Original Article

Correlation of enhanced oxidative stress with altered thyroid profile: Probable role in spontaneous abortion

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Abstract

Background: Spontaneous abortion or miscarriage is defined as the loss of a clinically recognized pregnancy that occurs before 20 weeks of gestational age. Changes in thyroid function can impact greatly on reproductive function before, during, and after conception. Oxidative stress affects both implantation and early embryo development by modifying the key of transcription. Malondialdehyde (MDA) is a major breakdown product of split off from lipid peroxidation. Superoxide dismutase (SOD) is responsible for detoxification of superoxide anion and required for normal health and reproduction. **Aim:** The aim of this study was to define the involvement of thyroid hormones, MDA and SOD levels and to establish MDA levels as an index of lipid peroxidation in women with spontaneous abortion by comparing the results with healthy pregnant females as controls. **Materials and Methods:** A cross-sectional case–control study was designed with two groups of women with 30 each in healthy pregnancy and with spontaneous abortion. **Results:** Demographic characteristics such as maternal age, paternal age, gestational age, body mass index, waist-hip ratio as well as biochemical parameters such as blood pressure, hemoglobin (Hb), sugar levels were found to be similar in both the participating groups. Characteristics like gravida and parity were found to be higher in the study group and differ significantly from control group. Spontaneous abortion before 24 weeks of gestational age was found to be associated with significant increase in mean serum thyroid stimulating hormone (TSH) (P = 0.0115) and MDA (P = 0.0001) levels and a significant decrease in mean serum T3 (P = 0.0003) and SOD (P = 0.0005) levels. The linear (Pearson) correlation analysis demonstrated a significant positive correlation of TSH with MDA and negative correlation with SOD in women with spontaneous abortion. **Conclusion:** The study demonstrates that altered thyroid profile, increased lipid peroxidation in terms of increased MDA levels and decreased SOD levels might be involved in the termination of otherwise wanted pregnancy.

Key words: Malondialdehyde, oxidative stress, spontaneous abortion, superoxide dismutase, thyroid hormones

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Introduction

The term spontaneous abortion or miscarriage refers to pregnancy loss at <20 weeks of gestation in the absence of elective medical or surgical measures to terminate the pregnancy.[1] It occurs in 10%–15% of clinically recognized pregnancies. As many as 80% of miscarriages occur before 12 weeks of gestation, with miscarriage rates decreasing sharply after the first trimester.[2] There are many causes of miscarriage including chromosomal abnormalities, uterine abnormalities, infection, endocrinological abnormalities, and autoimmune diseases.[3]

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Thyroid hormones have been found to be crucial in brain development, maturation, and normal function. Optimal maternal thyroid hormone concentrations can play a critical role in maintaining a balanced inflammatory response in early pregnancy to prevent fetal immune rejection and promote normal placental development through the regulation of the secretion of critical cytokines and angiogenic growth factors by human decidual cells.[4] Thyroid dysfunction, in particular hypothyroidism, can affect the health of both mother and fetus during pregnancy. Hypothyroidism has a higher prevalence than hyperthyroidism (2.5% vs. 0.2%) during the gestational period[5] and has a statistically significant relationship with recurrent pregnancy loss in the first trimester.[6]

Free radicals or reactive oxygen species (ROS) are reactive molecules with an unpaired electron, and they are continuously produced in cells deliberately or as accidental by-products of metabolic routes.[7] A series of oxidation-reduction reactions in the metabolic transformation of dietary proteins, carbohydrates and fats take place in the mitochondria of animal cells. These reactions are called oxidative phosphorylation.[8] The end products are oxygen and its derivatives such as superoxide and hydroxyl radicals.[7]

A sophisticated defense mechanism against these ROS exerts its effect on balancing the harmful consequences of oxidative stress.[9] Superoxide dismutase (SOD) is found in the cytoplasm of aerobic cells and converts superoxide anion to hydrogen peroxide (H_2O_2). On the other hand, glutathione peroxidase has an important function in the detoxification of H_2O_2 into water and nonreactive oxygen molecule.[10] Malondialdehyde (MDA) is a major breakdown product split off from lipid peroxides and can be used to assess the degree of lipid peroxidation.[11]

