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Original Article

Biochemistry Section

Evaluation of Osteopontin and Malondialdehyde Level and its Correlation with Iron Status in Hypothyroidism Patients: A Case-control Study

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ABSTRACT

Introduction: Hypothyroidism is a clinical condition characterised by abnormally low thyroid hormone production. Hypothyroidism is associated with increased Malondialdehyde (MDA), Total Iron Binding Capacity (TIBC) and decreased Osteopontin (OPN), iron and ferritin level.

Aim: To evaluate OPN and MDA level and its correlation with iron status in hypothyroidism subjects also to compare the OPN, MDA, iron, ferritin and TIBC levels in cases with the controls.

Materials and Methods: This case-control study was done at Santosh Medical College, Ghaziabad, in collaboration with Muzaffarnagar Medical College, Muzaffarnagar, Uttar Pradesh, India from September 2018 to September 2020. The current study involved a total of 240 female participants. There were 120 female hypothyroidism (cases) and 120 female normal healthy (control) participants of the same age group in the current study (30-60 years). Blood samples were collected from all participants and analysed for OPN, MDA, iron, ferritin and TIBC. OPN was estimated using sandwich- Enzyme Linked Immunosorbent Assay (ELISA) method. MDA was estimated by Kei satoh method. Serum ferritin was estimated by Immuno-turbidimetric on Access 2 Immunoassay Beckman Coulter and serum iron and TIBC were estimated by Beckman Coulter AU480 Clinical Chemistry Analyser. Pearson's correlation was used to evaluate the association between these parameters, which were represented as mean with Standard Deviation (SD).

Results: Female hypothyroidism subjects had significantly (p-value <0.001 for all) increased body mass index, waist circumference, hip circumference, and waist hip ratio than controls. Study showed increased levels of MDA and TIBC and decreased levels of serum OPN, ferritin and iron in hypothyroid subjects as compared to controls. A significant positive correlation was found between OPN vs ferritin (r=0.465, p-value <0.001), OPN vs iron (r=0.521, p-value <0.001) , MDA vs TIBC (r-value=0.591, p-value <0.001), whereas significant negative correlation was found between OPN vs TIBC (r=-0.454, p-value <0.001), OPN vs MDA (r=-0.501, p-value <0.001), MDA vs ferritin (r=-0.543, p-value <0.001) and MDA vs iron (r=-0.573, p-value <0.001).

Conclusion: The decreased levels of OPN and increased level of MDA in hypothyroidism subject, which increases risk of iron deficiency anaemia in hypothyroidism patients. Furthermore, OPN and MDA were well correlated with ferritin, iron and TIBC in hypothyroidism subjects. As a result, these aspects should be considered while assessing risks of iron deficiency anaemia in hypothyroidism.

Keywords: Body mass index, Lipid peroxidation, Reactive oxygen species, Thyroid hormone, Total iron binding capacity

INTRODUCTION

Hypothyroidism is a clinical condition in which the thyroid gland produces insufficient thyroid hormone. Hypothyroidism continues to be a major health issue in both developing and developed countries [1]. In the developed world, hypothyroidism affects 4% to 5% of the population. In urban India, hypothyroidism affects 10.95% of the population [2]. Females and the elderly are more likely to develop hypothyroidism [3]. Hypothyroidism can be primary or secondary in nature. Primary hypothyroidism is caused by thyroid gland abnormalities, while secondary hypothyroidism is caused by hypothalamic or pituitary dysfunction. There is an increase in serum Thyroid Stimulating Hormone (TSH) combined with decreased levels of serum T4 and T3 is called as overt hypothyroidism whereas, there is an increase in serum TSH associated with a normal concentration of serum T4 and T3 is called as sub-clinical hypothyroidism [4].

Osteopontin (OPN) is a bone formation and calcification molecule that was initially discovered in osteoblasts in 1986 [5]. OPN is a negatively charged phosphoglycoprotein with 300 amino acids and a cell binding sequence of arginine, glycine, and aspartic acid. It can be located on the long arm of chromosome 4 region 13 (4q13) and is found in different forms throughout the body [6]. Proinflammatory cytokines promote the transcription and expression of the OPN gene. Osteopontin has an important role in both physiological and pathological circumstances such as glomerulonephritis, cancer, obesity, and atherosclerosis, where it regulates bone metabolism, tissue repair, and cell signalling, including proliferation and invasion. Osteopontin has a wide range of biological functions depending on its structural changes and the environment in which it is produced [7].

