Correlation between Serum Sialic Acid, Serum Lipids, Cardiac Troponin I and Myoglobin among Women on Combined Oral Contraceptives

Idris Y. Mohammed¹, Usman Datti², Hadiza L. Abdullahi², Akeem O. Busari³

¹Department of Chemical Pathology, Bayero University Kano, Kano State, 700233, Nigeria
²Department of Medical Laboratory Science, Bayero University Kano, Kano State, 700233, Nigeria.
³Department of Medical Laboratory Science, Ladoke Akintola University of Technology, Ogbomoso, 210214, Nigeria

Abstract

Introduction

Globally, cardiovascular diseases (CVDs) are the leading cause of death, accounting for more deaths each year than all other causes combined (1). More than 80% of the world burden of CVD occurs in developing countries (2). In Nigeria, the burden of Non-communicable Diseases (NCDs) is attributable primarily to cardiovascular diseases and continues to show a rising trend (3–5). Cardiovascular related deaths were estimated to account for 51.1% of out of hospital deaths in Nigeria (4). A recent 20-year review of CVD in Nigeria revealed an increasing trend (6). CVD biomarkers include a wide array of substances found in the blood, urine, or tissue such as enzymes (AST, LDH & Creatine Kinase) and Cardiac proteins (TnI, TnT & Myoglobin). Other inflammatory markers including adhesion molecules, cytokines, acute phase reactants have various roles in risk assessment, diagnosis and prognostication. An association between serum Total Sialic Acid (TSA) and cardiovascular mortality in the general population has been demonstrated (7).

In healthy arteries, Sialic acid (SA) contributes to the overall net negative charge of vascular endothelial cells and low-density lipoprotein (LDL) surface receptors (8). The overall negative charge of cell surface and glycoproteins imparting cell to cell repulsion (antiadhesion effect). Due to the shielding effect, sialylated glycans protect parts of a glycoprotein from proteolytic attacks. LDL with lower SA concentration tends to form aggregates that are easier for smooth muscle cells to absorb (9), thereby promoting reverse cholesterol transport. SA, an acute-phase reactant, can be found at terminal oligosaccharide chains of acute phase proteins. Sialic acids are terminal sugar components of the oligosaccharide chains of glycoproteins and glycolipids. Sialic acids

Corresponding author: Idris Yahaya Mohammed

Department of Chemical Pathology, Bayero University Kano, PMB 3011, Kano, Kano State, Nigeria.
Email: idrismoore@gmail.com
Phone: 08133394293
Contribute significantly to the functioning stability and survival of glycoproteins in blood circulation and cell-to-matrix interactions. Due to the shielding effect, sialylated glycans protect parts of a glycoprotein from proteolytic attacks. Membrane sialic acids assist in cell – cell recognition serving as chemical messengers in tissues and body fluids. Serum TSA is a candidate marker of acute phase reaction in CVD (8). Another study showed that serum SA levels reflect acceleration of the atherosclerotic process by increased blood pressure, which is a known independent risk factor for atherosclerosis. Age and the usage of hormonal birth control tablets were both recognized to raise serum SA levels. (10).

Combined oral contraceptive (COC) use is prevalent in Nigeria with rates varying zonally from 2.7% in the northwest to 28.5% in the southwest and from 0.3% in Jigawa to 41.6% in Lagos (11). COC use increases the CVD risk (12), cause thromboembolic effect, affect lipid metabolism, cause an increase in inflammatory markers, rise in blood pressure and increased weight (13, 14). Serum SA has been shown to be elevated in cardiovascular disease (15) and proposed to be a long-term predictor of coronary heart disease (16). SA content of platelet, erythrocyte (RBC) and low-density lipoprotein (LDL) has also been shown to play important role in the development of atherosclerotic complications (17). Some studies showed that individuals with high serum triglycerides or cholesterol have a higher level of SA, while those with high HDL cholesterol have a substantially lower level. (18, 19). In addition, SA has been demonstrated to substantially correlate with female body mass index, fasting serum cholesterol, and triglycerides (20). Men’s serum total sialic acid strongly associated inversely with hip/waist ratio and considerably favorably with fasting blood cholesterol and triglycerides concentration (20).

