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A STUDY ON ESTIMATION OF SERUM LIPID PROFILE AND APOLIPOPROTEIN A-1 LEVELS IN PREGNANCIES WITH INTRAUTERINE GROWTH RESTRICTION

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ABSTRACT

Intrauterine growth restriction (IUGR) is one of the major causes of perinatal mortality and morbidity. Pregnancies with IUGR are associated with an altered lipid profile which in turn affects the fetal growth. The study was conducted on 60 age matched female individuals divided into three groups. Group A consisted of 20 normal pregnant women,Group B was formed by 20 women diagnosed as IUGR pregnancies and Group C included 20 normal nonpregnant women. Lipid profile and Apolipoprotein A1(ApoA1) were estimated and compared among the groups. There was a significant decrease in the levels of Total Cholesterol (TC), Triglycerides (TG), Very Low Density Lipoprotein Cholesterol (VLDL-C) and Apo A1 in pregnancies with IUGR when compared with normal pregnancies. Hence this study was done to indicate that Lipid profile and ApoA1 can be helpful in diagnosing pregnancies with IUGR.

KEYWORDS: **Apolipoprotein A1, Lipoproteins, Pregnancies, Intrauterine growth restriction.**

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INTRODUCTION

Intrauterine growth restriction (IUGR) represents a pathological inhibition of fetal growth and failure of the fetus to attain its predetermined growth potential. [1] According to an estimate, approximately 30 million newborns per year are affected by IUGR in developing countries. This rate is six times higher than that in developed countries.[2] IUGR is considered as a placental insufficiency disorder where sufficient nutrients are not provided by the placenta to the baby to sustain normal growth The increased nutritional requirement of the fetoplacental unit and the endocrine profile during pregnancy could explain the biochemical changes in lipid metabolism of pregnancy. Normal pregnancy is associated with hyperlipemia. All lipid levels are raised but the greatest increase is in triglyceride rich components [3]. Ideally sufficient maternal nutrients provided across the uteroplacental circulation function efficiently to meet the demands of the growing fetus. If balanced interaction between mother and fetus is disturbed it leads to IUGR (birth weight of 2500grams or less) with higher perinatal mortality and morbidity [4]. Apolipoproteins provide the structural element to the lipoprotein particle thus maintaining stability, act as ligands for specific receptors and also as activators or inhibitors of specific enzymes involved in lipoprotein metabolism[5]. Apo A1, a single polypeptide chain of 245 amino acids with glutamic acid as the C terminal residue and aspartic acid as the N terminal residue[6], is the major protein component of High density lipoprotein(HDL) with the molecular weight of 28.3 kDa[7]. Civeria F, has stated that ApoA1 levels in plasma are related to hepatic ApoA1 mRNA levels and that transcription of ApoA1 mRNA from ApoA1 gene seems to be an important factor in the control of ApoA1 production.The gene for ApoA1 has been mapped to chromosome 11q23[8] The modifications in lipid metabolism in pregnancy start soon after conception and increase in the second half of pregnancy coinciding with increasing fetal requirements for growth[4]. There is a progressive rise in Total Cholesterol(TC), Very Low Density Lipoprotein Cholesterol (VLDL-C) , Low Density Lipoprotein Cholesterol(LDL-C) and High Density Lipoprotein Cholesterol(HDL-C) towards term. A prominent increase is in Triglyceride (TG) concentrations reaching two to four times pre-pregnancy levels by the third trimester[9]. This is due to elevated estrogen levels during gestation which result in an increased hepatic synthesis of triglyceride rich VLDL. Also removal of lipoprotein triglyceride is reduced due to low activities of hepatic lipase and lipoprotein lipase[10]. Placental lactogen stimulates lipolysis in adipose tissue and the steroid hormones induce an insulin resistant state[11]. Despite the lack of a direct placental transfer of triglycerides, diffusion of their fatty acids to the fetus is ensured by means of lipoprotein receptors, lipoprotein lipase activity and intracellular lipase activities in the placenta. As both term and preterm infants can synthesize long chain polyunsaturated fatty acids from parental essential fatty acids, the fetus which receives the nutritional supply from the mother has a growth proportional to the nutritional state of the mother[4,12]. ApoA1 levels were significantly elevated in the second and third trimesters, reaching their highest levels in the second trimester followed by a fall after 33 weeks[13]. Certain studies showed that pregnancies with IUGR are associated with an altered lipid profile, with a decrease in TC, TG, LDL-C and Apo A1 when compared with normal pregnancies[13]. As there is a dearth of literature on the clear role of lipid metabolism in the pathogenesis of IUGR, the present study was undertaken to determine the serum lipid profile and ApoA1 levels in normal healthy pregnant women and pregnancies with IUGR.

