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Research Article

Study of Changes in Lipid Profile, Lipid Peroxidation and Superoxide Dismutase during Normal Pregnancy

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ABSTRACT

Altered metabolic and hormonal status of the body in pregnancy leads to changes in lipid profile and oxidative stress. The present study was conducted to assess serum total cholesterol (TC), low density lipoprotein (LDL), high density lipoprotein (HDL), very low density lipoprotein (VLDL) and triglycerides (TG) in healthy pregnant females and correlate these values with markers of oxidative stress; serum malondialdehyde (MDA), a marker for lipid peroxidation and superoxide dismutase (SOD), an antioxidant enzyme in the same subjects during the three trimesters of pregnancy. In this case control study, 54 pregnant females were assessed and compared with 60 healthy non-pregnant females who served as controls. Five pregnant females developed pregnancy related complications in the 2nd trimester & were dropped out and three more were not available for follow-up in the 3rd trimester. The outcome of the study revealed increased serum total cholesterol, low density lipoprotein, very low density lipoprotein and triglycerides values as gestation progressed. Serum high density lipoprotein showed a progressive rise till 2nd trimester and tapered off in the 3rd trimester. Serum MDA and SOD levels also increased during the entire course of pregnancy. Conclusively, increased lipid profile parameters can account for increased lipoperoxidation seen in the form of rising MDA which is counterbalanced by a rise in antioxidant enzyme SOD. As lipid excess and oxidative stress can provoke endothelial dysfunction, lipid profile estimation and monitoring should be made a part of routine investigations during antenatal period and the pregnant subjects should be supplemented with antioxidants to prevent overwhelming of oxidative stress.

Key words: Lipid profile, oxidative stress, MDA, SOD

INTRODUCTION

Changes in the plasma lipids during pregnancy have been recognized and thought to be mostly due to alterations in the hormonal milieu in the form of rise in insulin, progesterone, 17-B estradiol and human placental lactogen (Cunningham et al., 2001) (Alvarez *et al.,* 1996). These changes occur as a result of increased metabolic demands by the mother.

In early pregnancy, there is increased body fat accumulation associated with both hyperphagia and increased lipogenesis, while in late pregnancy, there is accelerated breakdown of fat depots which play an important role in fetal development (Herrera, 2002). The increase in maternal lipid profile during pregnancy differs with trimesters. The concentration of serum total cholesterol, serum triglycerides, serum HDL and serum LDL in normal pregnant women increase with increasing gestational age**.** The increase in maternal lipid profile is in response to the maternal switch from carbohydrate to fat metabolism, which is an alternative pathway for energy generation due to high demand. This increase has been attributed to decrease in maternal glucose insulin sensitivity (Sivan *et al.*, 1999).

The process of oxidative conversion of unsaturated fatty acids to primary products known as lipid hydroperoxides and a variety of secondary products is referred as free radical process of lipid peroxidation (Aurousseau *et al.,* 2006). Increased lipid levels in pregnancy may increase the susceptibility of polyunsaturated fatty acids (PUFA) to peroxidation damage by free radicals that may lead to increased production of malondialdehyde (MDA), a marker for lipid peroxidation (Ciragil et al., 2005). In health, Reactive Oxygen Species (ROS) and antioxidants remain in balance but this balance is disrupted in cases of oxidative stress (Aurousseau et al., 2006). Pregnancy, being a physiological state, is accompanied by a high energy demand of many body functions and an increased oxygen requirement because of which augmented levels of oxidative stress would be expected (Desai *et al.,* 2003).

The weight increase during pregnancy most probably causes blood lipidemia and increased lipid autoperoxidation (Bayhan *et al.*, 2002). Normal pregnancy is associated with oxidative stress causing increase in lipid peroxidation products, but this peroxidation is balanced by adequate anti oxidative responses (Chaudhari *et al.*, 2003). The anti oxidant enzyme SOD (Superoxide dismutase) activity increases throughout pregnancy. This occurs in response to normal oxidative stress due to pregnancy (Nakai *et al.*, 2000).

