2 Lipids And Hscrp As Markers Of Coronary Heart Disease Risk In HIV Infected Adults

Danai Tavonga Zhou, Vitaris Kodogo, Munyaradzi Dzafitita, Olav Øktedalen, Rudo Muswe, Babill Stray-Pedersen

Abstract: Life-expectancy among HIV-infected patients has risen due to use of antiretroviral drugs. However there are new concerns as accompanying metabolic and morphologic changes predispose HIV-infected individuals to cardiovascular disease. Progression of cardiovascular disease is a slow process marked by both changes in lipid levels and low grade systemic inflammation, assessed using highly sensitive C-reactive protein (hs-CRP). The aim of the study was to evaluate the risk of cardiovascular disease in HIV-infected patients using lipids and hs-CRP levels. Serum and plasma samples were collected from HIV-infected adults who were antiretroviral therapy-experienced (n=87) or antiretroviral therapy-naive (n=10). Samples were analysed using Siemens® Dimension Xpand analyser. Mean hs-CRP for all participants suggested that they had some risk of cardiovascular disease in spite of antiretroviral therapy history. Of all 97 participants, 57.7% had elevated hs-CRP levels (>3mg/dL) suggesting high risk, 26.8% had levels between 1 and 3mg/dL (average risk) and 34% had levels below 1mg/dL (low risk). Lipid levels were normal in all participants and there was no correlation between hs-CRP and lipid levels. There was no significant difference in hs-CRP levels by sex, age and antiretroviral therapy history. Elevated hs-CRP levels of participants suggest that HIV-infected patients may be at risk of cardiovascular disease through low-grade inflammation.

Index Terms: antiretroviral therapy, cardiovascular disease, HIV, hsCRP, inflammation, lipid.

1 INTRODUCTION

Treatment outcomes amongst HIV-infected individuals have greatly improved due to the use of antiretroviral therapy (ART) [1]. However studies have shown that the use of antiretroviral drugs is also accompanied by metabolic and morphological changes, which may lead to accelerated atherosclerosis and increased risk of coronary heart disease [2]. The risk of coronary heart disease is thought to double in HIV-infected patients, and of more serious concern is the fact that coronary heart disease has been reported as major non-AIDS causes of death in HIV-infected individuals [3]. Metabolic and morphological changes that may occur in HIV-infected individuals include dyslipidemia, insulin resistance and visceral fat deposition, which may all contribute to coronary heart disease [1, 3] and are thought to be related to medication [4].

Further, traditionally recognised risk factors such as: smoking, hypertension, old age and male sex are thought to contribute to formation of atherosclerotic lesions which predisposes to coronary heart disease in the era of HIV/AIDS [5]. Development of atherosclerotic lesions is a slow progressive process marked by low grade inflammation [4]. From a pathological viewpoint initiation, growth and complication of the atherosclerotic plaque might be considered to be an inflammatory response to injurious factors that promote atherogenesis [6]. Risk factors, including antiretroviral therapy, give rise to an increase in pro-inflammatory cytokines (e.g. interleukin-1, tissue necrosis factor-α); adhesion molecules (e.g. selectins); inflammatory stimuli with hepatic effects (e.g. interleukin-6) or other products of hepatic stimulation (e.g. serum amyloid A, and C-reactive protein) [7]. New patho-physiological insights have provided potential targets for measurement as a means to identify and monitor ongoing inflammatory process [8], [9]. New analyses have examined analytes such as highly sensitive C-reactive protein (hs-CRP), serum amyloid A and other acute-phase reactants or cytokines [2], [10], [11]. hs-CRP is thought to predict future coronary events in asymptomatic (healthy) individuals with no previous history of coronary heart disease [10]. Several studies now support a strong link between baseline elevation of hs-CRP and future coronary events [9]. It has been suggested that hs-CRP may offer more reliable estimate of coronary heart disease in well controlled HIV infections than standard risk markers and may detect early risk of coronary heart disease independently of traditional risk factors [5], [12]. The ability of hs-CRP to add to the predictive capacity of other established risk factors has been examined in many studies and hs-CRP retains an independent association with incidence of coronary heart disease [5], [12].