MATERIALS AND METHODS

The present study was conducted in 60 female individuals between the age group of 20-35 years with their consent. 40 women were selected from those admitted to the Government Kasthurbha Gandhi Hospital for women and children, Triplicane, Chennai,

during the year 2005 - 2006. Among them the group A was formed by 20 normal pregnancies with gestational age greater than 35 weeks and ultrasound findings corresponding to their gestational age. The remaining 20 women with similar gestational age formed the group B of pregnancies with IUGR having ultrasound findings showing the discrepancy between the gestational age and actual size of the uterus. The group C was formed by age matched 20 healthy nonpregnant women. Pregnancies with pregnancy induced hypertension, maternal diabetes, fetal congenital anomalies or malformations, maternal hepatic/renal/thyroid diseases and other confounding factors which may affect fetal nutrition and growth were excluded from the study. Fasting blood samples of about 8ml were drawn; serum was separated and analyzed for Total Cholesterol, Triglyceride, HDL-C using an enzymatic colorimetric method with Autopak kit. VLDL-C and LDL-C were calculated using the Friedwald equation[14] (LDL-C = TC – HDL-C – VLDL-C ; VLDL-C = TG/5). ApoA1 was estimated by the turbidimetric test using Spinreact[15] Kit method. A calibration curve was plotted with the absorbance and the ApoA1 concentration of each calibrator dilution. ApoA1 concentration in the sample was calculated by interpolation of its absorbance in the calibration curve using the ERBA Chem -5 plus semi-autoanalyzer. The data were analyzed, descriptive statistics like mean, standard deviation were calculated for all variables by groups. Using students' t' test, p value < 0.05 was considered statistically significant.The normal range of the respective biochemical parameters has been provided in parentheses in the tables and graphs.

RESULTS

Table I

Lipid profile and ApoA1 in normal pregnancies and nonpregnant women

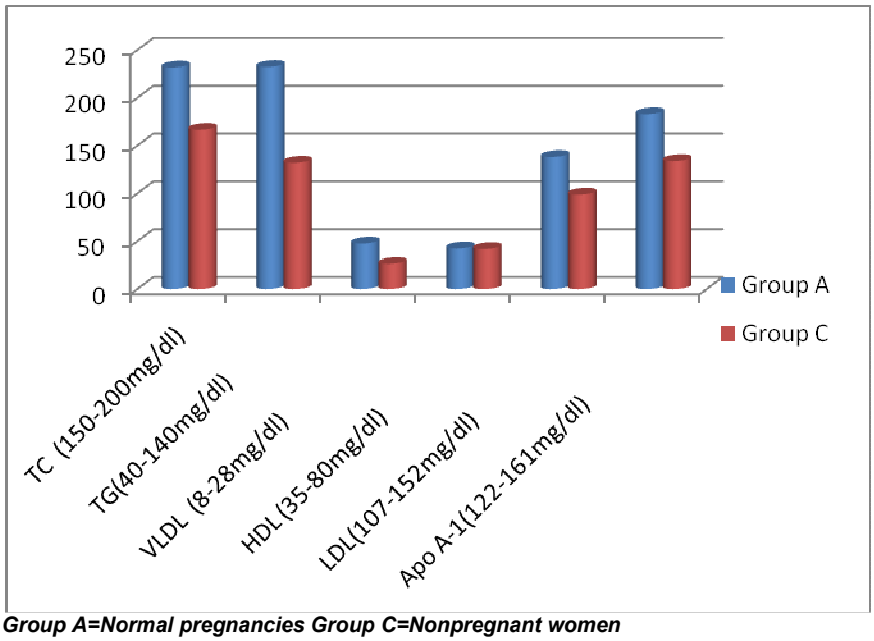
Lipid profile and ApoA1	Group A Mean±SD	Group C Mean±SD	p value	Significance
Total Cholesterol (TC) (150-200mg/dl)	231.8±32.9	166.6± 23.1	0.001	HS↑
Triglycerides (TG) (40-140mg/dl)	232.4±70.6	132.1±38.7	0.001	HS↑
VLDL-C (8-28mg/dl)	47.7±14.1	26.4±7.7	0.001	HS↑
HDL-C (35-80mg/dl)	42.5±12.0	41.9±2.5	0.83	NS
LDL-C (107-152mg/dl)	138.1±30.3	98.8±24.0	0.001	HS↑
Apo A-1 (122-161mg/dl)	183.0±52.3	133.9±45.6	0.003	MS↑

