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ORIGINAL ARTICLE

Oxidative Stress and Decreased Serum Levels of Superoxide Dismutase (SOD) as a Potent Risk Factor for Thyroid Disease among Local Pregnant Females in Punjab

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ABSTRACT

Aim: To assess the potent role of oxidative stress biomarkers in pregnant females with thyroid disease.
Study design: Cross sectional comparative study.
Place and duration of study: Institute of Molecular Biology & Biotechnology (IMBB), The University of Lahore from 1st July 2019 to 31st July 2021.
Methods: The study was designed to compare serum levels of oxidative stress markers and serum antioxidant levels between pregnant females who had thyroid disease as comorbidity and euthyroid pregnant controls. The markers were measured in fifty cases and fifty control serum samples using various laboratory tests and ELISA kits. Samples were collected from PINUM, Faisalabad and Obstetrics & Gynaecology Department, DHQ Chiniot, Faisalabad division.
Results: Mean ages of cases and controls were 29.19±4.17 and. 26.07±4.88 years. The serum levels of SOD were significantly decreased ($p \leq 0.001$) in pregnant females having thyroid dysfunction (0.05± 0.01 nmol/ml) as compared to the control group (0.06±0.01nmol/ml). None of the other oxidative markers showed a significant difference between cases and controls ($p>0.05$).
Conclusion: The lower serum SOD levels may be a potential risk factor for the development of thyroid disease in local pregnant females.
Keywords: Oxidative stress, Thyroid-stimulating hormone, Thyroid dysfunction, Superoxide dismutase, malondialdehyde,

INTRODUCTION

During pregnancy, thyroid abnormalities are associated with oxidative stress that could significantly affect placental implantation, early embryonic growth, and developmental phase via modulating various transcription factors. This in turn, may result in poor fetal outcome and decreased intelligence quotient (IQ) levels in children. In the current study we investigated the following markers of oxidative stress; malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). MDA can be used to assess the degree of lipid peroxidation while, SOD, catalase CAT and GPx are fundamental antioxidant enzymes that have a crucial role in neutralizing oxidative stress. There are many factors responsible for the development of thyroid disorders including genetic predisposition, exogenous factors, and various endogenous pathogenic precursors, and immune system alterations[1]. Hypothyroxinemia in the first trimester may affect fetal brain development and fetal neural tube defects. In past years, numerous researches have reported that subclinical hyperthyroidism and hypothyroidism can provoke deficits and complications similar to overt hyper and hypothyroidism.[2] Maternal thyroid dysfunction during pregnancy is one of the chief causes of fetal and placental anomalies, premature and false labor, low birth weight babies, gestational hypertension, eclampsia, still birth as well as many other complications.

In the west, the prevalence for subclinical hypothyroidism (elevated serum levels of TSH with normal serum concentration of freeT4) is estimated to be about 2-3% and overt maternal hypothyroidism (elevated serum concentrations of TSH with a low concentration of freeT4) about 0.3-hypothyroidism and 11% for overt hypothyroidism[3]. The prevalence of hyperthyroidism reported in India is 12%, whereas hypothyroidism is found in 1.25% of pregnant women[4]. According to the results of another study, the prevalence of hypothyroidism and thyroid autoimmunity was higher in Asian-Indian pregnant women than western women accounting for hypothyroidism about 4.8% and TAI 12.4%. In the west, the prevalence of hypothyroidism is estimated to be 2-5% according to the western literature and that of thyroid autoimmunity is around 5-10%[5,6].

Reactive oxygen species (ROS) production to a normal extent in thyroid tissue is essential for the biosynthesis of thyroid hormones. However, increased production and accumulation of ROS promotes the pathological destruction of thyrocytes and the expansion of autoimmune, iodine-induced, and inflammatory thyroid dysfunctions. Reactive oxygen species mediated oxidative stress also participates in the pathogenesis of autoimmune thyroid diseases (AITD) as well as iodine-induced thyroid autoantibody generation. Overproduction of reactive oxygen species and subsequent oxidative stress are interlinked with production and activation of a variety of growth factors and inflammatory markers. The ROS could arise from either the infiltrated B lymphocytes or thyrocytes themselves. Many studies revealed suppression of Sodium Iodide Symporter (NIS), TPO, and TG expression following cytokine stimulation (by IL-1β, IL-1α, TGF-β, IL-17, IL-6, TNF-α, TNF- β, TNF-γ) in thyrocytes[7].