The hs-CRP test is a qualitative analysis of very low levels of C-reactive protein in blood measured in mg/L. Risk is categorised as low, average and high with hs-CRP levels...
concentration of less than 1mg/L, 1-3mg/L and greater than 3mg/L, respectively [11]. Furthermore, hs-CRP test is cost effective and has a generally acceptable precision and reproducibility in coronary heart disease risk profiling [13], de Luka et al also pointed out that high hs-CRP is associated with coronary heart disease independent of traditional risk markers, viral load, CD4 count and the combination of antiretroviral therapy [14]. Use of hs-CRP in the HIV-infected population is however subject to debate, since levels may be elevated pre-antiretroviral therapy and during therapy regardless of regimen [15]. hsCRP levels also seem to remain elevated despite normalised CD4 cell count and suppressed viral load [15]. A cohort study done amongst HIV infected patients in South Africa showed elevated levels of hs-CRP and other biomarkers pre-antiretroviral therapy [16]. hs-CRP levels may be falsely elevated due to tissue injury, recent infection or general inflammation, and in individuals taking non-steroidal inflammatory drugs such as: aspirin, ibuprofen and naproxen [2]. Levels are also elevated in arthritic patients while anti-inflammatory drugs and statins reduce hs-CRP levels [2]. In some studies, changes in regimen have shown to be associated with increases in hs-CRP therefore use of hs-CRP may be more useful in those on stable antiretroviral therapy [17], [18]. In contrast to some of the editorial commentary that has expressed concerns about the robust predictive value of hs-CRP testing for risk assessment, in a comprehensive 2007 review of available published CRP study data, Ridker supported the use of hs-CRP as a consistently additive and independent inflammatory predictor of future coronary heart events, while refuting some of the critical commentary directed at certain ‘negative’ studies [19]. Results from an analysis in a cohort study done by Boger et al. [20], also gave evidence for use of hs-CRP as a more reliable estimate of coronary heart disease risk in people with well-controlled HIV infections than standard risk markers [20]. The study also traced significant correlation between hs-CRP and triglycerides, cholesterol, and body mass index (BMI). The study by Boger et al. also showed the following prevalence (as %): hs-CRP >3mg/L (high risk)-47%, hs-CRP between 1 and 3mg/L (average risk)-26%, and hs-CRP <1mg/L (low risk)-25% [20]. Though BMI were less predictive of coronary heart disease in HIV-infected patients there were indications that higher BMI is correlated with hs-CRP [20]. The aim of the current study was to evaluate the risk of coronary heart disease in both antiretroviral-naïve and antiretroviral-experienced HIV-infected patients using highly-sensitive C-reactive protein and lipid profiles.

2. MATERIALS AND METHOD

2.1 Study design and setting

A cross-sectional study was conducted at an HIV Clinic, in Harare, Zimbabwe where eligible HIV-infected patients attending clinic between March and June 2013 were recruited. Male and female patients aged 18 years and above were targeted but those taking non-steroidal inflammatory drugs, anti-inflammatory drugs and statins and those with documented history of coronary heart disease, arthritis, history of treatment failure were excluded.

2.2 Ethical considerations

Ethical clearance to carry out the project was given by the Joint Research Ethics Committee (JREC) of the Parirenyatwa Group of Hospitals and University of Zimbabwe College of Health Sciences, Medical Research Council of Zimbabwe (MRCZ) and Research Ethics Committee (REK), Norway after they were satisfied that the research would be carried out in accordance with the Declaration of Helsinki (1964).

2.3 Sample size determination

Sample size was determined using the Z test method viz n = Zp x (1-p)/d², where n = minimum number of samples, Z = test statistic at 95% confidence interval, p = population proportion=5%, d = standard deviation=0.05 n = [(1.96)² x 0.05] x (0.95)/0.05² = 73

97 participants were recruited into the study 73 were female and 24 were male.

2.4 Sample analysis

Plasma samples collected from patients visiting the HIV clinic were stored in a freezer at -20°C and thawed once before analysis. The Diazyme® hs-CRP assay used was an immunoturbidimetric method, in which an antigen antibody reaction occurs between hs-CRP in sample and anti-CRP antibody which has been sensitised to latex particles. The resulting agglutination is detected as an absorbance change, with the magnitude of the change being directly proportional to quantity of hs-CRP in the sample [21].

2.5 Data Analysis

Statistical analysis was carried out using the Stata® Statistical Package v 13.

