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Dissertation on

SERUM APOLIPOPROTEIN A-1 LEVELS IN PREGNANCIES WITH INTRAUTERINE GROWTH RESTRICTION

INSTITUTE OF BIOCHEMISTRY

MADRAS MEDICAL COLLEGE

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BONAFIDE CERTIFICATE

This is to certify that this dissertation work entitled "**Serum Apolipoprotein A-1 levels in Pregnancies with Intrauterine Growth Restriction"** submitted by **Dr.Uma Unnikrishnan**, is a work done by her during the period of study in this institute from August 2003 to September 2006.

.....

Dr.A. Manamalli, M.D.,
Director and Professor
Institute of Biochemistry
Madras Medical College
Chennai - 600 003.

.....

Dr. Kalavathy. Ponniraivan, B.Sc. MD
Dean
Madras Medical College
Chennai - 600 003

Place : Chennai

Date :

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CONTENTS

INTRODUCTION

Hyperlipidemia is commonly found in normal population associated with pathologies such as hypertension and atherosclerosis. In pregnant women however, hyperlipidemia has serious implications. The increased nutritional requirement of the fetoplacental unit and the endocrine profile during pregnancy could explain the biochemical changes in lipid metabolism of pregnancy.

The major lipids of human body are phospholipids, cholesterol, triglycerides and cholesteryl esters. These insoluble lipids are transported through blood as lipoproteins consisting of lipids and one or more specific proteins called apolipoproteins. In pregnancy, increase in female sex hormones, and lipolysis due to placental lactogen, cause an increase in lipid synthesis and metabolism resulting in increased cholesterol, triglycerides and phospholipid concentrations.

Ideally, sufficient maternal nutrients provided across uteroplacental circulation function efficiently to meet the demands of the growing fetus. If balanced interaction between mother and fetus is disturbed it leads to Intrauterine Growth Restriction (IUGR) which is associated with higher perinatal mortality and morbidity. Growth restricted fetuses are those born with birth weight 2500g or less.

More studies on biochemical changes in pregnancy are necessary to characterise the excessive gestational lipolytic responses. This would enable us to determine if the changes in lipoprotein profile during pregnancy imply a greater atherogenic risk with possible repercussions on fetal growth and development. Haemorrheological modifications in growth restricted pregnancies are partly secondary to changes in high density lipoprotein metabolism. Changes in apolipoprotein A-1 could be a good marker for the early detection of intrauterine growth restricted pregnancies.

In this study, the lipid fractions and apolipoprotein A-1 in normal pregnancies and pregnancies with intrauterine growth restriction were determined and compared.

REVIEW OF LITERATURE

Apolipoproteins are proteins found on the surface of the lipoprotein complex. Apolipoproteins bind to specific enzymes or transport proteins on the cell membranes and direct the lipoproteins to the proper site of metabolism.

LIPOPROTEINS

Lipoproteins are large molecules that transport lipids, primarily triglycerides and cholesterol through the blood. A typical lipoprotein consists of a lipid core of mainly non polar triacylglycerol and cholesteryl ester surrounded by a single surface layer of amphipathic phospholipids and cholesterol molecules. These are oriented such that the polar groups face outward to the aqueous medium[1]. Lipoprotein molecules differ in their relative lipid and apolipoprotein composition, size, density and function. Lipoproteins can be separated depending on their density using ultracentrifugation or on their electrophoretic mobility[2]. The five major groups of lipoproteins and their functions are given below

1) Chylomicrons (CM) –Transport of exogenous triglycerides.
2) Very low density lipoproteins (VLDL or pre beta lipoproteins) Transport of endogenous triglycerides.
3) Intermediate density lipoproteins (IDLor broad beta lipoproteins) Precursor of Low density Lipoproteins

4) Low density lipoproteins (LDL or beta lipoproteins) Cholesterol transport

5) High density lipoproteins (HDL or alpha lipoproteins) Reverse cholesterol transport.[3]

The source, size and composition of the various lipoproteins are given in chart 1

CHART 1

Composition of the Lipoproteins in plasma of humans

Lipo proteins	Source	Diameter (nm)	Density (g/ml)	Composition		Main Lipid	Apolipo proteins %
				% Protein	% Lipid		
CM	Intestine	90-1000	< 0.95	1-2	98-99	TGL	A-1 : 7.4 A-11 : 4.2 A-IV, B48:22.5 CI, CII, CIII : 66
CM remnants	CM	45-150	< 1.006	6-8	92-94	TGL PL C	B48, E
VLDL	Liver (intestine)	30-90	0.95-1.006	7-10	90-93	TGL	B 100 : 36.9, CI,CII,CIII :49.9
IDL	VLDL	25-35	1.006-1.019	11	89	TGL CE	B100 : 50-70, E CI,CII,CIII : 5- 10
LDL	VLDL	20-25	1.019-1.063	21	79	CE	B100 : 98
HDL, HDL1	Liver, intestine, VLDL, CM	20-25	1.019-1.063	32	68	PL CE	A I : 67 ,A II : 22, A IV, B100 - Trace; C-I,C-II, C-III : 5 - 11 D, E;
HDL 2 HDL3		10-20	1.063-1.125	33	67		
Pre βHDL		5-10	1.125-1.210	57	43		
		< 5	>1.210				A-1

Courtesy : Harper 26th Edition
Tietz - 3rd edition

The physiological functions of the lipoproteins involve a series of complex metabolic processes in which changes and exchanges occur

continuously between the various lipoproteins. This is referred to as the lipoprotein cascade.[4] As the percentage of apolipoprotein A-1 is found to be highest in High density lipoprotein, a detailed description of the lipoprotein and its metabolism, in relation to its function has been discussed below.

High density Lipoproteins

High density lipoproteins are secreted from both the liver and intestine. About 50% of high density lipoprotein mass is protein, 30% is phospholipid and 20% cholesterol[7]. The high density lipoprotein fraction comprises of several distinct subclasses, differing in their density particle size, or apolipoprotein composition. At least three well defined high density lipoprotein subgroups have been studied. These include high density lipoprotein[1] (HDL1) high density lipoprotein[2] (HDL2) and high density lipoprotein[3] (HDL3) [4].

High density lipoprotein[1] (HDL1)

High density lipoprotein[1] (HDL1) is found in the blood of diet induced hypercholesterolemic animals. It is rich in cholesterol and contains only apolipoprotein E. It is taken up by the liver via apolipoprotein E remnant receptor and also by low density lipoprotein receptor. It is for this reason that the latter are also called B100 E receptors. [5]

High density lipoprotein^{2} (HDL2).

It is rich in cholesterol ester. It is involved in reverse cholesterol transport. Its concentration in the blood is inversely proportional to atherosclerosis. High density lipoprotein^{2} (HDL2) contains more lipid and apolipoprotein A-1 levels than high density lipoprotein^{3} (HDL3) [4]

High density lipoprotein^{3} (HDL3)

It is considered to be the smaller and denser precursor of high density lipoprotein^{2} (HDL2).

Reverse Cholesterol Transport

High density lipoproteins are the main mediators of the reverse cholesterol transport, whereby cholesterol synthesized or deposited in peripheral cells is returned to the liver.

The process begins with the removal of free cholesterol from the cell membranes to nascent high density lipoproteins secreted by the liver and intestine. The nascent high density lipoproteins consist of discoid phospholipid bilayers containing apolipoprotein A-1 and free cholesterol. Lecithin cholesterol acyl transferase (LCAT) binds to the disk and is activated by apolipoprotein A-1. Surface phospholipid is hydrolysed by LCAT converting phospholipid into lysolecithin and free cholesterol into ester cholesterol. The

non polar cholesteryl esters move into the hydrophobic interior of the bilayer whereas lysolecithin is transferred to plasma albumin. In this process, the nascent high density lipoprotein is converted to the spherical lipid rich high density lipoprotein^{3} (HDL3) [1,5].

The smaller high density lipoprotein^{3} (HDL3) accepts cholesterol from the tissues via the ATP binding cassette transporter – 1 (ABC - 1). ABC – 1 is a member of a family of transporter proteins that couple the hydrolysis of ATP to the binding of the substrate cholesterol enabling it to be transported across the membrane to be taken up by the high density lipoprotein. As more and more cholesterol is accepted by high density lipoprotein^{3} (HDL3) the lipoprotein particle increases in size to form the less dense high density lipoprotein^{2} (HDL2). Mature high density lipoprotein^{2} (HDL2) are taken up by the liver and steroidogenic tissues where the receptor for high density lipoprotein namely class B scavenger receptor B1 (SR – B1) are found. High density lipoprotein^{2} (HDL2) binds to the receptor via apolipoprotein A-1 and cholesteryl ester is selectively delivered to the cells, but the particle itself including apolipoprotein A-1 is not taken up.[1]

The cycle is completed by the reformation of high density lipoprotein^{3} (HDL3) either after selective delivery of cholesteryl ester to the liver via the scavenger receptor B1 or by hydrolysis of high density lipoproteins$_2$ (HDL_2) phospholipid and triacylglycerol by hepatic lipase. In addition, free

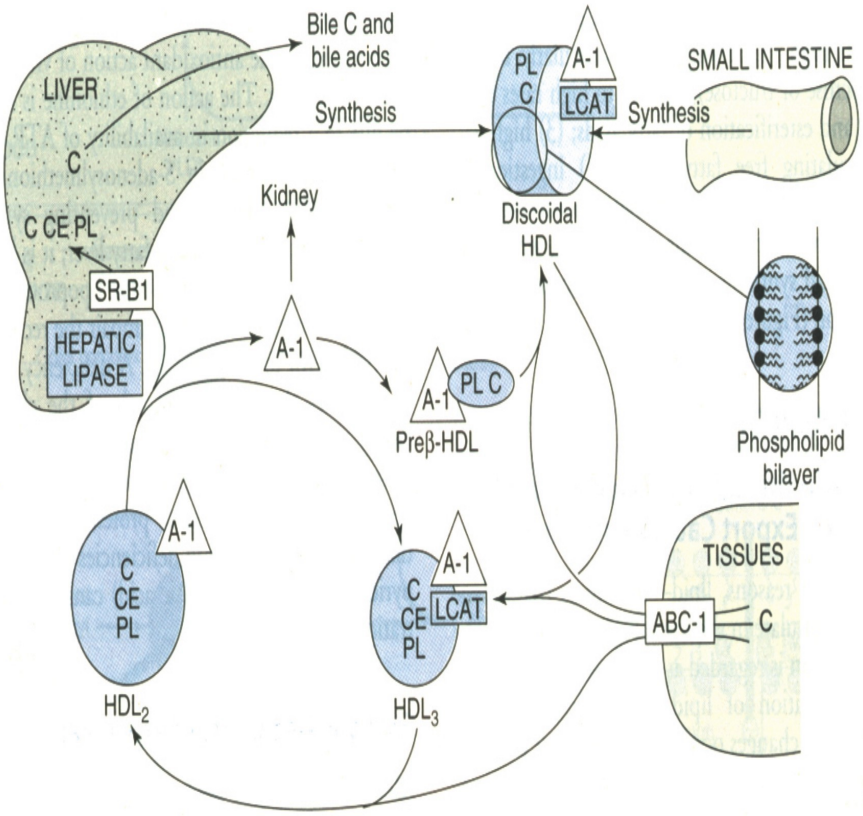
apolipoprotein A-1 released by this process, forms pre Beta high density lipoprotein after associating with a minimum amount of phospholipid and cholesterol. Pre Beta high density lipoprotein is the most potent form of high density lipoproteins in inducing cholesterol efflux from the tissues to form discoidal high density lipoprotein. Surplus apolipoproteins A-1 is destroyed in the kidney.[1,5]

An alternate pathway is present which transfers the cholesteryl esters formed in High density Lipoprotein to Very Low Density Lipoproteins and its remnants. Cholesterol ester transfer protein is a protein found in association with High density lipoprotein that facilitates the transfer of cholesteryl ester from high density lipoprotein to very low density lipoprotein, intermediate density lipoprotein and low density lipoprotein in exchange for triacylglycerol. This, thus prevents the product cholesteryl ester inhibiting the LCAT activity in high density lipoprotein.[4] By this alternate pathway also, the cholesteryl ester formed by LCAT finds its way to the liver via the VLDL remnants namely IDL and LDL.

The above said metabolism of HDL involving reverse cholesterol transport is clearly depicted in Fig.1.

FIG : 1

METABOLISM OF HIGH DENSITY LIPOPROTEIN(HDL) IN REVERSE CHOLESTEROL TRANSPORT



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APOLIPOPROTEINS

Apolipoprotein is the protein part of the lipoprotein molecule. Apolipoproteins have several roles and their distribution characterizes the lipoprotein. Apolipoproteins have three general functions.

