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Estimation of malondialdehyde and catalase activity in pregnant women at IIMS&R Hospital, Lucknow, India

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ABSTRACT

Background: During pregnancy, the physiological production of reactive oxygen species (ROS) is associated with a variety of maternal, placental, and fetal developmental functions. These functions are disrupted by excessive amounts of ROS, resulting to pregnancy complications. Different stages of pregnancy require a balance between oxidant and antioxidant production.

Objective: The aim of this study is to investigate the status of malondialdehyde (MDA) and catalase (CAT) activity in pregnant and age matched non-pregnant women.

Methods: In this cross-sectional study, a total of 74 participants were enrolled, including 37 pregnant and 37 age-matched non-pregnant women. The age range of participants was 18 to 40 years. MDA and CAT levels were measured spectrophotometrically. A p-value 0.05 was statistically significant.

Results: Compared to non-pregnant women, the mean plasma level of MDA was considerably elevated in pregnant women ($p<0.0001$). However, the mean level of CAT activity in pregnant women was significantly lower than in non-pregnant women ($p<0.0001$) In pregnant women, there is a significant strong negative correlation between MDA levels and CAT activity ($p<0.01$).

Conclusion: Pregnant women have high level of oxidative stress, indicating that the pregnant women are more susceptible to oxidative damage and may develop pregnancy-associated complications.

Keywords: catalase, oxidative stress, malondialdehyde, pregnancy, reactive oxygen species

Introduction

Pregnancy is a stressful condition that alters numerous metabolic and physiological functions and is characterized by a drastic increase in energy and oxygen demand for adequate fetal development and growth. Consequently, remarkable and drastic events occur during this period for sustaining mother and fostering the growth and maintenance of fetus [1]. In late pregnancy, negative energy equilibrium may be responsible for the development of oxidative stress, leading to increased lipid peroxidation and decreased antioxidant activity as contributing factors for complications in pregnancy [2]. In normal pregnancy, there is increase in oxidative stress because of high energy demand and increased requirements for tissue oxygen. Thus, both the mother and fetus are likely to experience oxidative stress at the time of pregnancy [3]. During a normal pregnancy, oxidative stress stimulates antioxidant mechanisms through enzymatic activity and non-enzymatic free radical quenchers. However, pregnancy is a condition in which this adaptation and balance may be disrupted [4].

Oxidative stress can be defined as a state of imbalance between reactive oxygen species (ROS)

and the mechanisms of detoxification and repair [5]. ROS react with essential cellular structures and molecules and alter their biological functions. Correspondingly, reactive nitrogen species (RNS) such as nitrous oxide (NO) or peroxynitrite (ONOO–) also have an impact on cells or produce excess of toxic products. In recent years the role of decreasing antioxidants and increasing oxidative stress is gaining significance as they are a threat for normal pregnancy. Certain biochemical indices are useful in assessing the progression of pregnancy [1].

During pregnancy, various maternal and placental functions and fetal developmental functions results in physiological generation of ROS. Abnormal overproduction of ROS disrupts these functions leading to pregnancy associated complications which may interfere in oocyte maturation, luteolysis, and embryo implantation [6]. The production and neutralization of ROS are balanced by enzymatic and non-enzymatic antioxidants systems in the human body [7].

Catalase (CAT) is an enzymatic antioxidant that converts hydrogen peroxide (H_2O_2) into water and oxygen. The H_2O_2 produced is reduced to superoxide radical ($O_2^{\cdot-}$) by the enzyme superoxide dismutase (SOD) [8]. This enzyme activity may define oxidative stress that occurs in the body [9]. Catalase is a hemoprotein containing four heme groups, and is found in both animals and plants. Catalase is evolutionary conserved protein and in found in almost all organisms. However, most organisms have more than one type of catalase. This enzyme is produced in the blood, bone marrow, mucous membranes, kidney, and liver of humans. [10]. Oxidative stress increases during pregnancy due to imbalance between oxidants and antioxidants [11]. It is reported that the levels of oxidants increase and the antioxidants decrease during pregnancy [12]. A marker of oxidative lipid degradation is malondialdehyde (MDA), which can be measured by the thiobarbiturate assay (TBA).

Catalase and MDA can be used as parameters to assess oxidative stress. The MDA levels and catalase enzyme activity in pregnant women have not been widely reported from hospitals in India. Therefore, the present study aimed to investigate the oxidative stress biomarker (MDA) and enzymatic antioxidant (CAT) in pregnant women with age matched non-pregnant women to correlate the MDA and CAT activity in pregnant women.

