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ORIGINAL ARTICLE

Oxidative stress a potent risk factor for thyroid diseases in young pregnant females.

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ABSTRACT… Objective: To evaluate the potent role of oxidative stress in thyroid disease development and progression in young pregnant females during 1st and second trimesters compared with matched healthy pregnant females of the same gestational age. **Study Design:** Cross-sectional Comparative study. **Setting:** Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Pakistan. Samples were collected at the Punjab Institute of Nuclear Medicine (PINUM), Faisalabad, and the Department of Obstetrics at DHQ Hospital, Chiniot. **Period:** July 2019 to July 2021. **Material & Methods:** Thyroid disease- comorbid pregnant women and euthyroid pregnant women were compared for serum levels of oxidative stress markers and antioxidants. The markers were evaluated between 68 healthy subjects in Group A and 43 subjects in Group B known for having thyroid diseases. Blood samples were taken under highly sterile conditions after gaining written consent from all subjects and stored at –8°c to –30°c. A spectrophotometer at 532nm was used to measure serum concentrations of oxidative stress markers. ELISA kits were used to determine serum levels of triiodothyronine, thyroxin, and thyroid-stimulating hormone. Data was analyzed using independent student t-test, multiple logistic regression, and receiver operating characteristic curve (ROC) analysis using SPSS version 25. **Results:** Group A and Group B had a mean age of 26.07±4.88 and 29.19±4.17 years, respectively. Pregnant women having thyroid dysfunction had decreased superoxide dismutase levels (0.05±0.01 nmol/ml) than group A (0.06±0.01). Other indicators of lipid peroxidation showed a non-significant mean difference compared between both groups ($p>0.05$). Multiple linear regression studies revealed healthy pregnant women with a positive thyroid medication history had higher SOD levels among group B than those without antithyroid medicine, with a Beta coefficient of +0.26 and $p=0.04$. According to ROC curve analysis, the Superoxide Dismutase biomarker area under ROC was 67.25% at $p=0.002$. Thus, it may be used as a diagnostic and predictive biomarker. A cutoff value of 51.5pg/ml was proposed, at which sensitivity is 79% and specificity is 69%. It can be a diagnostic and predictive biomarker. **Conclusion:** The results of the current study concluded that oxidative stress can be considered a potent risk factor for the development and progression of thyroid disease in young pregnant females.

Key words: Catalase, Goiter, Iodine Deficiency, Malondialdehyde, Oxidative Stress, Reactive Oxidative Species, Superoxide Dismutase, Thyroglobulin, TSH Receptor Protein, Thyroid Dysfunction, Young Pregnant Females.

INTRODUCTION

Thyroid disorders are pervasive in females, especially during their reproductive age. A study found that Asian-Indian pregnant women were more likely than Western women to have hypothyroidism (4.8% and 12.4%, respectively). Western literature puts hypothyroidism at 2-5% and thyroid autoimmunity at 5-10%.[1,2,3] Thyroid disorders correlate with oxidative stress as a causative or subsequent modulator, which modulates transcription factors and affects placental implantation, early embryonic growth and development, poor fetal outcomes, and lower child IQ levels.[4,5] Thyroid hormone biosynthesis requires balanced production of ROS.[6,7] In thyroid follicles, programmed cell death and necrosis cause lymphocyte invasion and iodine-induced Hashimoto's thyroiditis.[8] Untreated pregnant women with autoimmune Hashimoto's thyroiditis are vulnerable to developing persistent hypothyroidism after delivery due to immunological recommencement.[9,10]

SOD, Catalase, and glutathione (GSH) are

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considered among potent enzymatic and non-enzymatic antioxidants.[11] Pregnancy is a normal physiological process that facilitates ROS production increment.[12] Fatty acid metabolism is the mother's primary energy source; therefore, increased basal metabolic rate, oxygen intake, and fatty acid catabolism cause lipid peroxidation.[13,14] Increased erythropoiesis increases fetal iron supply by catalyzing large amounts of reactive hydroxyl (OH) ion generation by transitional metals like iron through the Fenton reaction.[15,16]

Oxidative stress causes gestational and postpartum complications in mothers and developmental abnormalities in children. Oxidative stress may cause thyroid illness. However, there is no plausible mechanism of action, and we have limited clinical trial data available in the literature.[17] The relationship between oxidative stress and thyroid illness in young pregnant women must be evaluated. Conventional oral antithyroid medications' effects on oxidative stress must be revisited to establish a better treatment efficacy.

