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# OXIDATIVE MODIFICATION OF LIPOPROTEINS AS A MEASURE OF MACROSOMIA IN DIABETIC PREGNANCY

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**ABSTRACT**

The present study is centered on the concept that Macrosomia is a frequent complication of pregnancy in diabetes mellitus. In this article, we shed light on the abnormalities in lipoprotein fractions in Diabetic pregnancy can act as a measure of macrosomia. We also raise the question of the possible beneficial effects of *n*-3 PUFAs in diabetic pregnancy in the prevention and treatment of long-term metabolic abnormalities associated with macrosomia.

The cases for this study were selected from the Gynaecology and Obstetrics 'Out-patients' and 'In-patients' clinic of Sassoon General Hospital,Pune. In all 130 cases belonging to the age group of 20-45 years could be studied. Out of the 130 subjects, 50 subjects belonging to the III trimester of pregnancy were studied. The study could evaluate 30 subjects belonging to the III trimester diabetic pregnancy group. 50 healthy non-pregnant women constituted the control group Ill trimester diabetic pregnant women exhibited elevated levels of Malondialdehyde levels (MDA) as compared to III trimester normal pregnant women. Lipid peroxides in lipoprotein fractions i.e. HDL, LDL, VLDL exhibited a significant increase in diabetic pregnant women as compared to normal III trimester pregnant women.Free fatty acid levels were significantly increased in III trimester diabetic pregnant women as compared to III trimester normal pregnant women.

## INTRODUCTION

### Diabetic Pregnancy

In patients with diabetes mellitus the oxygen free radicals are produced in excess and may play a role in several diabetic complications such as Anephropathy, neuropathy and retinopathy. It was suggested that maternal oxygen free redials may be involved in the induction of foetal anomalies (Eriksson et al 1993).

Recently, it has been suggested that in diabetes the increased load of glucose in the mitochondria may acclerate the flow of electron through the respiratory chain inducing mitochondrial leakage of free radicals (Eriksson et il et al (1991).

Mikhali et at (1994) hypothesized that an enhanced production of such radials in embryonic tissues may be directly related to the increased risk of congential malformations in diabetic pregnancies. Thus, the site of generation of oxygen free radicals may be a factor in determining the nature or extent of free radical damage energy fuel to glucose (Knopp 1978).

### Metabolic Changes in Diabetic Pregnancy

The mechanism for the macrosomia in the infant of the diabetic mother was postulated Pedersen who suggested that the increased delivery of glucose to the foetus would stimulate the foetal endocrine pancreas to secrete increased amounts of insulin, thereby normalizing the blood sugar in the foetal circulation, but in the process storing the increased quantity of nutrients crossing the placenta to the foetus in adipose tissue and other tissues responsive to the growth stimulus of insulin.

That the pancreas of foetus and the infant of the diabetic mother is hyperplastic and secrets increased amounts of insulin to glycemic loads, is now well established in human, rat and primate. It is possible, however, that an increase flux of other nutrients across the placenta to the foetus could also stimulate increased insulin secretion. Thus, amino acids are known to stimulate insulin secretion. Under certain circumstances, the idea that an increase in maternal glucose concentration can lead to foetal macrosomia , which shows that the glucose transfer is increased in both the early and late gestation, but particularly in the late gestation. It is suggested that triglyceride fatty acids and potentially other fuels can cross the placenta in the increased amounts particularly in the type II (adult onset) diabetic. In this connection it is interesting that the most marked cases of foetal macrosomia are not associated with the juvenile-onset diabetes, but with those of adult onset diabetics with the early severe gestational diabetes or moderate insulin requirements.

### lipid metabolism in macrosomia

Diabetes has been considered as an important factor, altering maternal metabolism and complicating fetal development, regardless of diabetic type.Changes in lipoprotein metabolism during normal pregnancy are reflected by increased serum concentrations of nonesterifiedfatty acids (NEFAs; free fatty acids), triacylglycerols (TAGs; triglycerides), cholesterol, phospholipids and apolipoproteins (apos) .Diabetes mellitus is also associated with alterations in lipid levels and with changes in serum lipoproteins. It may be hypothesized, therefore, that diabetes during pregnancy may alter lipoprotein metabolism further.