In our present study, we have tried to determine the following during the course of normal pregnancy:

a) Changes in lipid profile.

b) Changes in MDA and SOD levels as markers of oxidative stress.

c) Whether oxidative stress if present, is related to changes in the lipid profile.

MATERIALS AND METHODS

The present study was conducted on healthy pregnant females attending the antenatal clinic of Deptt. of Obstetrics and Gynaecology, Govt. Medical College, Amritsar. Various physiological and biochemical parameters were assessed in the Department of Physiology in collaboration with the Department of Biochemistry of the same institute. The appropriate institutional board approved the protocol used in this study.

Subjects

The study was conducted on a total number of 114 female subjects in the age group of 20-40 years, out of which, 60 were healthy non-pregnant females who served as controls and 54 were selected in first trimester and assessed in the second and third trimesters. The subjects were assessed for lipid profile, MDA and SOD along with certain physiological parameters like height, weight and body mass index (BMI) in the three trimesters. The subjects were divided into the following groups: group A- 60 healthy non-pregnant females, group B- healthy pregnant females further sub-divided into three sub-groups: group B-I (1^{st} trimester), group B-II (2^{nd} trimester) and group B-III (3^{rd} trimester). Out of the 54 pregnant females enrolled, five were dropped out in the 2^{nd} trimester due to pregnancy related complications and further three more were unavailable for follow-up in the 3^{rd} trimester. Table 1 shows the distribution of subjects in various groups.

Inclusion criteria

Healthy non-pregnant and pregnant females and consumers of mixed diet/food.

Exclusion criteria

It included risk factors for oxidative stress such as pre-eclampsia, AIDS, DM, TB, smoking and alcohol consumption. Also excluded were females with family history of obese children and multifetal pregnancies or any history of familial hyperlipidemia.

A detailed history including obstetric history was taken. A complete physical examination including weight, height and BMI (body mass index measured as weight/(height(m))^2) were done. An informed consent was taken from the subjects.

Fasting blood samples were taken and the following parameters were estimated in both cases and controls:

Serum MDA levels (Satoh, 1998)

Serum SOD levels (Marklund and Marklund, 1974) (Nandi and Chatterjee, 1988)

Lipid profile was done as follows:

i) Total serum cholesterol (CHOD-PAP Method)

ii) Serum triglycerides (GPO-Trinder Method)

iii) Serum High density lipoproteins (HDL) (Phosphotungstic Acid Method)

iv) Serum Low density lipoproteins (LDL) (Freidwald equation)

v) Serum Very low density lipoproteins (VLDL) (Freidwald equation)

Statistical analysis

Statistical analysis was done by using one way ANOVA with Post Hoc Tukey HSD comparing mean values of all the variables between the four groups. Pearson's co-efficient was used to find out the co-relation between the two variables. SPSS 17.0 software was used.

RESULTS

In the present study, a total number of 54 healthy females were assessed for the evaluation of oxidative stress and changes in lipid profile during the three trimesters. The observations were compared with 60 healthy controls as per the groups divided. According to table 2, mean values for age and height showed variation in group B-II and group B-III. This was because of drop out of subjects in the 2^{nd} and 3^{rd} trimesters. The increase in mean value for weight showed statistically highly significant ($p<0.001$) results between group B-I & group B-III, between group B-II & group B-III and between group A & group B-III. This increase was statistically significant ($p<0.05$) in women between group B-I & group B-II and between group A & group B-II and insignificant ($p>0.05$) between group A & group B-I.

