CrossMark

ORIGINAL ARTICLE

Serum iron, Folate, Ferritin and CD4 Count in HIV Seropositive Women

Simmi Kharb[1] · Manjulata Kumawat[1] · Meenakshi Lallar[2] · P. S. Ghalaut[3] ·
Smiti Nanda[2]

Abstract HIV infects cluster of differentiation 4 (CD4) T-lymphocytes, monocytes and macrophages resulting in decreased number and function of CD4 cells, changes that affect both cell mediated and humoral immunity. Hematological abnormalities are a common complication of human immune virus (HIV) infection and these abnormalities increase as the disease advances. Anemia is the most common haematological abnormality in HIV seropositive patients and its incidence is strongly associated with the progression of the disease. The aim of present study was to assess the haematological profile of HIV seropositive women and compare them with CD4 count. Two hundred seropositive females (age 18–25 years) attending antiretroviral therapy clinic were selected. Routine gynaecological and haematological investigations were carried out, study samples were drawn and serum iron, folate and ferritin were analysed by chemiluminiscence and CD4 count was determined by using flow-cytometry. Anemia was prevalent in seropositive women especially in those with low CD4 levels. Serum folate and ferritin levels were significantly lower in females with lower CD4 levels. Serum iron levels were higher at low CD4 levels. The mean CD4 count in HIV seropositive anaemic women were lower as compared to non anaemics suggesting that anaemia improves with higher CD4 cell counts. Plasma folate and ferritin levels are sensitive predictor of anaemia in early HIV infections and these patients should have a regular monitoring of their folate and ferritin levels especially with lower CD4 levels.

Keywords Iron · Folate · Ferritin · CD4 count · HIV seropositive women · Anaemia · ART clinic

Introduction

By 2015, the world is within reach of providing antiretroviral therapy to 15 million people. Under the 2013 WHO guidelines, the HIV treatment coverage in low- and middle-income countries represented only 34 % of the 28.6 million people eligible in 2013. The world has the potential to reach at least 90 % of pregnant women living with HIV with antiretroviral interventions by 2015 [1]. An estimated 35.3 million people worldwide were living with HIV and Sub-Saharan Africa was the region most heavily affected at the end of 2012 [2].

Hematological abnormalities are common complications of HIV infection and these abnormalities increase as the disease advances. Anemia is the most common haematological abnormality in HIV seropositive patients and its incidence is strongly associated with the progression of the disease. HIV itself is a cause of anaemia. Iron deficiency anaemia is common but other factors that may also contribute to the development of anemia in HIV infected patients includes nutritional deficiencies, infections, AIDS-related malignancies, drug treatment and a direct effect of HIV on the bone marrow [3–5]. HIV infects CD4 T-lymphocytes, monocytes and macrophages resulting in decreased number and function of CD4 cells, changes that affect both cell mediated and humoral immunity [3].

✉ Manjulata Kumawat
drmanjulata@yahoo.co.in

1 Department of Biochemistry, Pt. B.D. Sharma University of Health Sciences, PGIMS, House No. 22/8FM, Medical Campus, Rohtak, Haryana, India

2 Department of Obstetrics and Gynecology, Pt. B.D. Sharma University of Health Sciences, Rohtak, India

3 Department of Medicine, Pt. B.D. Sharma University of Health Sciences, Rohtak, India

Plasma folate and ferritin levels are sensitive predictor of anaemia. Both high and low ferritin levels have been reported to be highly prevalent in moderately immunosuppressed HIV-positive patients [6]. Serum folate is a short term indicator of folate status and lack of dietary folate leads to folate deficiency and macrocytic anaemia. Derangement in iron metabolism with resultant increase in plasma and total body iron concentrations may contribute to the depletion of CD4 T cell population. Studies of markers of iron deficiency are limited, hence the present study was planned to study markers of iron deficiency and CD4 counts in HIV seropositive females and to analyze gynaecological and haematological profile of HIV seropositive women and compare them with CD4 count in these women.

Materials and Methods

Two hundred seropositive females (age 18–25 years) attending antiretroviral therapy (ART) clinic of Medicine Department in collaboration with Department of Biochemistry and Obstetrics and Gynaecology, Pt. B.D. Sharma PGIMS, Rohtak were selected. Pregnant females and patients already diagnosed to have any invasive or preinvasive cancer were excluded. The study was approved by the institutional ethical committee. An informed written consent was obtained from the patients and all the ethical issues were duly taken care of.