1) They provide the structural element to the lipoprotein particles and thus are important in maintaining stability

2) They act as ligands for specific receptors

3) They act as activators or inhibitors of specific enzymes involved in lipoprotein metabolism[1]

Apolipoprotein A-1

Apolipoprotein A-1 is the major protein component of high density lipoprotein and comprises approximately 70% of the total protein mass ranging from 32% to 57% of the high density lipoprotein particle. The molecular weight of Apolipoproteins A-1 is reported to be 28.3KDa[6].

In humans Apolipoprotein A-1 is synthesized both in the liver and intestine. Civeria F, has stated that Apolipoprotein A-1 levels in plasma are related to hepatic Apolipoprotein A-1 mRNA levels, and that transcription of apolipoprotein A-1 mRNA from the apolipoprotien A – 1 gene seems to be an important factor in the control of apolipoprotein A-1 production[7].

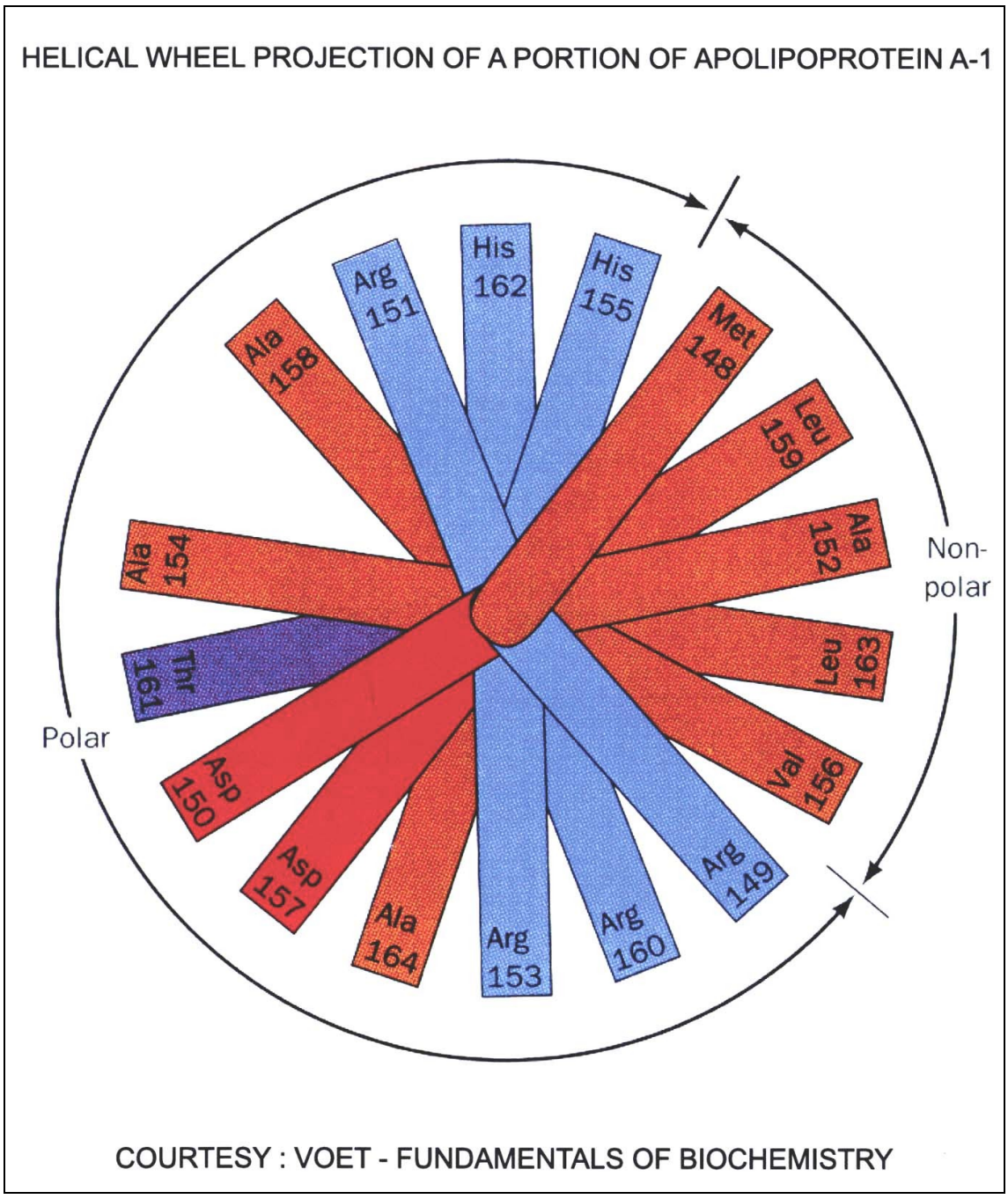
The gene encoding apolipoprotein A-1 is a member of the apolipoprotein multigene super family which includes genes encoding exchangeable apolipoproteins (apolipoproteins A-1, A-11, C's and E). The gene for apolipoprotein A-1 has been mapped on chromosome 11q 23 [8].

Structure of Apolipoprotein A-1

Apolipoprotein A-1 was one of the first apolipoproteins to be identified and characterized. It is a single polypeptide chain consisting of 245 amino acids with glutamic acid as the C terminal residue and aspartic acid as the N terminal residue. The N terminal domain (1 - 99) residues is highly conserved while the central (100 - 186) and C terminal domain (187 - 245) residues shows conservative substitutions between species.[8]

Apolipoprotein A-1 shown in Fig.2 has a high content of alpha helix structure. It consists largely of six tandem 22-residue segments of similar sequence that each has a high propensity for forming an alpha helix followed by a beta turn. These putative alpha helices have their hydrophobic and hydrophilic residues on opposite sides of the helical cylinder. The polar helix face has a dipolar character, because its negatively charged residues project from the centre of the face, whereas its positively charged residues are located at its edges. The phospholipids are arranged with their charged groups bound to oppositely charged residues on the polar face of the helix and with the first few

methylene groups of their fatty acid residues in hydrophobic association with the non polar face of the helix.[12]



Proline present at the first position of almost every 22 amino acid residues often form beta turns or kinks between helices and are therefore essential for the secondary structure of apolipoproteins. The presence of a proline residue appears necessary in defining the structural and functional properties of apolipoprotein A-1, which associates mostly with small lipoproteins (high density lipoprotein)[8].

Functions of individual domains

1) The N terminal domain (residues 1-43) may be important for the stability of the protein in the lipid free state and indirectly modulate its interaction with lecithin cholesterol acyl transferase.

2) The central domain has a function in the regulation of the interaction with phospholipid and is responsible for the plasticity of the molecule by allowing its association with varying amounts of phospholipid. The domain formed by residues 100 – 143 and within it by the helix containing residues 100 – 121 has a very significant role in the binding of phospholipids both in vitro and vivo. A part of central domain formed by residues 144 – 186 forms the site involved in lecithin cholesterol acyl transferase activation.

3) The C terminal domain appears crucial for the ability of apolipoprotein A-1 to promote cholesterol efflux from cholesterol loaded macrophages by the efficient binding of C terminal domain to the cell. Lecithin cholesterol acyl

transferase activation requires a certain affinity for phospholipids contributed by the C terminal domain (187 – 243) and within it probably the last helix (220 – 241)[8].

Lipid free form of Apolipoprotein A-1.

Lipid free form of apolipoprotein A-1 is a loosely folded and relatively flexible structure which may allow rapid lipid interaction of exposed hydrophobic portions of the protein. The N terminal sequence residues 1 – 43 of apolipoprotein A-1, apparently not required for lipid binding may be important for the stabilization of lipid free form of apolipoprotein A-1 in solution. Lipid free apolipoprotein A-1 typically exhibits an alpha helix content of 40 – 50%, while in association with phospholipid the alpha helix structure increases approximately 75% depending on the type of lipids and complexes formed[8].

Interaction of Apolipoprotein A-1 with Lipids- Role of amphipathic helices

The properties of synthetic peptides that correspond to each of the predicted apolipoprotein A-1 helical segments shows that peptides corresponding to helices 44;-65 and 220;-241 have been found to associate with lipids with a significant affinity. This result has been attributed to a deeper penetration of these two helices into the lipid interface, as compared to other helices. The two extreme helices of apolipoprotein A-1 may initiate the binding

to phospholipid, followed by a cooperative binding of the other helices. However, recent in vitro studies showed that the central domain of apolipoprotein A-1, the helix formed by residues 100;-121 is very important for the stabilization of the lipid apolipoprotein complex, while helices spanning residues 122;-186 contribute to the initial rates of lipid apolipoprotein association.

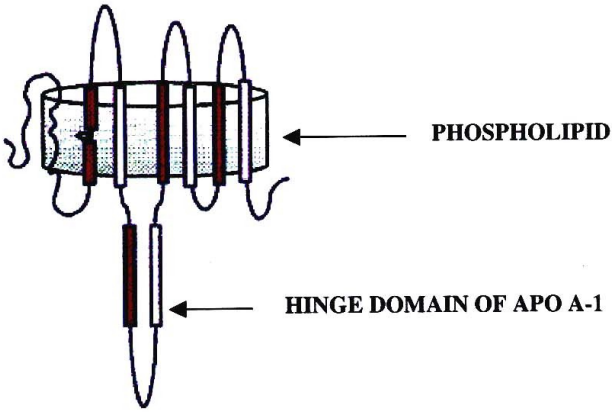
Lp A-1 complexes (Apolipoprotein A-1 containing lipoprotein complexes)

Apolipoprotein A-1 can form discoidal as well as spherical complexes. Discoidal complexes could contain two, three or more molecules of apolipoprotein A-1 per particle. The disc formed by a phospholipid bilayer can accommodate apolipoprotein A-1 amphipathic alpha helices, parallel to the phospholipid acyl chains. In small discoidal apolipoprotein A-1 containing lipoproteins, two helices do not interact with the lipid interface possibly because of a reduced affinity for lipids. However in large discoidal complexes, these helices which have been termed as hinge domain, interact with lipids and can therefore allow one molecule of apolipoprotein A-1 to associate with varying amount of lipid[8] as shown in Fig.3.

FIG. 3 **INTERACTION OF APO A-1 WITH LIPIDS IN LIPO PROTEIN A-1, COMPLEXES**

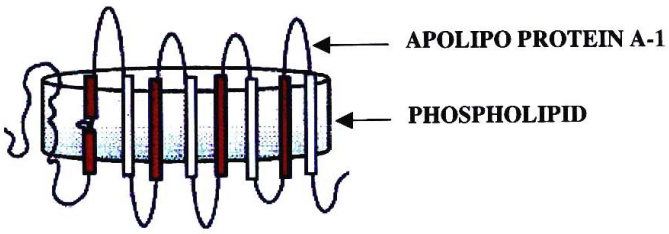
SMALL DISCOIDAL LpA-1 COMPLEXES

A



LARGE DISCOIDAL LpA-1 COMPLEXES

B



COURTESY : APOLIPOPROTEIN A-1 STRUCTURE FUNCTION RELATIONSHIP JLR2000; 41 : 853 - 872

Functions of Apolipoprotein A-1

1) Lecithin Cholesterol Acyl Transferase activation

The lecithin cholesterol acyl transferase glycoprotein consisting of 416 amino acids displays two actitivties, a phospholipase A2 activity since it can hydrolyse the sn_2 fatty acid from phosphatidyl choline and an acyl transferase activity.

Apolipoprotein A-1 was the first described activator of lecithin cholesterol acyl transferase and is its most potent in vivo activator. This reaction requires three steps. First lecithin cholesterol acyl transferase binds to the substrate (high density lipoprotein) and / or the activator, and in a second step lecithin cholesterol acyl transferase hydrolyses the fatty acid at the sn2 position of the phospholipid. The third and final step is the transesterification of cholesterol and concomitant release of cholesteryl ester.

Other apolipoproteins also activate lecithin cholesterol acyl transferase but below 20% of that observed for apolipoprotein A-1. The mechanism by which apolipoprotein A-1 activates lecithin cholesterol acyl transferase is still unknown, but may involve a direct interaction between 2 helices of apolipoprotein A-1 and residues 152 -169 of lecithin cholesterol acyl transferase. Positively charged residues present in both the helices 144 – 165

and 166 – 186 of apolipoprotein may interact with negatively charged residues found on the lecithin cholesterol acyl transferase helix[8].

