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Bone marrow macrophage iron stores in patients with HIV infection and AIDS-associated Kaposi's sarcoma

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Summary

Several observations have been made suggesting that excess iron is harmful to patients with HIV/AIDS disease. Bone marrow macrophage iron stores of 30 anaemic HIV infected patients (median age 32.7 years) and 20 anaemic AIDS-associated Kaposi's sarcoma patients (median age 37 years) were studied at the haematology department of the University of Maiduguri Teaching Hospital. Macrophage iron stores were assessed as either normal, decreased or increased by using grades ranging from 0 to 6. Marrow iron stores was increased in 16 (80%) of the patients with Kaposi's sarcoma and normal in 4 (20%) patients. Three of the 4 patients with normal iron stores were females of reproductive age. Regression analysis of iron status and opportunistic infection showed a positive correlation (p-value= 0.001). Of the 30 patients with HIV infection, 22 (73.3%) had normal iron stores and 8 (26.7%) had decreased iron stores. All the 8 (26.7%) patients with no stainable iron in the marrow were females of reproductive age group. Iron deficiency anaemia can complicate anaemia of HIV infected patients. In view of the documented risk associated with iron supplementation in anaemic patients with HIV/AIDS disease, little caution should be exercise as regards the use of haematinics and/or blood tonics in anaemic HIV- infected or AIDS-associated Kaposi's sarcoma patients. The fact that noninvasive evaluation for iron deficiency is compromised in many individuals due to the presence of chronic inflammatory process and/ or malignancy, bone marrow evaluation for iron stores still remains an important tool often underutilized by many clinicians attending to patients living with HIV/AIDS.

Keywords: *Bone marrow iron stores, HIV/AIDS.*

Résumé

Plusieurs observations ont été faite suggérant que l'excès de fer est dangereux aux patients avec le VIH/SIDA. Les macrophages de la moelle osseuse chez 30 patientes anémiés infectés du VIH (age médian de 32.7ans) et 30 patients anémiés ayant le SIDA associés au sarcome de Kaposi (age médian de 37 ans) étaient étudiés dans le département de d'Hématologie du Centre Hospitalier de l'Université de Maiduguri. Les dépôts de fer des macrophages étaient évalués comme normal, décroissant ou croissant en utilisant les degrés de 0 à 6. Les dépôts osseuses était croissant chez 16(80%) aux patients ayant le sarcome de Kaposi et normal chez 4(70%) patients. Trois de quatre patients ayant le dépôt de fer étaient femelle a l'age reproductive. L'analyse de régression du statut du fer et l'infection opportuniste montraient une corrélation positive (P=0.001). Sur les 30 patients ayant le HIV 22(73.3%) avaient un dépôt normal de fer et 8 (26.7%) avaient décrû le dépôt du fer. Tous les huit (26.7%) patients sans aucune de taux de fer soutenable dans les moelles étaient des femelle a l'age reproductive. L'anémie liée au deficie en fer peut compliquée l'anémie aux patients infectés du VIH. L'attention doit être exercée par l'utilisation des hematiniques et /ou des tonic sanguins en anémie chez les infectés du VIH ou le sarcome de Kaposi associé aux patients ayant le SIDA. L'évaluation non intensifiée du deficie du fer compromit chez plusieurs individus du a la présence d inflammation chronique, évaluation de la moelle osseuse du fer reste un important outil très souvent moins utilisé by les médecins recevant les patients ayant le VIH-SIDA.

Introduction

Several observations have been made suggesting that iron may be one of the cofactors involved in the pathogenesis of Kaposi's sarcoma [1-5]. Pathogenetic mechanisms such as activation of ribonucleotide reductase; a key enzyme involved in DNA synthesis, formation of mutagenic hydroxyl radicals and inhibition of CD4 positive lymphocytes have been described as well as increase production of HIV viral replication[6-8]. Iron supplementation in the presence of higher bone marrow iron stores was also found to be associated with increased frequency of microbial infections and shorter survival in human immunodeficiency virus (HIV) positive patients [9-11]. In the Western countries,

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anaemia of HIV infection and acquired immune deficiency syndrome (AIDS) is predominantly anaemia of chronic disease characterised by decreased serum iron, decreased total iron- binding capacity and increased bone marrow iron stores [12]. However, in Nigeria it is a combination of multiple nutrient deficiencies as a result of chronic anorexia, diarhoea, malabsorption and opportunistic infections [13]. The aim of this study is to examine the pattern of bone marrow macrophage iron stores in our anaemic HIV- infected and AIDS-Associated Kaposi's sarcoma patients and to see if there is coexistence of iron deficiency anaemia in our anaemic patients with HIV infection and AIDS-associated Kaposi's sarcoma.