Women with various gynaecological disorders including menstrual irregularities, vaginal infections, STD's, cervical dysplasias and haematological abnormalities were recruited. All the cases were subjected to general physical examination, detailed menstrual, medical, treatment history and Pap smear, pelvic examination. Cervicovaginal Pap smears of all subjects were taken using Ayre's spatula and cytobrush and immediately wet fixed in 95 % ethanol. Papanicolaou staining was done by the standard technique. Under all aseptic conditions, 6 ml of venous blood sample was collected from each subject for analysing HBsAg, STS status, Hb, PBF, serum iron, serum folate, serum ferritin and CD4 lymphocyte count. Also, CD4 lymphocytes count were done using standard flow cytometric technique. Serum iron, folate and ferritin were analysed by chemiluminiscence by competitive immunoassay using direct chemiluminiscence technology. Data so obtained was analysed using SPSS ver.20 and student's t test was applied, also, regression analysis was carried out.

Results

In the present study, anemia was prevalent in seropositive women especially in those with low CD4 levels. Serum folate and ferritin levels were significantly lower in females with lower CD4 levels. Serum iron levels were higher at low CD4 levels. The mean CD4 count in HIV seropositive anaemic women were lower as compared to non-anaemics suggesting that anaemia improves with higher CD4 cell counts. Thirty percent of women with CD4 count <200 cells/μl had vaginal infections while only 17.4 % of those with CD4 count >200/μl had vaginal infections ($p < 0.05$). Thus, CD4 count <200/μl was significantly associated with vaginal infection (Table 1).

HIV and HBsAg co-infection was present in 5 % of HIV seropositive females and HIV seropositive females with syphilis were 7 %. One patient had both HBsAg and STS status positive. In the study group 23.5 % patients had menstrual disorders while 76.5 % patients had regular menstrual cycles. Out of 47 patients with menstrual disorders, 17.5 % had oligomenorrhoea, 3.5 % had amenorrhoea, 2 % had menorrhagia and 0.5 % had polymenorrhoea. Menstrual disorders were found in 5.7 % patients who were not on ART and 33.1 % patients who were on ART. Thus the patients on ART had more menstrual disorders. Candida albicans and bacterial vaginosis infections were found 43.2 and 47.7 % respectively, while trichomoniasis accounted for just 3 % of vaginal infections. In our study 18.6 % patients had vaginal infections and were not on ART, while 21.3 % were on ART for less than a year and had infections. Vaginal infections were 19.1, 29.4 and 29.2 % in patients taking ART 1-2, 2-3 and more than 3 years respectively. Thus implying no beneficial effect of ART on prevention of vaginal infections in our study. Eleven percent patients had cytological abnormalities on Pap smear and Cytological abnormalities on Pap smear included 72.7 % ASCUS (atypical squamous cells of undetermined significance), 18.2 % LSIL (low-grade squamous intraepithelial lesion) and 9.1 %HSIL (high-grade squamous intraepithelial lesion). Total incidence of ASCUS, LSIL, and HSIL in our study was 8, 2 and 1 % respectively.

Only 3.5 % of HIV seropositive females had decreased serum iron levels, while 90 % had iron levels within the normal range. Six percent patients had increased iron levels. Eighty-three percent HIV seropositive women had

Table 1 Comparison of various parameters as per CD4 count

CD4 count (cells/μl)	Hb (gm %)	Serum Iron (μg/dl)	Serum Folate (ng/ml)	Serum Ferritin (ng/ml)
Up to 200 (n = 68)	8.98 ± 1.35	91.73 ± 32.06	1.85 ± 0.94	141.36 ± 134.59
≥200 (n = 132)	11.15 ± 1.48	90.12 ± 33.42	4.42 ± 1.48	189.40 ± 188.76
Statistical significance*	t = 10.31, p < 0.001 (VHS)	t = 0.331, p > 0.05 (NS)	t = 14.84, p < 0.001 (VHS)	t = 2.07, p < 0.05 (HS)

Table 2 Comparison of various parameters as per Hb status

Hb (gm %)	Serum Iron (µg/dl)	Serum Folate (ng/ml)	Serum Ferritin (ng/ml)
<12	88.48 ± 32.68	3.12 ± 1.72	155.17 ± 164.77
≥12	98.23 ± 32.87	5.01 ± 1.15	234.73 ± 189.70
Statistical significance*	$t = 1.75$, $p > 0.05$ (NS)	$t = 8.63$, $p < 0.001$ (VHS)	$t = 2.54$, $p < 0.01$ (HS)

serum folate less than 5.38 ng/ml and 16.5 % had serum folate levels more than 5.38 %. Mean serum iron levels in anaemic seropositive patients was 88.48 ± 32.68 µg/dl and 98.23 ± 32.87 µg/dl in non anemics which was not statistically significant ($p > 0.05$).

