

# Elevations Of Serum Total Cholesterol Differ By Trimester In Pregnant Women At Chitungwiza Central Hospital Ante-Natal Clinic In Zimbabwe

Lynet Nyakudya, Danai Tavonga Zhou

**Abstract:** A physiological increase in serum total cholesterol levels occurs during pregnancy. This hypercholesterolaemia may lead to metabolic disorders in the foetus and the mother. Little information is available on pregnancy-induced lipid metabolism disturbances in the Zimbabwe setting. A cross-sectional study of total serum cholesterol levels in pregnant women who attended Chitungwiza Central Hospital Antenatal Clinic between 24 January 2013 and 14 February 2013 was carried out. All recruited women were either in the second or third trimester of pregnancy. Samples were analysed using a BS120® Chemistry Analyser. The paired t test proportion was used to test the hypothesis at 5% significance level. There were a significant proportion of women with moderately elevated total serum cholesterol compared to pregnant women reference ranges but it could not be determined whether the elevations were pathological or physiological hence further studies are required to determine whether this could develop into overt hypercholesterolaemia post-partum. Of significance, in this study the third trimester women had significantly higher mean total cholesterol levels compared to the second trimester women, p value = 0.0033 ( $p < 0.05$ ).

**Key words:** total cholesterol, pregnancy, trimester, hypercholesterolaemia, Chitungwiza, Zimbabwe

## 1 INTRODUCTION

CHOLESTEROL is a lipid composed of a hydroxyl group, tetracycline rings and a hydrocarbon chain [1]. Cholesterol has many physiological roles: for example it is a precursor in the synthesis of Vitamin D and steroid hormones – cortisol, aldosterone, progesterone, oestrogen and their derivatives [2], [3]. It is also structural component of the plasma cell membranes; regulating membrane fluidity over a wide range of temperature. This is because the hydroxyl group on the cholesterol molecule interacts with the phosphate groups of the membrane phospholipids, while the carboxyl groups interact with the carboxyl groups of the phospholipids [4]. This increases membrane packing at the same time reducing membrane fluidity. Cholesterol also reduces the permeability of the membrane to neutral solutes, protons and sodium ions [5]. In the cell membrane, cholesterol is important for receptor mediated endocytosis [6]. This is particularly important in phagocytosis by cells of the immune system.

Cholesterol is also important in cell signaling as it aids in the formation of lipid rafts. Lipid rafts bring receptor proteins into close contact with high concentrations of secondary messenger molecules such as cyclic adenosine monophosphate (cAMP). This makes cholesterol important in the propagation of signaling cascades [7], [8], [9]. Cholesterol is also important in the development of the central nervous system and is particularly important in development of the brain and spinal cord of the foetus [10], [11]. Cholesterol is synthesised from acetyl-coA and this occurs mostly in hepatic cells of the liver although all cells of the body are capable of synthesizing it. Biosynthesis is regulated by homeostatic mechanisms which involve enzymes such as sterol regulatory element binding protein (SREBP) and 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) [12], [13]. SREBP responds to low intracellular cholesterol levels by up-regulating expression of lipogenic proteins such as HMGCR and low density lipoprotein receptor (LDLR) which promotes uptake of cholesterol into cells [13]. Cholesterol is oxidized in the liver and conjugated to glycine, glucuronic acid and sulfate to form bile salts; a component of bile [14], [15]. Bile is important for emulsification of dietary fats before digestion by lipases. If cholesterol is highly concentrated in the bile it leads to the formation of gall stones [16]. Cholesterol is not soluble in blood hence the need for it to be coupled to lipoproteins for transportation [1]. There are six major sub-fractions of lipoproteins which are: chylomicrons, very low density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) [17], [18], [19], [20], [21], [22]. Chylomicrons are the least dense of all the lipoproteins. They carry cholesterol from the gut to the muscles and other tissues that use fatty acids as a source of energy or for fat production. Chylomicrons not utilized by the muscle are absorbed by the liver. VLDLs are synthesized by the liver and contain excess cholesterol not required for bile salts production [17], [18], [19], [20], [21], [22]. IDLs are taken up by the LDL receptors on the liver surfaces or continue to lose triacylglycerols in the blood circulation until they form LDLs [18][19]. LDLs transport cholesterol from the liver to other cells of the body that have need of extracellular amounts. High levels of LDL result in deposition of cholesterol in arteries (atherosclerosis) which can result in coronary heart

