Oxidative Stress Parameters in Women with HIV and HIV/hepatitis B and/or C Co-infection

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Abstract

The pathogenesis of HIV/hepatitis B and/or C co-infection is far from being understood; yet, some studies have shown its relationship with oxidative stress. Because of oxidative stress (conjugated dienes and thiobarbituric acid reactants) and antioxidant defense systems (superoxide dismutase activity, α-tocopherol, reduced and oxidized glutathione) had different parameters in 26 women with HIV-monoinfection and 27 women with HIV/hepatitis B and/or C co-infection (with no signs of AIDS), they were evaluated. Spectral fluorofotometric methods were used. Statistical analysis was performed by parametric and non-parametric methods. The evaluation found that while conjugated dienes levels were significantly higher, superoxide dismutase (SOD) activity and α-tocopherol levels were significantly lower in women with HIV/hepatitis B and/or C co-infection than in those with HIV-monoinfection. Concurrently, during the highly active antiretroviral therapy (HAART), conjugated dienes, thiobarbituric acid reactants mean levels were lower; SOD activity and α-tocopherol levels were higher in HIV-monoinfected patients than in those with HIV/hepatitis B and/or C co-infection (with no signs of AIDS). This outcome was characterized by more expressed oxidative stress.

Keywords: HIV/hepatitis B and/or C co-infection; HAART therapy; Oxidative stress; Antioxidative defense

Background

According to data from the Russian Federation Ministry of Health, more than 600,000 HIV-infected people are presently registered in Russia. Thus, the majority of newly registered cases (60%) are diagnosed among women at ages of 20-30 years old. Among the routes of HIV expansion among the population, drug use (63.4%) and heterosexual contacts (20.3%) prevail. The numbers of new cases of infection, including unprotected sexual acts and HIV transmission from mother to the child, have increased annually. The Irkutsk region shows a particularly unfavorable epidemiological situation of HIV infection: there are more than 30 thousand people currently infected and 1300 new cases with HIV were registered by the end of year 2013. A sexual route of infection prevails in 73.4% of cases.

An increase in the number of patients with HIV/hepatitis B and/or C co-infection during the past decade has been seen [1]. Viral hepatitis taking the prominent place in the list of death causes of HIV-infected patients however, this influence on HIV infection course has been insufficiently studied even though there is an opinion that hepatitis leads to rapid HIV infection and AIDS progression [2,3]. The possibility of extra hepatic virus replication has been proved, in particular, in immune competent cells which may become a reservoir of infection, and the hepatocyte source of reinfection causes persistent immune deficiency. Faster reduction of CD4+ cells which impedes the achievement of sustainable response for viruses in patients with hepatitis have been registered [2]. The most important sequel of chronic HIV/hepatitis C infection is progressive liver fibrosis leading to cirrhosis as liver disease end-stage and in some cases for hepatic carcinoma [4].

Antimicrobial protection is provided through the combined impact of cellular and humoral factors [5-7]. Non-specific resistance of the organism largely determines the development and outcome of any infectious process [8].

Several studies have attributed oxidants as playing critical roles in the genesis of AIDS [9]. A number of researches have suggested that the mechanisms responsible for AIDS progression could be reversed through the administration of antioxidant reducing agents [10]. Oxidative stress development usually can be measured by relationship between antioxidant parameters (superoxide dismutase, reduced and oxidized glutathione, α-tocoferol) and parameters of lipids peroxidation (conjugated dienes and thiobarbituric acid reactants [11]. Of great interest are data on oxidative stress (OS) development and antioxidant defense (AOD) level in patients with HIV/hepatitis B and/or C [12-14]. It is probable, that these patients will experience more severe violations in their antioxidant defense system than in patients with HIV monoinfection. These studies are required to assign an adequate antioxidant therapy to patients.

Objective

To reveal of oxidative stress parameters in reproductive age women with monoinfection (HIV) and co-infection (HIV/hepatitis B and/or C) with or without HAART therapy

Methods

The study was conducted at the Scientific Centre for Family Health and Human Reproduction Problems, Russian Academy of Medical Sciences (Irkutsk, Russia), in Irkutsk infections hospital and in Irkutsk regional AIDS Center in 2012-2014 according to ethical standards of Helsinki Declaration (2008). This study was approved by the Ethic
Committee of Scientific Centre of Family Health and Human Reproduction Problems (Siberian Branch of RAMS), and all involved patients signed the Informed Consent agreement for participation in our study. The comparative analyses included HIV-monoinfected and HIV/hepatitis B and/or C co-infected 18-40 years old women with no signs of AIDS. The inclusion criteria were: 18-40 years of age, confirmed HIV carriers, and informed consent of this research. Exclusion criteria were: excessive weight, the presence of tuberculosis, and/or diabetes mellitus.