**Serum Lipid Peroxide Levels in Lipoprotein Fractions**

Pre-eclampsia and eclampsia are the leading causes of maternal and foetal death for the last 40 years. In the development of the matrenal manifestations of pre-eclampsia, dysfunction of the vascular endothelial cells is considered to play a major role. Currently, the attention has been focussed on the potential endothelial activator such as lipid peroxides and cytokines, but there is an increasing recognition that marked perturbations in the lipid and lipoprotein metabolism may also be of fundamental importance in the pathogenesis of Diabetic pregnancy associated complications.

Branch et al (1994) measured the serum antiobodies to oxidised low density lipoproteins (IgG of OX-LDL), malondialdehyde conjugated low density lipoprotein (MDA-LDL) in sera of healthy pregnant and pre-eclamptic subjects. They observed that OX-LDL and MDA-LDL was increased in the sera of pre-eclamptic patients. Such patients had significantly higher mean titers of antiobodies to MDA-LDL than healthy pregnant women. In a multiple regression model, pre-eclamptic patients still had a significantly higher mean titer. This finding indirectly supports the hypothesis that the decidual vascular lesions of pre-eclampsia and the arterial lesions of atherosclerosis share components of a common pathophysiologic pathway which involves the enhanced lipid peroxidation. These findings are consistent with the hypothesis that the oxidation of LDL and/or the generation of other lipid peroxidation products contributes to the foam cell formation in the decidua and in the pathogenesis of pre-eclampsia.

**Free Fatty Acid**

The numerous changes in morphology and the functions which occur in pregnancy and energy requirement for foetal growth presuppose important modifications in the carbohydrate metabolism. Several considerations also suggest that fat metabolism may also undergo some changes in pregnancy, but this subject has received comparatively less attention so far by physiologists and obstetricians.

Persson and Lunell (1975) studied the change in FFA, glycerol and ketone bodies in pregnant gestational insulin-dependant diabetic patients and control subjects. They found that in control subjects, FFA,glycerol and ketone bodies were not above normal pregnant values, but diabetic patients showed great variations in all parameters measured. FFA and ketone bodies were significantly above normal. In gestational diabetic patients all these parameters varied in parallel with a similar pattern in diabetic patients.

Knopp et al (1979) reported that the free fatty acid concentration of gestational diabetes not treated with insulin is significantly increased as compared to control subjects.

**MATERIAL METHODS**

The cases for this study were selected from the Gynaecology and Obstetrics 'Out-patients' and 'In-patients' clinic of Sassoon General Hospital,Pune. In all 130 cases belonging to the age group of 20-45 years could be studied. Out of the 330 subjects, 150 cases belonged to the normal uncomplicated pregnancy group. 50 subjects belonging to the III trimester of pregnancy were studied. The study could evaluate 30 subjects belonging to the III trimester diabetic pregnancy group. 50 healthy non-pregnant women constituted the control group

The subjects belonged to the lower socio-economic strata and each patient was subjected to a detailed history and thorough physical examination as follows:

1. Detailed history including the name of the patient, address, income, age, registration number of O.P.D. and/or I.P.D.
2. Clinical history including blood pressure, oedema on feet, convulsions etc. was noted.
3. General history about the primi or multigravida, history of any abortion was also recorded.
4. Routine investigations e.g. Blood Sugar, Urine Sugar, Hemoglobin etc. were done.

The subjects were further divided into different groups as per clinical diagnosis. The patients were selected and blood samples were collected before starting any of the treatment including Vitamin C and Vitamin E. Venous blood samples were collected in a plain sterile bulb in the morning after an overnight fast. Blood Sugar levels and proteinuria was determined.