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disease [19]. HDL is responsible for reverse cholesterol transport (RCT) that is transport of excess cholesterol from the cells and circulation to back to the liver [20], [21], [22]. High levels of HDL correlate with good cardiac health whilst high levels of LDL increase the risk of coronary heart disease [17], [18], [19], [20], [21], [22]. HDL and LDL are the two major fractions of lipoprotein cholesterol. Serum total cholesterol is a measure of all the cholesterol (HDL, LDL and VLDL) circulating in the blood. During pregnancy, maternal physiological and hormonal changes occur that are concerned primarily with the nutrition of the growing foetus [23]. One of the changes is the increase in the levels of total cholesterol in the blood [24]. Maternal LDL cholesterol is important for the synthesis of placental progesterone [25]. Structural cholesterol in plasma membranes plays a critical role in implantation and the formation of blood vessels in the utero-placental area [25], [26], [27]. Placental cholesterol levels changes affect transportation of molecules across the placenta and this varies with the gestational period [25], [26], [27]. HDL cholesterol is involved in maintaining a balance in the placental cholesterol levels [20], [21], [22], [23], [24], [25] [26], [27]. Maternal cholesterol is thought to be transported to the foetus for development of foetal and placental tissue [25], [26], [27]. Studies elsewhere have established that total cholesterol levels rise with increasing gestational age [20], [21], [22], [23], [24], [25] [26], [27], [28]. This hypercholesterolaemia (high blood cholesterol levels) has been attributed to the hormonal effects of progesterone and oestrogen [17], [18], [23]. Studies have shown that the daily production of progesterone increases thirtyfold, while that of oestrogen increases tenfold during pregnancy [29]. Progesterone increases plasma levels of LDL cholesterol and total cholesterol while lowering HDL-cholesterol but oestrogen has an opposite effect [20], [21], [22], [23], [24], [25] [26], [27], [28], [29]. Various studies have shown the attribute of diet to this hypercholesterolaemia during pregnancy to be insignificant [27] [28], [29]. Some studies have documented changes in maternal total cholesterol and the fractions of cholesterol [20], [21], [22], [23], [24], [25] [26], [27], [28]. HDL cholesterol increases from the second semester of pregnancy and remains high throughout the rest of pregnancy in response to oestrogen levels. Maternal LDL and total cholesterol increase progressively from the second semester of pregnancy and even remain high after delivery [24], [25] [26], [27], [28]. In the first trimester of pregnancy, cholesterol levels are thought not to change but several studies have shown that they actually fall when compared to pre-pregnancy levels [17], [18], [23]. Total cholesterol levels rise in the second and peak in the third trimester [24], [25] [26], [27], [28]. The placenta is an anatomical barrier that prevents contact of the maternal and fetal blood and is composed mainly of multinucleated trophoblast [25]. It is thought that maternal cholesterol is able to cross the placenta and enter fetal circulation and is taken up in the form of lipoproteins by the trophoblast on the maternal side by both receptor mediated and receptor independent mechanisms [26], [27]. Studies using animal models have shown that there is a direct association between maternal and fetal total cholesterol levels [26]. According to the National Health Institution of the United Kingdom, the optimum total cholesterol levels should be less than 5.2mmol/liter irrespective of sex and age. The USA National Institute of Health's National Human Genome Research (NIH-NHR) came up with reference ranges of total cholesterol levels for

pregnant women. NIH-NHR proposed that a low serum total cholesterol level lies below 4 mmol/liter, moderate total cholesterol lies between 4 and 6.8 mmol/liter and high level is above 6.8 mmol/liter [30]. Differences in maternal total cholesterol levels have been linked to age, sex, race and ethnicity [23], [29], [30]. This makes it difficult to recommend optimal maternal levels across all ethnic groups and races.

## 2 METHODOLOGY

### 2.1 Study Design

Cross sectional analytical study

### 2.2 Study Setting

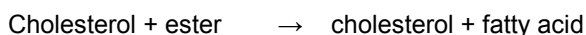
The study participants were recruited from Chitungwiza Central Hospital Antenatal Clinic. Chitungwiza is a peri-urban town that is 25km from Harare City and has a population of about 1 million residents. Some of the Chitungwiza Town residents are working class, while some are self-employed. Chitungwiza Central Hospital Antenatal Clinic serves women from Harare City, Chitungwiza Town, and Chitungwiza's rural environs.

### 2.3 Sample Processing

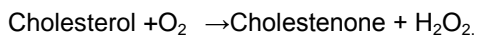
Blood was collected in plain tubes from pregnant women visiting Chitungwiza Central Hospital Antenatal Clinic who consented and fit the inclusion criteria. The blood samples were centrifuged at 1500 revolutions per minute for 5 minutes. Serum was separated and placed in serum pots which were stored at -20°C until the day of processing. On the day of processing, samples were thawed at room temperature and analyzed for total cholesterol using the Mindray® BS120 (Mindray Medical International Limited, Shenzhen, China) after calibration. The statistical package Stata 11 (Statacorp, Texas United States of America) was used in all statistical analysis of the data.