Oxidative attack on essential cell components by ROS as a result of a disturbed oxidant-antioxidant system balance is recognized in the pathogenesis of placental deficiency syndromes such as preeclampsia and fetal growth restriction.[12,13] Elevated ROS levels can influence the oocytes and embryos in their environments, i.e., follicular or peritoneal fluid. Oxidative stress affects both implantation and early embryo development by modifying the key of transcription and hence modifying the gene expression.[14] Recently, it has been found that oxidative stress plays a key role in uterine contractions by stimulating free radicals induced prostaglandins which subsequently results in pregnancy loss.[15]

Although the relationship between spontaneous abortions and some circulating biomarkers of oxidative stress has been evaluated in some animal and human studies, sadly the levels are estimated only in women after two or more episodes of miscarriage while the data regarding their imbalance in one pregnancy loss is inadequate. Moreover, there are very few researches which demonstrate the association of thyroid hormones and oxidative stress in the process of spontaneous abortion. The aim of this study was to assess the status of serum MDA, SOD, thyroid stimulating hormone (TSH), T3, T4 and their association with each other in women with spontaneous abortion of one episode before 24 weeks of gestation.

Materials and Methods

The subjects were enrolled from the Department of Obstetrics and Gynecology, of a tertiary care hospital attached to a medical college, between September 2014 and June 2015. This study was approved by Institutional Ethical Committee. Thirty women with spontaneous abortion were enrolled as study group. Thirty women with an ongoing healthy pregnancy served as control group. They were matched in their age and gestational age with study group.

All the enrolled participants were subjected to demographic, anthropometric, and biochemical analysis. A detailed history with full clinical examination was performed in all the participants. Furthermore, information about socioeconomic status, personal history like hypertension/diabetes mellitus/smoking/alcohol/drug abuse was taken. An informed written consent was obtained from all of the participants. Patients with a history of diabetes mellitus, thyroid dysfunction, heart disease, hypertension, uterine fibroid, infectious disease, smokers, and alcoholics were excluded from the study.

Five milliliters of blood samples were obtained before administration of any medication and before any medical or surgical intervention by venipuncture under all aseptic conditions. Samples were allowed to clot at room temperature. Serum was separated by centrifugation at 3500 ×*g* for 10 min. The separated serum samples were immediately processed for MDA and SOD analysis and remaining serum samples were stored at −20°C for hormonal analysis.

TSH, T3, and T4 levels in serum were estimated using ST AIA-Pack on AIA-360 fully automatic analyzer, TOSOH, Japan.[16,17] MDA level in serum was estimated by method of Satoh.[18] SOD activity in serum was estimated by method of Marklund and Marklund.[19]

All statistical analysis, study, Microsoft Excel 2007 software and various online calculators. The results of laboratory tests in the study and control groups were summarized as mean ± standard deviation. Comparison between subjects (both participating groups) was done using Student's unpaired *t*-test. 95% confidence interval was taken into consideration and

$P < 0.05$ was regarded as statistically significant. Correlation (Pearson) analysis was used to test the linear relationship between parameters.

Results

Demographic characteristics and biochemical parameters of the study population are shown in Table 1. The mean maternal age, paternal age, gestational age, body mass index, blood pressure, Hb, and sugar did not differ significantly in both the participating groups. On the other hand, gravida (2.23 ± 1.14) and parity (1.67 ± 1.18) were found to be significantly ($P = 0.0146$, 0.0320) higher in women with miscarriage on comparison with controls (1.6 ± 0.77, 0.6 ± 0.77), respectively.

Table 2 shows a comparison of the measured thyroid profile and oxidative stress results between both the participating groups. These results showed that mean serum TSH levels were higher, while mean serum T3 levels were found to be lower in the study group than the control group and difference was found to be statistically significant ($P = 0.0115$, 0.0003). Serum T4 levels were found to be lower than control group, but the difference was not significant. Serum MDA levels were found to be higher (6.846 ± 1.5) than controls (4.588 ± 1.17) while SOD levels were found to be lower in women with miscarriage (2.26 ± 0.66) than that of control group (3.16 ± 1.13) and difference were found to be highly significant ($P = 0.0005$, <0.0001), respectively.