In the present study, serum folate and ferritin levels are significantly higher in patients with Hb more than 12 gm/dl or the non-anaemics. A significant positive correlation of coefficient was found when compared Hb with serum iron, serum ferritin, serum folate and CD4 count (r = 0.142, 0.242, 0.626, and 0.596 respectively). Also, similar results were found when CD4 count were compared with Hb, serum iron, serum folate and serum ferritin (0.595, 0.140, 0.673, and 0.165 respectively; Table 2). Serum folate and serum ferritin had higher serum levels at higher CD4 counts meaning a lesser degree of anaemia at higher CD4 cell counts.

Discussion

The pathogenesis of HIV-associated anaemia is complex and involves factors other than iron deficiency, such as direct inhibition of haematopoiesis and stem cell differentiation by HIV. In the present study, HIV seropositive women had a higher incidence of menstrual disorders, vaginal infections and cervical dysplasia as compared to the general population that further increased with the severity of immunosuppression (depicted by the lower CD4 cell counts) [7].

Several studies have suggested that a relationship exists between iron status and progression of HIV disease. Increased deposition of iron-ferritin and/or hemosiderin has been noted in the organs of patients with AIDS [8, 9]. A study of bone marrow macrophage iron grades in aspirates obtained from HIV-infected adults suggests that high macrophage iron grades were more common in patients infected with Candida, Pneumocystis, and Mycobacterium species, and high macrophage iron grade was associated with shorter duration of survival. Haptoglobin, a plasma antioxidant with hemoglobin-binding capacity, was associated with altered iron metabolism and mortality in HIV-infected adults [10]. It has been hypothesized that iron loading in tissues is harmful because of direct cytotoxicity to immune effector cells, predisposition to neoplasia and

enhancement of some infections because of iron overload of macrophages [8].The concern that iron supplementation may be harmful for HIV-infected patients has important implications, because daily oral iron supplementation is recommended for pregnant women and is used widely to treat iron deficiency anemia led the authors to undertake the present study in Indian women.

In the present study, there was a higher prevalence of anaemia in HIV seropositive women especially at lower CD4 levels. Massad et al. [7] concluded that in HIV-seropositive women higher CD4 counts were associated with fewer problems. Greenblatt et al. [11] showed that the severity of CD4 depletion was associated with higher prevalence rates of vaginal infections. In contrast, Shah et al. [12] demonstrated that there is no correlation between CD4 lymphocyte count and menstrual loss or dysmenorrhoea.

Serum folate and serum ferritin levels in non-anaemic were significantly higher. Mean serum iron was significantly higher at CD4 less than 200 cell/µl as compared to CD4 more than 200 cell/µl. Serum ferritin was lower at CD4 less than 200 cell/µl as compared to CD4 more than 200 cell/µl, implying better iron stores at higher CD4 levels. CD4 T-lymphocyte cell count showed a negative correlation with serum iron levels. Kamagate et al. [13] reported that iron stores (serum ferritin) in HIV positive women were significantly elevated as compared to control women with higher CD4 and Hb levels.

Previous studies have suggested that iron overload is more severe in adults with more advanced HIV disease [13, 14]. In contrast, Semba et al. [15] reported that iron status is not related to markers of HIV disease severity (plasma HIV load and CD4 T lymphocyte count) in HIV-infected pregnant women in Malawi. Elevated ferritin concentrations have been associated with increased HIV disease progression, but ferritin is a positive acute-phase reactant and can be elevated in inflammatory states [10]. HIV disease, especially when advanced, may up-regulate expression of several pro-inflammatory cytokines. The association of elevated serum ferritin concentrations with increased HIV disease severity might be due to underlying infection and inflammation, rather than to alterations in iron metabolism.

In the present study only 3.5 % of HIV seropositive females had decreased serum iron levels while 90 % had

iron levels within the normal range and 6 % patients had increased iron levels. Mean serum iron was 88.48 ± 32.68 µg/dl in anaemics and 98.23 ± 32.87 µg/dl in non-anaemics which was not statistically significant. Mean serum iron was higher 91.73 ± 32.068 µg/dl at CD4 less than 200cell/µl than 90.12 ± 33.42 µg/dl at CD4 more than 200 cell/µl, which was statistically significant signifying higher iron levels associated with lower CD4 cell counts.