The imbalance between the generation of oxidants and the concentration of antioxidants is known as oxidative stress. It causes lipid peroxidation and oxidative Deoxyribonucleic Acid (DNA) damage [8]. Malondialdehyde (MDA), a natural product generated in all cells as an end product of lipid peroxidation, and has been considered as indicators of oxidative stress [9]. The increased generation of free radicals and the impaired capacity of the antioxidative defence result in hypothyroidism-related Reactive Oxygen Species (ROS). TSH overproduction may affect oxidative stress pathways [10]. Thyroid hormones play an important role in oxidative stress and oxygen utilisation. Overproduction of ROS causes thyroid hormones to use more oxygen, disrupting the pro-oxidant/antioxidant balance, resulting in oxidative stress and damage to cellular structures, lipids,

proteins, and DNA [11]. Measurement of serum ferritin, iron and total iron binding capacity which assess the percent saturation of transport from transferrin with iron, could be very crucial in hypothyroidism [12]. Hypothyroid patients have been found to have low ferritin levels [13]. Iron containing enzyme including Thyroid Peroxidase (TPO), which is involved in the first two steps of thyroid hormone synthesis [14]. Hypothyroidism can cause low iron levels due to poor gut absorption due to a lack of digestive acids and enzymes in the body [15]. Hypothyroidism and iron deficiency are intimately linked. In patients with hypothyroidism, estimating the iron profile may be beneficial because the underlying reason could be an iron deficit [16].

The present case-control study was designed to evaluate the OPN, MDA and iron status and to find out the correlation between OPN, MDA and iron status in hypothyroidism subjects.

MATERIALS AND METHODS

The present case-control study was conducted with the collaboration of Department of Biochemistry, Santosh Medical College, Ghaziabad and Department of Biochemistry, Muzaffarnagar Medical College, Muzaffarnagar, Uttar Pradesh, India, from September 2018 to September 2020. The study protocol was checked and accepted by the Ethics Committee of Santosh Medical College, Ghaziabad {F. No SU/2018/528(33), dated 25.05.2018} and Muzaffarnagar Medical College, Muzaffarnagar (MMC/IEC/2019/225, dated 27.03.2019). Verbal and written informed consent was taken from all the participants.

Inclusion criteria: Patient with age between 30-60 years from age and sex matched subjects, hypothyroidism subjects based on detailed history and laboratory confirmation of thyroid profile were included as cases and all healthy female subjects of same age group (30-60 years) were considered as control and patients who were willing provide the written informed consent were included in the study.

Exclusion criteria: Subjects with type 2 diabetes mellitus, asthma, Chronic Obstructive Pulmonary Disease (COPD), malignancy, sexually transmitted disease, cardiac disease, renal diseases, hepatic diseases, gout and arthritis, pregnancy or lactating female and all anaemic patient other than iron deficiency anaemia, hyperthyrodism, hashimotos disease, patients taking drugs known to affect with thyroid hormone metabolism and iron metabolism, as well as participants not willing to give consent or reject to participate in the study, were excluded from the study.

Sample size calculation: The prevalence rate of hypothyroidism in Western Uttar Pradesh (UP) is around 8.4% accordingly [17], the minimum sample size has been calculated using appropriate sample size formula:

$n=z^2 \times pq/d^2$

Where, z=1.96 at 95 % confidence interval,

p=0.084 and

q=1-p=0.916

d=absolute error 5%

$n=(1.96)^2 \times 0.084 \times 0.916/(0.05)^2=118$

Hence, minimum sample size for cases=118, the current study included a total of 240 female subjects. There were 120 female hypothyroidism (cases) and 120 female normal healthy (control) subjects of the 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 profile analysis and patients willing to give written consent and healthy control individuals taken those who were in and around Muzaffarnagar Medical College.