### 2.4 Principle of the Method

The Mindray® BS120 chemistry automatic analyzer uses an enzymatic method for the measurement of total cholesterol. About 50 microliters of patient's serum was mixed with the commercial reagent for total cholesterol. The reagent used a bacterial cholesterol ester hydrolase to cleave cholesterol esters.



The 3-OH group of the cholesterol was oxidized to a ketone using cholesterol oxidase enzyme.



Hydrogen peroxide then reacted with 4 aminoantipyrine in the presence of phenol to form the colored compound, quinoneimine.



The absorbance of quinoneimine was measured and used to calculate the total cholesterol concentration in the original sample.

**2.5 Inclusion Criteria**

1. Pregnant women between 16 and 45 years in the second trimester during the recruitment period
2. Pregnant women between 16 and 45 years of age in the third trimester during the recruitment period

**2.6 Sample Size Calculation**

The sample size was calculated using the following equation:

$$N_i = (\Sigma_1^2 + \Sigma_2^2) (Z_{\alpha/2} + Z_{\beta})^2 + (\mu_1 - \mu_2)^2$$

Where:

$$\alpha = 0.05$$

$$Z_{0.025} = 1.96$$

$$Z_{\beta} = Z_{0.2} = 0.84$$

From the Nigerian study:  $\mu_1 = 175.4$        $\Sigma_1^2 = (38.3)^2$

$\mu_2 = 157.2$        $\Sigma_2^2 = (37.8)^2$

$$N_i = (38.3^2 + 37.8^2) (1.96 + 0.84)^2 / (1.754 - 157.2)^2 = 69$$

Sample size = 138 (69 second trimester and 69 third trimester)

**2.7 Ethical Considerations**

Permission to use antenatal women’s specimens was sought from the Clinical Director of Chitungwiza Central Hospital, the Sister-in-charge of the Ante-natal Clinic and the Chief Medical Laboratory Scientist heading the Laboratory. The study obtained ethical clearance from the Joint Research Ethics Committee (JREC) of Parirenyatwa Hospital–University of Zimbabwe. Samples were assigned research numbers to maintain patient confidentiality.

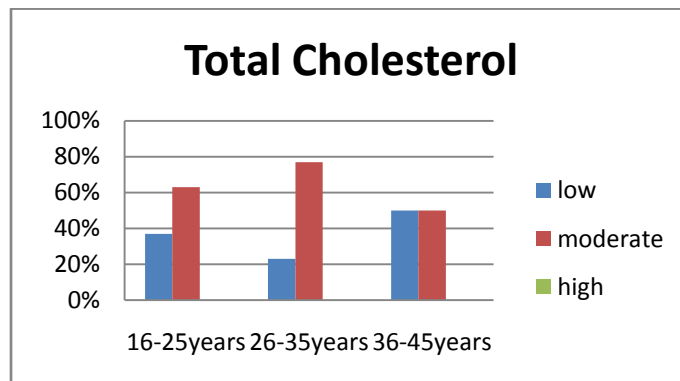
**3 RESULTS AND ANALYSIS**

Using the USA-NIH-NHR total cholesterol reference ranges for pregnant women the results were grouped into three categories: low (<4mmol/liter); moderate (4 - 6.8mmol/liter) and high (>6.8mmol/liter) [30].The results show that there is significant difference in the respective proportions of women in the second and third trimester with low, moderate and high total cholesterol levels (Table 1).More second trimester women than third trimester women had low total cholesterol values when using the USA-NIH-NHR established reference range. For the third trimester women, more (4.3% of the women) had elevated serum total cholesterol, than their second trimester counterparts (0%).

**Table 1: Distribution of Serum Total Cholesterol Levels by Age in the Second and Third Trimester Women of Chitungwiza Antenatal Clinic, Zimbabwe**