In thyroid tissue at the follicular cell level, the cell injury along with apoptosis and necrosis, preceding the invasion of lymphocytes, are some initial pathogenic steps for the evolution of iodine-induced Hashimoto's thyroiditis. Inflammatory markers and reactive oxygen species mediate the destruction during the pathogenesis of Hashimoto's thyroiditis.[8] Pregnant women having autoimmune thyroiditis (HT) are also at great risk to develop permanent hypothyroidism after delivery as exposed to acute exacerbation of disease as a result of immune resumption during the postpartum phase if left untreated[9].

Oxidative stress is always increased physiologically during pregnancy that leads to an imbalance between the antioxidant defense system and ROS generation followed by consequent lipid peroxidation and oxidative DNA destruction involving NF-κB signaling pathway in thyrocytes especially in the second and third trimester[10]. Antioxidants are categorized as enzymatic antioxidants including SOD, CAT, GSH-Px, and non-enzymatic antioxidants including glutathione (GSH)[11]. Pregnancy-induced physiological alterations support the increased generation of ROS especially in the second trimester of gestational amenorrhea[12]. This response is

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primarily due to increased basal metabolic rate and high oxygen consumption, as well as increment in catabolism of fatty acids, as fatty acids metabolism is primary energy resource by mother's body[13,14]. Increased erythropoiesis enhances fetal iron supply that results in increased iron availability by catalyzation of hefty sums of reactive hydroxyl (·OH) radicals production by transitional metals like iron through Fenton reaction[15,16].

Oxidative stress is unquestionably strongly associated with the pathogenesis of gestational complications and developmental disorders in the mother and the offspring. However, it is difficult to establish oxidative stress as a causative factor for thyroid disease due to several limitations and the lack of a convincing mechanism of action as well as very limited clinical trial-based data availability. The current study is conducted to recognize and analyze the correlation of oxidative stress as a risk factor for the development of thyroid diseases in local pregnant females belonging to iodine deficit areas of Punjab, Pakistan. The role of anti-thyroid drugs in favor of improving oxidative stress or anti-oxidant and oxidative species imbalance would also be analyzed. The therapeutic role of antioxidant supplementation is also studied in normal healthy pregnant females and the diagnosed cases for thyroid dysfunctions and thyroid autoimmunity.

MATERIALS AND METHODS

We conducted a cross-sectional comparative study in two years duration from July 2019 to July 2021 after getting approval from institutional ethical and review board for advanced research including total 100 pregnant females, 50 cases and 50 matched controls. Sampling was done using non-probability-convenient method. Women having BMI more than 35 and those who had a history of thyroid carcinoma and another anomaly i.e., cancer, diabetes were excluded. Samples were collected from PINUM Hospital, Faisalabad, Punjab and Obstetrics and Gynecology department, DHQ Chiniot, Faisalabad division, Punjab. Females undergoing prenatal examination were taken from Gynecology and Obstetrics Department, D.H.Q. District Chiniot, Faisalabad Division, Punjab. The whole experimental work was done at the Institute of Molecular Biology & Biotechnology (IMBB), The University of Lahore. The healthy pregnant females were considered as the control group while the diseased group was further categorized as a high-risk group and low-risk group depending upon the risk factors history using ATA 2017 guide for detection of high risk and a non-high-risk group of females for thyroid dysfunction.