Anthropometric Measurements

The standard device was used to measure both weight and height in light clothing and without shoes. Prior to eating in the morning, the weight was recorded on calibrated electronic weighing scales, and the height was measured to the nearby centimetre on a portable stadiometer. While in a standing position at the end of moderate expiration, the Waist Circumference (WC) was recorded using an anthropometric tape at a level on the skin midpoint between both the mean point of iliac peak and the inferior border of the last rib at the level of the umbilicus. In a standing position, the Hip Circumference (HC) was recorded over the broadest area of the gluteal region at the level of the pubic tubercle. Waist-to-Hip Ratio (WHR) was calculated by dividing the waist circumference (cm) by hip circumference (cm) [18]. Body Mass Index (BMI) of the subjects was calculated as body weight (kg) divided by the square of height (m^2) [BMI=Weight (Kg)/[Height (m)]^2 [19].

Biochemical Measurements

After an overnight fast of at least 10-12 hours, around 5 mL of venous blood was drawn from each participant and transferred into a plain tube for examination of osteopontin, malondialdehyde, serum ferritin, iron, and Total Iron Binding Capacity (TIBC). The serum/plasma was obtained by centrifuging the collected blood samples at 3000 rpm for 10 minutes.

- OPN was estimated by Sandwich-ELISA method using commercially available kit Elabscience, USA (Catalog Number, E-EL-H1347).
- Malondialdehyde in serum was estimated by the method described by Kei S (1978) [20].
- Serum ferritin was estimated by immuno-turbidimetric method by using commercially available kit from Beckman, USA (Catalog Number, 971209) on Access 2 Immunoassay chemistry analyser [21].
- Serum iron was estimated by photometric method using commercially available kit from Beckman, USA (Catalog Number, 922731) on AU480 chemistry analyser.
- Serum TIBC was estimated by photometric method using commercially available kit from Beckman, USA (Catalog Number, 923035) on AU480 chemistry analyser [22].

STATISTICAL ANALYSIS

Descriptive statistics were presented as mean±Standard Deviation (SD) for continuous variables, frequencies (percentage) for categorical variables. Tests of normality namely the Kolmogorov-Smirnov test were used. The student's t-test was conducted to compare the findings of two groups for all the parameters. Pearson's correlation analysis was used to evaluate the possible relationship between the parameters tested. A p-value <0.05 was considered to be statistically significant. IBM Statistical Package for the Social Sciences (SPSS) Statistics for Mac, Version 25.0, IBM Corp., Chicago, IL, was used to statistically analyse data.

RESULTS

[Table/Fig-1] demonstrates some of general characteristics of the studied subjects. All the patients were female hypothyroidism

Variable	Control subjects (n=120)	Hypothyroidism subjects (n=120)	p-value
Age (years)	40.96±4.15	40.64±4.84	0.58
BMI (Kg/m^2)	22.61±4.15	32.19±7.16	**<0.001**
WC (cm)	84.06±9.76	106.32±8.26	**<0.001**
HC (cm)	99.35±8.47	115.44±8.03	**<0.001**
WHR	0.85±0.12	0.92±0.09	**<0.001**

[Table/Fig-1]: Some of general characteristics of the studied subjects.
BMI: Body mass index; WC: Waist circumference; HC: Hip circumference; WHR: Waist to hip ratio; p-value <0.05 considered as statistically significant, Student's t-test was used to compare the findings of two groups

subjects. There was not significant difference between age in female hypothyroidism subjects as well as control subjects (40.64±4.84 vs 40.96±4.15). Female hypothyroidism patients had significantly higher mean BMI (32.19±7.16 kg/m^2 vs 22.61±4.15 kg/m^2, p-value <0.001), WC (106.32±8.26 cm vs 84.06±9.76 cm, p-value <0.001), HC (115.44±8.03 cm vs 99.35±8.47 cm; p-value <0.001), and WHR (0.92±0.09 vs 0.85±0.12, p-value <0.001) than control subjects.

[Table/Fig-2] demonstrates OPN, MDA and iron profile status in control and hypothyroidism subjects. Hypothyroidism subjects had significantly decreased biochemical marker namely OPN (4.45±0.39 vs. 7.22±1.53, p-value <0.001) compared to controls. Also, there was a significantly increased oxidative stress marker namely MDA (4.09±1.92 vs 1.50±0.41, p-value <0.001) compared to controls. Similarly, there was decreased iron profile markers namely ferritin and iron (9.26±0.84 vs 45.71±4.96, p-value <0.001, 50.91±3.37 vs. 88.22±5.64, p-value <0.001) compared to controls except for TIBC (461.94±47.09 vs 349.09±33.67, p-value <0.001) which was found to be significantly increased in hypothyroidism subjects.

