Iron status and anaemia of chronic disease in HIV-infected African women in Mangaung, Bloemfontein

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Abstract

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Background: Anaemia occurs widely among people living with HIV/AIDS. The aim of this study was to investigate the effect of HIV status on iron status, more specifically to investigate the nutritional health of women between 25 and 44 years of age.

Methods: An epidemiological study was undertaken in Mangaung, a black residential community of Bloemfontein in the Free State (South Africa). A random sample consisted of 500 women in two age groups (25–34 [n = 273] and 35–44 years [n = 215]). Blood specimens were collected in ethyldimethylacetic acid collection tubes according to standard procedures. Respondents fasted overnight, abstained from exercise and avoided consuming alcohol and caffeine for 24 hours prior to collection of the blood specimens. All specimens were taken in the morning. A full blood count was performed using a Coulter Microdiff 18 Cell Counter. The metabolic variables haematocrit (Hct), haemoglobin (Hb), serum iron, ferritin and transferrin were determined. The red blood cell count was performed to calculate the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Age and HIV-status groups were described and compared by non-parametric methods. A p-value lower than 0.05 was considered significant. HIV-infected and -uninfected groups were compared by 95% confidence intervals for the difference in the percentage of women with parameters below or above the normal range.

Results: Sixty-one per cent of the younger women and 38% of the older women were HIV infected. The percentage with serum ferritin levels below 20 µg/L was higher in HIV-uninfected women, ranging from 0% in older HIV-infected women to 10.4% in younger HIV-uninfected women. A large percentage of women had elevated transferrin values, ranging from 23.9% in older HIV-infected women to 44.8% in older HIV-uninfected women. A large percentage of women had anaemia of chronic disease, with HIV-infected women afflicted more often.

Conclusion: The results of the study indicate that prevalence of HIV infection in Mangaung is high, especially among women between 25 and 34 years of age. Although the parameters of iron status on average did not indicate iron deficiency in the different age and HIV-status groups, a large percentage of women did have anaemia of chronic disease, with HIV-infected women afflicted more often. Knowledge of the HIV status of a patient is of paramount importance in evaluating laboratory results of iron levels to determine future treatment or nutritional recommendations. HIV-infected and -uninfected individuals might not be comparable regarding their laboratory results to interpret iron store depletion, with consequences for further therapeutic actions in these two groups. The progression rate to AIDS might also be enhanced by certain interventions.

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Introduction

Nutrition is an important factor in the course of HIV infection[1] and is generally accepted as a major determinant of immune functioning.[2] Nutritional factors, although not the most important aetiological determinants, may change immune function to facilitate disease progression, influence viral expression, and play a significant role in disease processes and related morbidity and mortality.[3]

Although the reasons are not fully understood,[4] anaemia occurs widely among people living with HIV/AIDS.[5–7] Some researchers associate anaemia with HIV-disease progression and an increased risk of death,[7,8] even independent of CD4 count and viral load.[9] On the other hand, others find no relationship between indicators of iron status and disease severity in HIV-infected pregnant African women.[10] Anaemia can be associated with various conditions, including infection, neoplasms, dietary deficiencies, blood loss and medication.[7,9] Iron deficiency and anaemia may contribute to reduced energy levels, lower aerobic capacity, decreased endurance and fatigue.[11] A possible sign of early iron deficiency is reduced immunocompetence, which may lead to an increased propensity for infection.[12] Olsen et al[13] recommend that the role of iron in HIV infection be clarified, since iron is commonly administered to both anaemic patients and pregnant women, and as knowledge of HIV status is not actively sought by victims due to social stigmatisation, iron supplementation is a common phenomenon even in areas with a high HIV prevalence.

