Menstrual and Reproductive Function in Women with HIV-infection and Antioxidant Vitamins Deficiency

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Abstract

Reproductive age women proportion with HIV-infection increasing, but relationship of antioxidant deficiency and reproductive disorders in this category of patients remain unclear. Objective: To identify antioxidant vitamins deficiency and frequency of reproductive disorders interrelations in women with HIV-infection. 53 women of reproductive age with HIV-infection were divided for 4 groups according to blood serum vitamins concentration: 36 women - α-tocopherol level less than 12 µmol/L; 17 women - α-tocopherol level in the range 12-24 µmol/L; 35 women with retinol level less than 1.05 µmol/L and 18 women - retinol level in the range 1.05-2.8 µmol/L. Spectral fluorofotometric methods were used. Statistical analysis was performed by parametric and non-parametric methods. High incidence of menstrual disorders (63%), infertility (36.1%), and luteal phase deficiency was found in the group of women with HIV-infection and α-tocopherol deficiency. Elevated levels of primary lipid peroxidation (LP) products - diene conjugates, with the absence of significant differences in the content of the thiobarbituric acid reactants in this group were registered. The results obtained showed a close relationship of α-tocopherol deficiency and disorders of the reproductive system in women with HIV-infection. It is a significant reason to recommend including of antioxidant drugs in HIV-women patients of complex pathogenetic therapy to regulate adequately their reproductive function and possibly to preserve reproductive ability in these women.

Keywords: HIV-infection; Reproductive disorders; Antioxidant vitamins; Tocopherol; Retinol

Background

According to the Federal Research and Methodological Center for Prevention and Control of AIDS in the Russian Federation currently registered 246,000 HIV-infected women and 4398 HIV-infected children born by infected mothers. In the age groups 15-19 and 20-24 years there excess cases of HIV-infection among women compared with men registered since 2009. Since 2005 percentage of heterosexually acquired HIV among women is constantly growing - 61%. Irkutsk Region has unfavorable epidemiological situation of HIV-infection (more than 30 thousand people), with dramatically increasing proportion of HIV-infected women, sexual route of infection prevails [1].

There are numerous studies showing increased frequency of disturbances of the menstrual cycle and ovulation [2,3], decrease in ovarian reserve [4], endocrine pathology and hypogonadism among HIV-positive women compared to HIV-negative women [5,6]. Number of ovulatory cycles and coital frequency correlate with severity of HIV/AIDS clinical status and obviously could impact fertility, and may reflect the degree of wasting and disturbances of the menstrual cycle and ovulation [2,3], decrease in ovarian reserve [4]. Ovarian reserve is one of the key systems responsible for protecting the body from the adverse effects of both internal and external factors [17-19]. The most potent natural antioxidants are considered lipid soluble vitamins - α-tocopherol and retinol [20]. These vitamins, being elements of AOS, prevent the development of oxidative stress (OS). Several studies show the relationship of antioxidant deficiency and the reproductive system of women, due to their involvement in the work of all parts of the regulation of pituitary gonadotrophic function [18,19]. α-tocopherol, as antioxidant effects the reproductive system state, stimulates ovaries steroidogenesis, endometrium protein biosynthesis and influence other steroid hormones synthesis target organs [19].

Objective

To identify antioxidant vitamins deficiency and frequency of reproductive disorders interrelations in women with HIV-infection...
Methods