2. Cholesterol efflux from cells

Apolipoprotein A-1 bound to high density lipoprotein is important in cholesterol efflux from cells via reverse cholesterol transport. The phospholipid content of high density lipoprotein could affect cholesterol efflux between cells and lipoproteins. The ability of apolipoprotein A-1 to interact with lipid surfaces suggests a possible role for its amphipathic alpha helices to promote cellular cholesterol efflux from the plasma membrane. The central domain of apolipoprotein A-1 is more labile and could interact more easily with the plasma membrane to promote cellular cholesterol efflux.

A second mechanism which involves the direct binding of apolipoprotein A-1 to the cell membrane and causes the transfer of intracellular cholesterol to the plasma membrane is called binding and translocation dependant cholesterol efflux.

3) Solubility of lipoproteins:

The apolipoprotein imparts water solubility to the hydrophobic central core of the cholesteryl esters and triglycerides in the high density lipoprotein particle.

Serum levels of Apolipoprotein A-1 range from 120 to 150 mg/dl in males and 130 to 160 mg/dl in females.

Mutations of Apolipoprotein A-1

Naturally occurring Apolipoprotein A-1 mutations

a. Mutations of the N-terminal domain – several naturally occurring mutations in the N-terminal domain have been identified and these mutations are often associated with amyloidosis. This disease is a disorder of protein metabolism in which autologous proteins or their fragments associate with amyloid precursor proteins and are deposited as fibers in the tissues. Small N-terminal fragments of Apolipoprotein A-1 variants have been found in some individuals with amyloidosis. The presence of an extra positive charge in the N-terminal domain may be responsible for the formation of amyloid deposits.

b. Mutations of the central domain. Several naturally occurring mutations in the central domain of Apolipoprotein A-1 have been described. However. few of them have been associated with clear defects in lipid binding properties. Only when the secondary structure of the protein was clearly modified, were impaired lipid binding properties observed.

c. Mutations of the C-terminal domain – very few natural mutations have been observed in the C-terminal domain of Apolipoprotein A-1. Funke

et al has described a mutant in which a frame shift mutation leads to a modification of residues 203-229 and a smaller mature protein (229 residues) was formed. This patient revealed corneal opacity and reduced plasma lecithin cholesterol acyl transferase activity.[8]

Apolipoprotein A-1 Deficiency

Familial apolipoprotein A-1 defects may be caused by complete deficiency of the apolipoprotein A-1 gene, or by mutations in the apolipoprotein A-1 gene. Genetic deficiency of apolipoprotein A-1 may be due to the deletion of the gene or to nonsense mutations that prevent the synthesis of apolipoprotein A-1. Patients with this disorder sometimes display cutaneous xanthomas. The risk of premature cardiovascular disease in patients with apolipoprotein A-1 deficiency may be increased but the onset of symptoms varies from the third to the seventh decade.[16]

Apolipoprotein A-1 Milano

The amino acid substitution of cysteine for arginine at position 173 has the effect of changing the physical property of one of the amphipathic helical regions involved in lipid binding and also allows disulphide bonding to other proteins. The apolipoprotein A-1 Milano genotype is associated with reduction in high density lipoprotein levels but paradoxically is protective against atherosclerosis.[19]

Apolipoprotein A-11

It has a molecular weight of 17,000 daltons and has 154 amino acids, with a structure consisting of two identical monomers joined by a disulphide bridge. Its inhibition of apolipoprotein A-1 and lecithin cholesterol acyl transferase is questionable. It is associated with high density lipoproteins and chylomicrons[5].

Apolipoprotein AIV

It is secreted with chylomicrons but transferred to high density lipoproteins. The molecular weight is 46,000 daltons. Apolipoprotein A-IV is associated with the formation of triacylglycerol rich lipoprotein. It is synthesized by the intestine.[2]

Apolipoprotein B100 :

It is the main apolipoprotein of low density, very low density and intermediate density lipoprotein. It is the longest single polypeptide with 4536 aminoacids and molecular weight 5,50,000 daltons.[2]

Apolipoprotein B48:

Apolipoprotein B48 is synthesized from the same mRNA as apolipoprotein B100, but due to RNA editing mechanism a smaller polypeptide

is formed. Chylomicron and chylomicron remnants contain this apolipoprotein.[2]

Apolipoprotein CI CII CIII are smaller polypeptides freely transferable between several different lipoproteins. Apolipoprotein C1 is a possible activator of lecithin cholesterol acyl transferase and Apolipoprotein CII is an activator of lipoprotein lipase. Apolipoprotein CIII may inhibit the activation of lipoprotein lipase by Apolipoprotein CII.

Apolipoprotein E is an arginine rich apolipoprotein found in very low density lipoprotein and acts as a ligand for chylomicron remnant receptor in liver and low density lipoprotein receptor.[5]

METABOLISM IN PREGNANCY

Pregnancy is hyperlipidemic and glucosuric. These physiological changes in lipid and carbohydrate metabolism are accompanied by related alterations in amino acids. Together they increase the availability of glucose for the fetus while the mother utilizes lipids. These metabolic modifications start soon after conception, increase and become most marked in the second half of pregnancy coinciding with increasing fetal requirements for growth. The uterus and placenta also require carbohydrate fat and amino acids for work as well as for structure[22].

Carbohydrate Metabolism

Glucose passes freely across the placenta so its increased availability in the maternal circulation is of direct benefit to the fetus. Glucose also passes freely across the glomerulus, exceeding the tubular threshold, with glycosuria detected significantly often in pregnant women. Accompanying the glycosuria are other apparent abnormalities like insulin sensitivity increasing in first half and decreasing in second half of pregnancy. This increased sensitivity stimulates glycogen synthesis and storage, deposition of fat, and transport of amino acids into cells.

After 20 weeks of gestation, resistance to the action of insulin develops progressively and plasma levels of insulin rise. Likewise, plasma glucose level

also increases with a delay in return to fasting level in Glucose Tolerance Test (GTT). Fasting plasma insulin levels reach their maximum at around 32 weeks but the decrease in sensitivity to its action persists until delivery. This reduces maternal utilisation of glucose and induces glycogenolysis and gluconeogenesis, as well as the utilisation of lipids as energy sources. This can cause rapid development of ketosis during labour.[22]

Insulin resistance maybe a consequence of increased levels of human placental lactogen, oestrogen and progesterone. Oestrogen increases cortisol binding globulin which stimulates the adrenal to produce sufficient cortisol to produce insulin resistance. Progesterone increases the level of serum insulin without altering the concentration of glucose. Human placental lactogen stimulates insulin release and inhibits glucose uptake[22].

Amino acids : The plasma concentration of most amino acids usually glucogenic amino acids like alanine falls during pregnancy. This may be due to transport across the placenta and increased utilisation of amino acids by the mother for gluconeogenesis.

Proteins: The concentration of proteins in the maternal serum falls markedly, by 20 weeks of gestation. The total protein concentration falls from 7.0g to 6.0g per 100ml. Most of this is due to fall in serum albumin from 3.5g to 2.5g per 100ml, globulins may even rise by 0.2g per 100ml. The fall in albumin

concentration reduces the colloid osmotic pressure in the plasma, which is one of the factors predisposing to edema in pregnancy.

Lipid Metabolism in Pregnancy

Normal pregnancy is hyperlipaemic. All lipid levels are raised but the greatest increase is in triglyceride rich components. Plasma concentrations tend to underestimate the magnitude of the total increase because plasma volume doubles during pregnancy.

Total plasma cholesterol falls by 5% early in pregnancy, reaching its lowest point at 6-8 weeks. Following the initial fall there is a progressive rise to term. Total cholesterol increases by 24-206%, in very low density lipoproteins by 36% and in low density lipoproteins by 50 -90%. High density lipoproteins is raised by 10-23% at term, having risen to 30% in mid gestation and fallen. The most prominent change was a 2.7 fold increase in triglycerides with concentrations raised by 90-570% at term[22,24].

Two mechanisms specific for pregnancy seem to be responsible for this phenomenon. First elevated estrogen levels during gestation result in an increased hepatic synthesis of triglyceride rich very low density lipoprotein. Secondly, removal of lipoprotein triglycerides is reduced due to low activities of lipoprotein lipase, and hepatic triglyceride lipase, the effect being more striking for hepatic lipase than for lipoprotein lipase. The abundance of very

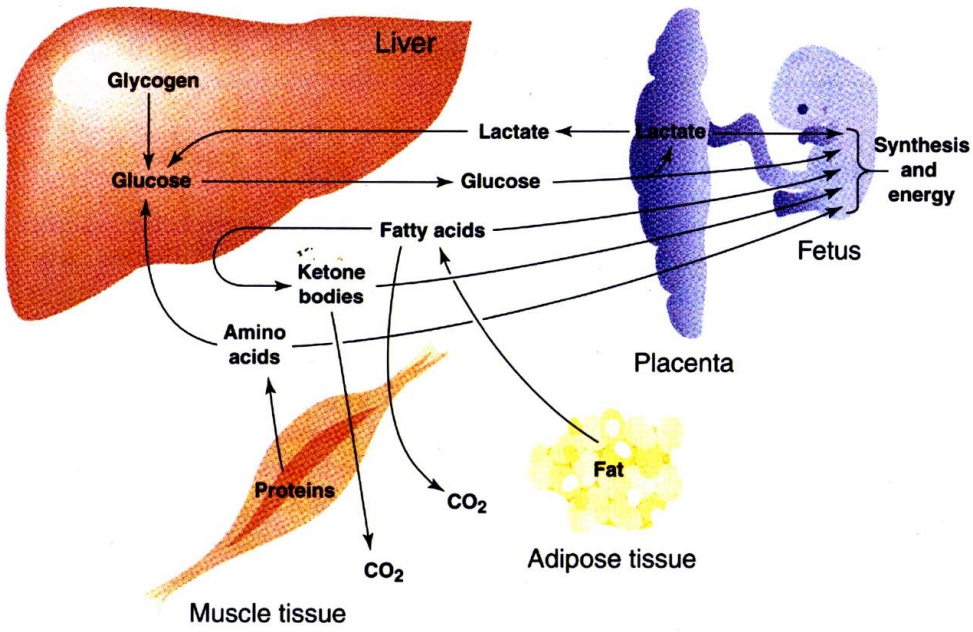
low density lipoprotein triglycerides drives an accelerated transfer of triglycerides to lipoproteins of higher density by the cholesteryl ester transfer protein. Thus the reduced hepatic lipase activity appears to be responsible for the shift of high density subclasses toward larger triglyceride rich and more buoyant species in late gestation. The low density lipoproteins also become enriched in triglycerides, but these particles become smaller and denser[27].

Maternal low density lipoprotein cholesterol is an important precursor of placental steroids (estradiol and progesterone). During pregnancy the placenta secretes a polypeptide hormone placental lactogen and two steroid hormones estradiol and progesterone. Placental lactogen stimulates lipolysis in adipose tissue and the steroid hormones induce an insulin resistant state. Thus in the post prandial state, pregnant women enter the starved state more rapidly than do nonpregnant women. This results from increased consumption of glucose and amino acids by the fetus. Plasma glucose, amino acids and insulin levels falls rapidly and glucagon and placental lactogen levels rise and stimulate lipolysis and ketogenesis. The consumption of glucose and amino acids by the fetus may be great enough to cause maternal hypoglycemia. On the other hand, in the fed state pregnant women have increased levels of insulin and glucose and demonstrated resistance to exogenous insulin[30].

Long chain polyunsaturated fatty acids (LCPUFA) circulate in maternal plasma, associated to lipoprotein triglycerides, and in a minor proportion in the

form of free fatty acids. 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. Maternal plasma free fatty acids are also an important source of long chain polyunsaturated fatty acids to the fetus, and their placental uptake occurs via a selective process of facilitated membrane translocation involving a plasma membrane fatty acid binding protein. This mechanism together with a selective cellular metabolism determine the actual rate of placental transfer and its selectivity, resulting even in an enrichment of certain long chain polyunsaturated fatty acids, in fetal circulation as compared to maternal. The degree to which the fetus is capable of fatty acid desaturation and elongation is not clear, although both term and preterm infants can synthesise long chain poly unsaturated fatty acids from parental essential fatty acids. Therefore the fetus which receives the nutritional supply from the mother has a growth proportional to the nutritional state of the mother. The nutritional supply to the fetus from the mother is illustrated in Fig.4.

NUTRITIONAL SUPPLY TO FETUS



COURTESY : DEVLIN - TEXTBOOK OF BIOCHEMISTRY

After delivery, plasma levels of lipids return to normal but this may take 6 months for some lipids and is affected by whether or not the mother breast feeds and by the use of oral contraceptives[22,35].