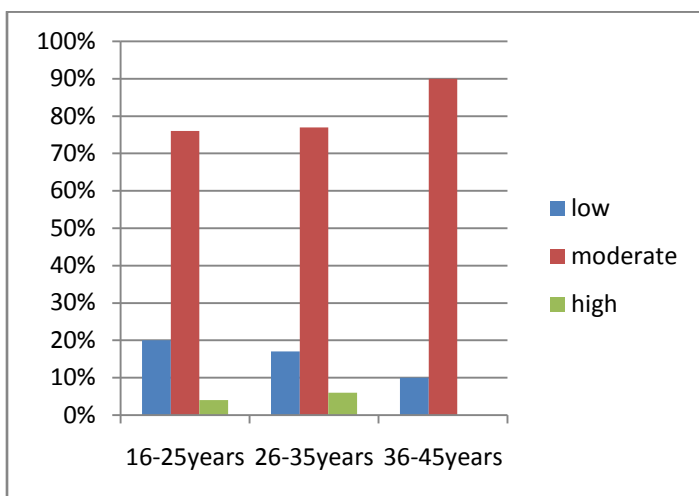
Age group	Second trimester women n=70			Third trimester women n=70		
	LowN/Proportion	Medium N/Proportion	HighN/Proportion	LowN/Proportion	Medium N/Proportion	HighN/Proportion
16-25yrs	12/37.5%	20/62.5%	0/0%	5/20%	19/76%	1/4%
26-35yrs	8/24%	26/76%	0/0%	6/17%	27/77%	2/6%
36-45yrs	2/50%	2/50%	0/0%	1/10%	9/90%	0/0%
Total number of women	22/31%	48/69%	0/0%	12/17%	55/79%	3/4%

For women in the second trimester of pregnancy, there is a general increase in the serum total cholesterol levels between 16 years and 35 years (Figure 1). For women in the third trimester of pregnancy, there is a general increase in the serum total cholesterol levels across all age groups studied (Figure 2).



**Figure 1: Distribution of Total Cholesterol by Age Group in the Second Trimester**

Serum total cholesterol levels were generally higher in the third trimester in each respective age group. There is also a general rise in the total cholesterol with a peak in the 36-45 year age groups (Figure 2).



**Figure 2:** Distribution of Total Cholesterol by Age Group in Third Trimester

Table 3 shows the differences in the statistical values obtained in these two distinct trimesters of pregnancy. The total cholesterol values were more widely spread in the third trimester as compared to the second trimester as illustrated by the higher standard deviation (sd) of 1.14 in third trimester than the sd of 0.87 in the second trimester.

**Table 3: Statistical Parameters Obtained From the Study**

Parameter	Second trimester values	Third trimester value
Minimum TC value	2.47mmol/liter	3.22mmol/liter
Maximum TC value	6.17mmol/liter	9.65mmol/liter
Mean TC value	4.55mmol/liter	5.13/liter
Standard deviation	0.87	1.14
P value	0.0033 (significant <0.05)	

All statistical analysis of the data was carried out using Stata 11 statistical software package. The data was tested using the Shapiro Wilk test and was found not to be normally distributed in both the second and third trimesters evaluated. The p value was calculated by the two sum Wilcoxon rank-sum (Mann-Whitney) method and was found to be less than 0.05. This shows that the difference in total cholesterol levels of third trimester and second trimester women is statistically significant.

## 4 DISCUSSION

The data showed that 0% of the second trimester women and 4% of the third trimester women had total cholesterol levels that were higher than that of the recommended levels. This suggests that there may be an increase in total cholesterol that occurs during pregnancy as many previous studies have shown [23], [24], [25] [26], [27], [28], [29]. The mean total cholesterol value of 4.88mmol/liter in the second trimester was within the desirable range of the normal reference range. The mean total cholesterol value of 5.13mmol/liter in the third trimester was very close to the borderline high of 5.18mmol/liter. In one study carried out on Nigerian women, the mean total cholesterol levels were higher than in our study: 5.37mmol/liter in the second trimester and 6.43mmol/liter in the third trimester [29]. In a similar study done on various ethnic groups, mean total cholesterol levels were: Ghanaian women – 4.65mmol/liter, Moroccan women – 5mmol/liter while for African Caribbean women the mean was 4.96mmol/liter [23]. The study of the Ghanaian, Moroccan and African Caribbean women however did not group the participants according to trimester. The differences in the mean total cholesterol levels in these various groups may be due to confounding by factors such as: trimester, age distribution together with ethnicity [23], [29], [30].

## 5 CONCLUSIONS

There was a physiological increase in mean total cholesterol levels by trimester of pregnancy in women attending antenatal clinic at Chitungwiza Central Hospital between January and March 2013. This suggests that there is a need to do routine tests for total cholesterol during pregnancy in order to differentiate between a physiological increase and a pathological one and to establish a national reference range for our Zimbabwean population.

## 6 RECOMMENDATIONS

The study participants were from only one antenatal clinic and sample collection was done over a three week period; reducing the sample size and power of the study. Total cholesterol levels are affected by several other factors such as diet, body mass index (BMI), maternal weight gain, maternal nutrition and pre-pregnancy lipid levels which were not accounted for in this study. There was also no control group of non-pregnant women in our study. There is also a need to measure specific important sub-fractions of total cholesterol such as: HDL, LDL and triacylglycerols. All these shortcomings can be addressed if a larger country-wide study is carried out. However our study does give evidence of progressive elevations in mean total cholesterol levels by trimester of pregnancy.

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