The serum was separated by centrifugation at 3000 rpm. and the following biochemical investigations were performed :

1. MDA (Malondialdehyde as an index of lipid peroxidation).
2. Lipid peroxide levels in various lipoprotein fractions namely HDL, LDL & VLDL.
3. Free fatty acids.

OBSERVATION TABLE II

Serum Lipid Peroxide (Total), Serum Lipid Peroxide fractions VLDL fractions, LDL fractions and HDL fractions and Serum Free Fatty Acids in Normal Non-Pregnant, Normal Pregnant (I, II, HI trimester), Pre-eclampsia (III trimester), Eclampsia (IIItrimester) and Diabetic Pregnancy (III trimester) Groups

| Study Group | Lipid Peroxides (Total) nmol MDA/ml | Lipid Peroxides (VLDL) nmol MDA/ml | Lipid Peroxides (LDL) . nmol MDA/ml | Lipid Peroxides (HDL) nmol MDA/ml | Free Fatty Acids fimol/Iit |
|---|---|---|---|---|---|
| Non-pregnant | ^2.08 ±0.71 | 0.49 ± 0.07 | 0.92 + 0. 16 | 0.67 ±0.08 | 500 + 40.13 |
| Normal Pregnant<br><br>III trimester (27-38 Weeks) | 3.92 + 0.58** | 1.53 ±0.41" | 1.52 + 0.19* | 0.87 ±0.14* | 961.10 + 25.54" |
| Diabetic Pregnancy (III trimester) | 7.19 + 0.30" | 1.80 ±0.70" | 4.14 + 0.51" | 1 25 + 0.29* | 982.18 + 14.42* |

Table II represents the values for serum total lipid peroxides and those in the lipoprotein fractions (VLDL,LDL and HDL) and also the concentration of serum free fatty acids in non-pregnant, normal pregnant(I,II,III trimester), pre-eclamptic (III trimester), eclamptic (III trimester) and diabetic pregnant (III trimester) women.

**Lipid peroxides - Total and Lipid peroxide fractions**

Appreciable oxidative stress can be illustrated by way of a statistically highly significant increase ($p<0.001$) in the serum level of circulating total lipid peroxides in the III trimester of normal pregnancy as compared to the non-pregnant group. A similar observation can be made for the III trimester pre-eclamptic, eclamptic and diabetic pregnant group on comparison to the III trimester normal pregnant group.

A generalized observation by way of the lipid peroxide levels being higher in the LDL fraction than the HDL fraction and being highest in the VLDL fraction (as circulating levels of TG are high) can be made for the physiological and pathological pregnancy groups.

The III trimester pre-eclamptic, eclamptic and diabetic pregnant groups exhibit a statistically highly significant increase ($p<0.001$) in the values of lipid peroxides associated with VLDL and LDL fractions on comparison to the same associated with the III trimester normal pregnant group.

A statistically significant increase ($p<0.01$) can be observed for the lipid peroxides associated with the HDL fraction of III trimester pre-eclamptic, eclamptic and diabetic pregnant groups when compared with the same for the III trimester normal pregnant group.

**Free Fatty acids**

Statistically highly significant ($p<0.001$) elevations in the levels of free fatty acids can be noted throughout the gestation period (I,II and III trimester) as compared to the values associated with the non-pregnant group.

A statistically highly significant increase ($p<0.001$) in the levels of free fatty acids can be observed in III trimester pre-eclampsia, while a statistically significant increase ($p<0.01$) can be observed in III trimester pre-eclampsia and diabetic pregnancy on comparison with the III trimester normal pregnant group.

**DISCUSSION**

Thus, estimation of lipid peroxides in fraction of lipoproteins, free fatty acids diabetic pregnancy will offer an insight into various metabolic disturbances and the onslaught of hyperoxidant stress in the pathology of pregnancy. Determination of lipid peroxides in lipnprotein fraction ocvn illustrate the phenomenon of oxidative modification of these lipoprotein fractions.

