malondialdehyde (MDA) levels were significantly increased in hypothyroid subjects, both with and without anemia, compared to healthy controls. Similarly, Decreased T3, T4, Total antioxidant capacity and Osteopontin levels were observed in hypothyroid subjects with or without anemia as compared to controls.

Table 2 shows the correlation analysis of the

Table 1 Biochemical Parameters of the Studied Subjects (mean ± SD)

Parameters	Controls	Hypothyroidism with Anemia	Hypothyroidism without Anemia
TSH (µIU/mL)	4.91 ± 0.56	33.43 ± 4.61 *	25.08 ± 1.53 *
T3 (ng/mL)	1.45 ± 0.30	0.41 ± 0.08 *	0.63 ± 0.09 *
T4 (µg/dL)	9.16 ± 1.10	2.72 ± 0.51 *	3.39 ± 0.55 *
MDA (nmol/mL)	1.50 ± 0.41	4.42 ± 1.16 *	3.45 ± 0.75 *
TAC (mmol/L)	1.99 ± 0.45	1.11 ± 0.22 *	1.49 ± 0.30 *
OPN (ng/mL)	7.22 ± 1.53	4.02 ± 0.56 *	4.45 ± 0.39 *

Table 2 Correlation of Studied Parameters with Osteopontin (OPN) in Hypothyroid Subjects with Anemia

Variables	TSH	T3	T4	MDA	TAC
OPN	r = -0.625	r = 0.439	r = 0.491	r = -0.426	r = 0.415
	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001

studied parameters in hypothyroid subjects with anemia. Osteopontin was significantly & negatively correlated with TSH & MDA and positively & significantly correlated with T3, T4 and TAC in hypothyroidism with anemia subjects.

Table 3 presents the correlation of the studied parameters in hypothyroid subjects without anemia. OPN was negatively and significantly correlated with TSH & MDA and positively and significantly correlated with T3, T4 and total antioxidant capacity in hypothyroid subjects without anemia.

Discussion

Hypothyroidism is the most prevalent among endocrine disorders. Variation in the measures of thyroid hormone leads to hypothyroidism. Thyroid hormones regulate the ferritin expression and decreases the levels of irons which can affect the metabolism of thyroid hormone.

In this study, A significant variation was recorded in the thyroid profile (TSH, T3 & T4) showing increased levels of TSH and decreased levels of T3 and T4. A decreased levels of T3 and T4 may be due the reduced TPO activity and reduced thyroid hormone production. Thyroid hormones promote erythrocyte precursor proliferation as well as erythropoietin synthesis and is dependent on iron levels for TPO activity. iron deficiency anemia has a negative impact on thyroid hormone status.[11] This is consistent with study by Sahana KR *et al*., reported increased level of TSH and decreased level of T3 and T4 in hypothyroidism subjects as compared to controls subjects. This may be the result of the body not making enough thyroid hormone, which is sometimes associated with a lack of iodine.[12]

Malonaldehyde is an oxidative stress marker and TAC reflects antioxidant status in this study. This study revealed significant changes in oxidative stress and antioxidant

Table 3 Correlation of Studied Parameters with Osteopontin (OPN) in Hypothyroid Subjects without Anemia

Variables	TSH	T3	T4	MDA	TAC
OPN	-0.397	0.562	0.474	-0.501	0.659
	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001

levels in the body. A significant low level of total antioxidant capacity and significant increase level of MDA were observed in hypothyroid subjects with anemia as compared to hypothyroid subjects without anemia**.** This alteration may be explained on both of increased levels of reactive oxygen species (ROS) that is associated in a variety of physiological and pathological conditions.[13] The oxidation of lipids by an increased MDA levels in hypothyroid subjects that may be aided by the increase in lipid level and caused by free radicals generated during oxidative stress. To neutralize ROS and minimize oxidative stress, healthy cells utilize a wide range of antioxidant defense mechanisms. These mechanisms play an important role in scavenging and converting ROS into less reactive or non-reactive molecules.[14]

Hypothyroidism has been associated with an increased oxidative stress and lipid peroxidation, which was believed to contribute to the development and progression of atherosclerosis and cardiovascular diseases. Highly increased production of ROS and oxidative stress in hypothyroidism subjects is might be due to the increased lipid peroxidation and failure of the antioxidant defense mechanism.

In the present study, serum total antioxidant capacity was decreased significantly in hypothyroid patients compared to control. These findings are consistent with the study done by Kalaivanam KN *et al*.[15] Kochman J *et al*. discussed the correlation between hypothyroidism and a decrease in the activity of antioxidant system components, indicating that thyroid hormones have an impact on oxidative stress and antioxidant systems. Oxidative stress is caused by an increase in oxidant levels and/or a decrease in antioxidant enzyme activity in iron deficiency anaemia. [8]

Osteopontin (OPN) is a glycoprotein that has been linked to bone growth and calcification, as well as processes such as inflammation, adhesion of cells and migration, and apoptosis prevention, due to its expression in a variety of body tissues. In this study, the level of OPN was significantly decrease in hypothyroid with anemic subjects compared to hypothyroid without anemic subjects. This is consistent with studies done by EL-Zawaw HT *et al*.[16] Sawaki D *et al*. studied Grave's disease patients and discovered elevated serum OPN levels, which were associated with an increase in OPN receptor co-expression and proinflammatory cytokine and chemokine production.

Osteopontin may be downregulated in hypothyroidism because of oxidative stress and a reduction of antioxidant defenses in the mitochondrial inner membrane, resulting in increased free radical production.[17] Numerous studies have suggested that OPN could be a useful predictive biomarker in patients with impaired thyroid function.

Serum OPN level was found decreased in hypothyroidism with or without anemic patients due to iodine deficiency.[18] The reduction in OPN levels might be attributed to various cellular processes occurring in the thyroid gland influenced by Osteopontin but exact mechanism of Osteopontin in iron deficiency anemia subjects is not known. However, this may be related to regulation at the level of Osteopontin gene expression.

The present study found that TSH & MDA levels increased significantly while T3, T4, total antioxidant capacity and Osteopontin levels decreased significantly. Results of the present study are in accordance with many previous studies conducted separately in hypothyroid subjects.[19] There was a significant reduction in Osteopontin in the hypothyroidism with or without anemia. This was further confirmed by correlation studies between the different parameters in all the studied groups. Correlation studies have found that OPN was positively and significantly correlated with T_3, T_4, and total antioxidant capacity whereas significantly negatively correlated with TSH and Malondialdehyde in hypothyroid subjects with or without anemia. The present study suggests a positive association between hypothyroidism and Osteopontin. However, there is negative correlation between TSH and Osteopontin. In associated condition of iron deficiency anemia, oxidative stress is increased causing a further decrease in the levels of Osteopontin.

In the present study, it was observed that hypothyroidism is negatively associated with Osteopontin. However, there is negative correlation between TSH and Osteopontin. Osteopontin, reflected that it could be used as prognostic biomarkers for the diagnosis of hypothyroidism. Osteopontin was Significantly negatively correlated with Malondialdehyde levels as well as positively correlated with total antioxidant capacity in both hypothyroid with or without anemic female subjects.

In conclusion, OPN appears to play a key role in modulating oxidative stress and antioxidant defenses in hypothyroid patients, with the condition exacerbated by anemia. The results highlight the potential use of OPN as a

biomarker for assessing oxidative imbalance in female hypothyroid patients. However, the study's limitations include a restricted sample size and the exclusive inclusion of female subjects, necessitating further research to validate these findings across broader populations.

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