The need to discover more reliable biomarkers is ongoing given the growing global burden of cardiovascular disease (CVD) and the rise in the usage of COC. Furthermore, data on the role of Sialic acid and its association with known cardiovascular risk factors in women using COC is scarce in our local environment. To the author’s knowledge, no research on serum sialic acid have ever been published using local data. Therefore, the purpose of this study was to ascertain the relationship between SA and some cardiovascular disease risk variables in women using combined oral contraceptives. This will give justification for deciding if SA could be used to estimate the risk of CVD.

Materials and Methods

Study design

This case-control study was conducted between July and December 2019 in the Kano metropolis at the Murtala Muhammad Specialist Hospital and Muhammadu Abdullahi Wase Specialist Hospital in Kano, Northwest Nigeria.

Study population and sampling

A total of 273 women between the ages of 18 and 40 make up the study population, including 173 women on COC and 100 age and sex matched women as controls. Women with irregular menstrual cycles, smokers, high blood pressure, recent and ongoing infections, renal disease, alcohol users, those with diabetes, cardiovascular disorders, and those on medication were all excluded. Sample size was calculated using Cochran (21) formula and a prevalence of 13% from a previous study (22). The control groups were selected from apparently healthy blood donors, volunteers, and students. Both study participants were recruited consecutively until the desired sample size was obtained. Ethical approval was obtained (ref: MOH/off/797/T4/883) from the Kano state research and ethics committee prior to the commencement of this study. This study complied with the provisions of the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from participants for inclusion into the study. The study aims and protocol was explained to the subject in the appropriate language.

Instrument and Data Collection

A pretested questionnaire was used to obtain information on sociodemographic characteristics, medical history, comorbidities, anthropometric measurements, combined oral contraceptive use and laboratory results of analysis. Data was collected with the help of trained research assistants.

Anthropometric Measurement

Height: This was measured by a vertical board with an attached metric rule and a horizontal headboard. The subjects were asked to stand barefooted, arms by their sides and to look straight backing the vertical board. Then the height was recorded in meters.

Weight: This was measured while the subjects are wearing light clothing with the subject standing in the center of a weighing scale’s platform. The weight was recorded in kilograms.

BMI: This was calculated as body weight divided by the square of height (kg/m²), with body weight expressed in kg and height in meters.
Specimen Collection, Processing and Storage
Venous blood was collected from the ante-cubital vein of participants. The skin overlying the vein was cleaned with methylated spirit swab. Using 10mls syringe and needle, blood sample was collected from each participant. Samples were collected in gel activator tubes (each tube well labelled with the participant’s details), centrifuged and the serum was separated into plain specimen bottles and stored at -20°C within 30 minutes after collection. These samples were utilized for the analysis of both serum sialic acid, serum lipids and cardiac biomarkers.

Equipment
The following equipment was employed: Cobas e 602 chemistry analyzer, Fine care chemistry analyzer, Centrifuge, UV-VISIBLE spectrophotometer, Water bath, Specimen containers (EDTA and plain sample bottles), Glass test-tubes, Plastic test-tubes, syringes. Chemicals
Reagents of analytical grade for the determination of Serum sialic acid viz: Thiobutyric acid, periodic acid and sodium arsenite, hydrochloric acid, sulphuric acid and butanol were procured from BDH®. Reagents for lipid profile and cardiac makers were procured from Randox Diagnostic Inc®.

Laboratory methods
Serum sialic acid was determined using Aminoff Method (23). Cardiac troponin I (cTnI), Myoglobin and CK-MB were determined using sandwich immunofluorescence assay technique (24) on Finecare analyser. Concentrations of serum total cholesterol (TC), triglycerides (TGs) and high-density lipoprotein cholesterol (HDL-c) were measured using enzymatic assays. Triglycerides TG was estimated by glycerol phosphate oxidase (25). High-density lipoprotein cholesterol by Phosphotungstics acid method (26) and Total cholesterol by colorimetric method (27). Low-density lipoprotein cholesterol (LDL-c) levels was calculated using the Friedewald formula (28).