Table 1: Comparison of demographic characteristics and biochemical parameters in study and control group

Parameters	Case	Control	*P*	*t*
Maternal age (years)	28.13±4.82	26.03±4.84	0.1023 (NS)	1.6551
Paternal age (years)	31.4±4.36	29.33±5.29	0.1106 (NS)	1.6223
Gestational age (weeks)	12.5±4.73	13.5±4.39	0.3990 (NS)	0.8494
Gravida	2.23±1.14	1.6±0.77	0.0146 (S)	2.529
Parity	1.67±1.18	0.6±0.77	0.0320 (S)	2.207
BMI (kg/m^2)	21±3.89	20.73±3.46	0.7691 (NS)	0.2949
WHR (cm)	0.91±0.04	0.9±0.04	0.2395 (NS)	1.189
SBP	113.66±14.64	111.4±11.65	0.5098 (NS)	0.6635
DBP	73.07±6.86	72.13±10.92	0.6935 (NS)	0.3965
Hb (g/dl)	10.32±2.17	11.02±1.5	0.1473 (NS)	1.472
Sugar (mg/dl)	96.58±19.18	91.88±13.49	0.2774 (NS)	1.098

BMI: Body mass index; WHR: Waist-hip ratio; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; Hb: Hemoglobin; NS: Not significant; S: Significant

Table 2: Comparison of serum thyroid hormone and serum malondialdehyde and superoxide dismutase status in study and control group

Parameters	Case	Control	*P*	*t*
TSH (μIU/ml)	3.8±3.5	2.04±0.89	0.0115 (S)	2.682
T3 (ng/ml)	1.33±0.68	2.08±0.83	0.0003 (HS)	3.869
T4 (μg/dl)	9.32±2.93	10.18±2.44	0.2223 (NS)	1.234
MDA (nmol/ml)	6.846±1.5	4.588±1.17	<0.0001 (HS)	6.488
SOD (U/ml/)	2.26±0.66	3.16±1.13	0.0005 (HS)	3.768

MDA: Malondialdehyde; SOD: Superoxide dismutase; TSH: Thyroid stimulating hormone; NS: Not significant; S: Significant; HS: Highly significant

Impact of TSH, T3, and T4 on the MDA and SOD levels in patients with miscarriage and women with normal pregnancy was verified using linear (Pearson) correlation analysis.

Table 3 shows significant positive correlation of TSH ($r = 0.6205$, $P = 0.0003$) with MDA levels and negative correlation of TSH ($r = -0.3474$, $P = 0.060$) with SOD levels in women with spontaneous loss of pregnancy. On the other hand, both T3 and T4 levels had negative correlation with MDA ($r = -0.1094$, -0.1507) and positive correlation with SOD levels ($r = 0.2655$, 0.1404), respectively.

A partial positive correlation of TSH with MDA ($r = 0.03497$, $P = 0.8544$) and negative correlation with SOD ($r = -0.1354$, $P = 0.4756$) has been found in the group of women with normal ongoing pregnancy as shown in Table 4. The results also depicted that T3 has a positive correlation with both MDA ($r = 0.08055$, $P = 0.6722$) and SOD levels ($r = 0.1905$, $P = 0.3133$), T4 has negative correlation with MDA ($r = -0.06225$, $P = 0.7838$) and positive correlation with SOD ($r = 0.2957$, $P = 0.1126$).

Table 5 shows that there is significant negative correlation of MDA with SOD levels in study ($r = -0.4512$, $P = 0.0123$) and control ($r = -0.4567$, $P = 0.0112$) group.