Group A= Normal pregnancies , Group C=Nonpregnant women

HS=Highly significant; NS= Not Significant; MS=Moderately Significant. ↑increase

Graph 1

Lipid profile andApoA1 in normal pregnancies and nonpregnant women



Group A=Normal pregnancies Group C=Nonpregnant women

Note : All the lipid parameters except HDL-C in Group A(normal pregnancies) were significantly elevated with a p value of 0.001 when compared to Group C(normal nonpregnant women) as shown in table I and graph 1.

Table II

Lipid profile and ApoA1in normal pregnancies and pregnancies with IUGR

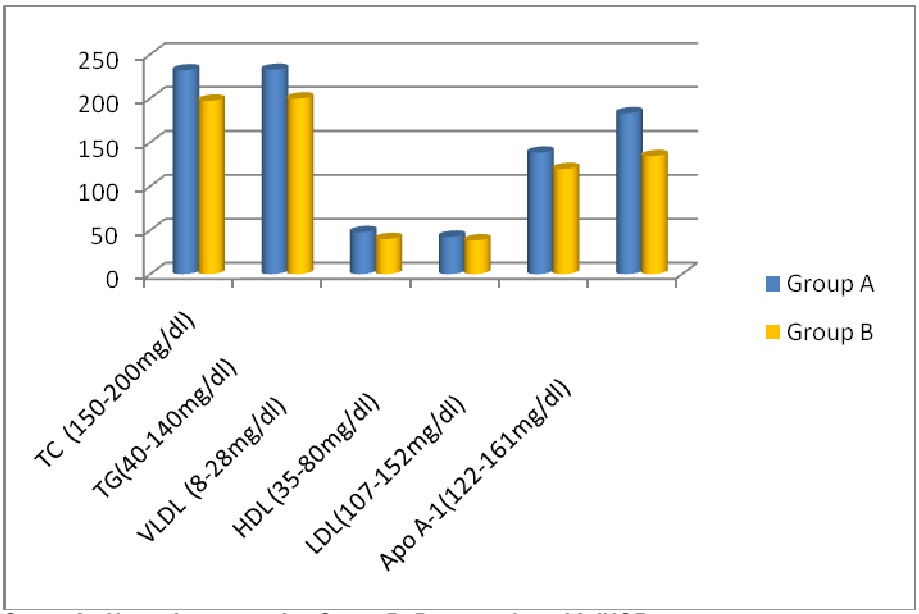
Lipid profile and ApoA1	Group A Mean±S.D	Group B Mean±S.D	p value	Significance
Total Cholesterol (TC) (150-200mg/dl)	231.8±32.9	197.20±55.5	0.02	S↓
Triglycerides (TG) (40-140mg/dl)	232.4±70.6	197.70±35.9	0.05	S↓
VLDL-C (8-28mg/dl)	47.7±14.1	39.94±10.50	0.05	S↓
HDL-C (35-80mg/dl)	42.5±12.0	37.3±11.4	0.16	NS
LDL-C (107-152mg/dl)	138.1±30.3	119.62±57.6	0.21	NS
Apo A-1 (122-161mg/dl)	183.0±52.3	134.13±37.6	0.001	HS↓

Group A= Normal pregnancies,Group B=Pregnancies with IUGR HS = Highly significant;

NS= Not Significant; S=Significant; ↓=Decrease.