Table 1: Distribution of subjects in various groups

Group A	Controls	60
Group B-I	1st trimester	54
Group B-II	2nd trimester	49
Group B-III	3rd trimester	46

Table 2: Mean values for all the parameters in four groups

Parameter	**Group A** Controls (N=60) (Mean±SD)	**Group B-I** 1ST Trimester (N=54) (Mean±SD)	**Group B-II** 2ND Trimester (N=49) (Mean±SD)	**Group B-III** 3RD Trimester (N=46) (Mean±SD)
AGE (years)	25.83±4.42	25.46±4.01	25.14±4.04	25.39±4.16
WEIGHT (kgs)	55.48±4.47	55.06±4.80	58.51±4.60	64.00±3.32
HEIGHT (cms)	152.22±6.95	151.20±7.89	151.29±7.21	151.83±7.13
BMI (kg/m^2)	24.09±2.90	24.26±3.22	25.74±3.22	27.95±3.11
SOD (U/ml)	1.61±0.57	3.20±0.47	3.70±0.46	6.12±0.95
MDA (nmol/L)	2.61±0.72	2.63±0.55	3.61±0.74	5.81±0.59
TOTAL CHOLES-TEROL (mg/dl)	149.80±8.26	166.90±9.81	212.59±18.40	229.78±14.02
TRIGLYCERIDES (mg/dl)	70.77±8.96	102.61±7.98	155.76±16.32	194.50±9.09
LDL (mg/dl)	77.72±5.94	100.41±5.50	112.12±7.68	129.61±7.63
HDL (mg/dl)	40.97±2.95	41.80±3.41	62.27±8.74	43.63±3.36
VLDL (mg/dl)	16.62±2.61	17.26±3.33	25.94±3.89	22.33±5.87

SD: Standard deviation

Table 3: *p*-Values for all the parameters

Parameter	**A vs B-I**	**A vs B-II**	**A vs B-III**	**B-I vs B-II**	**B-I vs B-III**	**B-II vs B-III**
WEIGHT	0.94***	0.002***	0.000*	0.001**	0.000*	0.000*
BMI	0.990***	0.03**	0.000*	0.079***	0.000*	0.001**
SOD	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
MDA	0.998***	0.000*	0.000*	0.000*	0.000*	0.000*
TOTAL CHOLESTEROL	0.000*	0.000*	0.000	0.000*	0.000*	0.000*
TRIGLYCERIDES	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
LDL	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
HDL	0.821***	0.000*	0.041**	0.000*	0.279**	0.000*
VLDL	0.827***	0.000*	0.000*	0.000*	0.000*	0.000*

Highly significant,Significant,***Not significant*
p>0.05: Not significant, p<0.05: Significant, p<0.001: Highly significant

The mean value for BMI also showed an increase from 1st trimester to 3rd trimester. This increase was not significant between group A & group B-I and between group B-I & group B-II, but highly significant between group A & group B-III and between group B-I & group B-III. The increase between group A & group B-II and between group B-II & group B-III was statistically significant (Table 3).

The oxidative stress markers also showed a rise from 1st to 3rd trimester. The mean values for SOD showed a highly significant rise (when all the groups were compared) from 1st trimester to 3rd trimester as well as when compared to controls. The mean values for MDA showed a progressive rise as the gestation progressed. The increase was highly significant between all the groups (i.e., between group A & group B-II, between group A & group B-III, between group B-I & group B-II, between group B-I & group B-III, between group B-II & group B-III) except between group A & group B-I which was not significant.

Mean values for serum total cholesterol increased throughout the three trimesters and this increase was highly significant amongst all the groups. Similar rise was seen for serum triglycerides and serum LDL as shown in table 2. The mean values for serum HDL showed a progressive rise till the 2nd trimester and a fall during the 3rd trimester. This rise was highly significant between group A & group B-II and between group B-I & group B-II and not significant between group A & group B-I and group B-I & group B-III. Serum VLDL values showed an increase in all the groups which was highly significant except between group A and group B-I.