The study was performed in 2012-2014 period according to ethical standards of Helsinki Declaration (2008) in the Scientific Centre for Family Health and Human Reproduction Problems, Russian Academy of Medical Sciences (Irkutsk, Russia), Irkutsk regional infections hospital and Irkutsk regional AIDS Centre. 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 the study. A random sample of women aged 18-40 years with HIV-infection from Irkutsk was carried out. The inclusion criteria: reproductive age, confirmed HIV carriers, informed consent for investigations. Exclusion criteria: excessive weight, the presence of tuberculosis, alcohol and drug abuse. Patients were divided into four groups depending on vitamins in blood serum content (36 women - α-tocopherol level less than 1.05 µmol/L (3 group) and 18 women - retinol level less than 12 µmol/L (1 group); 17 women α-tocopherol level in the range 1.05-2.8 µmol/L (4 group)); 12-24 µmol/L α-tocopherol level in the range 1.05-2.8 µmol/L (2 group); 35 women with α-tocopherol level in the range 12-24 µmol/L (2 group); 35 women with α-tocopherol level less than 1.05 µmol/L (3 group) and 18 women - retinol levels - were taken as standards [21].

Blood samples were obtained to confirm HIV, hepatitis B virus status, hepatitis C virus, to determine CD4 cell count, HIV viral load, complete blood cell count and blood biochemistry, including the serum concentrations of antioxidant parameters (α-tocopherol, retinol) and parameters of oxidative stress (plasma conjugated dienes (CDs) and thiobarbituric acid reactants (TBARs)) after overnight fasting. Lymphocyte phenotype was determined with a four-colored monoclonal antibodies immune phenotyping panel. Differential counts were determined with a cytometric method using a FacsCount (Becton Dickinson, USA). HIV viral load was obtained by reverse transcriptase-polymerase chain reaction. Patient’s blood samples centrifuged for 5 min at 1,500 g at 4°C; and erythrocytes were rinsed three times with NaCl 0.9% (wt/vol).

CD₃ absorbance detected in plasma heptanes extracts at 232 nm [22]. For conversion of absorption units to µmol/L coefficient of molar absorption (K=2.2105 M-1 C-1) was used. TBARs levels were detected by fluorometry [23] and estimated in µmol/L. α-tocopherol and retinol levels were detected in serum by fluorometry [24].

Body mass index (BMI) was calculated using the standard formula that divides weight in kilograms by the square of height in meters (kg/m²).

Levels of hormones in the serum were evaluated with ELISA and radioimmunoassay devices: “Immunoassay” (Russia), Elx808 Ultra Microplate Reader Bio-Tek Instruments, ins (USA). Several instrumental methods: colposcopy, pelvic, thyroid and mammary glands ultrasound investigations on the device “ALOKA-650” were used. Human papillomavirus was detected by the method of DNA amplification or the polymerase chain reaction.

Statistical analysis was performed by STATISTICA 6.1 software (Stat-Soft Inc., USA). To compare the frequency of occurrence - using z-test or Fisher’s exact test. 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. The level of significance was set at p<0.05.

Results

Group’s characteristics are shown in a Table 1. Occurrence frequency of patients with hepatitis was differences in groups 1 and 2. In group 1 HIV / hepatitis B+C combination was significantly more often - 12 cases (33.3%) compared to group 2 - 1 case (5.9%) (p<0.05). There were no significant differences in ages, BMI, alcohol consumption, cigarette smoking, and illicit drugs consumption (p>0.05). There were no significant differences in those who received highly active antiretroviral therapy (HAART) between groups (p>0.05).

| Parameter                        | α-tocopherol, µmol/L | P-value | Retinol, µmol/L | P-value |
|----------------------------------|----------------------|---------|----------------|---------|
| Age (years) (mean ± SD)          | 31.4 ± 3.9           | 0.467   | 31.6 ± 4.6      | 0.326   |
| BMI (kg/m²) (mean ± SD)          | 19.4 ± 2.9           | 0.035   | 19.7 ± 3.5      | 0.959   |
| Frequent alcohol use (>2 drinks daily) (% (n)) | 44.4% (8) | 0.239   | 35.3% (12)      | 0.155   |
| Frequent cigarette use (>1 pack daily) (% (n)) | 47.6% (10) | 1.000   | 23.5% (8)       | 0.064   |
| Injecting illicit drug use (% (n)) | 41.2% (7) | 0.197   | 17.1% (6)       | 0.101   |
| Hepatitis                        | B                    | 0.160   | 14.3% (5)       | 5.6% (1) |