Graph 2

Lipid profile and ApoA1 in normal pregnancies and pregnancies with IUGR



Group A= Normal pregnancies,Group B=Pregnancies with IUGR

Note : TC, TG and VLDL-C were significantly lower in the Group B (pregnancies with IUGR) when compared to Group A(normal pregnancies) as shown in Table II and Graph 2. No significant variation in HDL-C and LDL-C. Apo A1 in pregnancies with IUGR is lower than normal pregnancies with highly significant p value of 0.001.

DISCUSSION

In this study the lipid fractions and Apo A1 in normal pregnancies(Group A), pregnancies with IUGR(Group B) and nonpregnant women(Group C) were determined and compared. This would enable us to analyze if the changes in lipoprotein profile during pregnancy imply a greater atherogenic risk with possible repercussions on fetal growth and development. In our study, it is clear that the lipid parameters TC, TG, VLDL-C and LDL-C were increased with high significance in normal pregnant women when compared to the nonpregnant women. The TC, TG and VLDL-C in pregnancies with IUGR although higher than the normal range were significantly lower when compared to normal pregnancies. The HDL-C and LDL-C concentrations in normal pregnancies and pregnancies with IUGR were within the normal range with no significant difference between the two groups. In Piechota's[9] study where the lipids and apolipoproteins Apo A1 and Apo B in normal pregnancies and nonpregnant women were analyzed in the first, second and third trimesters, Apo A1 and Apo B were significantly elevated by 32% and 56% respectively in the third trimester. A rise of TC by 43% and LDL-C by 36% in the third trimester with an increase in HDL-C by 25% in the second trimester was seen. But the most prominent change was a 2.7 fold increase in TG in the third trimester[9]. However, in the present study lipid profile and ApoA1 was estimated only in the third trimester.Dabi et al[16] noted that serum TC,TG, LDL-C and VLDL-C in pregnancies with IUGR decreased with increasing gestational age. The levels of TC and LDL-C in their study were significantly lower (p value 0.001) in pregnancies with IUGR when compared with normal pregnancies, while TG,VLDL-C and HDL-C levels did not show any statistically significant difference between the two groups[16]. In Sattar's study women with pregnancies complicated by IUGR had significantly lower median TC, LDL-C , IDL and VLDL concentrations relative to control patients.There was no significant differences in the median concentrations of TG and HDL-C between the two groups[17]. Turgay et al[18] has reported in his study that TC,TG,HDL-C and