Patients underwent interviews that included demographic, medical, nutritional and recreational drug-related questions. A physical examination was completed and anthropometrics were measured. After overnight fasting, blood samples were obtained to confirm HIV status, hepatitis B virus (HBV) status, and hepatitis C virus (HCV) status, and to determine CD4+ cell count, HIV viral load, plus complete blood cell count and blood biochemistry, including the plasma concentrations of antioxidant parameters (superoxide dismutase (SOD), reduced (GSH) and oxidized (GSSG) glutathione, α-tocoferol) and parameters of oxidative stress (plasma conjugated dienes (CDs) and thiobarbituric acid reactants (TBARs)). Lymphocyte phenotype was determined with a four-colored monoclonal antibodies immunephenotyping panel. Differential counts were determined with a cytometry method using a FacsCount (Becton Dickinson, USA).

TBARs levels were detected by fluorometry and estimated in mmol/l. The concentration of CDs absorbance detected on plasma heptanes extract at 232 nm [15]. The coefficient of molar absorption (K = 2.2 · 10^3 M^-1 C^-1) for conversion of absorption units to m mol/l was used. TBARs levels were detected by fluorometry end estimated in mmol/l. SOD activity was measured in erythrocytes using a commercially available kit (Ransel; Randox Lab, Crumlin, U.K.) [16]. Fluorometry for GSH and GSSG levels in hemolysate were used. And, α-tocopherol levels were detected in plasma by fluorometry [17,18].

Body mass index (BMI) was calculated using the standard formula that divides weight in kilograms by the square of height in meters (kg/m^2).

Statistical analysis was performed by STATISTICA 6.1 software (Stat-Soft Inc., USA). Means and standard deviation (SD) of means were calculated and significance of differences between values was evaluated by Student (T-test) and Mann-Whitney (U-test) tests. Since the distribution of most of the indicators corresponds to normal, the level of significance was set at p<0.05.

### Results

Group’s characteristics are shown in a Table 1. There were no significant differences between the HIV-monoinfected and HIV/hepatitis B and/or C co-infected in ages (30.58 ± 5.35 years vs. 31.67 ± 3.51 years; p>0.05), BMI (19.19 ± 3.27 kg/m^2 vs. 20.19 ± 3.04 kg/m^2; p>0.05), alcohol consumption (30.8% vs. 22%; p>0.05), cigarette smoking (65.4% vs. 40.7%; p>0.05), illicit drugs (19.2% vs. 25.9%; p>0.05). There were no significant differences in those who received highly active antiretroviral therapy (HAART) (38.5% vs. 40.74; p>0.05).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HIV-monoinfected (n=26)</th>
<th>HIV/hepatitis B and C co-infected (n=27)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) (mean ± SD)</td>
<td>30.58 ± 5.35</td>
<td>31.67 ± 3.51</td>
<td>0.352</td>
</tr>
<tr>
<td>Receiving HAART (% (n))</td>
<td>38.5% (10)</td>
<td>40.74% (11)</td>
<td>0.911</td>
</tr>
<tr>
<td>BMI (kg/m²) (mean ± SD)</td>
<td>19.19 ± 3.27</td>
<td>20.19 ± 3.04</td>
<td>0.258</td>
</tr>
<tr>
<td>Frequent alcohol use (&gt;2 drinks daily) (% (n))</td>
<td>30.8% (8)</td>
<td>22% (6)</td>
<td>0.694</td>
</tr>
<tr>
<td>Frequent cigarette use (&gt;1 pack daily) (% (n))</td>
<td>65.4% (17)</td>
<td>40.7% (11)</td>
<td>0.128</td>
</tr>
<tr>
<td>Injecting illicit drug use (% (n))</td>
<td>9.2% (5)</td>
<td>25.9% (7)</td>
<td>0.800</td>
</tr>
</tbody>
</table>

Table 1: Characteristics of Patients of HIV-monoinfected and HIV/hepatitis B and/or C co-infected Groups. All values are mean ± SD.* - significant.