The current study aimed to evaluate oxidative stress as a potent risk factor for developing thyroid disorders in young pregnant females.

MATERIAL & METHODS

After receiving approval from the Board of Advanced Scientific Research (BAR), IMBB, University of Lahore(IMBB/UOL/23/086), this two-year cross-sectional comparative study was conducted from July 2019 to July 2021. One hundred eleven pregnant females during 1st and 2nd trimesters visiting antenatal care outdoor were included in groups A and B. All subjects were well informed of their data and clinical findings utilization for research purposes, and consent forms and questionnaires designed per American Thyroid Association (ATA) 2017 guidelines were provided and filled duly by all enrolled subjects.[18] Group A included 43 euthyroid healthy pregnant females less than 35 years old and 68 matched controls in the first and second trimesters of gestational age. Non-probability-convenient sampling was the method employed. All women visiting for antenatal follow-up were included; those pregnant females with a BMI over 35, aged over 35 years, to avoid effects of aging, thyroid cancer, diabetes mellitus, and pregnancy-induced hypertension were excluded. Samples were taken from the Punjab Institute of Nuclear Medicine (PINUM) Hospital, Faisalabad, Department of Gynecology and Obstetrics, DHQ hospital, Chiniot.

The entire experiment was conducted at the University of Lahore's Institute of Molecular Biology and Biotechnology lab. The serum concentrations of FT3, FT4, and TSH were measured by ELISA. Oxidative stress biomarkers were analyzed by using a spectrophotometer at 532nm wavelength. SPSS version 25 was used for statistical analysis. Variables were described as mean ±SD applicable. Multiple logistic regression and ROC curve analysis were used after the student-independent t-test showed significant differences. P-value < 0.05 was considered statistically significant.

RESULTS

We evaluated the correlation of serum antioxidant concentration in young pregnant females with the presence of thyroid disease in the current study. Sixty-eight pregnant females having healthy pregnancies with euthyroid status during 1st and the 2nd trimesters of gestational amenorrhea and 43 pregnant females with a history of thyroid diseases in 1st and 2nd trimesters of gestational amenorrhea were enrolled from the pregnant population and divided into two groups: A and B. Mean age and serum concentration of superoxide dismutase enzyme were significantly different in group A and B. The mean age in groups A and B was 26.07±4.86 and 29.18±4.17 years, respectively.

SOD level was significantly decreased ($p \leq 0.001$) in pregnant females with thyroid disorders in Group B (0.05± .01) as compared to group A (0.06± .01).

The results regarding stress biomarkers revealed the role of stress markers in pregnant females suffering from thyroid abnormalities. An overall reduction in GSH level was observed

in pregnant females with thyroid disorders in Group B (4.09±3.63nmol/ml) compared to Group A (4.57±3.52nmol/ml), with p=0.52 being considered insignificant.

The mean value of AOPPs in group A was 1.28±0.37nmol/ml compared to group B 1.34± 0.33 nmol/ml, respectively. A statistically insignificant increasing tendency was observed in Advanced Oxidative Protein Byproducts (AOPPS) levels between Group A and Group B with p=0.43.

Multiple Linear regression for factors affecting SOD levels

Multiple linear regression was applied to assess the effect of different variables on serum SOD levels to elucidate the findings further. The results revealed that a positive drug history for thyroid medication was linked to increased SOD levels with a Beta coefficient of 0.26 and p=0.04.

ROC Curve Analysis to Assess the Predictive Value of Serum SOD for Development of Thyroid Disease

ROC curve analysis revealed the area under the curve (AUC) for SOD as a biomarker as 67.25% at p=0.002. The area under the curve indicated it was a fair test to predict thyroid dysfunction. We propose a cut-off value of _51.5 picogram/ml at which sensitivity is 79% and specificity is 69%.