Discussion

The purpose of the present study was to evaluate the status of serum thyroid hormones, MDA and SOD in women with

Table 3: Correlation of thyroid profile with malondialdehyde and superoxide dismutase in study group

Thyroid hormones	Correlation	Correlation coefficient (*r*)	95% CI		*P*
			Lower	Upper	
TSH	Serum-TSH–serum-MDA	0.6205	0.3350	0.8016	0.0003 (HS)
	Serum-TSH–serum-SOD	−0.3474	−0.6290	0.01482	0.060 (NS)
T3	Serum-T3–serum-MDA	−0.1094	−0.4520	0.2612	0.5648 (NS)
	Serum-T3–serum-SOD	0.2655	−0.1048	0.5712	0.1561 (NS)
T4	Serum-T4–serum-MDA	−0.1507	−0.4847	0.2217	0.4267 (NS)
	Serum-T4–serum-SOD	0.1404	−0.2316	0.4767	0.4592 (NS)

MDA: Malondialdehyde; SOD: Superoxide dismutase; TSH: Thyroid stimulating hormone; NS: Not significant; HS: Highly significant; CI: Confidence interval

Table 4: Correlation of thyroid profile with malondialdehyde and superoxide dismutase in control group

Thyroid hormones	Correlation	Correlation coefficient (*r*)	95% CI		*P*
			Lower	Upper	
TSH	Serum-TSH–serum-MDA	0.03497	−0.3295	0.3904	0.8544 (NS)
	Serum-TSH–serum-SOD	−0.1354	−0.4727	0.2365	0.4756 (NS)
T3	Serum-T3–serum-MDA	0.0805	−0.2882	0.4285	0.6722 (NS)
	Serum-T3–serum-SOD	0.1905	−0.1824	0.5154	0.3133 (NS)
T4	Serum-T4–serum-MDA	−0.0623	−0.4133	0.3049	0.7838 (NS)
	Serum-T4–serum-SOD	0.2957	−0.07234	0.5929	0.1126 (NS)

MDA: Malondialdehyde; SOD: Superoxide dismutase; TSH: Thyroid stimulating hormone; NS: Not significant; CI: Confidence interval

Table 5: Correlation of malondialdehyde with superoxide dismutase in study and control group

Group	Correlation coefficient (*r*)	95% CI		*P*
		Lower	Upper	
Study group	−0.4512	−0.6980	−0.1085	0.0123 (S)
Control group	−0.4567	−0.7016	−0.1153	0.0112 (S)

CI: Confidence interval; S: Significant

spontaneous abortion and also to examine the association between serum thyroid hormones and levels of serum MDA and SOD.

Thyroid disease is the most common endocrine condition affecting the women of reproductive age. Thyroid hormones are essential for the sustenance of the developing fetus and fetus thyroid gland is not fully functional until after 12 weeks of pregnancy. Therefore, if the mother does not have sufficient thyroid hormones, she may be at increased risk of miscarriage.[6]

In the present study, altered thyroid profile was seen in women with spontaneous pregnancy loss. The results showed the significant elevation in serum TSH (3.8 ± 3.5, $P = 0.0115$) levels and significant decrease in serum T3 (1.33 ± 0.68, $P = 0.0003$). Although serum T4 were also found to be lower in women with loss of pregnancy, we found no significant difference on comparison with controls. These findings are in consistence with the study of Barapatre and Vaidya that elevated serum TSH levels without alteration in T3 and T4 during pregnancy increases the risk for miscarriage in both first and second trimester.[20] Allan *et al*. also reported that women with TSH levels above 6 mIU/ml has a significant increased risk for still birth[21] and further risk of miscarriage increases by 15% for each 1 mIU/ml elevation of TSH level.[22] Both overt hypothyroidism and subclinical thyroid dysfunction can have adverse effects on fetal and maternal outcome, but in women with subclinical hypothyroidism, gestational age is low at the time of abortion.[23]

Thyroid hormones play an important role in maintaining a viable pregnancy and contributing to healthy offspring. During pregnancy, increased thyroid hormone demand leads to increased iodine uptake and hence increased synthesis of thyroid hormones. Estrogen induces a rise in serum thyroid binding globulin, and the placenta releases several thyroid stimulatory factors in excess, for example, human chorionic gonadotropin, alpha subunit of which is identical to TSH and with a weak thyrotropic activity.[24,25] Maternal thyroxin is particularly critical early in pregnancy, because of reliance of fetus on transplacental passage of maternal thyroxine until about 12 weeks of gestation. From this time onward, maternal as well as fetal thyroid hormones seem to be necessary for normal neurodevelopment.[26] Thyroid disease interferes with human reproductive physiology, reduces the likelihood of pregnancy and adversely affects pregnancy outcome, thus becoming relevant in algorithm of reproductive dysfunction.[27] Subclinical hypothyroidism too appears to have an adverse impact on the pregnancy with increased incidence of miscarriage, gestational diabetes mellitus, gestational hypertension, and preeclampsia.[21]