LDL-C increased significantly in normal pregnancy .Turgay concluded that during pregnancy the percent change in TG is affected positively by the nutrition level of the mother. Festus et al[19] reported a very significant increase in TG concentration in the third trimester which he attributes to the switch from carbohydrate to fat metabolism for energy generation due to high energy demand. Edison et al[20] noted that mothers with low serum TC are associated with infants who weighed less than those born to control mothers. The following explanation corroborates well to the results obtained in our study. The changes in lipid levels during pregnancy result from the metabolic adaptation of the mother, mobilization of fat deposits, the increase in free fatty acids and the relationship between circulating progesterone and estrogen suggesting that these hormones are responsible for the lipid change observed. The hyperestrogemia in normal pregnancies enhances the hepatic biosynthesis of triglycerides and thus increases the supply of free fatty acids to the fetus[21] Normal fetal development needs the availability of both essential fatty acids and long chain polyunsaturated fatty acids, thus indicating a relationship between nutritional status of the mother during gestation which is reflected by her lipid profile and fetal growth [22] .The study by Gloria Alvino showed that fatty acid profile and plasma lipids were significantly altered in maternal circulation of IUGR pregnancies[23] . It is possible that lower concentrations of serum TC, TG and VLDL-C in the group of pregnancies with IUGR may have decreased the availability of glycerol, long chain polyunsaturated fatty acids and essential fatty acids to the fetuses of the above group of mothers, ultimately leading to intrauterine growth restriction[23] In this study Apo A1 level in normal pregnant women, were significantly higher than the non pregnant group giving a p value of 0.003. Similarly, high ApoA1 levels in normal pregnancies were seen in the work done by Munozetal[13] wherein the ApoA1 levels in this group were much higher than that obtained in our study, probably attributed to the racial variation and lower nutritional status of the women coming to our general hospital. However in our present study ApoA1 levels in pregnancies with IUGR were significantly lower than the levels in normal pregnancies (p =0.001). Similar observations were made in the study by Munoz.A and Uberos J[13]with a significant difference in ApoA1 levels between the above two groups (p value ≤0.05). Data shows that poor fetal growth permanently alters its cholesterol metabolism and that measurement of ApoA1 in pregnant women can help to identify children at increase risk of cardiovascular disease in adult life. Studies have shown that IUGR babies are associated with a late life increased prevalence of metabolic syndrome, a condition associating obesity with hypertension,type 2 diabetes mellitus and cardiovascular disease[24] . Fetal intrauterine growth restriction has been associated with adult disease as the intrauterine deprivation programs the fetus to develop increased appetite and obesity,hypertension and diabetes as an adult [25.] Accordingly, maternal metabolic indices could help in predicting their children's future risk of coronary heart disease The alternate view is that genetic factors influencing both birth weight and lipid profile could explain the relationship between these two factors. Genetic factors play an important role in the determination of serum lipids and to a lesser extent, birth weight. It was proposed by Christian Wadsauk[26] that alteration in expression levels of placental lipoprotein receptors could be associated with changes in their uptake or efflux functions and might contribute to altered lipid levels in the fetal circulation in pregnancies with IUGR . Hence the genotype responsible for an atherogenic lipid profile might itself cause restricted fetal growth in utero. To our knowledge ,only few studies have estimated lipid profile[1316,17] and Apo A1[13] in pregnancies with IUGR. . As the present study showed a significant decrease in TC,TG , VLDL-C and Apo A1 in pregnancies with IUGR similar to previous studies ,estimation of the lipid profile and Apo A1 can help to identify those mothers at a greater risk for IUGR thereby decreasing perinatal mortality and morbidity .

CONCLUSION

In the present study variations in lipid profile and Apo A1 levels were observed between the normal pregnancies and pregnancies with IUGR. However this investigation can only add up to the ultrasonographic diagnosis of IUGR.An increase in the sample size can authenticate the findings of the study. Estimating ApoA1 levels before and after nutritional supplementation of mothers during pregnancy and simultaneous determination of fetal ApoA1 levels in cord blood can further substantiate the study.