Methods and materials

An epidemiological study was undertaken in Mangaung, a black residential community of Bloemfontein in the Free State (South Africa). The main objective of the study was to investigate the nutritional health

of women between 25 and 44 years of age. This age group was chosen to coincide with the age range used in another study in the same geographical area, but done a number of years ago.[14] As part of the current epidemiological study, socio-demographic status, health status (determined by a medical examination), dietary intake (determined using a comprehensive food frequency questionnaire), levels of physical activity, body perception and attitude toward weight control, prevalence and risk of lifestyle diseases, anthropometry, micronutrient status and prevalence of HIV were determined. This paper reports on the iron status of HIV-infected and -uninfected women, and whether HIV status is linked to changes in iron stores.

Participants

A sample size of 500 was estimated to be representative of the Mangaung metropole. The respondents were from two built-up areas (Phahameng and Botchabela) and two informal settlements (Joe Slovo and Namibia). Post-pubertal but pre-menopausal black women were randomly selected from the two age groups 25 to 34 and 35 to 44 years, using township maps. The residential plots in the four selected areas were counted and numbered. Namibia had 2 995 plots, Phahameng 1 711, Joe Slovo 1 359 and Botchabela 2 308. A proportionate number of respondents were randomly selected from these plots. Twenty subjects were recruited per week over a 25-week period (March 2000 – November 2000). A randomly selected residence was approached by a community health worker. If no one was at home, the residence to the right was targeted, and if still unsuccessful, the residence to the left of the original address was approached. If all attempts failed, another plot was randomly selected.

The women participated voluntarily after giving written informed consent. Each participant received an amount of R40.00 to cover travelling and other logistical costs. The study was approved by the Ethics Committee of the Faculty of Health Sciences, University of the Free State (ETOVS no 02/00). The results of four women who were found to be pregnant during the medical examination were excluded. All participants were originally unaware of their HIV status, and were antiretroviral therapy (ART) naïve. Women who chose to be informed of their HIV status after testing (< 30%) were confidentially seen by a medical practitioner who referred HIV-infected patients for counselling and further follow-up.

Blood specimens

A registered nurse collected the blood specimens according to standard procedures. Respondents fasted overnight, abstained from exercise, and avoided consuming alcohol and caffeine for 24 hours prior to collection of the blood specimens. All blood specimens were taken in the morning. It was not possible to evaluate all the specimens due to clotting or haemolysis. Respondents were not requested to return for repeat specimens.

The methods and equipment that were used to determine the parameters evaluated in this study are listed in Table I.

Blood specimens were taken in ethyldimethylacetic acid (EDTA) collection tubes and a full blood count was performed using a Coulter Microdiff 18 Cell Counter. The metabolic variables haematocrit (Hct), haemoglobin (Hb), serum iron,

ferritin and transferrin were determined. The red blood cell count was performed in order to calculate the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Counting of red and white blood cells was performed sequentially.

Table I: Methods and equipment used to determine the parameters evaluated in the study

Parameter	Methods and equipment
HIV tests	Human Immunodeficiency Viruses (HIV-1/HIV-2: recombinant antigens and synthetic peptides) reagent pack (Abbott, Germany, catalogue no 3D41-20)
Total serum ferritin	Determined on the Hitachi 902 using an immunoturbidimetric immunoassay supplied by Randox (catalogue no FN 2467)
Total serum iron	Determined on the Hitachi 902 using a colorimetric assay method (catalogue no 11876996, Boehringer Mannheim-Roche Diagnostics, Mannheim, Germany)
Total serum transferrin	Determined on the Hitachi 902 using an immunoturbidimetric immunoassay supplied by Randox (catalogue no TF 7197)

Statistical analysis

Age and HIV-status groups were described and compared by non-parametric methods. Data were processed using the SAS software program.[15] All datasets were categorised into two age groups (25–34 years, and 35–44 years), and two HIV-status groups (HIV uninfected and HIV infected). For each group, continuous variables were described by medians (as data were not always equally distributed), and categorical variables were described by frequencies and percentages. A p-value lower than 0.05 was considered significant. HIV-infected and -uninfected groups were compared by 95% confidence intervals (CIs) for the difference in the percentage of women with parameters below or above the normal range.