Variable	Control subjects (n=120)	Hypothyroidism subjects (n=120)	p-value
Osteopontin (ng/mL)	7.22±1.53	4.45±0.39	**<0.001**
Malondialdehyde (nmol/L)	1.50±0.41	4.09±1.92	**<0.001**
Ferritin (ng/mL)	45.71±4.96	9.26±0.84	**<0.001**
Iron (µg/mL)	88.22±5.64	50.91±3.37	**<0.001**
Total iron binding capacity (µg/dL)	349.09±33.67	461.94±47.09	**<0.001**

[Table/Fig-2]: Osteopontin, malondialdehyde and iron status in control and hypothyroidism subjects.

[Table/Fig-3] shows correlation of OPN and MDA with body iron status in hypothyroidism subjects. OPN was positively and significantly correlated with ferritin and iron (r=0.465, p-value <0.001; r=0.521, p-value <0.001 respectively) and negatively and significantly correlated with TIBC (r=-0.454, p-value <0.001). Similarly, MDA was positively and significantly correlated with TIBC (r=0.591, p-value <0.001) and negatively and significantly correlated with ferritin and iron (r=-0.543, p-value <0.001; r=-0.573, p-value <0.001, respectively).

Parameters	Ferritin		Iron		Total iron binding capacity	
	r-value	p-value	r-value	p-value	r-value	p-value
Osteopontin	0.465	**<0.001**	0.521	**<0.001**	-0.454	**<0.001**
Malondialdehyde	-0.543	**<0.001**	-0.573	**<0.001**	0.591	**<0.001**

[Table/Fig-3]: Correlation of osteopontin and malondialdehyde with iron profile status in hypothyroidism subjects.
A p-value of less than 0.001 is considered statistically significant. The correlation between the studied parameters was analysed using Pearson's correlation analysis

[Table/Fig-4] shows correlation between OPN and MDA in hypothyroidism subjects. OPN was negatively and significantly correlated with MDA (r=-0.501, p<0.001).

Parameters	Malondialdehyde	
	r-value	p-value
Osteopontin	-0.501	**<0.001**

[Table/Fig-4]: Correlation between osteopontin and malondialdehyde level in hypothyroidism subjects.
p-value <0.001 considered as statistically significant. The Pearson's correlation analysis was done for the relationship between studied parameters

DISCUSSION

The levels of OPN, oxidative stress marker (MDA), and body iron status (ferritin, iron, and TIBC) in hypothyroidism patients were measured in the current study, and significant differences in these markers were observed between control and hypothyroidism subjects. Correlation analysis revealed a significant correlation between biochemical markers, oxidative stress markers as well as body iron status in hypothyroidism subjects. The anthropometric parameters BMI, WC, HC, and WHR were significantly increased in hypothyroidism patients as compared to controls, in the present study. This result is consistent with the findings of Savita and Mersiha M et al., also reported the significant increased BMI, WC, HC and WHR in hypothyroidism subjects, suggested that hypothyroidism is associated with a higher rate of general obesity, which could contribute to the development of cardiometabolic risk [23,24]. Similarly, Reinehra T et al., observed that thyroid function negatively associated with weight status and found increased TSH and free thyroxine (fT3) concentration in obese subjects [25]. Pesic M et al., observed that elevated level of TSH positively associated with BMI in hypothyroid patients [26]. WHR is a measure that reflects central obesity, and it has a strong relationship with hypothyroidism. Jung CH et al., concluded that patients with hypothyroidism exhibited higher WHRs [27].

OPN, a glycoprotein that can be identified in plasma. OPN was reported to be involved in the development and calcification of bone, as well as processes such as inflammation, cell adhesion and migration, and apoptosis prevention, because of its expression by several other tissues of the body [28]. In the present study, the level of OPN was found to be significantly lower in hypothyroidism subjects as compared with control subjects. This result is in accordance with that of El-Zawaw HT et al., who reported that OPN was significantly lower in the hypothyroid group than in the control group. Similar to TSH receptor antibodies (TRAbs), OPN demonstrated a significant positive correlation with TRAbs and a significant negative correlation with BMI [29]. OPN was discovered to be down-regulated in hypothyroidism patients. OPN was positively correlated with fT3 and free thyroxine (fT4), negatively correlated with TSH, and there was a significant association between the two. According to authors, OPN may be useful novel prognostic biomarker in subjects with reduced thyroid function. Serum OPN level was found decreased level of hypothyroidism patients due to iodine deficiency. Low level of OPN in hypothyroidism is independent of the aetiology and can be related to certain process going in thyroid cells [7].