Apolipoprotein A-1 in Pregnancy

Apolipoprotein A-1 is the major protein component of High density lipoprotein and comprises approximately 70% of the total protein mass in this lipoprotein. The high density lipoprotein concentrations in normal pregnancies are closely related to circulating progesterone and oestrogen, and to the pregnancy period, in which they are determined. The high density lipoprotein concentration increases through a rise in apolipoprotein A-1 synthesised predominantly in the liver when oestrogen levels are raised.

Apolipoprotein A-1 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. Apolipoprotein A-II also increases but it is not significant. Apolipoprotein B100 increases throughout pregnancy[36].

INTRAUTERINE GROWTH OF FETUS

Human fetal growth is characterized by sequential patterns of tissue and organ growth, differentiation, and maturation that are determined by maternal provision of substrate and fetal growth potential governed by the genome.

Fetal growth has been divided into three consecutive cell growth phases

First 16 weeks: phase of hyperplasia characterized by rapid increase in cell number

16 – 32 weeks : Cellular hyperplasia and hypertrophy

> 32 weeks : Cellular hypertrophy

During the third phase, most fetal fat and glycogen deposition takes place. The corresponding fetal growth rates during these cell growth phases are from 5g/day at 15weeks, 15 to 20g/day at 24 weeks and 30to 35g/day at 34 weeks[38].

Under ideal conditions sufficient amounts of maternal nutrients are provided across utero placental circulation which functions efficiently to meet the demands of the growing fetus. An appropriate hormonal and endocrine milieu for both mother and fetus enables optimal growth, with balanced interaction of many factors. This allows normal development and maturation and ultimately a smooth transition from intrauterine to extrauterine life. Metabolic shift occurs in virtually all maternal nutrients in pregnancy. The placenta and the uteroplacental blood flow play a key role in regulation and delivery of fuel to the fetus. [40]

INTRAUTERINE GROWTH RESTRICTION

According to World Health Organisation low birth weight is defined as 2500 grams or less and prematurity as birth at 37 weeks or less. Low birth weight defined as less than 2500 grams has been modified to describe very low birth weight infants weighing 1500 grams or less, extremely low birth weight those who weigh 1000 grams or less. Nowadays the term restriction has largely replaced retardation as the latter may erroneously convey mental delay rather than only the intended suboptimal fetal growth [38].

Risk factors of intrauterine growth restriction are constitutionally small mothers, poor maternal weight gain, social deprivation, fetal infections, congenital malformations, chromosomal abnormalities, chemical teratogens, vascular disease, chronic renal disease, chronic hypoxia, maternal anaemia, and placental and cord abnormalities.

Screening and identification of Intrauterine growth restriction

These include

- Early establishment of gestational age
- Monitoring maternal weight gain
- Uterine fundal height between 18 and 30 weeks. The uterine fundal height in centimeters conincides with weeks of gestation.

- Ultrasonic measurement: Routine ultrasound screening at 16 to 20 weeks to rule out visible anomalies, and follow up at 32 to 34 weeks to evaluate fetal growth.

- Doppler velocimetry – abnormal umbilical artery Doppler velocimetry characterised by absent or reversed end diastolic flow signifying increased impedance has been uniquely associated with fetal growth restriction.

Growth appears to be the best indicator of long term fetal well being and might be associated with development of abnormal states later in life. Distinct from their appropriately grown counterparts, growth restricted fetuses have physiologic characteristics that are believed to alter permanently the development and metabolic function of organs. In addition to increased morbidity and mortality, growth restriction has been associated neonatally with hypoglycemia, hypocalcemia, polycythemia and thrombocytopenia. Hypoinsulinemia decreased thyrotropin levels, and diminished insulin like growth factor-1 have been described in small for gestational age infants.[38]

APOLIPOPROTEIN A-1 AND LIPOPROTEINS IN INTRAUTERINE GROWTH RESTRICTION

Pregnancies having intrauterine growth restriction are associated with an abnormal lipid profile, with significant decreases in levels of serum cholesterol, serum triglyceride, low density lipoprotein and high density lipoprotein when compared with normal pregnancies. Although high density lipoprotein values were not statistically different in normal when compared with pregnancies complicated with intrauterine growth restriction, apolipoprotein A-1 level was significantly lower in women with intrauterine growth restricted pregnancies.[36]

Studies from different areas have shown that indices of fetal growth, such as birth weight are inversely associated with cardiovascular morbidity and mortality in men and women in adult life. Although the mechanisms are not known it has been suggested that abnormalities in the metabolism of serum lipids may, in part, explain these associations. Several recent studies have demonstrated that total cholesterol, low density lipoprotein, and apolipoprotein B, known risk factors for cardiovascular disease are inversely related to size. In addition, there is some evidence that small size at birth is associated with decreased levels of high density lipoprotein cholesterol and apolipoprotein A-1. These associations have been attributed to a programmed response to intrauterine malnutrition that induces permanent changes in the structure and function of organs, which cause an atherogenic lipid profile in adult life. This theory is supported by a study demonstrating that exposure to the Dutch famine

in utero influenced lipid levels in later life. The alternative view is that genetic factors influencing both birth weight and lipid profile could explain the relationships between these two factors. Genetic factors play an important role in the determination of serum lipids, and to a lesser extent, birth weight. It could be proposed that the genotype responsible for an altherogenic lipid profile might itself cause restricted fetal growth in utero.[41]

The concurrent decrease in apolipoprotein A-1 and increase in apolipoprotein B in pregnancies with intrauterine growth restriction could partly explain the viscosity changes that occur in this complicated pregnancy. The affinity of both apolipoprotein B and plasminogen for the same endothelial receptors leading to competitive inhibition of fibrinolysis, could explain the increase in plasma viscosity observed in the case of intrauterine growth restriction[36].

Apolipoprotein B increases throughout pregnancy both in normal and in pregnancies with restricted fetal growth. Apolipoprotein A-1 shows a non significant decrease after 33 weeks in normal pregnancies but in women with intrauterine growth restriction a decline to pregestational levels is observed. Haemorrheological modifications in the intrauterine growth restricted group are in part secondary to changes in high density lipoprotein metabolism, mainly serum levels of Apolipoprotein A-1. Along with thorough clinical assays, changes in Apolipoprotein A-1 and more specifically high density lipoprotein / apolipoprotein A-1 ratio could be a good marker of intrauterine growth restricted pregnancies.[41,42]

AIM OF THE STUDY

A review of literature on previous studies in normal pregnancies and pregnancies with intrauterine growth restriction revealed variations in the lipid profile between the two groups. Among the lipid parameters, Apolipoprotein A-1 was significantly different in the pregnancies with intrauterine growth restriction when compared to normal pregnancies. However, these studies were not able to clearly define the role of lipid metabolism in the pathogenesis of intrauterine growth restriction, and there is still a dearth of literature on this aspect. So the present study was undertaken with the following aim.

1. To determine total cholesterol, triglycerides, high density lipoprotein, low density lipoprotein, and very low density lipoprotein in healthy non pregnant women, in normal pregnancies and pregnancies with intrauterine growth restriction.

2. To establish the serum level of Apolipoprotein A-1 in apparently normal healthy non pregnant women of the child bearing age, in normal healthy pregnant women and in pregnant women with intrauterine growth restriction with a view to assess the role of Apolipoprotein A-1 in intrauterine growth restriction.

3. To determine whether a cut off level of Apolipoprotein A-1 can be established between normal pregnancy and pregnancy with intrauterine growth restriction.

MATERIALS AND METHODS

The study was conducted on 80 female individuals in the age group 20-35 years. Among them 20 healthy non pregnant women selected from friends and relatives formed the control group. The remaining 60 women were selected from those admitted to Government Kasthurbha Gandhi Hospital for Women and Children, Triplicane, Chennai – 5. Among them 20 women with gestational age greater than 35 weeks, formed the study group of normal pregnancies with ultrasound findings corresponding to their gestational age. The remaining 40 women with similar gestational age formed the study group of pregnancies with intrauterine growth restriction.

The pregnancies with intrauterine growth restriction group had ultrasound findings showing discrepancy between the gestational age and actual size of the uterus. Pregnancies with pregnancy induced hypertension, maternal diabetes, fetal congenital anomalies or malformations, maternal hepatic / renal / thyroid diseases and any other confounding factor which may affect fetal nutrition and growth were excluded from the study.

BLOOD COLLECTION

About 8ml of blood was collected after an overnight fast. The blood was allowed to clot and the serum separated. After centrifugation clear serum was obtained. The levels of serum cholesterol, triglycerides, and high density lipoprotein cholesterol were measured within 3 hours of blood collection.

About 1 ml of serum was stored in eppendorfs at – 20^0C for the estimation of apolipoprotein A-1.

LIPID ASSAYS

1. TRIGLYCERIDES

Serum triglycerides were measured using the enzymatic colorimetric method.

Kit used - Autopak

PRINCIPLE Enzymatic determination of triglycerides was done based on the following reactions

Triglycerides + H_2O $\xrightarrow{\text{LIPOPROTEIN LIPASE}}$ Glycerol + Fatty acid

Glycerol + ATP $\xrightarrow{\text{GLYCEROL KINASE}}$ Glycerol 3 phosphate +ADP

Glycerol. 3 phosphate+O_2 $\xrightarrow{\text{GPO}}$ Dihydroxy acetone phosphate + H_2O_2

$2H_2O_2$ + 4-Aminoantipyrine + ADPS $\xrightarrow{\text{PEROXIDASE}}$ Red quinone +$4H_2O$

GPO = Glycerol 3 phosphate oxidase.

ADPS = N Ethyl – N- Sulfopropyl– n- ansidine

The intensity of the coloured complex produced which was directly proportional to concentration of triglycerides was measured at 546nm.

REAGENTS

Reagent 1(Enzymes / Chromogen)

Lipoprotein Lipase	-	1100 U/L
Glycerol Kinase	-	800 U/L
Glycerol 3 phosphate oxidase	-	5000 U/L
Peroxidase	-	350 U/L
4-Aminoantipyrine	-	0.7 mmol/L
ATP	-	0.3 mmol/L

Reagent 1A (Buffer);

Pipes buffer, ph 7.50	-	50 mmol/L
ADPS	-	1mmol/L
Magnesium salt	-	15 mmol/L

Standard

Triglycerides 200mg/dl

Preparation of working reagent

The contents of one bottle of Reagent 1 was mixed with one bottle of Reagent 1A and mixed by gentle swirling.

Procedure

1ml of working reagent and 10µl of sample / standard were mixed and incubated for 5 minutes at 37^0C. The absorbance was measured at 546 nm.

	Blank	**Standard**	**Sample**
Working Reagent	1000 µl	1000 µl	1000 µl
Distilled water	10 µl	-	-
Standard	-	10 µl	-
Sample	-	-	10 µl

Calculation

Concentration of triglycerides = $\frac{\text{OD Sample}}{\text{OD Standard}}$ x 200

Linearity upto 1000mg / dl

Reference range Male : 60 – 165 mg / dl

Female : 40-140 mg / dl

2. ESTIMATION OF CHOLESTEROL – TOTAL

Serum cholesterol was estimated using the enzymatic cholesterol oxidase method.

Kit used - Autopak

Principle

Enzymatic determination of cholesterol was done based on the following reactions

Cholesterol Ester $+H_2O$ CHOLESTEROL ESTERASE Cholesterol+Fatty acids

Cholesterol + O2 CHOLESTEROL OXIDASE Cholestenone + H_2O_2

$2H_2O_2$ + Phenol + 4 Aminoantipyrine PEROXIDASE Red quinone + 4H2O

The intensity of coloured complex produced which was directly proportional to concentration of cholesterol content was measured at 505 nm.

REAGENTS

Reagent 1 (Enzymes / Chromogen)

Cholesterol Esterase	-	200 U/L
Cholesterol Oxidase	-	250 U/L
Peroxidase	-	1000 U/L
4-Amino antipyrine	-	0.5 mmol/L

Reagent 1A (Buffer)

Pipes buffer, pH 6.90	-	50 mmol/L
Phenol	-	24 mmol/L
Soduim cholate	-	0.5 mmol/L

Standard – cholesterol standard 200mg / dl

Preparation of working reagent

Reagent 1 was dissolved in 50ml of Reagent 1A and mixed gently, and stored in amber coloured bottles at 2-8^0 C

Procedure

1ml of working reagent and 10µl of sample / standard were mixed and incubated for 10 min at 37^0C. The absorbance was measured at 505mn against a reagent blank.