Materials

Characteristic of subjects

In this cross sectional study, a total of 74 subjects (37 pregnant and 37 age matched non-pregnant women) aged between 18-40 years were enrolled from the outpatient department, Obstetrics & Gynaecology, IIMSR, Integral University, Lucknow, India (Table 1). The gestational age of pregnant subject were not recorded. This study was approved by the Institutional Research and Ethical Committee (IEC/IIMS&R/2017/11) and followed the ethical standards of the 1964 Helsinki Declaration and its later amendments or comparable ethical standards [13]. Detailed medical history and written informed consent were taken from each subject enrolled for the study. The pregnancy of women were diagnosed by using urine human chorionic gonadotropin (HCG) pregnancy test kit (PregaNews, Mankind Pharma Ltd, New Delhi, India) and further confirmed by the physician. Subjects with diabetes, ischemic heart disease, angina, myocardial infarction (MI), electrocardiogram (ECG) abnormalities, anemia (Hb of 8.0 g/dl or less), chronic liver and kidney diseases, hypothyroidism or those on drugs for antihypertension and diuretics were excluded for both pregnant and non-pregnant groups. Subjects with a smoking history and exposure to biomass fuels were also excluded.

Estimation of MDA by thiobarbituric acid reactive substances (TBARS) method

MDA was estimated by the TBARS method. Plasma was deproteinised and the precipitate was treated with TBA at 90°C for 1 hour [11]. The pink color indicated the concentration of thiobarbituric acid reactive substance (TBARS), which was measured using a UV-Visible double beam spectrophotometer at 530 nm. (Systronics-2205, Systronic India Ltd.

Table 1: Clinical parameters of pregnant and non-pregnant women

Parameters	Pregnant women (n=37)	Non-pregnant women (n=37)	p value
Age (years)	26.03±4.64	27.41±4.12	0.18
Weight (kg)	52.86±6.30	48.70±5.36	0.003*

Data was represented as mean ± SD (Standard deviation); *p < 0.05, considered as statistically significant; CAT: catalase, MDA: malondialdehyde

Table 2. Correlation of clinical and oxidative stress parameters among pregnant women

Parameters	Age (years)	Weight (kg)	MDA (µmol/L)	CAT (Units/mg Hb)
Age (years)	1	0.443**	-0.097	-0.079
Weight (kg)	-	1	0.034	-0.127
MDA (µmol/L)	-	-	1	-0.543**
CAT (units/mg Hb)	-	-	-	1

*Correlation is significant at the 0.05 level (2-tailed)
**Correlation is very significant at the 0.01 level (2-tailed); CAT: catalase, MDA: malondialdehyde

Gujarat, India). The MDA concentration was calculated according to the following formula: MDA (µmol/l) = A (532 nm) × 1.75/0.156.

Determination of catalase activity

The catalyse activity was determined by the published method [14]. The enzyme activity was measured by the chromic acetate and hydrogen peroxide method. Catalase enzyme was allowed to split H_2O_2 for different time periods. The reaction was stopped at different time intervals by the addition of dichromate acetic acid mixture and the remaining H_2O_2 was determined by measuring chromic acetate colorimetrically [14]. The chromic acetate formed was measured at 590 nm using UV-Visible double beam spectrophotometer (Systronics-2205, Systronic India Ltd. Gujarat, India). The activity of catalase was expressed as µmoles of hydrogen peroxide consumed/min/gm/Hb or unit/gm Hb and calculated by calibration curve.

Statistical analysis

Data analysis was performed using the IBM SPSS software version 20.0 (Armonk, NY, USA). The p-values among the groups were determined using analysis of variance (ANOVA). Values arerepresented as the mean ± SD (standard deviation). Pearson correlation coefficient was calculated among pregnant women. A p-value <0.05 was considered as statistically significant for the data analysed.

Results

Oxidative stress can be measured by MDA levels, a biomarker of lipid peroxidation. In this cross sectional study, the mean of MDA levels was 2.71 ± 1.29 µmol/L. This value was significantly higher among pregnant women than non-pregnant women (p<0.0001). However, the mean of CAT activity was found significantly low in pregnant women as compared to non-pregnant women (p<0.0001) (Table 2, Figure 1). Similarly, the catalase activity levels were found significantly low in pregnant women. The levels of MDA and CAT activity have shown a significant negative correlation among pregnant women (p<0.01) (Table 2).