Hence, it is evident that increased TSH levels and inadequate thyroid hormone supply might be one of the causative factors for spontaneous abortion. Therefore, it is suggested that the universal screening for detection of thyroid dysfunction among pregnant women attending antenatal clinic should be done compulsorily and the treatment of thyroid dysfunction should be started as early as possible after the diagnosis of thyroid dysfunction to reduce thyroid manifestations in maternal complications.

Oxidative stress of placenta plays an important role in pathogenesis of many pregnancy complications including miscarriages, preeclampsia, and preterm labor.[8] The current study showed that serum MDA levels were found to be significantly higher (6.846 ± 1.5, $P \leq 0.0001$) in women with loss of pregnancy than control group (4.588 ± 1.17) consistent with earlier studies.[28-30] Study of Sane *et al*. also reported elevations of serum MDA levels in women with either induced or spontaneous abortions compared with controls.[31] Since MDA is a byproduct of lipid peroxidation, therefore, an elevation in MDA levels may reflect an overproduction of lipid peroxides and/or impaired antioxidant defense mechanism.

These lipid peroxides are produced mainly in the placenta due to membrane disruption[32] and concentration of lipid peroxide increases in the deciduas of women undergoing early pregnancy loss.[28] Here also a significant positive correlation (r = 0.6205, P = 0.0003) was found between serum TSH and serum MDA levels indicating that raised oxidative stress with consequent enhanced lipid peroxidation as the TSH levels increased further increase the risk of miscarriage.

Increased levels of antioxidants have been documented in normal pregnancy, whereas loss of antioxidant defense has been observed in patients with recurrent spontaneous abortion as a result of their increased consumption.[33] In the present study, there was a significant reduction in serum SOD levels (2.26 ± 0.66, P = 0.0005) in women with spontaneous loss of pregnancy on comparison to the healthy pregnant controls (3.16 ± 1.13) consistent with earlier studies.[34,35] This oxidative stress in terms of decreased SOD activity might be involved in the termination of spontaneous abortions through stimulating prostaglandins synthesis.[28] Saad *et al.* also reported that elevation in plasma levels of MDA along with decreased levels of SOD and other antioxidant enzymes was associated with enhanced lipid peroxidation, which may end in spontaneous abortion.[36] Serum SOD activity is reported to be important for corpus luteum activity, embryonic development, and maintenance of early pregnancy. In the gestational corpus luteum, theca interna cells stain heavily for SOD activity.[37] Hence, SOD activity might be an important factor for the maintenance of fertility and early pregnancy and reduced SOD levels may results in loss of otherwise wanted pregnancy. This depletion of SOD could also be as a result of increased free radical production in terms of raised levels of MDA as pregnancies that went successfully to term were reported to be associated with increased plasma levels of SOD early in the first trimester.[35] The present investigation also pointed out the negative correlation of TSH with levels of SOD indicating that increased TSH levels might be involved in the decrease of SOD levels resulting in enhanced oxidative stress, imbalance in antioxidant/oxidant ratio and hence loss of pregnancy.

Conclusion

Increased serum TSH and serum MDA levels and decreased serum T3 and SOD levels in women with spontaneous abortion up to 24 weeks of gestational age indicate that alteration in thyroid profile results in enhanced oxidative stress and loss of antioxidant defense, which may subsequently result in the termination of pregnancy and expulsion of products of conception out of the uterine cavity. Thus, there is need of hour to look at this arena of research to decrease the oxidative stress, increase the levels of antioxidants in pregnant women with disturbed thyroid profile.

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Conflicts of interest

There are no conflicts of interest.

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