	Blank	**Standard**	**Sample**
Working Reagent	1000µl	1000 µl	1000 µl
Standard	-	10 µl	-
Sample	-	-	10 µl

Calculation

$$\text{Cholesterol concentration mg /d1} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 200$$

Linearity upto 500mg / dl

Reference range depends on age, sex, diet, race and geographical location. Serum level 150 – 200 mg /dl

3. HDL CHOLESTEROL

Serum HDL cholesterol was estimated using the phosphotungstate method.

Kit Used - Autopak

Principle – PHOSPHOTUNGSTIC ACID METHOD

Chylomicrons, VLDL and LDL fractions in serum were separated from HDL by precipitating with phosphotungstic acid and magnesium chloride. After centrifugation, the cholesterol in the HDL fraction which remained in the supernatant was assayed with cholesterol enzymatic method

REAGENTS

Reagent 1 (Enzymes / Chromogen)

Cholesterol esterase	-	200 U/L
Cholesterol oxidase	-	250 U/L
Peroxidase	-	1000 U/L
4 Aminoantipyrine	-	0.5mmol/L

Reagent 1- A (Buffer)

Pipes buffer. pH 6.90	-	50 mmol/L
Phenol	-	24 mmol/L
Sodium Cholate	-	0.5 mmol/L

Reagent 2 (Precipitating Reagent)

Phosphotungstic acid	-	2.4 mmol/L
Magnesium chloride	-	39 mmol/L

Standard HDL cholesterol 50mg / dl

PROCEDURE

Precipitation

200µl of the sample was mixed with 200µl of the precipitating reagent 2, allowed to stand for 10min at room temperature and then centrifuged at 4000 rpm for 10min to obtain a clear supernatant. The supernatant was used to determine the concentration of HDL cholesterol in the sample.

Assay procedure

1ml of cholesterol working reagent was mixed with 20µl of the supernatant / standard and incubated for 5 min at 37^0C. The absorbance was measured at 500 nm against a reagent blank.

	Blank	Standard	Test
Reconstituted reagent	1ml	1ml	1ml
Standard	-	20ul	-
Supernatant	-	-	20ul

Calculation

$$\text{HDL cholesterol} = \frac{\text{OD Sample}}{\text{OD Standard}} \times \text{conc. standard}$$

The standard of 50mg /dl is fed as 100mg /dl to account for the dilution of the sample in the precipitation step.

Reference Range

In males : 30 – 65 mg/dl

In females : 35 – 80 mg/dl

4. VLDL cholesterol and LDL cholesterol

VLDL cholesterol and LDL cholesterol were calculated using the

FRIEDEWALD EQUATION

(LDL cholesterol) = (Total cholesterol)- (HDL Cholesterol) - Triglyceride/5

VLDL cholesterol = Triglyceride/5

As per the reference range of the Autopak Kit utilised for TC and HDL cholesterol this should vary from VLDL : Males 12 - 33 mg / dl; Females 8 - 28 mg / dl. LDL : Male 108 - 162 mg / dl; Females 107 - 152 mg / dl.

APOLIPOPROTEIN A-1

Turbidimetric Test

Kit used - Spinreact

Principle

Anti Apo A-1 antibodies when mixed with samples containing Apo A-1, formed insoluble complexes. These complexes caused an absorbance change dependent upon the Apo A-1 concentration of the patients sample, which was quantified by comparison from a calibrator of known Apo A-1 concentration.

Reagents.

Reagent (R_1). Diluent	Tris buffer 100mmol/L
	Polyethylene glycol 4000
	pH 7.2 sodium azide 0.95g/L
Reagent (R_2) Antibody	Goat serum, anti human Apo A-1, tris 100 mmol /L
	pH 7.2 sodium azide 0.95g/L

Preparation of working reagent

The working reagent was prepared by diluting antiserum Apo A-1 (R_2) 1:41 with buffer solution (R_1)

Samples were diluted 1:21 with saline solution (NaCl 0.9%).

Apo CAL calibrator dilutions in NaCl 9g/L as diluent was prepared.

Procedure

Spectrophotometer semi autoanalyser.

Wavelength 600nm

Temperature 37^0C

Cuvette light path 1 cm

Measurement

1ml of the working reagent and 20µl of the diluted sample / calibrator dilution was mixed and incubated at 37^0C for 10 minutes. The absorbance was read. at 600nm.

	Blank	Sample
Working Reagent	1.0ml	1.0 ml
Saline Solution (0.9%)	20 µl	-
Sample / Calibrator	-	20 µl

Calculation

A calibration curve was plotted with the absorbance and the Apo A-1 concentration of each calibrator dilution. Apo A-1 concentration in the sample was calculated by interpolation of its aborbance in the calibration curve.

Linearity - 600 mg/dl

Reference range - Between 122-161 mg/dl.

RESULTS

The level of serum apolipoprotein A-1 and other biochemical parameters namely serum total cholesterol, serum triglycerides, high density lipoprotein, very low density lipoprotein and low density lipoprotein in the control and study groups are tabulated in tables I to III Table I consists of the biochemical levels in the control group namely, the normal non pregnant women. The biochemical parameters in normal pregnancies are tabulated in table II, and the biochemical levels of pregnancies with IUGR are tabulated in table III.

The mean and standard deviation for the parameters analysed in each group is also given in the respective tables. The same has been elaborated separately in table.IV The mean level of each biochemical parameter in the different groups along with its standard deviation are also shown as bar diagrams from Chart II to Chart VIIs.

TABLE – I

Normal non - pregnant women

Sl. No	Total Cholesterol (mg/dl)	TGL (mg/dl)	VLDL (mg /dl)	HDL (mg/dl)	LDL (mg/dl)	ApoA-1 (mg/dl)
1.	138	100	20	45	73	105.1
2.	122	129	25.8	40	56.2	91.2
3.	187	200	40	40	107	176.7
4.	186	173	34.6	44	107.4	213.3
5.	170	196	39.2	42	88.8	162.1
6.	140	178	35.6	42	62.4	185.7
7.	165	180	36	40	89	131.3
8.	170	190	38	44	88	196.1
9.	122	90	18	40	64	102.6
10.	209	113	22.6	42	144.4	69.3
11.	185	101	20.2	44	120.8	121.7
12.	140	122	24.4	42	73.6	106.2
13.	160	94	18.8	44	97.2	107.7
14.	175	131	26.2	46	102.8	125.0
15.	182	127	25.4	42	114.6	161.7
16.	174	100	20	44	110	220.9
17.	171	93	18.6	38	116.4	124.5
18.	180	98	19.6	44	126.4	115.5
19.	186	101	20.2	40	125.8	67.3
20.	170	126	25.2	36	108.8	95.8
Mean	**166.6**	**132.1**	**26.4**	**41.9**	**98.8**	**133.9**
S.D	**± 23.1**	**± 38.7**	**± 7.7**	**± 2.5**	**± 24**	**± 45.6**

TABLE –II
Normal Pregnant women

Sl. No	Total Cholesterol (mg/dl)	TGL (mg/dl)	VLDL (mg /dl)	HDL (mg/dl)	LDL (mg/dl)	ApoA-1 (mg/dl)
1.	293	196	39.2	30	179.8	184.6
2.	244	231	42.2	28	169.8	215.4
3.	226	210	42	52	132	229.7
4.	201	119	28.8	34	143.2	187.2
5.	245	257	51.4	32	161.6	263.4
6.	246	323	64.6	48	133.4	328.1
7.	220	198	39.6	40	140.4	117.6
8.	232	405	81	51	100	225.7
9.	155	128	25.6	40	89.4	130.9
10.	207	188	37.6	30	139.4	171.9
11.	291	296	59.2	30	201.8	175.4
12.	234	224	44.8	50	139.2	149.2
13.	253	126	25.2	60	167.8	135.4
14.	204	276	55.2	44	104.8	182.2
15.	280	231	46.2	66	167.8	216
16.	193	321	64.2	42	86.8	169.7
17.	237	190	64.2	46	126.8	127.2
18.	230	234	46.8	62	121.2	176.5
19.	225	246	48	40	137	122.8
20.	220	250	50	50	120	152.7
Mean	**231.8**	**232.4**	**47.7**	**42.5**	**138.1**	**183.0**

Sl. No	Total Cholesterol (mg/dl)	TGL (mg/dl)	VLDL (mg /dl)	HDL (mg/dl)	LDL (mg/dl)	ApoA-1 (mg/dl)
S.D	± 32.9	± 70.6	± 14.1	± 12.0	± 30.3	± 52.3

TABLE - III

Pregnancies with IUGR

Sl. No	Total Cholesterol (mg/dl)	TGL (mg/dl)	VLDL (mg /dl)	HDL (mg/dl)	LDL (mg/dl)	ApoA-1 (mg/dl)
1.	131	248	49.6	32	49.4	76.14
2.	252	246	49.2	44	158.8	130.64
3.	159	372	74.4	30	54.6	165.87
4.	226	237	47.4	33	145.6	120.51
5.	194	135	27	28	139	120.21
6.	174	337	67.4	44	62.6	130.4
7.	249	188	37.6	40	171.4	170.2
8.	122	169	33.8	30	58.2	110.1
9.	192	168	33.6	36	122.4	140.2
10.	216	167	33.4	24	158.6	150.1
11.	168	196	39.2	34	94.8	136.3
12.	162	92	18.4	30	113.8	81.3
13.	167	221	44.2	30	92.8	130.1
14.	173	194	38.8	36	98.2	133.6
15.	277	121	24.2	62	190.8	160.1
16.	232	205	41	50	141	165.9

Sl. No	Total Cholesterol (mg/dl)	TGL (mg/dl)	VLDL (mg /dl)	HDL (mg/dl)	LDL (mg/dl)	ApoA-1 (mg/dl)
17.	200	159	31.8	56	112.2	170.1
18.	208	176	35.2	46	126.8	90.3
19.	167	156	31.2	44	91.8	100.4
20.	225	287	57.4	24	143.6	170.3
21.	191	116	23.2	48	119.8	139.4
22.	239	229	45.8	32	161.2	170.2
23.	295	175	35	30	230	151.2
24.	235	163	32.6	40	162.4	160.1
25.	367	238	47.6	44	275.4	170.3
26.	179	135	27	45	107	150.5
27.	398	358	71.6	40	286	140
28.	141	70	14	42	85	154.9
29.	167	144	28.8	40	98.2	149.2
30.	195	176	35.2	38	121.8	79.2
31.	202	168	33.6	42	126.4	107.7
32.	178	156	31.2	44	102.8	176.3
33.	212	206	41.2	32	138.8	184.3
34.	187	172	34.4	36	116.6	160.8
35.	193	184	36.8	40	116.2	174.4
36.	186	176	35.2	38	112.8	121.1
37.	194	190	38	40	116	198.8
38.	201	186	37.2	36	127.8	82.3

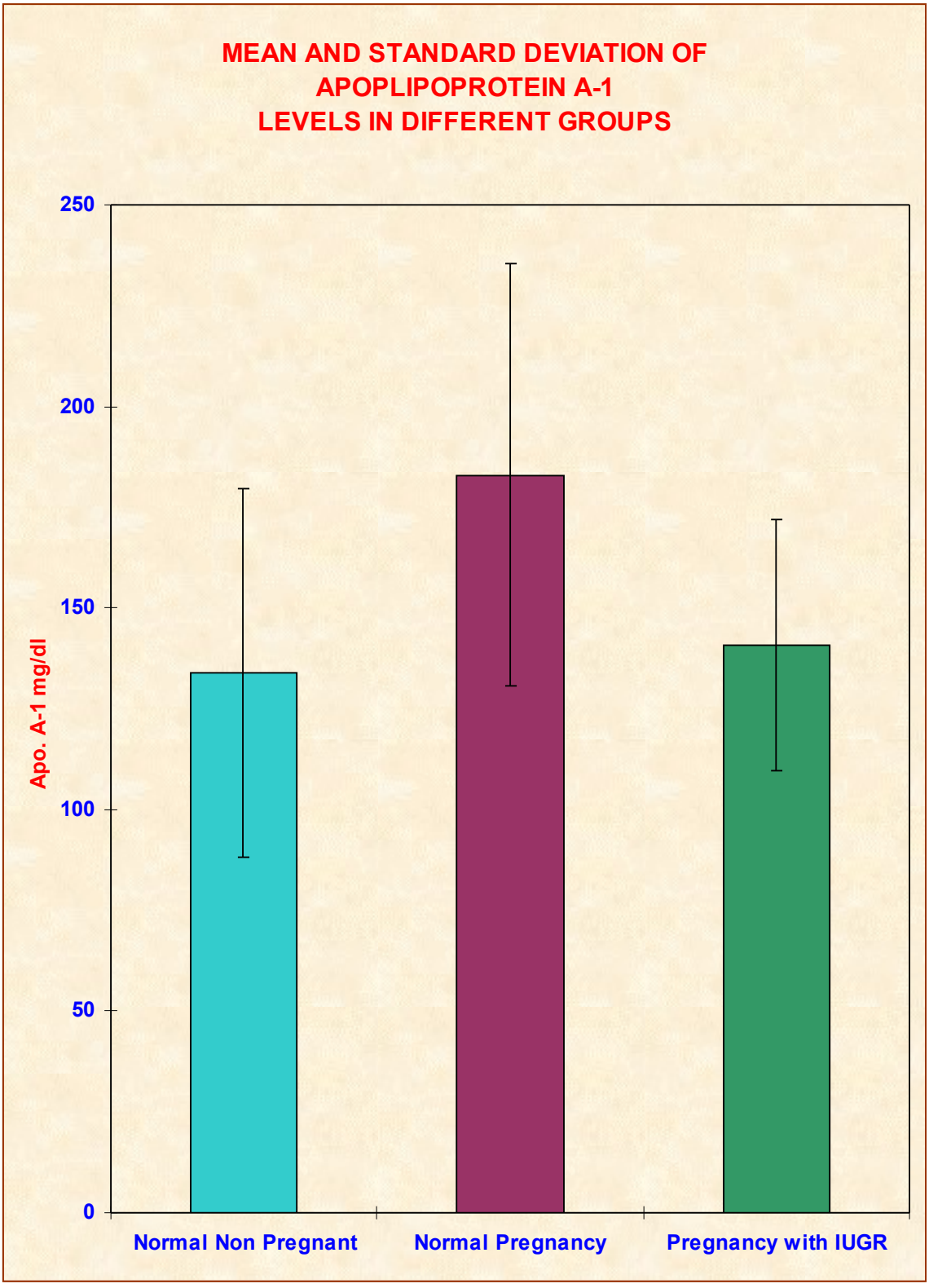
Contd...