Results

In this sample the prevalence of HIV infection was higher in the younger (25–34 years) than in the older women (35–44 years). Sixty one per cent of younger women and 38% of older women were HIV infected. An overview of the micronutrient intake of participants has been published previously[16] and showed that the median total iron intake of women participating in this study did not differ significantly between those with low or high parameter values, independent of their age or HIV status.[16]

Iron status parameter median values

The median iron status parameters for the entire group are given in Table II.

The median values for all iron status parameters of HIV-infected and -uninfected women for both age groups fell within the normal reference ranges. Within these ranges, however, the younger HIV-infected women had significantly lower Hb ($p = 0.0002$) and Hct ($p = 0.0003$) median values than the HIV-uninfected group.

In the older group, the HIV-infected women had significantly lower median transferrin ($p = 0.04$) but higher median serum ferritin (s-ferritin) values ($p = 0.02$) than the HIV-uninfected women.

Iron status parameter percentages

The parameter percentages for the younger women are given in Table III and for the older women in Table IV.

Few women had low s-ferritin levels. However, more HIV-uninfected women (in both age groups) had low s-ferritin levels than infected women (younger group: 10.4% vs 5.3%; older group: 6.5% vs 0%). Although the difference was not significant, a trend was observed in both age groups, indicated by a 95% Wilcoxin CI [3.1; 14.4] for the younger group and [-2.2; 15.4] for the older group.

Table II: Median values for iron status parameters of HIV-uninfected and HIV-infected women in both younger and older age groups

Parameter	Normal range	25–34 years				35–44 years			
		HIV -*		HIV +*		HIV -		HIV +	
		n	Median	n	Median	n	Median	n	Median
Serum ferritin (µg/L)	20–200	77	63.9	95	74.1	62	57.4†	46	80†
Serum iron (mg/L)	0.7–1.8	93	0.9	116	0.9	62	0.9	53	0.9
Transferrin (g/L)	2.0–3.0	68	2.9	85	2.8	58	3†	46	2.8†
Transferrin saturation (%)	20–50	68	25.4	85	26	58	24.1	46	23.8
Haemoglobin (g/dL)	11.7–16.0	106	14.1†	166	13.6†	131	13.7	82	13.5
Haematocrit (L/L)	0.35–0.47	106	40.9†	166	39.2†	131	39.5	82	39.8
MCV (fL)	81–99	106	89.7	166	89.9	131	91.5	82	91.5
MCH (pg)	27–34	106	30.5	166	30.9	131	31.3	82	31.2
MCHC (g/dL)	32–36	106	33.9	166	34.1	131	34.2	82	34.1

*HIV -: HIV-uninfected; HIV +: HIV-infected; †statistically significant

Table III: Iron status parameter percentages for HIV-infected and HIV-uninfected women in the 25- to 34-year age group

Parameter	HIV -*		HIV +*		95% CI
	n	%	n	%	
Serum ferritin (µg/L):	(n = 77)		(n = 95)		
< 20	8	10.4	5	5.3	-3.1;14.4
20–200	67	87	87	91.6	
> 200	2	2.6	3	3.2	
Serum iron (mg/L):	(n = 93)		(n = 116)		
< 0.7	28	30.1	42	36.2	-16.6;4.8
0.7–1.8	59	63.4	68	58.6	
> 1.8	6	6.5	6	5.2	
Transferrin (g/L):	(n = 68)		(n = 85)		
< 2.0	1	1.5	4	4.7	-8.7;2.3
2.0–3.0	45	66.2	55	64.7	
> 3.0	22	32.4	26	30.6	-12.6;16.5
Transferrin saturation (%):	(n = 68)		(n = 85)		
< 20	22	32.4	26	30.6	-10.4;14.2
20–50	41	60.3	51	60	
> 50	5	7.4	8	9.4	
Haemoglobin (g/dL):	(n = 106)		(n = 166)		
< 11.7	5	4.7	16	9.6	-11.0;2.0
11.7–16.0	95	89.6	148	89.2	
> 16.0	6	5.7	2	1.2	
Haematocrit (L/L):	(n = 106)		(n = 166)		
< 0.35	5	4.7	22	13.3	-14.0;-2.5†
0.35–0.47	95	89.6	144	86.8	
> 0.47	6	5.7	0	0	
MCV (fL):	(n = 106)		(n = 166)		
< 81	10	9.4	14	8.4	-5.7;8.8
81–99	92	86.8	144	86.8	
> 99	4	3.8	8	4.8	
MCH (pg):	(n = 106)		(n = 166)		
< 27	10	9.4	12	7.2	-4.3;9.9
27–34	91	85.9	147	88.6	
> 34	5	4.7	7	4.2	
MCHC (g/dL):	(n = 106)		(n = 166)		
< 32	9	8.5	5	3	0.0;12.6
32–36	91	85.9	158	95.2	
> 36	6	5.7	3	1.8	