In the 1st trimester, we observed a positive correlation between SOD and serum TC & TG which was highly significant for TC. Serum MDA showed a positive correlation with HDL. In the 2nd trimester, SOD showed a positive correlation with LDL, HDL & VLDL which was significant for LDL. MDA showed a positive correlation with TC & LDL levels and it was significant for LDL. In the 3rd trimester, SOD showed a positive correlation with TC, TG, LDL & VLDL and it was significant for TC. MDA showed a positive correlation with TG & VLDL.

DISCUSSION

Pregnancy is a stressful condition in which many physiological and metabolic functions are altered to a considerable extent (Walsh, 1994). Metabolic adaptations of pregnancy include insulin resistance, hemodilution, hyperlipidemia and weight gain with increased fat deposition in the trunk as well as thighs (Gunderson, 2003). Raised levels of oxidative stress would be expected during pregnancy because of high energy demand and increased oxygen requirement (Desai et al., 2003). Free radical oxidation during pregnancy becomes activated. The intensity of oxidative stress adjusts to the dynamic physiology, mother`s body weight and changes of blood lipid concentration (Oþeraitienë et al., 2005).

We observed an increase in mean values in weight of the pregnant subjects. Weight increase during pregnancy most probably caused blood lipidemia and increased lipid auto peroxidation (Bayhan et al., 2002). The increase in mean values for BMI follows a pattern somewhat similar to that seen in normal pregnancy (Chen *et al.*, 2005). The findings of increase in weight and BMI are associated with an increase in body fat percentage levels (Pasupathi et al., 2009).

The rise in mean values for MDA with increasing gestation are consistent with studies where increase in lipid peroxidation occurred as pregnancy progressed and placental lipoperoxidation increased with an increase in total serum lipids (Kharb and Singh, 2004) (Patil et al., 2007). The elevation of mean values of SOD with each trimester falls in accordance with studies by Gitto et al and Desai et al (Gitto et al., 2002) (Desai et al., 2003). These changes occur in response to the oxidative stress during pregnancy.

In our study, the serum levels for total cholesterol, LDL and triglycerides increased throughout gestation. These findings are supported by a study by Munoz et al (Munoz et al., 1995). This study also states that LDL and HDL levels are related to circulating estrogen and progesterone. The principal modulator of hypertriglyceridemia is estrogen as pregnancy is associated with hyperestrogenemia. Estrogen induces the hepatic biosynthesis of endogenous triglycerides which is carried by VLDL. This process is modulated by hyperinsulinism found in pregnancy (Adegoke et al., 2003). High HDL levels till 2nd trimester and a further decline during 3rd trimester are consistent with findings of Mankuta et al (Mankuta et al., 2010). Another study also supports the rise in total cholesterol, triglycerides, LDL and VLDL (Takahashi et al., 2008).

Oxidative stress may arise from intracellular accumulation of triglycerides having an impact on mitochondrial efficiency, resulting in accumulation of electrons in the electron transport chain which reacts with oxygen to form superoxide radical. The combination of high lipid levels and oxidative stress leads to production of lipid peroxides and oxidized

lipoproteins (Bjorkhem and Diczfalusy, 2002) (Vejux and Lizard, 2009).

With the progression of a normal pregnancy, gradual suppression of lipid peroxidation takes place through the activated production of endogenous antioxidants such as SOD to protect the fetus from toxic oxygen effects (Qanungo and Mukherjea, 2000). Both lipid peroxidation and antioxidation were enhanced during pregnancy.

We conclude that lipid peroxidation increases with a rise in lipid profile during normal pregnancy and a parallel rise in antioxidant defense. The enhanced antioxidation counterbalances lipoperoxidation. The fall in HDL levels in the 3rd trimester can be a potential risk factor for atherosclerosis as certain studies have incriminated the role of abnormal lipid metabolism and oxidative stress in the pathogenesis of atherosclerosis and hypertension. Hence we recommend the estimation and monitoring of lipid profile to be a part of routine investigation during pregnancy and also additional supplementation in the form of antioxidants to the pregnant females to protect them from oxidative stress.

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