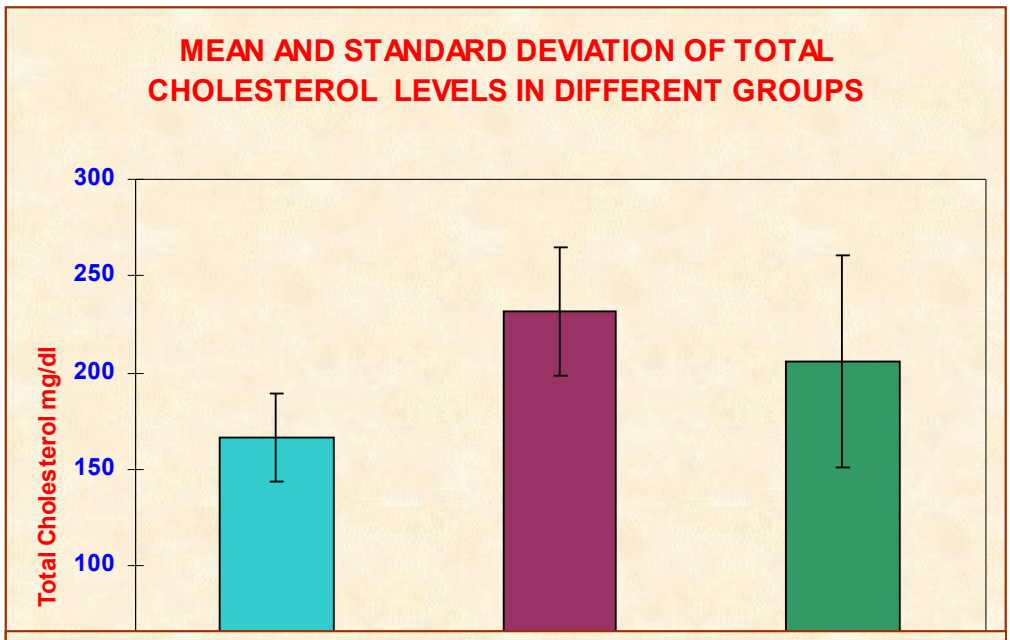
Sl. No	Total Cholesterol (mg/dl)	TGL (mg/dl)	VLDL (mg /dl)	HDL (mg/dl)	LDL (mg/dl)	ApoA-1 (mg/dl)
39.	188	174	34.8	38	115.2	148.1
40.	197	188	37.6	34	119.4	162.0
Mean	**205.9**	**192.7**	**38.9**	**38.3**	**129.1**	**140.8**
SD	**± 54.5**	**± 62.9**	**± 12.6**	**± 7.9**	**± 50.2**	**± 31.3**

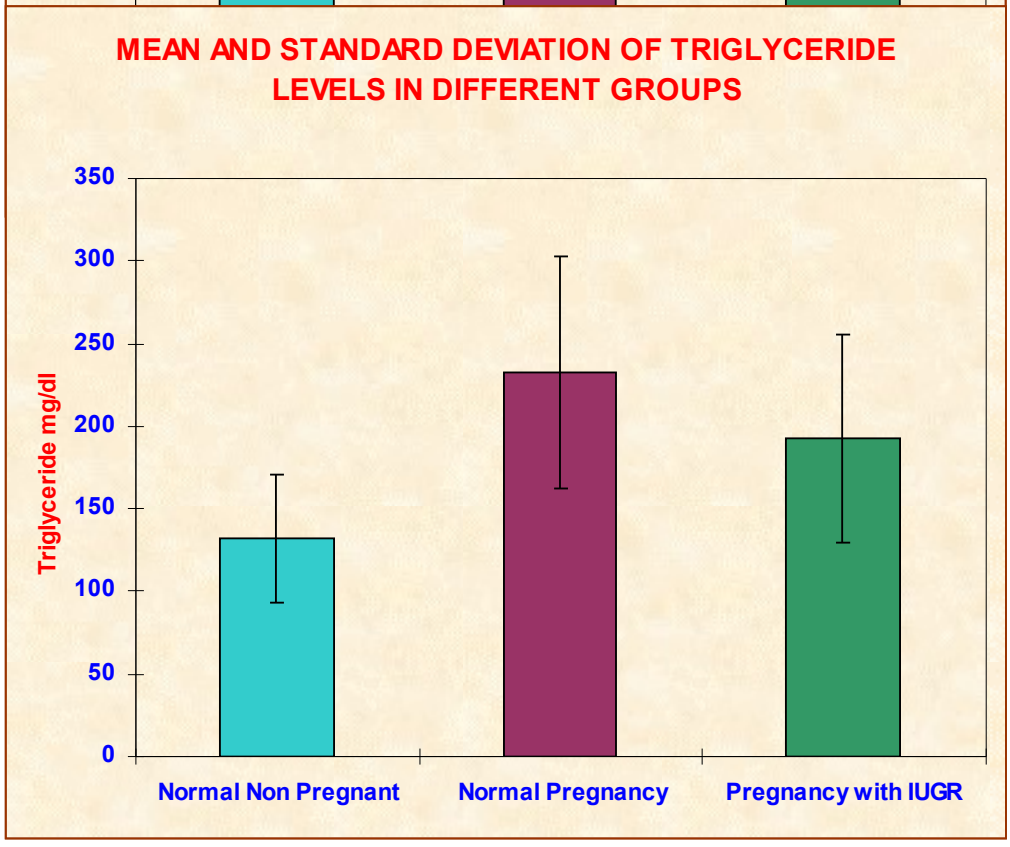
Table - IV

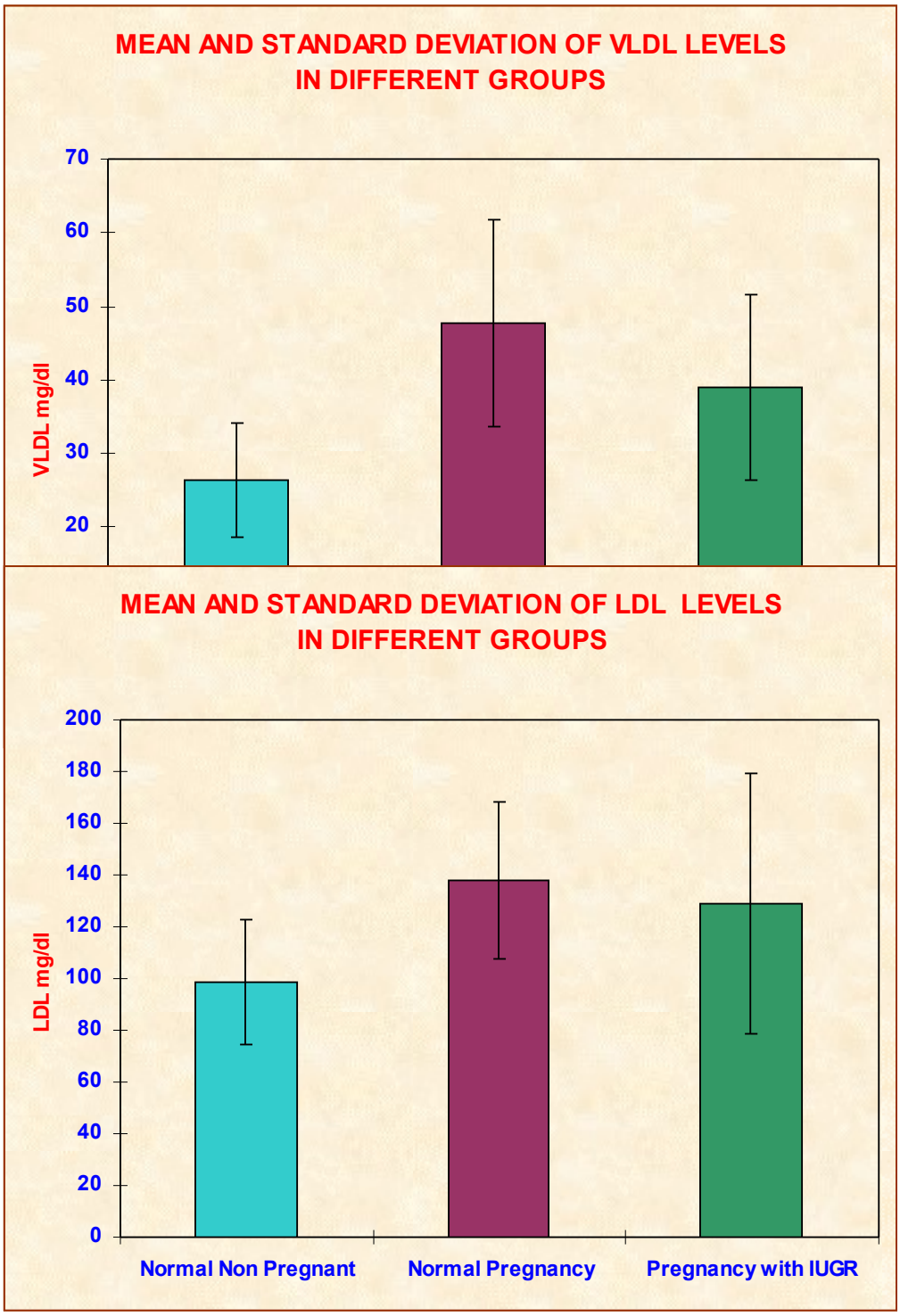
Mean and Standard deviation of the biochemical parameters in the different groups

Variables	Normal Nonpregnant	Normal Pregnancies	Pregnancy with IUGR
TC mg/dl	166.6 ± 23.1	231.8 ± 32.9	205.9 ± 54.5
TGL mg/dl	132.1 ± 38.7	232.4 ± 70.6	192. 7 ± 62.9
VLDL mg/dl	26.4 ± 7.7	47.7 ± 14.1	38.9 ± 12.6
HDL mg/dl	41.9 ± 2.5	42.5 ± 12.0	38.3 ± 7.9
LDL mg/dl	98.8 ± 24.0	138.1 ± 30.3	129.1 ± 50.2
Apo A-1, mg / dl	133.9 ± 45.6	183.0 ± 52.3	140 .8 ± 31.3


MEAN AND STANDARD DEVIATION OF
APOPLIPOPROTEIN A-1
LEVELS IN DIFFERENT GROUPS
Apo. A-1 mg/dl
250
200
150
100
50
0
Normal Non Pregnant
Normal Pregnancy
Pregnancy with IUGR


MEAN AND STANDARD DEVIATION OF TOTAL CHOLESTEROL LEVELS IN DIFFERENT GROUPS
Total Cholesterol mg/dl
300
250
200
150
100


MEAN AND STANDARD DEVIATION OF TRIGLYCERIDE LEVELS IN DIFFERENT GROUPS
Triglyceride mg/dl
350
300
250
200
150
100
50
0
Normal Non Pregnant
Normal Pregnancy
Pregnancy with IUGR


MEAN AND STANDARD DEVIATION OF VLDL LEVELS
IN DIFFERENT GROUPS
VLDL mg/dl
70
60
50
40
30
20
MEAN AND STANDARD DEVIATION OF LDL LEVELS
IN DIFFERENT GROUPS
LDL mg/dl
200
180
160
140
120
100
80
60
40
20
0
Normal Non Pregnant
Normal Pregnancy
Pregnancy with IUGR

DISCUSSION

On scrutinisation of the mean obtained for the lipid parameters in the control group (tables I) namely 166.6 ± 23.1mg/dl for total cholesterol, 132.1 ± 38.7 mg / dl for triglycerides, 26.4 ± 7.7 mg/dl for VLDL, 41.9 ± 2.5 mg/dl for HDL and 98.8±24.0 mg/dl for LDL, it is seen that total cholesterol, triglycerides and HDL fall well within the reference range of the kit method employed for their analysis. VLDL and LDL mean correlate well with the reference range given in standard reference books.