Plasma folate and ferritin levels are sensitive predictor of anemia in early HIV infections and these patients should have a regular monitoring of their Hb, folate and ferritin levels especially with lower CD4 levels since their lower levels can accurately predict development of anaemia in the near future. As iron levels are relatively higher in these patients even when all other parameters depicting anaemia and iron supplementation should be avoided or kept to minimum levels. Early treatment of anaemia based on observations in the present study can lead to a better quality of life and longer morbidity free survival in the HIV seropositive women.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interests.

Ethical Approval The study was approved by the institutional ethical committee.

Informed Consent An informed consent was obtained from the patients and all the ethical issues were duly taken care of.

References

1. UNAIDS, Global Report: UNAIDS Report on the Global AIDS Epidemic 2013, 2013.
2. Bekolo CE, Nguena MB, Ewane L, Bekoule PS, Kollo B. The lipid profile of HIV-infected patients receiving antiretroviral therapy in a rural Cameroonian population. BMC Public Health. 2014;14:236. doi:10.1186/1471-2458-14-236.
3. Levine WC, Pope V, Bhoomkar A, Tambe P, Lewis JS, Zaidi AA, et al. Increase in endocervical CD4 lymphocytes among women with non-ulcerative sexually transmitted diseases. J Infect Dis. 1998;177(1):167–74.
4. Volberding PA, Levine AM, Dieterich D, Mildvan D, Mitsuyasu R, Saag M. Anemia in HIV infection: clinical impact and evidence-basedmanagement strategies. Clin Infect Dis. 2004;38(10):1454–63.
5. Obirikorang C,IssahakuRG, OsakunorDNM, Osei-Yeboah J. Anaemia and iron homeostasis in a cohort of HIV-infected patients: a cross-sectional study in Ghana. AIDS Res Treat 2016; 2016:1623094. doi: 10.1155/2016/1623094. (**Epub 2016 Mar 22**).
6. Minkoff HL, Eisenberger MD, Feldman J, Burk R, Clarke L. Prevalence and incidence of gynecological disorders among women infected with human immunodeficiency virus. Am J Obstet Gynecol. 1999;180(4):824–36.
7. Massad LS, Evans CT, Minkoff H, Watts DH, Greenblatt RM, Levine RM, et al. Effects of HIV Infection and its treatment on the self-reported menstrual abnormalities in women. J Women Health (Larchmt). 2006;15(5):591–8.
8. Boelaert JR, Weinberg GA, Weinberg ED. Altered iron metabolism in HIV infection: mechanisms, possible consequences, and proposals for management. Infect Agents Dis. 1996;5(1):36–46.
9. Savarino A, Pescarmona GP, Boelaert JR. Iron metabolism and HIV infection: reciprocal interactions with potentially harmful consequences? Cell Biochem Funct. 1999;17(4):279–87.
10. De Monyé C, Karcher DS, Boelaert JR, Gordeuk VR. Bone marrow macrophage iron grade and survival of HIV-seropositive patients. AIDS. 1999;13(3):375–80.
11. Greenblat RM, Bacchetti P, Barkan S, Augenbraun M, Silver S, Delapenha R, et al. Lower genital tract infections among HIV infected and high risk uninfected women: findings of the women's interagency HIV study (WIHS). Sex Transm Dis. 1999;26(3):143–51.
12. Shah PN, Smith JR, Wells C, Barton SE, Kitchen VS, Steer PJ. Menstrual symptoms in women infected by the human immunodeficiency virus. Obstet Gynecol. 1994;83(3):397–400.
13. Kamagate S, Bleyere M, Koukou L, Mama K. Alteration of iron stores in women of reproductive age with HIV in Abidjan. Int J Biosci. 2012;2:11–22.
14. Delanghe JR, Langlois MR, Boelaert JR, Van Acker J, Van Wanzeele F, Van der Groen G, et al. Haptoglobin polymorphism, iron metabolism and mortality in HIV infection. AIDS. 1998;12(9):1027–32.
15. Semba RD, Taha TE, Kumwenda N, Mtimavalye L, Broadhead R, Miotti PG, et al. Iron status and indicators of human immunodeficiency virus disease severity among pregnant women in Malawi. Clin Infect Dis. 2001;32(10):1496–9.