Informed consent was obtained from all patients before their sample collection and all the participants were well informed that their clinical data would be used for scientific purposes. Besides undergoing routine prenatal registration, all the subjects were interviewed by the obstetrician regarding the obstetrical history (e.g. miscarriages, preterm deliveries, and infertility), personal and family history of thyroid disorders, clinical signs consistent with thyroid disorders, personal history of type 1 diabetes or other autoimmune diseases, history of anti-thyroid drugs use (thyroxin or amiodarone treatment) and history of any therapeutic head or neck irradiation. As per the 2011 guideline of the ATA for diagnosis and management of thyroid disease during pregnancy and postpartum[17,18].

Blood samples were acquired in the morning from each subject. Blood was obtained through venipuncture by antiseptic technique and centrifuged at 4000 rpm for 10 minutes and serum was separated. Blood samples were collected into EDTA tubes. Serum was stored at -8°C until testing. The concentration of TSH and FT4 was detected by an ELISA diagnostic kit. All oxidative stress biomarkers were analyzed and detected by using spectrophotometry at the wavelength absorbance rate of 532nm.

Statistical analysis was performed using SPSS-25.0. Statistical comparisons were made using the student t-test and were significant, so variables were further analyzed through multiple logistic regression and ROC curve analysis. P-value ≤0.05 was taken statistically significant.

RESULTS

The mean age of the case group was 29.18±4.17 years and the mean age of the control group was 26.07±4.86 years (Table 1). Other than SOD, the biomarkers of oxidative stress were not significantly associated with thyroid disease in pregnant females (p> 0.05). The serum levels of SOD were significantly decreased (p≤0.001) in pregnant females having thyroid dysfunction (0.05±0.01) as compared to the control group (0.06±0.01). The mean MDA of control subjects is 0.06±0.02 nmol/ml and that of the diseased group is 0.05±0.01 nmol/ml. The mean catalase of controls and patients is 0.48±0.12 nmol/ml and 0.50±0.12 nmol/ml respectively. An overall decreasing trend was observed in GSH levels between diseased pregnant females (4.09±3.63 nmol/ml) and controls (4.57±3.52 nmol/ml) but is not statistically significant (p=0.52). Likewise, the mean value of AOPPs in controls was 1.28±0.37 nmol/ml and in pregnant females having thyroid anomaly is 1.34±0.33 nmol/ml respectively. An increasing drift was observed in AOPPs levels between diseased patients and controls but this did not reach statistical significance (p=0.43) [Table 2].

Table 1: Demographic and clinical data of the females (n=100)

Variable	Controls	Cases	P value
	Mean±SD	Mean±SD	
Age (years)	26.07±4.88	29.19±4.17	0.001*
Serum T3 nmol/L	34.74±8.39	42.61±10.19	<0.001*
Serum T4 nmol/L	1.37±1.25	1.29±0.22	<0.001*
Serum TSH mIU/L	0.22±0.31	0.45±1.52	0.163
	Median QR	Median QR	
No. of pregnancies	3 2	2.72 1.56	0.58**

*t-test; ** Mann Whitney U test

Table 2: Serum concentration of oxidative stress biomarkers in case and control groups

Variable	Controls	Cases	P value
	Mean±SD	Mean±SD	
MDA (nmol/ml)	0.06±0.02	0.05±0.01	0.40
SOD (nmol/ml)	0.06±0.01	0.05±0.01	<0.001*
Catalase (nmol/ml) after 5 seconds	0.48±0.12	0.50±0.12	0.51
Catalase (nmol/ml) after 1 minute	0.47±0.10	0.49±0.12	0.29
Catalase (nmol/ml) after 2 minutes	0.47±0.10	0.50±0.13	0.30
Advanced Oxidative protein (AOPPs) nmol/ml	1.28±0.37	1.34±0.33	0.43
GSH in nmol/ml	4.57±3.52	4.09±3.63	0.52

*Independent sample two tailed 't' test

DISCUSSION

The relationship of thyroid hormones with the oxidative status of the body has been in studies for many years. Normal serum concentration and conversion from T4 to T3 by deiodinase enzymes and adequate supply of thyroid hormones to the cellular level is essential for normal basal metabolic rate of the human body[19-21]. The fluctuation in the normal levels of thyroid either synthesis or secretion as the result of some pathological condition can alter the metabolic process of the body. Many studies have revealed that hypothyroidism is associated with decreased antioxidants levels and functions, that may lead to oxidative stress in animal models, however, the reports on oxidative status in hypothyroid females is very limited and controversia[22,23].