Data Analysis
Data collected were entered into an excel spreadsheet which was subsequently exported to Statistical Package for Social Sciences (SPSS) software package version 16.0. (IBM Corporation, Armonk, NY, USA). Data was summarized as mean and standard deviations with results presented as ranges and percentages in figures and tables. Student t-test and ANOVA were used to determine differences in the means of various groups while Pearson’s correlation was used to determine correlation between variables at P < 0.05.

Results
Baseline Characteristics
The mean age of the patients and controls were 29.19±6.45 years and 26.42±7.64 years, respectively. No statistically significant difference was observed in the mean age (p>0.05) (Table 1). The mean BMI for cases was higher than that of controls with values of 25.20±4.58 kg/m² and 21.95±2.65 kg/m², respectively, and the difference was statistically significant (p<0.001) (Table 1). The mean systolic blood pressure (SBP) of cases (116.9±11.2 mmHg) was significantly higher than that of controls (110.1±11.2 mmHg) (p<0.001) (Table 1). Figure 1 showed that TSA correlates poorly with systolic blood pressure (r=+0.129). The mean diastolic blood pressure (DBP) was 77.3 ±7.3 mmHg for patients and 77.7±7.2 mmHg for control, and when compared, the difference was statistically significant (p<0.001) (Table 1).

Comparison of TSA and Lipid Profile of Combined Oral Contraceptive Users and Controls
The mean TSA was found to be significantly higher (p<0.0001) in the COC group (1.070±0.117) when compared to the control group (0.837±0.272). Also, a significantly higher (p<0.0001) levels of TC (3.944±0.918 vs 3.106±0.635), LDL-C (2.408±1.085 vs 1.088±0.392), HDL-C (1.049±0.421 vs 0.902±0.542) and TGs (1.620±0.703 vs 1.052±0.514) were observed in the COC group in comparison to the control group.

Cardiac Biomarker Profile of Combined Oral Contraceptive Users and Controls
The mean level of the assayed cardiac biomarkers Myoglobin, cTnI and CK-MB in women on COC and controls is shown in Table 3. There was no statistically significant difference in levels of Myoglobin (p = 0.204), cTnI (p=0.151) and CK-MB (p=0.196) between the two groups.
Table 4 showed the relationship between TSA and various serum lipids. The results show a very weak inverse relationship between TSA and TC (r= -0.136), LDL-C (r= -0.146), HDL-C (r= -0.006) while TSA and TGs showed a very weak but positive relationship (r= +0.089).

Table 5 also showed a significant strong positive relationship between TSA and cTnI (r= 0.424, P= 0.020), creatine kinase (r= 0.441, P= 0.015) and myoglobin (r= 0.403, P= 0.027) among women using COCs.

Discussion
Cardiovascular diseases and their complications are the main cause of mortality and morbidity in developed countries, and they are emerging as prominent public health problems in developing countries (29). In women, combined oral contraceptives affect the cardiovascular system through their impact on lipid profile, blood pressure and body mass index (30). The rising incidence of these known risk factors in women on combined oral contraceptives underlines the need to identify other potential risk factors such as Sialic acid (31). Approximately 64% and 30% of women worldwide and in Nigeria who use combined oral contraceptives, respectively, are in the 18–30 age range, which is consistent with the average age of the participants in our study (29.19±6.450) years.