Discussion

The normal pregnancy collates with increasing oxidative stress because of high demand of metabolic and increased requirement of tissue oxygen [15]. MDA is a stable end product which is produced by lipid peroxidation and is a reliable marker for

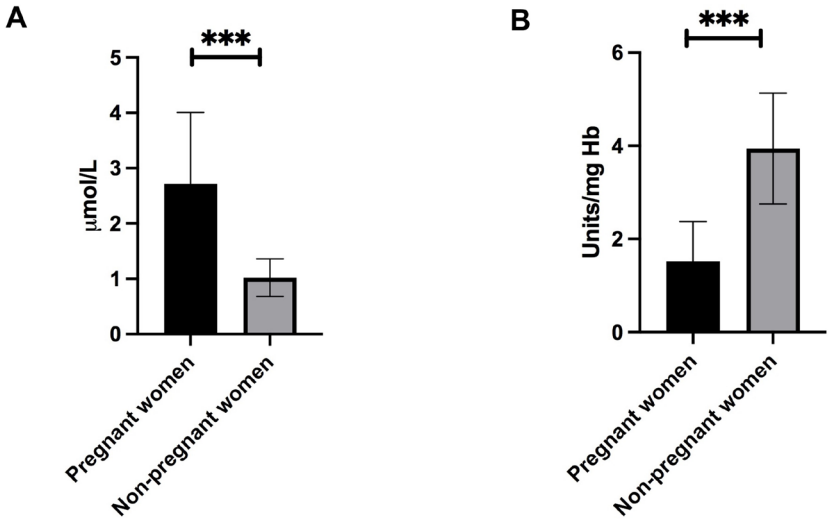

Figure 1. Oxidative stress parameters of pregnant and non-pregnant women. (A) MDA; (b) catalase activity

the assessment of free radical induced damage to tissues. Lipid peroxidation is increased during the progression of normal pregnancy [12]. The balance between the production of ROS and activation of antioxidant mechanisms protects tissues from damage and prevents disorders. During pregnancy the antioxidant system was stronger than peroxidation. Status report shows that $PM_{2.5}$ particulate matter with diameter ≤ 2.5 µm is associated with adverse pregnancy because oxidant mechanism occurs during the pregnancy [16].

Placental lipid production is controlled by placental antioxidant system [17]. ROS function as signal transduction molecule in normal physiology. However, their overproduction may result in numerous human health problems. Although the body's own mechanism plays a crucial role in controlling the level of these free radicals, the levels of antioxidants that counterbalance these oxidative radicals get impaired themselves [6]. In this study, we assess the serum MDA and catalase levels and the values of these markers were compared between pregnant and non-pregnant women. The result of this study show that the mean level of MDA was found significantly higher in pregnant women as compared to non-pregnant women ($p<0.0001$).

Physiologically, the concentration of total cholesterol, low density lipoprotein-cholesterol, and triglycerides increases during pregnancy. With oxidative stress, the lipid peroxidation including the concentration of MDA also increases after 25 weeks of pregnancy in pregnant women [18]. The pregnant women with iron deficiency anaemia are likely to have elevated pro-oxidant and diminished antioxidant status, which may increase the risk for both the pregnant women and fetus [19].

Thus, suppression of oxidative stress by antioxidant system, such as catalase, may play an important role in preventing preterm labor [20]. Overall, we found reduction in individual antioxidant levels in normal pregnancy. The decreased catalase activity in our study can be attributed to the decline in various antioxidants during pregnancy. Therefore, even though individual antioxidants rise during pregnancy, the net result maybe a lower antioxidant capacity [15]. In our study, the decrease in catalase and increase of MDA were statistically significant.

Pre-pregnancy body mass and weight gain during pregnancy are useful measures of maternal nutrition. These factors interact to determine birth weight: on average, women with lower pre-pregnancy body mass needs to gain more weight during pregnancy to deliver infants of the same birth weight as women who start pregnancy with higher body mass [21]. The first estimates of pre pregnancy body mass and weight gain during

pregnancy in India as compared to sub-Saharan Africa: 42.2% of Indian women are underweight when they begin pregnancy compared with 16.5% of African women. In both regions, women gain little weight during pregnancy, but because of pre pregnancy deficiency, Indian women end pregnancy weighing less than African women [22].

Conclusion

The MDA level is significantly increased in pregnant women when compared with non-pregnant women. The level of enzymatic antioxidant i.e. catalase is significantly decreased in pregnant women as compared to non-pregnant women. According to the Karl Pearson's correlation coefficient, there is a negative correlation between MDA and catalase i.e., MDA is increased and catalase is decreased in pregnant women. Hence, the increased concentration of MDA and decreased concentration of catalase in pregnant women supports the hypothesis that oxidative stress occur in pregnant women.

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Author contribution

NS: conducted data collection, wrote the manuscript. SK: supervised study, contributed to the completion of the manuscript. MMK: supervised study, contributed to the completion of the manuscript. HA: reviewed and finalized the manuscript, contributed to the completion of the manuscript. RA: guided to study design, supervised study, reviewed and finalized the manuscript.

Conflict of interest

No conflict of interest.

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