Demographic Data	Group A (n=68)		Group B (n=43)		P-Value
	Mean	Std. Deviation	Mean	Std. Deviation	
Age in years	26.07	4.88	29.19	4.17	0.001*
Serum T3 nmol/L	42. 61	8.39	34.74	10.19	<0.001*
Serum T4 nmol/L IU	1.37	1.25	1.29	.22	<0.001*
Serum TSH mIU/L	0.22	0.31	0.45	1.52	0.163
	Median	QR	Median	IQR	
Number of Pregnancies	3	2	2.72	1.56	0.58**

Table-I. Demographic and lab data
***t-test; ** Mann Whitney U test**

Markers of Oxidative Stress	Group A (n=68)		Group B (n=43)		P-Value
	Mean	Std. Deviation	Mean	Std. Deviation	
MDA (nmol/ml)	.06	.02	.05	.01	0.40
SOD (nmol/ml)	.06	.01	.05	.01	<0.001*
Catalase (nmol/ml) after 5 seconds	.48	.12	.50	.12	0.51
Catalase (nmol/ml) after 1minute	.47	.10	.49	.12	0.29
Catalase (nmol/ml) after 2 minutes	.47	.10	.50	.13	0.30
Advanced Oxidative protein products (AOPPs) nmol/ml	1.28	.37	1.34	.33	0.43
GSH in nmol/ml	4.57	3.52	4.09	3.63	0.52

Table-II. Serum concentration of oxidative stress biomarkers in Group A and Group B
***Independent sample, two-tailed test**

Model Summary		Unstandardized Coefficients		Standardized Coefficients	P-Value
		B	Std. Error	Beta	
1	(Constant)	0.03	0.01		0.002
	Age in years	<0.001	<0.001	0.10	0.42
	Body Mass Index (kg/m2)	<0.001	<0.001	0.13	0.22
	Number of pregnancies	<0.001	0.001	-0.03	0.77
	Trimester at the time of presentation	<0.001	0.002	0.02	0.87
	Drug history	0.002	0.001	0.26	0.04*
	Serum T3 levels in nmol/L	-3.396E-6	<0.001	-0.003	0.98

Table-III. Factors affecting serum SOD levels in pregnant females with thyroid disease
ANOVA: p=0.04; Adjusted R2=0.07

Test Result Variable(s): Serum SOD levels in nmol/ml				
Area Under the Curve	Std. Error	P-Value	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
67.2%*	0.05	.002	.57	.78
*Fair to good test as per conventions of ROC curve analysis				

Table-IV. ROC curve analysis to see if serum SOD levels can be a good predictive biomarker for development of thyroid disease in pregnant females

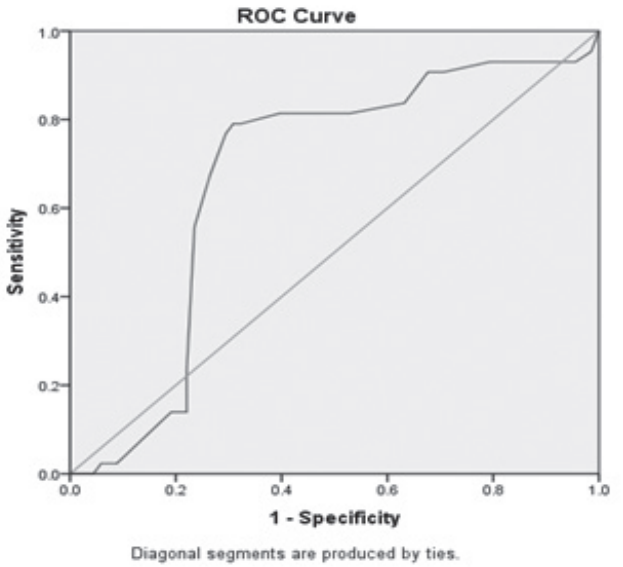

Figure-1. ROC curve for SOD as biomarker for thyroid disease in pregnancy

DISCUSSION

Studies have observed the association between thyroid hormones and oxidative metabolism for years. Homeostatic basal metabolic rate requires appropriate serum levels, appropriate deiodinase enzyme conversion of T4 to T3, and thyroid hormone delivery to cells for normal metabolic and endocrinological functions[19,20,21], Aging, obesity, hormonal status, stress, and organ specificity affect antioxidant activity. Since hormones signal and regulate metabolism, hormonal instability significantly affects ROS production.[22,23,24,25] Thyroid hormones regulate oxidative metabolism and produce ROS as oxidants.[26]