Frequent cigarette use (>1 pack daily) (% (n))
Table 1: Characteristics of Patients of Four Groups. *significant differences.

| Parameter | α-tocoferol, µmol/L | P-value | Retinol, µmol/L | P-value |
|-----------|----------------------|---------|----------------|---------|
| Viral load (log10 copies/mL) (mean ± SD) | 5.4 ± 2.4 | 1.8 ± 0.5 | 3.2 ± 1.1 | 4.8 ± 1.2 | 0.382 |
| CD4+ count (cells/mL) (mean ±SD) | 89.8 ± 47.9 | 114.6 ± 110.7 | 102.3 ± 95.1 | 88.8 ± 52.8 | 0.632 |
| AST (IU/L) (mean ±SD) | 78.3 ± 68.1 | 45.5 ± 20.0 | 57.7 ± 69.3 | 49.3 ± 26.1 | 0.103 |
| ALT (IU/L) (mean ±SD) | 55.9 ± 22.8 | 45.5 ± 37.5 | 62.0 ± 57.2 | 41.8 ± 20.1 | 0.140 |
| Albumin (g/dL) (mean±SD) | 39.5 ± 5.1 | 44.9 ± 14.7 | 39.7 ± 52.4 | 44.2 ± 15.2 | 0.100 |
| Bilirubin (mg/dL) (mean±SD) | 19.9 ± 11.6 | 13.1 ± 8.9 | 17.9 ± 11.2 | 17.5 ± 12.3 | 0.955 |
| Hgb (g/dL) (mean±SD) | 110.3 ± 14.1 | 113.5 ± 10.4 | 111.7 ± 14.1 | 110.5 ± 12.3 | 0.746 |

Table 2: Laboratory Parameters Value in Four Groups. *significant differences.

Features of the menstrual and reproductive function of women surveyed are presented in Table 3. Menstrual cycle disorders were significantly more common in patients of group 1 compared with group 2 (63.9% vs. 35.3%, p<0.05). Reproductive history of women with HIV revealed high incidence of total infertility cases in group 1 (36.1% vs. 11.8% in the 2 group, p<0.05), in this case, the prevailing primary infertility - in 22.2% (p<0.05). Significant differences were recorded in the frequency of luteal phase deficiency - 61.1% vs. 47.1%, p<0.05 - between the 1 and 2 groups. Significant differences in frequency of amenorrhea, oligomenorrhea, secondary infertility and structure of the causes of infertility in group 1 compared to group 2 data were not established (p>0.05). 3 and 4 groups had no differences in both the total number of women with menstrual irregularities, and individual diseases (p>0.05). There were also no significant differences in the frequency of presence infertility and structure of the causes of infertility (p>0.05).

Discussion

It was suggested that OS may have pathological roles in development of HIV-infection [25]. It has been established that OS induces the initial stages of apoptosis, which contributes to the depletion of T cells and the progression of HIV-infection [26]. OS often occurs due to decreased activity of the antioxidant deficiency system that is typical for HIV-infection endogenous intoxication syndrome. We found primary LP products - diene conjugates increasing in women with HIV-infection and α-tocopherol deficiency. Formation and accumulation of conjugated dienes affects the permeability of membranes, and membrane-associated activity of enzymes and ion transport. This effect is the loss of membrane barrier functions which is the basis of the pathogenesis of reproductive disorders, including infertility [27]. It is believed that LP products acting on the diaphragm gametes violate its fluidity apoptotic processes in a cell and may cause infertility [28,29].

