

THE RELATION BETWEEN CHOLESTEROL AND PLASMA N-3, N-6 AND SATURATED FATTY ACIDS OF PREGNANT SUDANESE WOMEN**Dr. Khalil A. K. H.***

Department of Basic Medical Science, College of Medicine, Dar Al Uloom University, Riyadh, Kingdom of Saudi Arabia.

Corresponding Author: Dr. Khalil A. K. H.

Department of Basic Medical Science, College of Medicine, Dar Al Uloom University, Riyadh, Kingdom of Saudi Arabia.

Article Received on 23/04/2018

Article Revised on 14/05/2018

Article Accepted on 04/06/2018

ABSTRACT

Cholesterol is a member of steroids group. It is found naturally in animals and rarely in plants, it has two forms either esterified (cholesterol esters) or free. Essential fatty acids are polyunsaturated fatty acids (PUFA) which contain more than one double bond. The purpose of this study was to investigate and correlate between the level of fatty acid composition in plasma phosphatidylcholine and cholesterol of pregnant Sudanese women. Blood samples were obtained from the pregnant women at delivery time in Khartoum state hospitals. Full and detailed history and examinations were performed in the study groups. Plasma lipids were extracted by Folch method and separated by Gas Liquid Chromatography (GLC). Blood cholesterol level were calculated. A positive co relation was found between cholesterol with saturated fatty acids and omega-6 fatty acids, and negative with omega-3 and omega-3/omega-6 ratio among the pregnant Women.

KEYWORDS: Cholesterol, Essential fatty acids, Gas liquid chromatography, phosphatidylcholine.**I. INTRODUCTION**

Cholesterol is a member of steroids group. It is found naturally in animals and rarely in plants, it has two forms either esterified (cholesterol esters) or free state.

It is found mainly in cell membranes where it has a vital role of maintaining fluidity. It has a rigid ring system and short-branched hydrocarbon tail. Although it is largely hydrophobic it has one hydroxyl polar group, making it amphipathic.^[1]

Membranes are composed of two layers of amphipathic lipids of which phospholipids is the major component (61%) in humans, with proteins (enzymes, transporters or receptors etc) located at the internal or external face of the membrane. The lipid fatty acid composition of the membranes influences their physical properties, particularly the membrane fluidity (i.e. packing of lipids in the membrane bilayer), which is determined by; 1) the nature of fatty acids chains; 2) the amount of cholesterol and 3) interaction of both polar and non-polar lipids and between lipids and proteins.^[2]

According to Lauritzen et al., LCPUFA are acylated to membrane phospholipids often in sn-2 position, and make-up about 21-36% of the total fatty acid in cell membranes. DHA is specifically distributed in high concentrations in neuronal tissues such as photoreceptor membranes of the retina and in cerebral cortex of the brain. In contrast, AA is generally found in relatively

large amount in most tissues.^[3,4] Their distribution in different phospholipids classes also differs. Phosphatidylcholine (PC), which is predominantly present in the outer surface of the membrane bilayer, is rich in AA. About half of the total fatty acids content of phosphatidylethanolamine (PE) and phosphatidylserine (PS) in the outer segment of rat retina is comprised of DHA.^[5]

Distribution of the LCPUFA in plasma and red blood cell is influenced by various processes such as dietary intake, intestinal absorption, metabolism, storage, and exchange among compartments, however, the typical composition of each lipid class is distinctive.^[6] In human plasma, PC (69% of PLs), cholesterol esters (28% of total cholesterol) and triglyceride are dominant lipids classes, whereas in RBC, PC (29%), sphingomyelin (26%), PE (31%); and PS and Phosphatidylinositol (PI) (13.2 %) are the major classes of phospholipids.^[6]