*HIV -: HIV-uninfected; HIV +: HIV-infected; †statistically significant

Table IV: Iron status parameter percentages for HIV-infected and HIV-uninfected women in the 35- to 44-year age group

Parameter	HIV -*		HIV +*		95% CI
	n	%	n	%	
Serum ferritin (µg/L):	(n = 62)		(n = 46)		
< 20	4	6.5	0	0	-2.2;15.4
20–200	51	82.3	44	95.7	
> 200	7	11.3	2	4.4	
Serum iron (mg/L):	(n = 62)		(n = 53)		
< 0.7	25	40.3	13	24.5	1.3;29.2†
0.7–1.8	28	45.2	39	73.6	
> 1.8	9	14.5	1	1.9	
Transferrin (g/L):	(n = 58)		(n = 46)		
< 2.0	0	0	2	4.4	-12.4;1.0
2.0–3.0	32	55.2	33	71.7	
> 3.0	26	44.8	11	23.9	2.4;37.1†
Transferrin saturation (%):	(n = 58)		(n = 46)		
< 20	21	36.2	14	30.4	-9.6;20.4
20–50	30	51.7	30	65.2	
> 50	7	12.1	2	4.4	
Haemoglobin (g/dL):	(n = 131)		(n = 82)		
< 11.7	6	4.6	7	8.5	-12.4;2.7
11.7–16.0	121	92.4	72	87.8	
> 16.0	4	3.1	3	3.7	
Haematocrit (L/L):	(n = 131)		(n = 82)		
< 0.35	3	2.3	10	12.2	-17.2;-4.1†
0.35–0.47	123	93.9	69	84.2	
> 0.47	5	3.8	3	3.7	
MCV (fL):	(n = 131)		(n = 82)		
< 81	8	6.1	2	2.4	-3.1;9.4
81–99	112	85.5	75	91.5	
> 99	11	8.4	5	6.1	
MCH (pg):	(n = 131)		(n = 82)		
< 27	10	7.6	2	2.4	-1.7;11.3
27–34	108	82.4	75	91.5	
> 34	13	9.9	5	6.1	
MCHC (g/dL):	(n = 131)		(n = 82)		
< 32	10	7.6	1	1.2	0.0;12.3
32–36	108	82.4	76	92.7	
> 36	13	9.9	5	6.1	

*HIV -: HIV-uninfected; HIV +: HIV-infected; †statistically significant

Compared to s-ferritin levels, a larger percentage of women had serum iron levels below 0.7 mg/L. In the younger group there was no significant difference between the HIV-infected and -uninfected women (95% CI [-16.6; 4.8]). In the older group, however, significantly more HIV-uninfected women had low serum iron levels (95% CI [1.3; 29.2]).

Very few women had low transferrin levels. Although more HIV-infected women had low transferrin levels, the difference was not significant for either age group. More than 20% of all women, however, had elevated transferrin levels above 3.0 g/L. In the older age group the elevated transferrin was significantly more pronounced in the HIV-uninfected than in the HIV-infected group (95% CI [2.4; 37.1]).