Table 3: Level of some selected cardiac Biomarkers in Women on Hormonal Contraceptives

Table 2: TSA, Lipid Profile and Cardiac Biomarkers in Study Population and Controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study group</th>
<th>Control group</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSA (mg/ml)</td>
<td>n=173</td>
<td>n=100</td>
<td></td>
</tr>
<tr>
<td>TC Mean±SD</td>
<td>1.070±0.117</td>
<td>0.837±0.272</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lipid profile</td>
<td>n=173</td>
<td>n=100</td>
<td></td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>3.94±0.318</td>
<td>3.16±0.35</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.049±0.241</td>
<td>0.930±0.32</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>2.68±1.085</td>
<td>1.08±0.392</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TRIGs (mmol/l)</td>
<td>1.629±0.703</td>
<td>1.05±0.544</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

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A total of 121 (69.9%) contraceptive users have their mean body mass index (25.20 ± 4.583) significantly at (P = 0.0001) higher and in the overweight range which could be attributed to the impact of estrogen on the reduction of ability to burn energy after eating and thus resulting in increased storage of fat in the body (32). The estrogen component of combined oral contraceptives has been found to increase weight and body mass index in contraceptive users (13), which could account for the observed high body mass index in the group on combined oral contraceptives. Additionally, our data concurs with prior studies that found a substantial rise in body mass index among women of reproductive age who used oral contraceptives compared to the control group. (33,34). The raised body mass index, systolic and diastolic blood pressure observed in this study pose an increased risk of cardiovascular diseases (35). TSA showed an insignificant positive association with systolic blood pressure (r= 0.129, P= 0.090) and DBP (r= 0.120, P= 0.115). Use of combined oral contraceptives have been proposed to be responsible for increased blood pressure through its effect on smooth muscles and the rennin-angiotensin system (36). The mean total sialic acid was found to be significantly increased (p< 0.0001) in combined oral contraceptive users than in the control group which agrees with the...
theory that combined oral contraceptives elevate the level of inflammatory markers and acute phase proteins, and sialic acid is inherently an acute-phase reactant and moieties are also present at the end of acute phase proteins oligosaccharide chains (31).

<table>
<thead>
<tr>
<th>Variable</th>
<th>TC</th>
<th>TGs</th>
<th>LDL-C</th>
<th>HDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>-0.136</td>
<td>-0.089</td>
<td>-0.146</td>
<td>-0.006</td>
</tr>
<tr>
<td>p</td>
<td>0.074</td>
<td>0.247</td>
<td>0.055</td>
<td>0.943</td>
</tr>
</tbody>
</table>

- *r* = correlation coefficient. Correlation significant at 0.05 levels (2 tailed) 
- TSA: Total Sialic Acid 
- TC: Total Cholesterol 
- TGs: Triglycerides 
- LDL-C: Low Density Lipoprotein Cholesterol 
- HDL-C: High Density Lipoprotein Cholesterol

compared to the control group. Also, a significant increase (*P*= 0.0332) in high-density lipoprotein-cholesterol was observed in the combined oral contraceptive users when compared with control subjects. This agrees with a study (37) that reported that high-density lipoprotein-cholesterol level increases with contraceptive use due to inhibition of the hepatic lipase (enzyme responsible for clearing high-density lipoprotein-cholesterol from the circulation) activity by estrogen. Low-density lipoprotein-cholesterol is also significantly (*P* < 0.0001) increased in the combined oral contraceptive users compared to the control group. The level of triglycerides increased significantly (*P* = 0.0001) in the combined oral contraceptive users which agrees with a previous study (38) that reported that liver triglycerides synthesis is enhanced by estrogen and inhibited by androgen as triglycerides partly enter the circulation as very low-density lipoprotein.

A distinguished finding in this study is the significant positive correlation between TSA and cTnl (*r*=0.424, *P*= 0.020), CK-MB (*r*= 0.441, *P*= 0.015) and Myoglobin (*r*=0.403, *P*= 0.027). This observation agrees with previous work which demonstrated that TSA has been shown to correlate (*r* = 0.567, *P*= 0.001) well with high-sensitivity C-Reactive protein (42), cTnl and CK-MB (32). However, many of the acute-phase proteins are glycoproteins with sialic acid residues at the terminal position of their oligosaccharide side chains. TSA may suffer from lack of specificity to CVD as increased sialylation of plasma proteins has been reported in response to inflammation due increased protein bound to sialic acid (43).

**Conclusion**

This study demonstrated that combined oral contraceptive use is associated with significant increase in serum lipids. TSA shows a poor correlation with serum lipids implying a limited utility as a CVD marker.

**References**