Over production of free radicals or deficiency of multiple antioxidant defence systems can cause oxidative stress, which leads to oxidation of primary cellular macromolecules and subsequent molecular dysfunction and it leads to the lipid peroxidation and oxidative DNA damage [30]. MDA is a very important oxidative stress biomarker that is generated as a result of lipid peroxidation caused by ROS activity and is a successful player in hypothyroidism [31]. In the present study, significantly increased level of MDA was found in hypothyroidism subjects as compared to control subjects which indicates the increased oxidative stress. This result is consistent with findings of Chakrabarti SK et al., found MDA level is increased in treatment-naive primary hypothyroidism subjects [32]. Increase in malondialdehyde level is considered to indicate the physiological adaptation and response to hypothyroidism and thyroid hormones are play an important role in reducing the toxicity of the oxidative stress in human beings [33].

Iron is essential for the production of Reactive Oxygen Species (ROS) and extremely reactive hydroxyl radicals, which shifts the body's oxidative stress balance [34,35]. Ferritin is an iron storage protein that causes the body to produce inflammatory cytokines and lowers antioxidant levels. This could be due to the fact that hypothyroidism patients have lower ferritin levels, which have antioxidant qualities [36,37]. In the present study, decreased level of iron and ferritin and increased level TIBC were found in hypothyroidism subjects as compared to controls. Iron deficiency was found in a higher percentage of hypothyroid patients. These findings are consistent

with past research that has found that iron deficiency anaemia is usually related with low thyroid hormone levels [38]. According to Abnday TH et al., iron deficiency was found in a high majority of patients with primary hypothyroidism [39]. Similarly, at the same time, a study by Akhter S et al., suggested that abnormal thyroid hormone levels in iron deficient patients could be a result of abnormal thyroperoxidase activity. This enzyme plays a crucial part in thyroid hormone production [40].

In addition, an increase in serum MDA in hypothyroidism patients implies lipid peroxidation, which is a result of oxidative stress. In hypothyroidism patients, an increase in MDA level has a significant and positive correlation with iron and ferritin, as well as a significant and negative correlation with TIBC. This may be due to the deficiency of iron and ferritin along with low levels of thyroid hormones [38].

Furthermore, on correlation analysis, OPN was found to be significantly and positively correlated with ferritin and iron and significantly and negatively correlated with TIBC in hypothyroidism subjects. It is possible that the pattern of change in OPN is attributable to the many cell processes occurring in the thyroid gland as a result of OPN influence. Correlation analysis found significant and positive correlation of OPN with ferritin and iron and significant and negative correlation with TIBC whereas MDA showed significant positive correlation with TIBC and significant negative correlation with ferritin and iron. In the current study, we have also correlated the oxidative stress marker namely MDA with OPN in hypothyroid subjects. Pearson's correlation coefficient revealed a strong negative correlation between MDA and OPN in hypothyroidism subjects. This suggests that oxidative stress is associated with osteopontin due to decreased osteopontin levels, decreased iron and ferritin level in hypothyroidism subjects.

Limitation(s)

The patient group was small in the present study. Authors suggested further prospective population-based research in this way for finding the role of OPN in hypothyroidism subjects.

CONCLUSION(S)

Finally, the findings of the present study show that hypothyroidism is linked to increased oxidative stress (as measured by MDA) and decreased biochemical parameters (as measured by OPN), putting hypothyroidism patients at risk for iron deficiency anaemia. In hypothyroidism patients, oxidative stress markers and OPN were very well link with ferritin, iron, and TIBC. As a result, when assessing the risk of hypothyroidism, several parameters should be considered. However, further clinical research is required before the hypothesis may be accepted.

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- For any images presented appropriate consent has been obtained from the subjects. NA

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