Apolipoprotein A-1 whose mean level is 133.9 ± 45.6 mg/dl in the above group is well within the reference range of 122 to 166mg/dl of the kit method employed for its analysis, and that of Dr.Nobert Tietz who has quoted a reference range of 130 to 160 mg/dl for females. As the mean for all the parameters in the control group, correlate well with the reference range of the

kit method employed for their analysis, or that quoted in standard books they are found to be valid and accepted as reference range for the present study.

The mean levels of lipid parameters in normal pregnant women (table II) and those with IUGR (table III) correlate well with that given by Dabi DR[43]. Apolipoprotein A-1 in both the groups is lower to that obtained by Munoz et al[36]. To find out whether the mean values for the different parameters in normal pregnancies, and pregnancies with IUGR, varied from that of the reference range obtained for the study, the latter means are compared with that of the former in tables V and VI respectively. The 'p' value on their comparison has been obtained by using the students 't' test and the same along with its corresponding statistical significance has been given in the respective tables.

Table - V

Comparison of the levels of the biochemical parameters in normal pregnancies with reference range

Group	TC mg/dl	TGL mg/dl	VLDL mg/dl	HDL mg/dl	LDL mg/dl	Apo A-1 mg/dl
Non Pregnant Women	166.6 ± 23.1	132.1 ± 38.7	26.4 ± 7.7	41.9 ± 2.5	98.8 ± 24.0	133.9 ± 45.6
Normal Pregnancy	231.8 ± 32.9	232.4 ± 70.6	47.7 ± 14.1	42.5 ± 12.0	138.1 ± 30.3	183.0 ± 52.0
p value	0.001	0.001	0.001	0.83	0.001	0.003
Significance	↑ HS	HS ↑	HS ↑	NS	HS ↑	MS ↑

From table V it is clear that all the lipid parameters in normal pregnant women except HDLc are greatly elevated from their reference range resulting in high significance (P value 0.001). The absence of any statistical significance for HDLc, can be attributed to the brunt of increase in total cholesterol being shared only by, VLDLc and LDLc. As VLDLc is a component of VLDL, which is transporting endogenous triglycerides from liver to tissues, and on its enroute the triglyceride is hydrolysed by lipoprotein lipase; the released free fatty acids is taken up by tissues[1]. In pregnancy, this free fatty acids also goes to the fetus. Since the free fatty acids that supplies the fetus originates from VLDL it is probably the reason for the increase of this lipoprotein and its cholesterol fraction. LDL which is formed from VLDL naturally increases leading to the increase of its fraction LDLc.

There is absence of any statistical significance for HDL as its main function is only reverse cholesterol transport, and thereby probably not a source of fatty acids to the fetus. Apolipoprotein A1 level of 183.0 mg / dl in normal pregnant women, though higher than the reference range of the kit method employed is much lower than that of 263 mg / dl obtained by A. Munozetal in a similar group[36]. This can probably be attributed to the racial variation and to the lower nutritional status of the women coming to general hospital. As reviewed in literature the increase in apolipoprotein A-1 can be attributed to HDL metabolism[41,42].

The following explanation corroborate well to the results obtained in the study. The changes in lipid levels during pregnancy result from the metabolic adaptation of the mother, mobilisation of fat deposits, the increase in free fatty acids and the relationship between circulating progesterone and oestrogen suggesting that these hormones are responsible for the lipid change observed. The increase in triglyceride concentration in normal pregnancies indicates a progressive increase in the supply of free fatty acids to the fetus during pregnancy. Normal fetal development needs the availability of both essential fatty acids and long chain poly unsaturated fatty acids, thus indicating a relationship between nutritional status of the mother during gestation as reflected by her lipid profile and fetal growth.

Table - VI

Comparison of the levels of the biochemical parameters in pregnancies with IUGR with reference range.

Group	TC mg/dl	TGL mg/dl	VLDL mg/dl	HDL mg/dl	LDL mg/dl	Apo A-1 mg/dl
Non Pregnant Women	166.6 ± 23.1	132.1 ± 38.7	26.4 ± 7.7	41.9 ± 2.5	98.8 ± 24.0	133.9 ± 45.6
Pregnancies with IUGR	205.9 ± 54.5	192.7 ± 62.9	38.9 ± 12.6	38.3 ± 7.9	129.1 ± 50.2	140.8 ± 31.3
p value	0.003	0.001	0.001	0.05	0.01	0.49
Significance	MS ↑	HS ↑	HS ↑	S↓	S↑	NS

In table VI where the group of pregnancies with IUGR is compared with non pregnant women, triglycerides and VLDLc are greatly increased giving

rise statistically to high significance ($p<0.001$). On the other hand total cholesterol shows only a moderately significant increase unlike that in the previous table. This can be due to more of triglyceride synthesis in comparison to cholesterol. The increase in triglyceride and VLDLc can be attributed by the same explanation, as given for the increase in triglyceride in the previous table (table V). It can be said that the brunt of the moderate increase of total cholesterol is borne by VLDLc than LDLc which shows only a mere significant increase. Apolipoprotein A-1 unlike in previous table does not show any significant increase. This is reassured by a significant decrease of HDLc a component of HDL.

It is possible that the lower concentrations of serum cholesterol, serum triglycerides, VLDLc, and LDLc 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. Studies have suggested that deficiencies in essential fatty acids might be present in growth restricted fetuses[44]. Infusion of essential fatty acids to women with intrauterine fetal growth restriction have produced a marked gain in fetal biparietal diameter and estimated weight[39].

Table - VII

Comparison of the levels of the biochemical parameters in pregnancies with IUGR with normal pregnancies.

Group	TC mg/ dl	TGL mg/dl	VLDL mg/dl	HDL mg/dl	LDL mg/dl	Apo A-1 mg/dl
Normal Pregnancies	231.8 ± 32.9	232.4 ± 70.6	47.7 ± 14.1	42.5 ± 12.0	1381 ± 30.3	183.0 ± 52.3
Pregnancies with IUGR	205.9 ± 54.5	192.7 ± 62.9	38.9 ± 12.6	38.3 ± 7.9	129.1 ± 50.2	140.8 ± 31.3
p value	0.03	0.03	0.01	0.11	0.47	0.001
Significance	S↓	S↓	S↓	NS	NS	HS ↓

From table VII where the levels in pregnant women with IUGR and normal pregnant women are compared, it is clear that the elevation of the lipid parameters namely total cholesterol, triglyceride and VLDLc is more in normal pregnant women than the group with IUGR. Hence the above three lipid parameter levels are significantly lower (p value 0.03) in the group with IUGR, to that in normal pregnant group. There is absence of any significant variation in HDLc and LDLc. This can be attributed because these two lipoproteins probably are not a source of free fatty acids to the fetus.

Apolipoprotein A-1 in the group of pregnant women with IUGR is much lower than its normal counterpart giving rise statistically to a highly significant lower p value. The actual levels of apolipoprotein A-1 in the two groups with the obtained significance is elaborated below in table VIII

Table - VIII

Apolipoprotein A-1 levels in normal pregnancies and pregnancies with IUGR

Variable	Normal Pregnancies	Pregnancies with IUGR	p value	Significance
Apo A-1 mg/ dl	183.0 ± 52.3	140.8 ± 31.3	0.001	HS ↓

This finding correlates with the study of A. Munoz and J. Uberos conducted at the University of Granada Hospital, Spain, except for the fact that the level of significance is higher in this study. As this is the only biochemical parameter which varies statistically by high significance when the two groups of pregnant women are compared, it indicates that the absence of elevation in apolipoprotein A-1 level during pregnancy can be a prime contributing factor in IUGR. The ratio of HDL / APO A-1 in the two groups of pregnancies is elaborated in the following table IX.

Table - IX

Ratio of HDL/ApoA-1in normal pregnancies and pregnancies with IUGR.

Group	HDL mg/dl	Apo A - 1 mg/dl	HDL/Apo A-1
Normal Pregnancies	42.5± 12.0	183.0 ± 52.3	0.25 ± 0.09
Pregnancies with IUGR	38.3 ± 7.9	140.8 ± 31.3	0.28 ± 0.08
p value	0.11	0.001	0.18
Significance	NS	HS ↓	NS

The table confirms that determination of the ratio is of no added value in this study unlike that of Munoz et al [36].

According to Jeffrey N. Jones the lipid profile of the intrauterine growth restricted fetuses indicated markedly diminished fetal liver derived apolipoprotein A-1 levels and decreased absolute total lipid levels, but triglycerides were elevated and free fatty acids lowered. These alternations are consistent with an inability to hydrolyse circulating triglycerides, leading to diminished peripheral adipose deposition. The lipid values in these fetuses indicated a diminished ability to deposit serum triglycerides in sufficient amounts to provide adipose stores, diminished levels of free fatty acids and impaired formation or secretion of apolipoprotein A-1 all of which are critical in the transition to neonatal life.

According to A. Munoz, haemorrheological modifications in growth restricted pregnancies are partly secondary to changes in high density lipoprotein metabolism mainly levels of Apolipoproteins A-1. The concurrent decrease in Apolipoproteins A-1 and increase in Apolipoprotein B in pregnancies with intrauterine growth restriction could partly explain the viscosity changes that occur in this complicated pregnancy. The affinity of both Apolipoprotein B and plasminogen for the same endothelial receptors, leading to competitive inhibition of fibrinolysis could explain the increase in plasma viscosity observed in the case of intrauterine growth restriction. However, only Apolipoprotein A-1 levels were determined in the present study.

The alternative 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 would be proposed that the genotype responsible for an atherogenic lipid profile might itself cause restricted fetal growth in utero.

In this study as indicated by previous studies also, there was a significant decrease in apolipoprotein A-1 levels in pregnant women with intrauterine growth restriction. This finding consistent with a decrease in apolipoprotein A-1 levels in cord blood of intrauterine growth restricted fetuses as indicated by Jefferey N. Jones suggests that the early detection of apolipoprotein A-1 in pregnant women would be useful to identify those mothers at a greater risk for intra uterine growth restriction.

Hence attempt has been made in this study to ascertain whether a
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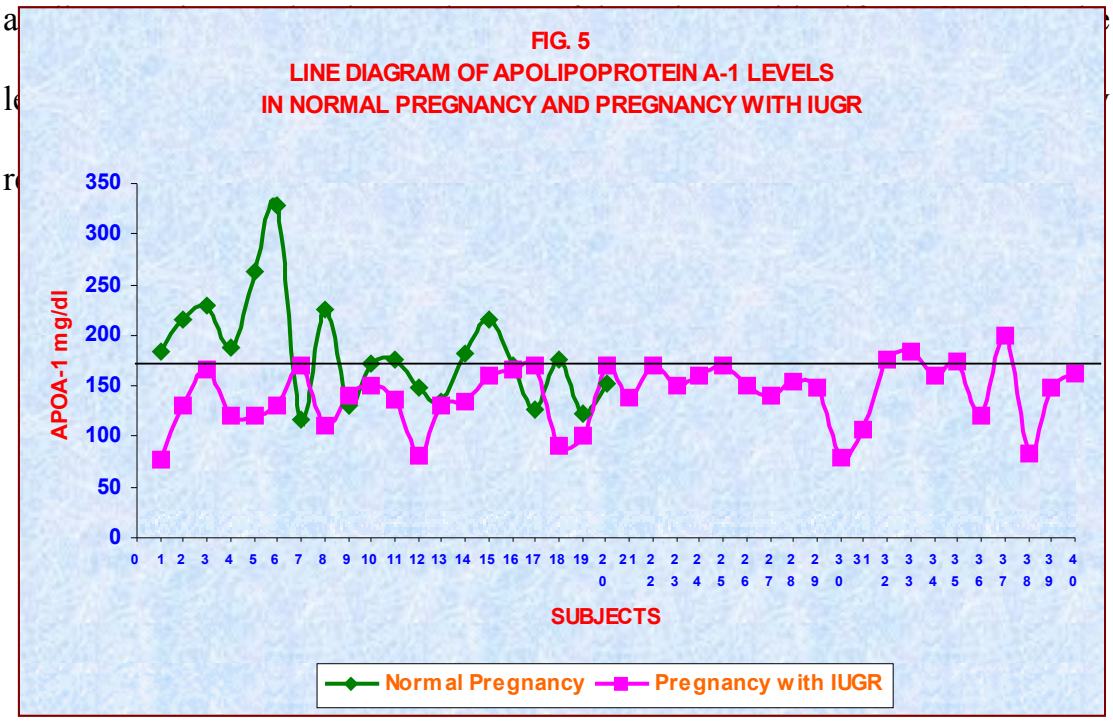

For the various cut off levels of apolipoprotein A-1 selected from the line diagram the sensitivity, specificity, positive predictive value and negative predictive value have been ascertained, which have been tabulated in table X.