3. RESULTS

3.1 Tests for normality

Ninety-seven participants were recruited in the study and box and whisker plots were used to determine disperser and skewness of data. As the hsCRP and lipid profile data was skewed to the right, medians and interquartile ranges (IQR) were used to describe the data. Pearson chi-squared tests and/or Fischer’s exact tests were used to check for differences.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ART-naive (n=10)</th>
<th>ART-experienced (n=87)</th>
<th>OR* CI **</th>
<th>p-value ***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender Frequency (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>4 (40%)</td>
<td>20 (23%)</td>
<td>-1.399-0.904**</td>
<td>0.238</td>
</tr>
<tr>
<td>Females</td>
<td>6 (60%)</td>
<td>67 (77%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Age (+SD) / years</td>
<td>37.2 (5.92)</td>
<td>43 (9.68)</td>
<td>-12.023-0.424**</td>
<td>0.067</td>
</tr>
<tr>
<td>Heart disease Yes (frequency/%)</td>
<td>0 (0%)</td>
<td>8 (9.2%)</td>
<td></td>
<td>0.317</td>
</tr>
<tr>
<td>No (frequency/%)</td>
<td>10 (100%)</td>
<td>79 (90.8%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Comparison of statistics by antiretroviral drug experience
shown in Table 3. When compared using hs-CRP levels falling in the range 0-3mg/dL, whilst 57.7% (n=55) had elevated hs-CRP (>3mg/dL), suggesting high risk of coronary heart disease. For the 41 participants with hs-CRP levels below 3, eleven (26.8%) had hs-CRP below 1 suggesting low coronary heart disease. Thirty of the participants (34%) had hs-CRP levels suggesting average risk of coronary heart disease (hs-CRP range 1-3mg/dL). There was significant difference (p=0.000) in hs-CRP for participants with hs-CRP below 3 and those with elevated hs-CRP (Table 2, Figure 2). When hs-CRP levels were compared by age, sex, antiretroviral therapy history there was no significant difference (p>0.05) (Table 2).

Table 2 Comparisons of hs-CRP levels

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>hs-CRP Median (IQR)</th>
<th>p-values sig&lt;0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levels of hs-CRP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>all (n=97)</td>
<td>3.80 (2.00-8.90)</td>
<td></td>
</tr>
<tr>
<td>elevated (&gt;3mg/dL) (n=56)</td>
<td>1.70 (0.90-2.40)</td>
<td></td>
</tr>
<tr>
<td>low [0-3mg/dL] (n=41)</td>
<td>7.95 (4.25-14.40)</td>
<td>0.000, sig</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>male (n=24)</td>
<td>2.80 (2.10-8.70)</td>
<td></td>
</tr>
<tr>
<td>female (n=73)</td>
<td>3.90 (2.00-8.90)</td>
<td>0.109</td>
</tr>
<tr>
<td>Age group/years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-28 (n=6)</td>
<td>2.35 (1.00-3.80)</td>
<td></td>
</tr>
<tr>
<td>29-39 (n=31)</td>
<td>2.40 (1.40-7.30)</td>
<td></td>
</tr>
<tr>
<td>40-50 (n=41)</td>
<td>4.10 (2.40-14.50)</td>
<td></td>
</tr>
<tr>
<td>51+ (n=19)</td>
<td>5.50 (2.40-9.90)</td>
<td>0.342</td>
</tr>
<tr>
<td>ART* history</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ART-naïve</td>
<td>2.2 (1.1-4.8)</td>
<td>0.487</td>
</tr>
<tr>
<td>ART-experienced</td>
<td>3.8 (2.2 -8.9)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 Comparison of prevalence of opportunistic infections by hs-CRP level

<table>
<thead>
<tr>
<th>Opportunistic infections</th>
<th>normal=0</th>
<th>elevated= 1</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes=1</td>
<td>30 (73.17)</td>
<td>36 (64.29)</td>
<td>66</td>
</tr>
<tr>
<td>No=0</td>
<td>11 (26.83)</td>
<td>20 (35.71)</td>
<td>31</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>56</td>
<td>97</td>
</tr>
</tbody>
</table>

Pearson chi2=0.8593, Fisher’s exact = 0.386, p = 0.354

Prevalence of participants’ opportunistic infections was compared to those with elevated hs-CRP and those with low hs-CRP levels as shown in Table 3. When compared using both Pearson chi squares and Fischer’s exact tests it was established that there was no significant difference prevalence of opportunistic infection amongst those with low hs-CRP and those with elevated hs-CRP levels (Table 3).

3.4 Participants’ lipid levels

Lipid measures of coronary heart disease risk were described using medians (IQRs) and were on average all within normal ranges as shown: HDL [46.79(38.28- 56.84) mg/dL], LDL [100.75 (88.35- 130.20) mg/dL] and total cholesterol OR*=Odds ratio at 95% CI, CI**=confidence interval, ***sig<0.05

3.2 Participants’ demographics

Mean±SD age was 42.40±9.50 years, 24 (24.74%) were male (mean ±SD age=43.03±9.00 years), 73 were female (mean ±SD age=42.18±9.72 years). Ten (10.31%) of the participants were antiretroviral therapy-naïve whilst 87 were antiretroviral therapy-experienced (Table 1).