Antioxidants may help thyroid hormone restoration, thus reducing hypothyroidism-induced oxidative stress. Oral thyroxin does not normalize blood transaminase activity in hypothyroid individuals. In thyroid dysfunctions, oral vitamins E and C neutralize blood transaminase activity and increase the antioxidant defense system. Thyroid disorders, notably autoimmune and inflammatory ones, require an adequate antioxidant balance and proper thyroid hormone levels to establish a balanced antioxidant-to-oxidant ratio.[27] As lipid peroxidation rises throughout pregnancy, the placenta's pro-oxidants and antioxidants can reduce it. Increased maternal oxidative stress and hormonal abnormalities damage placental function by altering gene methylation. Pregnancy weakens the non-enzymatic antioxidant defense mechanism due to the substantially reducing NADPH needed for GSSG regeneration in GSH.[28]

MDA as a potent indicator of lipid peroxidation in increasing oxidative stress is not verified across age and gender. A case-control research examined the amniotic fluid oxidant imbalance in pregnant hypothyroidism patients. Hypothyroid ladies had higher amniotic fluid superoxide anion levels than healthy females.[29] SOD converts superoxide anion into H2O2 biologically. Hypothyroidism caused superoxide anion buildup due to lower SOD activity in several studies. Pasupathi and Latha found in 2008 that pregnant women with hypothyroidism had lower amniotic fluid H2O2 concentrations than healthy pregnant women. Another study suggests that SOD, which converts superoxide anion into H2O2, may be less active in amniotic fluid.[30] According to the study conducted by Ramandeep et al. In 2017, pregnant women with thyroid alteration (hyper or hypothyroidism) had lower SOD levels than the control group. According to a study, MDA levels were high in hypothyroidism patients not using L-Thyroxin, but after treatment with thyroxin, MDA levels were significantly reduced. The current study found no significant difference in MDA levels between pregnant females with thyroid disease in Group B and Group A. 1989 antithyroid medications were tested for their effects on rat antioxidant enzyme

concentrations. Researchers administered pregnant rats methimazole (or diluent) for the last three days before premature or term delivery and, in the second series, propylthiouracil for the last 10 days, both antithyroid medications, which pass the placenta, dramatically reduced thyroid hormone levels in pregnant dams. Fetal offspring from methimazole and propylthiouracil-treated dams had higher lung superoxide dismutase and catalase and glutathione peroxidase activities at 20 and 21 days of gestation than control offspring.[31]

CONCLUSION

Healthy pregnant women had greater SOD levels (0.06±.01) than those with thyroid illness (0.05±.01) ($p < 0.001$). Drug history affected diseased SOD levels. Positive thyroid disease drug history in order to achieve and maintain normal circulatory thyroid hormone levels was strongly associated with elevated SOD levels with a Beta coefficient of 0.26 and $p=0.04$. SOD as a biomarker has an AUC of 67.25% at $p=0.002$, suggesting a cutoff value of 51.5 picogram/ml with sensitivity of 79% and specificity of 69%. GSH levels decreased between Group B (4.09±3.63nmol/ml) in comparison to Group A (4.57±3.52nmol/ml) but were not statistically significant ($p=0.52$). The case group had a mean age of 29.18±4.17 years, and the control group 26.07±4.86 years, indicating age as a demographic risk factor. The study we conducted found propylthiouracil, carbimazole, and methimazole increased SOD serum concentrations in the case group.

LIMITATIONS

There were a few limitations in the current study. Considering it as a cross-sectional study, which did not have sufficient predictive value. In the future, it is recommended the prospective cohort study be designed to further evaluate the evidence of SOD as a predictive biomarker for the development of thyroid diseases in pregnant females. We also suggest the use of antioxidants for better improvement and efficacy of antithyroid drugs as well as a preventive measure.

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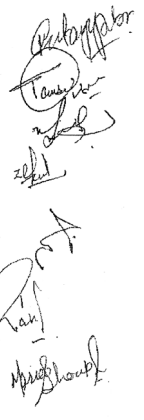
1	Mutayyaba Majeed	Questionnaire and design compilation, Proof reading lab processing.	
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