Human red blood cells cannot synthesise membrane lipids *de novo* and depend on two mechanisms to achieve this goal; lipids exchange and acylation of fatty acids. The phospholipids of the plasma lipoproteins exchange with the outer bilayer phospholipids (PC and SM) in an extremely slow rate (a turnover time of 5 days), however, phospholipids of inner leaflet of the bilayer (PS and PE) are non-exchangeable. In contrast free cholesterol (FC) in red cell exchanges readily with esterified cholesterol (CE) in the plasma lipoprotein (a half-life of 7 hours);

and FC can also be converted to CE by enzymatic process of lecithin: cholesterol acyltransferase (LCAT).^[6]

The acylation mechanism is essentially an energy-dependent process. The fatty acids are incorporated into lysophosphatides (mostly lyso-PC) to produce the natural phospholipids with two acyl chains. The enzyme acylase and the products (phospholipids) are present in the inner leaflet of the bilayer. Membrane lipids are therefore slowly replaced through this pathway. This process approximately takes about 30 days before lipids equilibrium is reached following a change in dietary fatty acids.^[7]

II. MATERIALS AND METHODS

Study design: Cross sectional prospective study.

Study area (setting): Khartoum, Maternal and Bahri Hospitals in Sudan.

Duration: From January 2010-to February 2014.

Subject and populations

Third trimester pregnant women (35-40) weeks having no organic conditions which may alter fatty acids level (HTN, DM).

Exclusion criteria

- Non pregnant or pregnancy less than 35 weeks.
- Lactating.
- Has organic conditions which may alter fatty acids level (HTN, DM).

Control: From healthy non pregnant mature Sudanese females (14-40 years) not suffering from organic conditions which may alter the fatty acids level.

Data collections: Written and oral consent were taken from the participants before filling the questionnaire which express the personal, medical, obstetrical history, socioeconomic etc.....

Procedure for determination of plasma phosphatidylcholine fatty acids

- ❖ 5 mls venous blood was dropped from the study group in lithium heparin tube.
- ❖ Separation of blood cells from plasma using centrifuge (2000rpm/15minuts).
- ❖ Add 50microL tissue homogenate to hexane (1ml) and (1ml) BF3/MeOH reagent 14%.
- ❖ Heat to 100c (1hour) and cooled to room temperature.

- ❖ Extraction of methyl ester after addition of H₂O (1ml).
- ❖ Fatty methyl ester then analyzed using GLC.

Cholesterol Quantification using a Spectrophotometric Assay^[8]

- ❖ We dilute the cholesterol standard to 0.5µg/µL by adding 20µL of cholesterol.
- ❖ Standard to 180µL of cholesterol reaction buffer, This will create 200µL of the 0.5µg/µL solution.
- ❖ Mix the 200µL solution well to ensure complete mixing. Add 0, 4, 8, 12, 16, and 20µL of the new standard solution into each microwell.
- ❖ Cholesterol Reaction Buffer was added to each well in order to have each well contain 50µL of solution (add 50µL of buffer into well #1, 46µL of buffer into well #2, etc. Generating a 0, 2, 4, 6, 8, and 10µg/well standard.
- ❖ Add 50µL of the unknown sample into an empty well.
- ❖ Then Add 50µL of the Reaction Mix to each of the respective wells.
- ❖ Incubate the 96-well plate for 37°C for 60 minutes under aluminium foil. To protect the solution from light.
- ❖ Measure the absorbance at 570nm wavelength using a microplate reader.

Statistical analysis

Data was analyzed by using SPSS version 16(SPSS, Chicago, IL, USA). The results are given as mean and standard deviation (mean ± SD). In addition to P values and correlation for some parameters.

III. RESULTS

Table (1): Represents the different parameters (weight, height, BMI and MUAC) of control and pregnant women.

Mean pregnant weight was (73 Kg) range(59-89 Kg), while for control the mean value was (61) Kg range (40-86 Kg). BMI for pregnant women was 29 range(20-43) whereas in control was (24) with a range of (14 – 39).

Weight and BMI of pregnant women was significantly higher than control (p≤0.05).