Our results are in line with a very popular study conducted by Sies et al[24] in 2017 at oxidative stress observing increased oxidative stress levels in hypothyroid females while some reports are in contradiction to these results. The production of ROS is largely dependent on aerobic metabolism. In shortage of appropriate oxygen levels, the metabolism shift towards anaerobic pathways that can cause the production of reactive oxygen species. These harmful oxygen species are highly reactive and oxidize the biological macromolecules present in their environment, thereby altering the cell functions that cause oxidative stress (OS).

In physiological conditions, the antioxidant is produced in the body that neutralizes these reactive oxidants and keeps the oxidative balance. However, under pathological states, an oxidative imbalance arises from reactive oxidants overproduction or deficit antioxidant defense system unable to cope with the excessive ROS. Chronic stress leads to cellular dysfunction. Antioxidant activity is mediated by some specific factors such as aging, obesity, hormonal state, and organ specificity. Hormonal imbalance has immense effects on the generation of ROS as hormones are responsible for proper signaling and regulating metabolic activities[25]. Thyroid hormones play a potent role in ROS production through the regulation of oxidative metabolism as they are considered as having properties like oxidants.[26] Maternal Hypothyroidism is a prevalent hypometabolic disease state involving reduced mitochondrial oxygen consumption rate and low tissue proliferation. Moderate chronic placental and fetal hypoxia are caused by poor uteroplacental circulation during maternal hypothyroidism in pregnancy. Many studies described the association of hypothyroidism with raised ROS production from the placenta as a result of placental hypoxia in hypothyroidism, enhanced lipid peroxidation and oxidative stress that might lead to progression of atherosclerosis.

Our study findings of increment in serum antioxidants levels are similar to many studies that have evidenced the beneficial role of antioxidant supplementation and thyroid hormone replacement in hypothyroidism-induced oxidative stress. However there is discrepancy between our study results and some studies describing that thyroxin administration leads to increased oxidative stress in several tissues in hypothyroid animal models. The possible reason for this difference can be ethnicity and biological environmental reasons. Apart from the fact, serum transaminase activity that has been elevated is not normalized in hypothyroid patients treated with oral thyroxin. Oral administration of exogenous antioxidants like vitamin E and vitamin C neutralizes elevated transaminase activity in serum and boosts up the down-regulated antioxidants defense system in thyroid dysfunctions.

Besides metabolic stress due to excessive amount of macronutrients, mitochondrial dysfunction and endothelial reticulum stress add to an increase in oxidative stress. Our study results revealing increased BMI as a demographic factor for development of thyroid disease are similar to that demonstrating that reactive species generated in mitochondria are signalling molecules regulating the secretion of pro-inflammatory cytokines, linking inflammation to OS. This correlation was precisely observed in cardiovascular diseases and autoimmune diseases in obese individuals. The normal levels of thyroid hormones play a noteworthy role in antioxidant modulation while an appropriate antioxidant defense system is required for prevention as well as treatment in thyroid disorders especially autoimmune and inflammatory thyroid illnesses[22,26,27].

Tissue hypothyroidism can increase oxidative stress. Oxidative imbalance and inflammation both have a major key role in initiation and advancement in subsequent autoimmune thyroid disease as well as hyperthyroidism or hypothyroid disease. Iodine deficiency is the major cause of thyroid disorders under development and iodine insufficient regions, as it may cause compensatory goitrogenesis. Some of them develop hypothyroidism due to a failure of iodine adaptative response (Wolff-Chaikoff effect. In addition to genetic predisposing factors, immunological factors, exposure to certain environmental exogenous factors, and the number of pregnancies in females, OS-triggered inflammation-inducing oxidative stress may also induce increased lipid peroxidation and has a potent role in the initiation of the persistent thyroid disease condition.