The HIV/hepatitis B and/or C co-infected group consisted of women with HIV/hepatitis B - 6 (22.2%), HIV/hepatitis C – 10 (37.1%), HIV/hepatitis B+C – 11 (40.7%). Clinical manifestations in patients with HIV co-infection presented more severe signs of the main symptoms and syndromes: weaknesses, fatigue, pain in the right upper quadrant, lymphadenopathy, and hepatitis splenomegaly. Patients who had no clinical symptoms significantly more often belonged to a group of HIV-mono infected patients.

Laboratory data are shown in a Table 2. CD4+ cell counts and HIV viral loads were not significantly different between the HIV-monoinfected and HIV/hepatitis B and/or C co-infected group (CD4+ counts 217.5 ± 99.3 vs. 177.3 ± 89.1 cells/ml (p>0.05); viral loads 2.5 ± 0.31 vs. 5.85 ± 1.53 log10 HIV-1 RNA copies/ml (p>0.05)). There were no statistically significant differences between the HIV-monoinfected and HIV/hepatitis B and/or C co-infected in their levels of alanine aminotransferase (ALT) (45.6 ± 39.1 vs. 59.4 ± 62.1 U/L; p>0.05), aspartate aminotransferase (AST) (55.7 ± 47.9 vs. 79.4 ± 67.8 U/L; p>0.05), albumin (40.6 ± 3.3 vs. 41.8 ± 13 g/dl; p>0.05), hemoglobin (111.2 ± 10.0 vs. 111.5 ± 16.0 U/L; p>0.05). However, plasma bilirubin was significantly higher in HIV/hepatitis B and/or C co-infected group (27.3 ± 6.3 mg/dl) than in those who were HIV-mono infected (8.2 ± 2.8 mg/dl) (p<0.05).

Plasma levels of CDs and TBARs prove the presence of oxidative stress. Table 3 demonstrates that the CDs level mean is significantly higher (1.9 ± 1.00; p<0.05) in patients with HIV/hepatitis B and/or C co-infected than in those who were HIV-mono infected (1.4 ± 0.9 µmol/l). There were no TBARs level differences between the HIV-monoinfected and HIV/hepatitis B and/or C co-infected group in (0.9 ± 0.5 vs. 1.1 ± 1.0 µmol/l) (Table 3). HIV/hepatitis B and/or C co-infected group also had significantly lower levels of antioxidants, including SOD (1.6 ± 0.04 U/mg Hb) and α-tocoferol (7.7 ± 3.1 µmol/l), than the HIV-mono infected group (1.8 ± 0.2 U/mg Hb and
9.7 ± 2.9 µmol/l, respectively (p<0.05) (Table 3). However, there were no differences in GSH and GSSG levels between mono infected and HIV/hepatitis B and/or C co-infected groups, respectively (p>0.05).

<table>
<thead>
<tr>
<th>Laboratory value</th>
<th>HIV-monoinfected (n=26)</th>
<th>HIV/hepatitis B and/or C co-infected (n=27)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viral load (copies/ml) (mean ± SD)</td>
<td>2.5 ± 0.31</td>
<td>5.85 ± 1.53</td>
<td>0.278</td>
</tr>
<tr>
<td>CD4+ count (cells/ml) (mean ± SD)</td>
<td>217.5 ± 99.3</td>
<td>177.3 ± 89.1</td>
<td>0.127</td>
</tr>
<tr>
<td>AST (IU/L) (mean ± SD)</td>
<td>55.7 ± 47.9</td>
<td>79.4 ± 67.8</td>
<td>0.146</td>
</tr>
<tr>
<td>ALT (IU/L) (mean ± SD)</td>
<td>45.6 ± 39.1</td>
<td>59.4 ± 62.1</td>
<td>0.442</td>
</tr>
<tr>
<td>Albumin (g/dl) (mean ± SD)</td>
<td>40.6 ± 3.3</td>
<td>41.8 ± 13</td>
<td>0.685</td>
</tr>
<tr>
<td>Bilirubin (mg/dl) (mean ± SD)</td>
<td>8.2 ± 2.8</td>
<td>27 ± 6.3</td>
<td>0.006*</td>
</tr>
<tr>
<td>Hgb (g/dl) (mean ± SD)</td>
<td>111.2 ± 10.0</td>
<td>111.5 ± 16.0</td>
<td>0.928</td>
</tr>
</tbody>
</table>

Table 2: Laboratory Parameters Value in Patients of HIV-monoinfected and HIV/hepatitis B and/or C co-infected Groups. All values are mean ± SD. * - Significant.