The mean cholesterol level of pregnant women was 169.4 mg/dl (Range(103-260 mg/dl) which was significantly higher than the control group 156.8 mg/dl. Both values are less than the mean international level of cholesterol (200- 240 mg/dl).^[9]

Table 1: Anthropometric and biochemical measurements of the pregnant and non pregnant groups.

Criteria	Control (66) Mean ±SD	Pregnant (66) Mean ±SD	P value
Weight(kg)	61.4±10.7	73.1±8.4	p≤0.05
Height (meter)	1.6±0.1	1.6±0.1	p≥0.05
BMI	24.00±5.7	29.00±7.7	p≤0.05
Cholesterol level mg/dl	156.8±34.5	169.4±33.0	p≤0.05

Table 2: Shows Correlation between saturated, omega-3, omega-6 fatty acids and omega-3/omega-6 ratio with the biochemical parameters among the neonates and pregnant women.

A positive co relation was found between cholesterol with saturated fatty acids and omega-6 fatty acids, and negative with omega-3 and omega-3/omega-6 ratio among the pregnant and neonates.

Table 2: Correlation between saturated, omega-3, omega-6 fatty acids and omega-3/omega-6 ratio with cholesterol among the neonates and pregnant women.

	neonates				Pregnant			
	SFA	w-3	w-6	w-3/w-6	SFA	w-3	w-6	w-3/w-6
Cholesterol	.01*	-.19*	.09*	-.23*	.08*	-0.32**	0.62*	-0.33**

Table 3: Correlation between saturated, omega-3, omega-6 fatty acids and omega-3/omega-6 ratio with the biochemical parameters among the control and pregnant women. A positive co relation was found between

cholesterol with saturated fatty acids and omega-6 fatty acids, and negative with omega-3 and omega-3/omega-6 ratio among the pregnant and controls.

Table 3: Correlation between saturated, omega-3, omega-6 fatty acids and omega-3/omega-6 ratio with the biochemical parameters among the control and pregnant women.

	Control				Pregnant			
	SFA	w-3	w-6	w-3/w-6	SFA	w-3	w-6	w-3/w-6
cholesterol	.02**	-0.32*	0.21**	-0.43*	.083*	-0.32**	0.62*	-0.33**

IV. DISCUSSION

The mean cholesterol level of pregnant women was 169.4 mg/dl \pm 33.0 which was significantly higher than the controls 156.8 mg/dl \pm 34.5. Both values were less than the mean international level of cholesterol, and this may be explained by the low fat content of our diet.^[10] Cholesterol is needed by the placenta of pregnant women to synthesize steroid hormones which help placental maturation, also needed by the neonates; it is a key constituent of the cell membranes, the structure of which is obviously important in cell-to-cell interactions, which are essential in embryonic differentiation.^[11] However, cholesterol not only plays a role in the physical structure of the membrane (eg, its viscosity and interference with phospholipids), but also makes up specific parts of this membrane, such as the caveolae (detergent-resistant domains), which are important for transduction and ceramide-induced apoptosis. In fact, 7-dehydrocholesterol reductase deficiency characterize by Smith-Lemli-Opitz syndrome (microcephaly, corpus callosum agenesis, holoprosencephaly, and mental retardation), male pseudohermaphroditism, finger anomalies, and failure to thrive.^[12] Increased cholesterol synthesis during pregnancy is enhanced by high levels of oestrogen.^[13]

A positive correlation was found between cholesterol, saturated fatty acids and omega-6 level among controls, pregnant women and neonates ($r=0.02, 0.08, 0.01, r=0.21, 0.62, 0.09$) ($p \leq 0.05$) respectively, and negative with omega -3 fatty acids and omega-3/omega-6 ratio of control, pregnant and neonates ($r= -0.32, -0.32, -0.19, r= -0.43, -0.33, -0.23$) ($p \leq 0.05$) respectively. There was no specific correlation between essential fatty acids and cholesterol level during pregnancy and neonates, but generally evidence is accumulating that long-chain

omega-3 fatty acids (DHA) can decrease the risk of cardiovascular disease by preventing arrhythmias that can lead to sudden cardiac death, decreasing the risk of thrombosis(a clot) through synthesis of eicosanoid that can lead to myocardial infarction (MI) or stroke, decreasing serum triglyceride and cholesterol levels by enhancing HDL synthesis, slowing the growth of atherosclerotic plaque, improving vascular endothelial function, lowering blood pressure slightly, and decreasing inflammation.^[14]