Patients and methods

Bone marrow macrophage iron stores of 30 anaemic HIV- infected patients and 20 anaemic AIDS-associated Kaposi's sarcoma patients seen at the Haematology Day Ward of the University of Maiduguri Teaching Hospital between October 2002 to August 2005 were reviewed. All the patients were referred for the purpose of evaluating the cause of anaemia and for possible commencement of chemotherapy for AIDS-associated Kaposi's sarcoma. Some of the patients seen had documented evidence of AIDS- defining illnesses such as pulmonary tuberculosis, oral thrush and herpes zoster in association with recurrent pyrexia and/or chronic diarhoea. Other relevant information was obtained from individual patients' case note.

All HIV antibody seropositive status and CD4+ cell count of the patients were already known by the referring clinicians and were all determined at the immunology department of the hospital which is a national reference centre for the diagnosis of HIV disease. The diagnosis of Kaposi's sarcoma in all the 20 patients was made by a pathologist through histological examination of biopsied specimens from multiple sites such as lower limbs, trunk and rectum as well as penis, lymph nodes, scrotum and oropharynx. Haematocrit were estimated for all the patients in Haematology departments using the micro-haematocrit method. Females and Males with haematocrit value of less than 36% and 40% respectively were classified as anaemic [14]. In all cases, adequate number of fragments was obtained either through the posterior superior iliac spine or the sternum using either the Klima or Salah aspiration biopsy needle under plain xylocaine local anaesthesia. Films were made immediately at bed side and allowed to dry. In the laboratory, the films were fixed in absolute methanol for 10-20 minutes. Two slides each was routinely stained with leishman's stain for the morphological assessment of the marrow cells for all the patients. Two sets of slides, one containing adequate fragment and another a squash marrow film were stained by perls's acid Ferro cyanide for the presence of haemosiderin in bone marrow macrophage [15]. A positive (a film of normal iron replete adult) and a negative (a film of iron deficient adult) control were set up along side the test films [15]. The perls's Ferro cyanide stained films were routinely examined under low power (x10 and x40) to assess the macrophage haemosiderin storage iron seen as bluish deposits within the marrow fragments and then under high power (x100) oil immersion for the haemosiderin. Macrophage haemosiderin iron stores were assessed as either normal, decreased or increased by using grades ranging between 0 to 6 (0= no stainable iron at low power, 1= iron positive only after examination with oil immersion, 2= iron positive at low power, 3= numerous small iron particles in reticulin, 4= large iron particles tending to aggregate, 5= dense large iron clumps, 6= very large iron clumps and extra cellular iron) [16]. Grades of 1 to 3 were classified as Normal, Grades of 0 as Decreased and Grades of 4 and above as Increased [16]. The data was analyzed using the Statistical Package for Social Sciences software version 11.0(Chicago, IL, USA) and a probability level of $p<0.05$ was taken as significant.

Results

The bone marrow macrophage iron stores of 50 patients, comprising 30 (60%) anaemic HIV- infected patients and 20 (40%) anaemic AIDS-associated Kaposi's sarcoma patients were studied.

Anaemic AIDS-associated Kaposi's sarcoma patients

A total of 20 patients, comprising 17 (85%) males and 3 (15%) females were studied. Their ages ranged between 21-45 years (median 37). Mean haematocrit and CD4+ cell count was 29.5% $\pm$ 7.5SD and 119.0cells/μl $\pm$ 91.4 SD respectively. Haemosiderin macrophage iron stores were increased in 16 (80%) patients and normal in the remaining 4 (20%) patients. Three of the 4 patients with normal iron stores were females of reproductive age. Fourteen (70%) patients had 4 iron grades in the marrow. Seven (35%) patients had pulmonary tuberculosis,

5 (25%) had oral thrush and 2 (10%) had herpes zoster whereas 1 (5%) had seborrheic dermatitis. Regression analysis of iron status and opportunistic infection showed a positive correlation (p-value= 0.001).