Diabetic pregnant women present a problem of grave concern to the Obstetricians and Gynaecologists. A systemic disorder like diabetes increases the chances of development of many a secondary complications, the implications of which can prove to be fatal at the time of delivery. Decompartmentalized transition metal metabolism and the phenomenon of antioxidant glycosylation associated with the generation of superoxide anion (O*) radical are the primary disposing factors

for hyperoxidant stress associated with diabetes. The present study which evaluated 30 III trimester diabetic pregnant women demonstrated a highly significant increase ($p<0.001$) in the level of MDA as compared to III trimester normal pregnant women ( Table 1).

Recent studies by Ericksson (1991) have suggested that in the diabetes the increased load of glucose in the mitochondria may accelerate the flow of electrons through the respiratory chain, inducing mitochondrial leakage of free radicals.

**Serum Lipid Peroxide Levels in Lipoprotein Fractions**

Lipid metabolism is dramatically altered during pregnancy. Normal pregnant women have hyperlipidemia, even more so in women with pre-eclampsia suggesting that abnormal lipid metabolism may have a role in the genesis or expression of toxemia. Since serum lipoprotein of various fractions could have different roles, the distribution of lipid peroxides in lipoprotein fractions could be significant in the pathogenesis of Diabetic pregnancy. Some studies have suggested that additional changes in the normal pattern are brought about by complications of pregnancy such as macrosomia which aggravates the degree of hyperlipidemia.

Our results showed a definite increase in lipid peroxide level and lipid peroxide of different lipoprotein fractions in patients with diabetic pregnancy.

Appreciable oxidative stress can be illustrated by way of a statistically highly significant increase ($p<0.001$) in the serum level of circulating total lipid peroxides in III trimester of normal pregnancy as compared to non-pregnant group. A similar observation can be made for III trimester of diabetic pregnant group in comparison with III trimester normal pregnant group (Table I).

A generalized observation by way of lipid peroxides being higher in LDL fraction than HDL fraction and being highest in VLDL fraction (as circulating levels of TG are diabetic pregnant groups exhibit a statistically highly significant increase ($p<0.001$) in the value of peroxides associated with VLDL and LDL fraction on comparison with the same associated with III trimester normal pregnant group (Table I).

A statistically significant increase ($p<0.01$) can be observed for lipid peroxides associated with HDL fraction of III trimester pre-eclamptic, eclamptic and diabetic pregnant groups when compared with the same for III trimester normal pregnant group (Table I).

Witztum (1993) showed that the increased MDA levels reflects oxidative modification of lipoproteins, a process occurs primarily in the vascular wall. Serum TG are bound to lipoproteins especially VLDL and thus a positive correlation between triglycerides and MDA concentrations may reflect increased lipoprotein availability for peroxidation. Alternatively, significant increase in the intrinsic susceptibility of lipoproteins to oxidation can occur in diseases associated with hyperlipoproteinemia or disorders in which the proportion of polyunsaturated lipids in lipoproteins is increased. It is also observed that lipid changes during diabetic pregnancy have been shown to be greater than in normal pregnancies.

**Free Fatty Acid**

Skryten et al (1976) and Knopp et al (1978) reported that free fatty acid concentration of gestational diabetes not treated with insulin are significantly increased as compared to control subjects.

Currently, attention has been focussed on potential endothelial activators such as lipid peroxides and cytokines, but there is an increasing recognition that marked perturbations in lipid and lipoprotein metabolism may also be fundamently important in the pathogenesis of macrosomia.

**CONCLUSIONS**

Thus, estimation of lipid peroxides in fraction of lipoproteins, free fatty acids in diabetic pregnancy will offer an insight into various metabolic disturbances and the onslaught of hyperoxidant stress in the pathology of pregnancy. Determination of lipid peroroxides in lipoprotein fraotins can illustrate the phenomenon of oxidative modification of these lipoprotein fractions.

In this original article , we have attempted to demonstrate that macrosomia is associated with abnormal metabolism of lipoproteins. The crucial question is whether *n*-3 PUFA supplementation can prevent or ameliorate lipoprotein status in macrosomic offspring of diabetic mothers.

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