The percentage of women with transferrin saturation below 20% was highest in the older age group (both HIV-infected and HIV-uninfected women). In both age groups, this percentage did not differ significantly between the two HIV-status groups. Similarly, no significant difference was seen in the percentage of women with elevated transferrin saturation above 50% between HIV-infected and -uninfected states.

The percentage of women with low Hb levels ranged from 4.6% in the older HIV-uninfected women to 9.6% in the younger HIV-infected women. Although the HIV-infected groups (both age intervals) had a higher percentage of low Hb values and the confidence interval indicated a trend, the difference was not statistically significant (younger group: 95% CI [-11; 2.0]; older group: 95% CI [-12.4; 2.7]).

Similarly, the percentage of women with low Hct levels ranged from 2.3% (older HIV-uninfected women) to 13.3% (younger HIV-infected women). In both younger and older women, significantly more women had low Hct values in the HIV-infected groups with 95% CI [-14.0; -2.5] (younger group) and 95% CI [-17.2; -4.1] (older group).

No significant differences between MCV and MCH of HIV-infected and -uninfected women could be found in either age group. The percentage of women with low MCHC, however, seemed more pronounced in the HIV-uninfected women. The difference was close to significant with 95% CIs [0.00; 12.6] and [0.0; 12.3], in the younger and older groups, respectively. The percentage of women with low MCHC ranged from 1.2% in the older HIV-infected women to 8.5% in the younger HIV-uninfected women.

Discussion

Prevalence of HIV in the random sample included in this study was very high. In developing countries, a disturbing trend of a faster growing rate of new infections in females than in males is being witnessed,[17,18] with women of childbearing age infected at the highest rate of all subgroups.[19]

Nutritional deficiencies may develop during any stage in the HIV-infected individual. Because the early signs and symptoms of nutritional deficiencies, such as fatigue, irritability and dry skin are non-specific, many deficiencies remain unnoticed until they have progressed to levels of expressive body depletion.[20]

S-ferritin levels are usually in equilibrium with body iron stores and measurements of s-ferritin may best reveal an early negative iron balance,[12] while high s-ferritin may indicate significant iron overload.[21] S-ferritin is the most sensitive parameter of a negative iron balance, decreasing only in the presence of true iron deficiency, as does transferrin saturation. More HIV-uninfected than -infected women had decreased s-ferritin levels, and the 95% CI in both younger and older women seemed to confirm a trend. Based on s-ferritin, most women in both age groups did not have an iron deficiency, and no significant difference existed between HIV-infected and -uninfected women.

In contrast, an increased s-ferritin is not prognostic of a positive iron balance. S-ferritin levels may also increase in chronic diseases unrelated to iron metabolism, such as inflammatory diseases.[12] It is possible that this acute phase protein was reactively elevated, explaining the higher median s-ferritin levels in HIV-infected subjects, and was therefore not a true reflection of increased iron stores compared to HIV-uninfected subjects.

Studies relating s-ferritin levels to bone marrow iron content in various chronic disorders suggest that s-ferritin in sick patients without liver disease may be interpreted as follows:[22]

< 30 µg/L:	Iron stores depleted
Between 30 and 50 µg/L:	Iron stores probably depleted
Between 50 and 150 µg/L:	Iron stores uncertain
> 150 µg/L:	Iron stores present

These parameter ranges could suggest that HIV-infected and -uninfected individuals are not comparable with regard to their laboratory results to interpret iron store depletion, with consequences for further therapeutic actions in these two groups.