REFERENCES

1. Mandruzzato G, Antsaklis A,Botet F, Chervenok FA, Figueras F, Grunebaum A, Puerto B, Skupski D, Stanojevic M, Intrauterine restriction (IUGR) .J Perinatal Med, 36(4):277-281,(2008)
2. De Onis M,Blossner M,Villar J, Levels and patterns of intrauterine growth retardation in developing countries.Eur J Clin Nutr,52 (l1) : 5-15,(1998)
3. William J.Marshall, S.K.Bangert.Lipids and Lipoproteins.In:William J Marshall.eds, Clinical Chemistry, 3rd edition,Mosby Elsevier,Philadelphia USA ,1995, pg 213-225.
4. Ian R.McFadyen.Maternal Physiology in Pregnancy.In: Geoffrey Chamberlain eds.Turnbull's Obstetrics 2nd edition, Churchill Livingstone,London, 1996,pg 97-114.
5. Peter A Mayes,Kathleen M.Botham.Lipid transport and storage.In:Harper's Illustrated Biochemistry 26th edition,McGraw-Hill,New York,2003,pg 205-218.
6. Libowang,David Atkinson, et al, The Inter facial properties of Apo A-1 and an amphipathic α helix consensus peptide of exchangeable apolipoproteins at the Triolein/Water interface. J Biol Chem, 280(6):4154-4165,(2005)
7. Philippe G.Frank and Yves L.Marcel, Apolipoprotein A-1: structure;-function relationships.Journal of Lipid Research,41:853-872,(2000)
8. Civeira F, Pocovi M,Cenarro A,Garces C et al, Adenine for guanine substitution-78 base pairs 5' to the Apolipoprotein (APO) A-1 gene : relation with high density lipoprotein cholesterol and APO A-1 concentrations.Clin Genet, 44:307-312.(1993)
9. Piechota W,Staszewski A, Reference ranges of lipids and apolipoproteins in pregnancy.Eur J Obstet Gynecol Reprod Biol, 45(1): 27-35,(1992)
10. Karl Winkler, Birgit Wetzka, Michael M. Hoffman, Low density lipoprotein(LDL) subfractions during pregnancy: Accumulation of Buoyant LDL with advancing gestation.The Journal of Clinical Endocrinology and Metabolism, 85 (12) : 4543-4550,(2000).
11. Robert A.Harris,David W. Crabb. Metabolic Interrelationships.In: Thomas M. Devlin. 4th edition, Text Book of Biochemistry with clinical correlations,1997,526-529
12. Uberos-Fernandez J, Munoz, Hoyos A et al, Lipoproteins in pregnant women before and during delivery: influence on neonatal haemorrheology. AJ Clinical Pathol, 49(2) : 120-3,(1996)
13. A Munoz, J Uberos,et al, Relationship of blood rheology to lipoprotein profile during normal pregnancies and those with intrauterine growth retardation. J Clinical Path, 148(6): 571-574,(1995)
14. Friedwald WT, Levy RI, Fredricson DS, Estimation of concentration of low density lipoprotein in plasma without use of preparative ultracentrifuge.Clin Chem,18(6):499-502,(1972)
15. NobertW Tietz,Clinical Guide to Laboratory tests,3 rd edition,W B Saunders:pg483,(1983)
16. Dabi Dr. Parakh Manish Goyal Vikas, A cross- sectional study of lipids and lipoproteins in pregnancies with intrauterine growth retardation. J.Obstet Gynecol Ind,54(5): 467-472,(2004) .

17. Naveed Sattar,Ian A Greer,Peter J. Galloway.Lipid and Lipoprotein concentration in pregnancies complicated by Intrauterine growth restriction.The Journal of clinical Endocrinology and Metabolism, 84(1):128-130,1999.
18. Turgay Emet, Isik Ustuner, Seda Guvendag,Plasma lipids and lipoproteins during pregnancy and related pregnancy outcomes.Archives of Gynecology and Obstetrics,288(1) pg 49-55,2013.
19. Festus O.Okojie,Idonije O.Blessing et al,Comparative study of lipid profile of normal pregnant women in the different trimesters.Archives of Applied Science and Research,(3):528-532,2011.
20. Robin J Edison,Kateberg,Alan Ramaley,Richard Kelley,Adverse birth outcome among mothers with low serum cholesterol.Pediatrics,120:723,2007
21. T.Sunitha,K.Sameera and G.Umarani,Risk factors of metabolic syndrome in pregnancy induced hypertension cases.Int J Pharm Bio Sci,3(4):(B):840-844,(2012)
22. Herrera E, Lipid metabolism in pregnancy and its consequences in the fetus and newborn.Endocrine 19:43-55,2002.
23. Gloria Alvino, Veronica Cozzi et al,Maternal and fetal fatty acid profile in normal and IUGR pregnancies with and without pre eclampsia.Pediatric Research,64(6): 2008.
24. George Valsamakis,Christina Kanaka,Causes of Intrauterine growth restriction and the postnatal development of the metabolic syndrome.Annals of the New York Academy of Sciences vol 1092,Womens Health and Disease: Gynecologic,Endocrine and reproductive issues pg 138-147, 2006.
25. Ross MG,Beal MH ,Adult sequelae of intrauterine growth restriction.Semin Perinatol Jun 32(3):213-8,2008.
26. Christian Wadsauk, Silvia Tabano , Intrauterine growth restriction is associated with alterations in placental lipoprotein receptors and maternal lipoprotein composition, American journal of Physiology –Endocrinology and Metabolism vol 292, 2007.