Anaemic HIV infected patients.

A total of 30 patients, comprising 17 (56.7%) females and 13 (43.3%) males were studied. Mean age for all the patients was 32.7 years ± 7.7SD. Median haematocrit and CD4 cell count for all the patients was 33% ± 4.5 SD and 320cells/μl ± 91.8 SD respectively. Haemosiderin iron stores were normal in 22 (73.3%) patients and decreased in 8 (26.7%) patients. All the 8 (26.7%) patients with no stainable iron in the marrow were females of reproductive age group.

Discussion

The results of this study demonstrate that bone marrow macrophage iron stores are increased in our patients with AIDS-associated Kaposi's sarcoma compared to our anaemic HIV- infected patients. Although there is scanty literature on the pattern of marrow iron stores in AIDS-associated Kaposi's sarcoma in our environment, several observations have been made suggesting that iron may be one of the cofactors involved in the pathogenesis of Kaposi's sarcoma [1-5]. The increased iron stores observed in our AIDS-associated Kaposi's sarcoma may not be unrelated to the fact that AIDS patients exhibit alteration in the metabolism of iron that may lead to induction and/or progression of Kaposi's sarcoma, increased deposition of this element in the tissues and increased susceptibility to opportunistic infection. Other Caucasian studies have also documented the role of iron in the increased susceptibility to infection by mycobacterium spp [17, 18].

The fact that three of the four patients with AIDS –associated Kaposi's sarcoma who had normal marrow iron stores were females provides a hormonal explanation for the lower prevalence of Kaposi's sarcoma among women, as they are known to have a lower iron reserves than men. Interestingly, disappearance of Kaposi's sarcoma lesions during or just after pregnancy has been reported [19]. However, genetic and environmental factors have been documented as the cause of high incidence rates of Kaposi's sarcoma among Bantu women in the South African Transvaal. This was attributed to dietary iron in traditional fermented beer that is home-brewed from local crops in steel drums [20, 21]. In this study, females of reproductive age group with human immunodeficiency infection were found to be iron deficient compared to their male counterpart having adequate marrow iron stores. This is contrary to the earlier Caucasian studies where anaemia of HIV infection and acquired immune deficiency syndrome (AIDS) is predominantly anaemia of chronic disease characterized by decreased serum iron, decreased total iron- binding capacity and increased bone marrow iron stores [12 22,23]. The differences may be partly because of the high prevalence of iron deficiency among females of reproductive age group in Africa couple with the regular monthly menstrual loss of iron. Similarly, in another study, Sagir *et al* [24] examined the bone marrow of 24 anaemic AIDS patients and observed that 8 (33.3%) had absent iron stores whereas 16 (66.7%) had increased marrow iron stores. Background effect of anorexia, chronic diarrhoea and malabsorption could also contribute to the nutritional deficiencies that may lead to iron deficiency anaemia. There is a need for a further study to look at the bone marrow macrophage iron stores of non-anaemic HIV infected males and non-anaemic premenopausal/postmenopausal females with HIV/ AIDS in our environment.

In conclusion this study has shown that bone marrow macrophage iron stores are increased in our patients with AIDS-associated Kaposi's sarcoma compared to 73.3% of our anaemic HIV- infected patients who had normal iron stores in the marrow. In view of the documented risk associated with iron supplementation in anaemic patients with HIV/AIDS disease, little caution should be exercise as regards the use of haematinics and/or blood tonics in anaemic HIV- infected or AIDS-associated Kaposi's sarcoma patients. The fact that noninvasive evaluation for iron deficiency is compromised in many individuals due to the presence of chronic inflammatory process and/ or malignancy, bone marrow evaluation for iron stores still remains an important tool often underutilized by many clinicians attending to patients living with HIV/AIDS.

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