Serum iron levels decrease once body iron stores (as indicated by s-ferritin) are depleted, and increase with iron overload. However, on its own, serum iron is not a valuable parameter of iron status as it is affected by many pathological factors other than the amount of iron in the body, such as chronic infections.[21] More than 30% of both HIV-uninfected and -infected women in this study had serum iron levels below 0.7 mg/L. In the older group, the percentage of women with low serum iron levels was significantly higher in the HIV-uninfected than in the HIV-infected women, which is contrary to what is expected in chronic infections. In the younger group, the expected trend was seen with more HIV-infected women with low serum iron values than HIV-uninfected women. However, this was not a significant difference. A possible explanation is that older HIV-infected women might have other nutritional deficiencies (e.g. pyridoxine deficiency), causing an inability to utilise the available iron. Another possibility is the fact that erythropoiesis fails to increase in chronic disease anaemia because iron release from mononuclear phagocytes, which is the primary source of iron for making new red blood cells does not increase.[22] HIV status could therefore have a similar effect on other related parameters (e.g. Hb and Hct) in both age groups (through a number of mechanisms), independent of the effect on serum iron *per se*. This is indeed seen in both age groups, where significantly more HIV-infected women had low Hct values compared to HIV-uninfected women. Furthermore, more HIV-infected women in this study had low Hb levels when compared to HIV-uninfected women, and the difference was close to significant. Hb alone is unsuitable as a diagnostic tool in cases of suspected iron deficiency anaemia because it is affected only late in the disease, it cannot distinguish iron deficiency from other types of anaemia, and Hb values in normal individuals vary widely.[12]

Serum transferrin is less labile than serum iron, and has a reciprocal relationship to iron stores – it increases in uncomplicated iron deficiency and decreases in iron overload. Transferrin increases as an adaptive mechanism to enhance iron absorption. It can however decrease in chronic illnesses associated with low serum iron concentrations, but remains unchanged in acute illness.[21] A large percentage of women had elevated transferrin levels. Significantly fewer older HIV-infected women had the expected high serum transferrin levels when compared to HIV-uninfected women, possibly due to the mechanisms described for low serum iron values.

Transferrin saturation decreases only in the presence of true iron deficiency.[12] In the older group there was no significant difference between HIV-infected and -uninfected women in terms of transferrin saturation to correlate with the difference between their serum iron levels. This might well indicate a masked effect on the serum iron levels in the HIV-infected women, as already discussed.

An MCV value below 81 fL indicates that the erythrocytes are microcytic (small), while a low MCH indicates that the erythrocytes are hypochromic (pale). A microcytic, hypochromic anaemia occurs when body stores are depleted and the iron deficiency is severe, but both the MCV and MCH remain normal in early iron deficiency. In contrast to iron deficiency anaemia, the anaemia of chronic disease is usually, but not always, characterised by a normocytic, normochromic blood picture, which could explain the relatively low percentage of especially HIV-infected women with decreased MCV and MCH. Furthermore, serum iron levels and transferrin saturation are low in chronic disease, while iron stores are normal,[23] as was found in a large percentage of patients included in this study.

Increased levels of iron intake have been associated with a significant decreased progression rate to AIDS.[24,25] Although iron supplementation may prevent or treat iron deficiency,[11,13] it may also activate HIV expression and possibly worsen immunosuppression,[9] thereby increasing the rate of progression of HIV infection.[13] It should therefore be administered with caution in HIV-infected patients.[11] Iron supplementation of 60 mg twice weekly over four months had no effect on the viral load of anaemic, pregnant, HIV-infected women.[13] In developing countries, where a high prevalence of both HIV infection and iron deficiency exists, Clark and Semba[10] recommend that the current practice of iron supplementation be continued. In the event of anaemia of chronic disease, iron supplementation is, however, often not effective, and treatment of the underlying pathology will usually result in improvement in the anaemia.[23]

The results of the study indicate that prevalence of HIV infection in Mangaung is high, especially among women between 25 and 34 years of age. Although, on average, the parameters of iron status did not indicate iron deficiency in the different age and HIV-status groups, a large percentage of women did have anaemia of chronic disease, with HIV-infected women afflicted more often.

Knowledge of the HIV status of a patient is of paramount importance in evaluating laboratory results in order to determine future treatment or nutritional recommendations and interventions. HIV-infected and -uninfected individuals might not be comparable regarding their laboratory results to interpret iron store depletion, with consequences for further therapeutic actions in these two groups. The progression rate to AIDS might also be enhanced by certain interventions.

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