Pregnancy is a state involving physiological alterations related to the development of the fetus, maternal adaptation to the conception and maternal homeostasis maintenance is accompanied by serious health risks. Pregnant women have been exposed to too many complications and disorders related to and caused by increased oxidative stress. The placenta is a potent source of pro-oxidants and antioxidants and can control lipid peroxidation to a normal limit as it is increased in pregnancy. Placental function is disrupted by increment in oxidative stress and hormonal disturbances in the maternal compartment through increased placental oxidative stress affecting placental gene methylation state. NADPH is a prerequisite to achieving a balanced redox ratio of GSSG/GSH that is a well-thought-out strong indicator of oxidative stress. Glutathione (GSH) functions as a strong antioxidant as it searches for free radicals as a co-substrate for glutathione peroxide, a well-defined mechanism of decreasing serum GSH concentrations with a gradual increase in oxidative stress, providing the logic for decreased intracellular GSH levels due to increased cellular requirement for NADPH. Due to the remarkable reduction in NADPH amount needed for regeneration of GSSG in GSH, the non-enzymatic antioxidant defense system is significantly weakened during pregnancy[28].

MDA as a strong biomarker of lipid peroxidation in increased oxidative stress does not undergo verification across the age and gender. A case-control study was conducted to evaluate the oxidative imbalance in the amniotic fluid of the pregnant females diagnosed with hypothyroidism and have low serum concentrations of free T4 in the first trimester as equal to the healthy normal pregnant females through measuring the serum superoxide concentration. Results showed significantly higher levels of superoxide anion in the amniotic fluid of hypothyroid females as compared to healthy females. This increased amniotic fluid's superoxide anion concentration is the major cause of oxidative stress resulting in DNA damage and cell proliferative disorders and can damage the normal amniotic fluid function through the changes in its constituent substances[29].

Superoxide anion has a short half-life and physiologically it is converted into H_2O_2 by an enzyme superoxide dismutase. Novakovic et al[30] supports the fact that this reduced concentration of H_2O_2 in amniotic fluid is may be due to the reduced activity of SOD that is responsible for the conversion of superoxide anion into H_2O_2. The current study has observed decreased SOD levels in pregnant females suffering from thyroid fluctuation either hyperthyroidism or hypothyroidism as compared to the control group which is in accordance with the study reported by Ramandeep et al.[15] The prevalence of subclinical hyperthyroidism, as well as hypothyroidism, is very high in the South Asian population of pregnant females, which may be due to iodine deficiency and exposure to certain environmental factors, and higher oxidative stress levels. Methimazole and propylthiouracil used in GD treatment cause immunoregulation, decrease antibody levels and decrease thyroid tissue inflammation; these actions facilitate the remission of the disease. According to a study result, MDA levels were high in patients diagnosed with hypothyroidism not using L-Thyroxin however, after treatment with thyroxin, MDA levels are significantly reduced resembling to our study findings of MDA levels that were not significantly varying between pregnant females with thyroid disease and control group.

These results show a significant connection between hypothyroidism and increased lipid peroxidation end products as well as increased oxidative stress. Long ago in 1989, a study was conducted to evaluate the modulatory role of antithyroid drugs on antioxidant enzyme concentrations in rats. They administered the antithyroid drug methimazole (or diluent) to pregnant rats for the final 3 days before premature or term delivery; in the second series of experiments, propylthiouracil was administered for the 10 days before delivery. Both antithyroid drugs, known to cross the placenta, produced significantly decreased thyroid hormone levels in the pregnant dams. Fetal offspring from methimazole and propylthiouracil-treated dams demonstrated significant increases in pulmonary superoxide dismutase activity at 20 and 21 days of gestation and catalase and glutathione peroxidase activities at 21 days compared with control offspring[31].

CONCLUSION

An increase in serum concentrations of SOD in case group associated with antithyroid drugs including propylthiouracil, carbimazole, and methimazole administration. The reduction in serum MDA levels in the hypothyroid diseased group with oral administration of thyroxin.

Conflict of interest: Nil

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