<table>
<thead>
<tr>
<th>Blood value</th>
<th>HIV-monoinfected with HAART (n=10)</th>
<th>HIV/hepatitis B and/or C with HAART (n=11)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ cells (µmol/l)</td>
<td>1.1 ± 0.41</td>
<td>2.2 ± 1.3</td>
<td>0.007*</td>
</tr>
<tr>
<td>TBARs (µmol/l)</td>
<td>0.7 ± 0.4</td>
<td>1.5 ± 1.1</td>
<td>0.041*</td>
</tr>
<tr>
<td>SOD (U/mg Hb)</td>
<td>1.9 ± 0.1</td>
<td>1.6 ± 0.2</td>
<td>0.001*</td>
</tr>
<tr>
<td>GSH (µmol/l)</td>
<td>2.2 ± 0.3</td>
<td>2.1 ± 0.4</td>
<td>0.667</td>
</tr>
<tr>
<td>GSSG (µmol/l)</td>
<td>2.1 ± 0.4</td>
<td>2.4 ± 1.2</td>
<td>0.174</td>
</tr>
<tr>
<td>α-tocoferol (µmol/l)</td>
<td>11.7 ± 2.5</td>
<td>8.1 ± 2.1</td>
<td>0.002*</td>
</tr>
</tbody>
</table>

Table 4: Differences in Oxidative Stress and AOD Parameters in HIV-monoinfected and HIV/hepatitis B and/or C co-infected Groups with HAART. All values are mean ± SD. * - Significant.

Discussion

It has been reported that HIVs induces OS by disturbing cellular antioxidant defense and initiating oxidative reactions. OS is associated with impaired balance of intracellular reactive oxygen species (ROS)/antioxidants and cellular redox status along with activation of ROS, growth and free radical peroxidation processes, degradation of cellular structures [19,20]. Viral exposure, depending on the strength and duration, can cause OS and cell death, or the initiation of adaptive defense mechanisms that lead to the growth of cellular redox status and new balance of ROS/antioxidants appearance.

Table 3: Differences in Oxidative Stress and AOD Parameters between the HIV-monoinfected and HIV/hepatitis B and C co-infected Groups All values are mean ± SD. * - Significant.

Notably, the HAART treatment outcomes (Table 4) show statistically significant differences between groups of patients who received this treatment. The CDs and TBARs mean levels were significantly lower (1.1 ± 0.41 µmol/l and 0.7 ± 0.4 µmol/l, respectively) in HIV-monoinfected patients than in HIV/hepatitis B and/or C co-infected patients (2.2 ± 1.3 µmol/l and 1.5 ± 1.1 µmol/l, respectively; p<0.05). AOD system changes were registered by 2 indicators – the raised SOD values (1.9 ± 0.1 U/mg Hb) and α-tocoferol (11.7 ± 2.5 µmol/l) in comparison with HIV/hepatitis B and/or C co-infected group of women (1.6 ± 0.2 U/mg Hb and 8.1 ± 2.1 µmol/l, respectively; p<0.05). There were no differences in GSH and GSSG levels in the HIV-monoinfected and HIV/hepatitis B and/or C co-infected groups with HAART, respectively (p>0.05).
Vitamin E has been suggested to have a protective role in cell membranes by preventing lipid peroxidation due to its lipophilic nature. Cell membranes may underlie the deficit of antioxidant protection in viral disease. Use of HAART does not improve the condition of patients more pronounced imbalance between the pro-oxidants and antioxidant factors compared to monoinfected women. HIV/hepatitis B and/or C co-infected is associated with increased oxidative stress and decreased antioxidant concentrations compared with HIV-monoinfection (both with and without HAART). This fact may lead to decreased intake of antioxidants in cell damaged by hepatitis in the human liver or HIV infection. Therefore, the focus of future research should be placed on antioxidants as additional agents for treatment of HIV and AIDS patients. In this respect, further research is needed.

Conclusions

Women with HIV/hepatitis B and/or C co-infection demonstrate more pronounced imbalance between the pro-oxidants and antioxidant factors compared to monoinfected women. HIV/hepatitis B and/or C co-infection is associated with increased oxidative stress and decreased antioxidant concentrations compared with HIV-monoinfection (both with and without HAART). This fact may indicate increased intake of antioxidants in cell damaged by hepatitis in the human liver or HIV infection. Therefore, the focus of future research should be placed on antioxidants as additional agents for treatment of HIV and AIDS patients. In this respect, further research is needed.

Acknowledgement

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References