The positive correlation between n-6 fatty acids and cholesterol level explained by the competition of n-6 and n-3 with the same desaturase enzyme, so high n-6 indicate low n-3(DHA) which enhance lowering of cholesterol. A higher intake of linoleic acid may protect against ischemic stroke, not by decreasing cholesterol level but possibly through potential mechanisms of decreased blood pressure and reduced platelet aggregation.^[15]

REFERENCES

1. Smith CA, Marks D and Lieberman M. Mark's Basic Medical Biochemistry: A Clinical Approach. 2nd ed. Philadelphia: Lippincot Williams & Wilkins; 2005.
2. Stillwell W, Wassall SR. Docosahexaenoic acid: membrane properties of a unique fatty acid. Chem Phys Lipids, 2003; 126(1): 1-27.
3. Crawford MA, Hassam AG, and Williams G. Essential fatty acids and fetal brain growth. Lancet, 2006; 1(7957): 452-3.
4. Fliesler SJ. and Anderson RE. Chemistry and metabolism of lipids in the vertebrate retina. Prog Lipid Res., 2003; 22(2): 79-131.
5. Stinson AM, Wiegand RD and Anderson RE. Recycling of docosahexaenoic acid in rat retinas

- during n-3 fatty acid deficiency. *J Lipid Res.*, 1991; 32(12): 2009-17.
6. Rise P. Fatty acid composition of plasma, blood cells and whole blood: relevance for the assessment of the fatty acid status in humans. *Prostaglandins Leukot Essent Fatty Acids*, 2007; 76(6): 363-9.
 7. Van Deenen LL, DeGier J. Lipids of red cell membrane in *The Red Blood Cell*, D.M. Surgenor, Editor. Academic Press: New York; 2004.
 8. Smith M, Haggard C. Cholesterol Quantification using a spectrophotometer Assay. *Biomed. engr. sc. ed.*
 9. Qureshi IA, Xi XR, Limbu YR, Bin HY, Chen MI. Hyperlipidaemia during normal pregnancy, parturition and lactation. *Ann Acad Med Singapore*, 1999; 28(2): 217-21.
 10. Nyuar KB, Khalil AK, Crawford MA. Dietary intake of Sudanese women: a comparative assessment of nutrient intake of displaced and non-displaced women. *nutr. health*, 2011; 11(6): 13-25.
 11. Charles Roux, Claude Wolf, Nicole Mulliez, Wassila Gaoua, Valerie Cormier, Françoise Chevy, and Danièle Citadelle. Role of cholesterol in embryonic development. *American j. of Clinical Nutrition*, 2000; 50(6): 98-123.
 12. Usui I. Fatty acid induced insulin resistance in rat-1 fibroblasts overexpressing human insulin receptors: impaired insulin-stimulated mitogen activated protein kinase activity. *Diabetologia*, 2007; 40: 894-901.
 13. Nancy F Butte. Carbohydrate and lipid metabolism in pregnancy: normal compared with gestational diabetes mellitus. *AMJ of Clini Nutri*, 2000; 11(9).
 14. Otto SJ. Maternal and neonatal essential fatty acid status in phospholipids: an international comparative study. *Eur J Clin Nutr.*, 1997; 51(4): 232-42.
 15. Last, Allen R, Wilson, Stephen A. Low-Carbohydrate Diets. *American Family Physician*, 2006; 45(2): 100-113.