3.3 Participants’ hsCRP levels

The average hs-CRP was elevated above normal for all participants [median (IQR) =3.8 (2.8-8.9) mg/dL]. When categorized by level of hs-CRP, 42.3% (n=41) of participants had hs-CRP levels falling in the range 0-3mg/dL, whilst 57.7% (n=55) had elevated hs-CRP (>3mg/dL), suggesting high risk of coronary heart disease. For the 41 participants with hs-CRP levels below 3, eleven (26.8%) had hs-CRP below 1 suggesting low coronary heart disease. Thirty of the participants (34%) had hs-CRP levels suggesting average risk of coronary heart disease (hs-CRP range 1-3mg/dL). There was significant difference (p=0.000) in hs-CRP for participants with hs-CRP below 3 and those with elevated hs-CRP (Table 2, Figure 2). When hs-CRP levels were compared by age, sex, antiretroviral therapy history there was no significant difference (p>0.05) (Table 2).
[167.95(144.40- 198.84) mg/dL]. When compared by antiretroviral therapy history there was no significant difference (p>0.05) between the antiretroviral-experienced and the antiretroviral- naïve participants (Table 1). There was no correlation between hs-CRP levels and total cholesterol, LDL cholesterol, HDL cholesterol and total cholesterol/HDL cholesterol ratio.

4. DISCUSSION
Many previous studies have shown that HIV-infected patient on antiretroviral therapy are at increased risk of coronary heart disease [22], [23], [24]. However, some studies have shown that HIV-infected patients who are not on antiretroviral therapy are also at risk of coronary heart disease [25], [26], [27]. This is in agreement with results from our study, in which both antiretroviral therapy-naïve and antiretroviral therapy-experienced participants had elevated hs-CRP levels indicating risk of coronary heart disease risk in spite of antiretroviral therapy history. The increased levels may be attributed to antiretroviral toxicity and the metabolic and morphologic changes that predispose to atherosclerosis [1], [3], [25]. In antiretroviral therapy naïve patients the elevated hs-CRP levels could be attributed to the pathologic effects of the virus as well the effects of cytotoxic T-cells action when trying to eliminate infected cell resulting in chronic inflammatory processes [28]. C-reactive protein (CRP) binds to phosphocholine expressed on the surface of dead or dying cells and levels increase in inflammatory conditions such as bacterial infections. Recently, many studies have suggested that there is an association between H. pylori infection and serum high levels of hs-CRP. When mean serum level of hs-CRP in 200 patients with H. pylori and 50 healthy control subjects were compared, levels were significantly higher in infected patients than in healthy controls [29]. A similar study was carried out on HIV-infected patients with various co-infections to determine the pattern of C-reactive protein (CRP) concentrations. Surprisingly, patients with the opportunistic infections: Pneumocystis carinii pneumonia (PCP) and Mycobacterium avium complex (MAC) had a significantly lower increase in CRP concentration than patients infected with common bacterial pathogens [30]. There is concordance between our study and that by S. Grützmeier et al. [30] as no significant difference was demonstrated in the prevalence of opportunistic infections by hs-CRP levels in this current study (Table 3). This study suggests that elevations in hsCRP are due to HIV status as both ART-experienced and ART-naïve patients had elevated levels of average hsCRP. Studies have shown that there is poor deposition and distribution of fat in HIV-infected patients on antiretroviral therapy [31], [32] viz increased concentration of LDL cholesterol and other lipids but decreased concentration of HDL cholesterol [33], [34]. In this study, that we carried out at an HIV treatment clinic, in Harare, Zimbabwe, lipid levels were on average within normal ranges as shown: HDL [46.79(38.28- 56.84), LDL [100.75 (88.35-130.20)] and total cholesterol [167.95(144.40- 198.84)]. When compared by antiretroviral therapy history there was no significant difference (p>0.05) in lipid levels between the antiretroviral-experienced and the antiretroviral- naïve participants (Table 1).

5. CONCLUSION
Based on mean hs-CRP levels of the participants, the study showed that HIV-infected patients have elevated hsCRP increasing their risk of coronary heart disease, in spite of their antiretroviral therapy history. The results of the study suggest that there is no correlation between high levels of hs-CRP and the concentration of lipids in HIV-infected patients. Since there is no significant difference in prevalence of opportunistic infections by hs-CRP levels there is no strong evidence linking increase in hs-CRP to co-infections in this group of participants.

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