TABLE - X
SENSITIVITY SPECIFICITY CHART FOR THE DIFFERENT CUT OFF LEVELS SELECTED

Cut off Level	Sensitivity	Specificity	Positive predictive value	Negative predictive value
160 mg/dl	62.5%	65%	78.1%	46.4%
170 mg/dl	77.5%	65%	81.5%	59.0%
176 mg/dl	92.5%	50%	78.7%	76.9%

From the table it is clear that a level of 176 mg / dl of apolipoprotein A-1 which has a sensitivity of 92.5% is the most appropriate level to demarcate the two groups. But as the above level of apolipoprotein A-1 has only 50 % specificity its reliability in confirming IUGR can be considered only next to ultrasonography in pregnant women.

CONCLUSION

Among the lipid parameters triglycerides, VLDL and total cholesterol were much higher in normal pregnancy compared to their non pregnant counterpart. In pregnancy with IUGR though the lipid parameters increased from the level in non pregnant women they do not reach the levels seen in normal pregnancy.

Apolipoprotein A-1 in normal pregnancies is much higher than in non pregnant women, this marked elevation of apolipoprotein A-1 is absent in pregnant women with IUGR. Hence apolipoprotin A-1 is a significant biochemical parameter in differentiating pregnant women with IUGR from those of normal pregnancy.

SCOPE FOR FURTHER STUDY

All the subjects in the present study belonged to the third trimester of pregnancy. Further advances in this study can be done by estimating the level of Apolipoprotein A-1 in all the three trimesters of pregnancy and comparing the corresponding changes in serum levels of Apolipoprotein A-1 in both the study and control group.

A complete nutritional assessment of the pregnant women can be done, and the level of Apolipoprotein A-1 can be estimated before and after nutritional supplements are given in pregnancies with ultrasound diagnosed intrauterine growth restriction.

Apolipoprotein A - 1 levels can also be estimated in the fetuses to rule out any intrauterine growth restriction.

Other biochemical parameters like Apolipoprotein B can be determined and its relation to Apolipoprotein A-1can be studied in pregnancies with IUGR.

ABBREVIATIONS

TC	-	Total Cholesterol
TGL	-	Triglycerides
VLDL	-	Very Low Density Lipo Protein.
HDL	-	High Density Lipoprotein
LDL	-	Low Density Lipoprotein
Apo. A-1	-	Apolipoprotein A-1
IUGR	-	Intra Uterine Growth Restriction.
VLDLc	-	VLDL Cholesterol
LDLc	-	LDL Cholesterol
HDLc	-	HDL Cholesterol
HS	-	Highly significant
MS	-	Moderately significant
S	-	Significant
NS	-	Not significant

BIBLIOGRAPHY

1. ***Peter A Mayes, Kathleen M. Botham***, Lipid Transport and storage, Harper's Illustrated Biochemistry, 26th edition. 205-218, 2003.

2. ***D.M.Vasudevan***, Cholesterol Lipoproteins and Cardiovascular disease, Text book of Biochemistry, 4th edition. 141 -155, 2005.

3. ***William J. Marshall***, Lipids and Lipoproteins, Clinical Chemistry 3rd edition, 213-225, 1995.

4. ***Evan A. Stein, Gary L. Myers***, Lipids. Apolipoproteins and Lipoproteins, Tietz, Fundamentals of Clinical Chemistry, 4th edition. 379-400, 1996.

5. ***Peter A. Mayes***, Lipid Transport and Storage, Harper's Biochemistry, 25th edition. 268 – 283,2002.

6. ***Libowang, David Atkinson, et al***. The Inter facial properties of Apo A-1 and an amphipathic α helix consensus peptide of exchangeable apo lipoproteins at the Triolein / Water interface, J Biol Chem, 2005; 280(6); 4154-4165.

7. ***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 lipoprotien cholesterol and APO A-1 concentrations, Clin Genet, 1993; 44: 307 – 312.

8. ***Philippe G. Frank and Yves L. Marcel***. Apolipoprotein A-1: structure; - function relationships, Journal of Lipid Research 2000; 41 : 853 – 872.

9. ***Brewer, H.B. Jr, Fairwell T et al***. The amino acid sequence of human Apo A-1 an apolipoprotein isolated from high density lipoproteins, Biochem. Biphys. Res. Commun. 1978; 80: 623 – 630.

10. ***Shoulders C.C. Baralle F.E.*** Isolation of the human HDL apolipoprotein A-1 gene, Nucleic Acids Res. 1982; 10: 4873-4882.

11. ***Li, W.H., Tanimura et al.*** The apolipoprotein multigene family : biosynthesis, structure – function relationships and evolution, J.Lipid research 1988; 29: 245-271.

12. ***Donald Voet, Judith G.Voet Charlotte W. Pratt***, Lipoproteins and receptors mediated endocytosis, Fundamentals of Biochemistry. 262-265, 1999.

13. ***Nadir Rifai, Paul S. Bachori K***, Lipids, Lipoproteins and Apolipoproteins, Tietz textbook of clinical Chemistry, 3rd edition. 809-862 1986.

14. ***Morlose JF, Jahoor F, Forrester TE***. Plasma Apolipoprotein A-1 and Birth weight, Lancet 1997; 350 : 1823 – 4.

15. ***Schaefer EJ, Heaton WH, Wetzel MG, Brewer HB Jr***. Plasma apolipoprotein A-1 associated with a marked reduction of HDL and premature artery disease, Arteriosclerosis, 1982; 2 : 16-26.

16. ***Norum RA, Lakier JB, Goldstein S, et al***. Familial deficiency of apolipoproteins and precocious coronary artery disease, N. Eng J. Med, 1982; 306 : 1513 – 1515.

17. ***Qureshi I.A. et al***. Hyperlipidemia during normal pregnancy, parturition and lactation, Anna Acad Med Singapore Mar; 28 (2) : 217 – 21.

18. ***Harvey D, Prince J, Burton J et al.*** Abilities of children who were small for gestational age babies, Pediatrics 1982; 69: 296-300.

19. ***C.B.Marenah***, Lipid metabolism, hyper and hypolipidemias and atherosclerosis, Clinical Biochemistry, Metabolic and Clinical aspects, edited by William J. Marshall, Stephen K. Bangert. 624-627, 1995.

20. ***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 1999; 84 (1) : 128-130.

21. ***Chard T, Costeloek, Leaf A***. Evidence of growth retardation in neonates of apparently normal weight, Eur. J.Obstet. Gyenocol. Reprod. Endocrinol 1992; 45 : 5962.

22. ***Iain R. McFadyen***, Maternal Physiology in Pregnancy, Turnbull's Obstetrics 2nd edition, Edited by Geoffrey Chamberlain. 97-114, 1996.

23. ***Brizzi P, Tonolo G, Esposits F***. Lipoprotein metabolism during normal pregnancy, Am. J Obstet Gynecol 1999; 181 (2) : 430-4.

24. ***Piechota W, Staszewski A***. Reference range of lipids and apolipoproteins in pregnancy, Eur J Obstet Gynecol Reprod Biol 1992; 45(1) : 27-35.

25. ***Lechtig A, Yarbrough C et al.*** Influence of maternal nutrition on birth weight, Am J Clin Nutr 1975; 28 (11) : 1223-33.

26. ***Neary RH, Kilby MD, et al.*** Fetal and maternal lipoprotein metabolism in human pregnancy, Clin Sci (Land) 1995; 88(3) : 311-8.

27. ***Karl Winkler, Birgit WetzKa, Michael M. Hoffmann.*** Low density lipoprotein (LDL) subfractions during pregnancy: Accumulation of Buoyant LDL with Advancing gestation, The Journal of Clinical Endocrinology and Metabolism 2000; 85 (12) : 4543-4550.

28. ***Jeffrey N. Jones, Cicek Gercel Taylor, Douglas D. Taylor.*** Altered cord serum lipid levels associated with small for gestational age infants, Obstetrics and Gynecology, 1999; 93:527-531.

29. ***Spencer JA, Chang TC et al***, Third trimester fetal growth and measures of carbohydrate and lipid metabolism in umbilical venous blood at term, Arch Dis Child Fetal Neonatal Ed 1997; 76 : F21-F25.

30. ***Robert A. Harris, David W.Crabb,*** Metabolic Interrelationships, Text Book of biochemistry with clinical correlations 4th edition, edited by Thomas M.Devlin; 526-529, 1997.

31. ***Sattar N. Bendomir A, et al***, Lipoprotein Subfractions in pre eclampsia, pathogenic parallels to atherosclerosis, Obstet Gynecol 1997; 89: 403-408.

32. ***Potter JM, Nestel PJ***, The hyperlipidemia of pregnancy in normal and complicated pregnancies, Am J, Obstet Gynecol 1979; 133 : 165 -177.

33. ***Kuzawa CW, Adair LS.*** Lipid profiles in adolescent Filipinos : relation to birth weight and maternal energy status during pregnancy, Am J Clin Nutr 2003; 77 : 960-6.

34. ***Emilo Herrea.*** Implications of dietary fatty acids during pregnancy on placental, fetal and postnatal development, - a review, Placenta 2002; 23 (1) : 942 – 945.

35. ***Uberos – Fernandez J, Munoz, Hoyos A et al.*** Lipoproteins in pregnant women before and during delivery: influence on neonatal haemorheology, AJ Clinical Pathol 1996; 49 (2) : 120-3.

36. ***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 1995; 48 (6) : 571 – 574.

37. ***Fahraeus L, Larsson, Cohn U,*** Plasma lipoproteins including high density lipoprotein subfractions during normal pregnancy, Obstet Gynecol 1995; 66: 468 – 72.

38. Fetal growth disorders, William's Obstetrics, 21st edition 744-760, 2001.

39. ***Herrera E, Lasuncion MA, Lopez Luna P et al.*** Role of lipoprotein lipase activity on lipoprotein metabolism and the fate of circulating triglycerides in pregnancy, Am J Obstet Gynecol 1988; 158 : 1575-1583.

40. ***Marconi AM, Cetin I, Davoli E, et al.*** An evaluation of fetal gluconeogenesis in intrauterine growth retarded pregnancies, Metabolism 1993; 42 : 860-864.

41. ***Fletcher AP, Alkjaersig NK, Burstein R.*** The influence of pregnancy upon blood coagulation and plasma fibrinolytic enzyme function, Am J Obstet Gynecol 1979; 134 : 743-751.

42. ***Huisman A, Aarnoudse JG et al.*** Whole blood viscosity during normal pregnancy, Br J Obstet Gynecol 1987; 94 : 1143 - 90.

43. ***Dabi Dr, Parakh Manish Goyal Vikas.*** A cross- sectional study of lipids and lipoproteins in pregnancies with intrauterine growth retardation, J.Obstet Gynecol Ind. 2004; 54 (5) : 467-472.

44. ***Richard G Ijzerman et al.*** Evidence for genetic factors explaining the association between birth weight and low density lipoprotein cholesterol and possible intrauterine factors influencing the association between birth weight and high density lipoprotein cholesterol. Analysis in twins, The Journal of clinical Endocrinology and Metabolism 2001; 86 (11) : 5479 – 5484.

45. ***Knopp RH, Warth MR et al***. Lipoprotein metabolism in pregnancy, fat transport to the fetus and the effect of diabetes, Biol Neonate 1996; 50 : 297-317.