Our study proved that women with HIV and α-tocopherol deficiency have higher level of the reproductive system disorders.
(incidence of menstrual disorders, infertility, and a high percentage of failure of the luteal phase) than in the group with normal vitamin content. It is known that α-tocopherol affects various parts of the reproductive system and its deficiency certainly has pathogenetic importance in the development of infertility [19,30]. Thus, reduced levels of α-tocopherol may contribute to the development of reproductive disorders in women with HIV-infection.

| Parameter | α-tocoferol, µmol/L | P-value | Retinol, µmol/L | P-value |
|-----------|---------------------|---------|----------------|---------|
| Menstrual cycle disorders | <12 µmol/L (n=36) (1) | 12-42 µmol/L (n=17) (2) | <1.05 µmol/L (n=35) (3) | 1.05-2.8 µmol/L (n=18) (4) |
| Menstrual cycle disorders | 63.9% (23) | 35.3% (6) | 48.6% (17) | 66.7% (12) |
| Menstrual cycle disorders | 19.4% (7) | 5.9% (1) | 11.4% (4) | 22.2% (4) |
| Menstrual cycle disorders | 36.1% (13) | 29.4% (5) | 34.3% (12) | 33.3% (6) |
| Menstrual cycle disorders | 19.4% (7) | 23.5% (4) | 14.3% (5) | 33.3% (6) |
| Menstrual cycle disorders | 36.1% (14) | 11.8% (2) | 28.6% (10) | 27.8% (5) |
| Menstrual cycle disorders | 22.2% (8) | (0) | 17.1% (6) | 5.6% (1) |
| Menstrual cycle disorders | 17.1% (6) | 11.8% (2) | 11.4% (4) | 22.2% (4) |

Table 3: Characteristics of Menstrual and Reproductive Function in Four Groups. * significant differences.

Levels of CDs and TBARs prove the presence of oxidative stress. Table 4 demonstrates that the CDs mean level is significantly higher (1.8 ± 0.9 µmol/L) in group 1 patients than those in group 2 (1.3 ± 0.7 µmol/L) (p<0.05). No TBARs levels differences between groups registered (p>0.05) (Table 4).

| Blood Parameter | α-tocoferol, µmol/L | P-value | Retinol, µmol/L | P-value |
|-----------------|---------------------|---------|----------------|---------|
| Blood Parameter | <12 µmol/L (n=36) (1) | 12-42 µmol/L (n=17) (2) | <1.05 µmol/L (n=35) (3) | 1.05-2.8 µmol/L (n=18) (4) |
| Blood Parameter | 1.8 ± 0.9 | 1.3 ± 0.7 | 1.6 ± 0.9 | 1.6 ± 1.0 |
| Blood Parameter | 1.03 ± 0.7 | 0.9 ± 0.5 | 1.0 ± 0.7 | 0.9 ± 0.6 |

Table 4: Groups Differences of Oxidative Stress Parameters. All values are mean ± SD. * - significant differences.

It is necessary specially emphasize the large number women with HIV/hepatitis B and C co-infection in the group with α-tocopherol deficiency. Information on combined effects of blood-borne viral hepatitis and HIV-infection on the reproductive health of women is scarce in the scientific literature [31-33]. Probably, the co-infection accompanying low antioxidant activity may contribute to the development of reproductive disorders in these patients and, moreover, HAART - therapy did not improve their status. We found downward trend CD4+ cells in this patients and lower albumin level due to the presence of α-tocopherol deficiency. The study also shows a tendency to viral load increase in women with HIV-infection and α-tocopherol deficiency, probably due to the presence the large number women with HIV/hepatitis B and C co-infection. Association between retinol levels and frequency of the menstrual and reproductive system disorders in women with HIV-infection was not found.

**Conclusions**

Our study found higher levels of the menstrual cycle and reproductive disorders were registered in women with HIV-infection.
and α-tocopherol deficiency. It is a significant reason to recommend including of antioxidant drugs in HIV-women patients of complex pathogenetic therapy (HAART) to regulate adequately their reproductive function and possibly to preserve reproductive ability in these women. Antioxidant vitamins deficiency correction proposed to avoid reproductive disorders progressing. In this respect, further research is needed.

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