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Causes of hyperferritinaemia classified by HIV status in a tertiary-care setting in South Africa

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SUMMARY

This study included all patients, with known HIV-1 status, admitted to hospital over a 5-year period with serum ferritin values exceeding 1500 μg/l. Markedly elevated serum ferritin levels are associated with a host of causes which poses a diagnostic dilemma, as the aetiology is often highly dependent on local epidemiology. We evaluated patients' records retrospectively to determine underlying causes of possible hyperferritinaemia. Aetiologies associated with hyperferritinaemia varied significantly depending on HIV-1 status. In patients infected with the HIV-1 virus, infectious causes predominated with *Mycobacterium tuberculosis* accounting for more than 50% of the patient population with an odds ratio of 17·98 (95% confidence interval 8·31–38·88) in HIV-positive compared to HIV-negative patients. Of the HIV-1-negative patients, hereditary haemochromatosis accounted for less than 2% of patients and chronic renal failure was the most common diagnosis. The finding of hyperferritinaemia should prompt determination of HIV-1 status, as this impacts significantly on aetiological epidemiology. In HIV-1-positive patients, aggressive investigation for mycobacterial infection should be undertaken in cases of combined hyperferritinaemia and positive HIV-1 serology.

Key words: HIV-1 positive, *Mycobacterium tuberculosis*, severe hyperferritinaemia.

INTRODUCTION

Ferritin is a high-molecular-weight ferrous protein with its main function being iron storage [1, 2]. Estimation of serum ferritin is a frequently requested special investigation [3]. Serum ferritin levels usually reflect total body iron stores, since its secretion into circulation is proportional to the amount of cellular iron in the form of cellular ferritin [1]. However, despite physiological regulation of iron homeostasis, the proportionality does not always hold true and a host of conditions have been implicated to cause markedly elevated serum ferritin levels [2–6]. Normal values for serum ferritin are typically considered as 12–300 μg/l in males and 12–150μg/l in females. Interpretation of an elevated serum ferritin value will vary significantly depending on the patient population and local epidemiology [2, 3]. The terms hyperferritinaemia and severe or extreme hyperferritinaemia are often loosely used to constitute levels of serum ferritin exceeding 1500 μg/l [3, 7] and 10000 μg/l [8, 9], respectively; however, there does not seem to be consensus in literature at present [10].

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Hyperferritinaemia seems to be a common finding in HIV-1-positive population groups, with an estimated prevalence of 16–36% [2, 11]. Within this clinical context, infectious diseases are often cited as the most common cause for elevated serum ferritin, with aetiological causes ranging from fungal (*Histoplasma capsulatum*) [9], bacterial (*Clostridium difficile*) [2, 9], mycobacterial (*Mycobacterium tuberculosis* [12] and *Mycobacterium avium-intracellulare complex* [9]) and viral (cytomegalovirus [2, 9]) infections. Of these pathogens, the greatest focus has been placed on *H. capsulatum*, and severe hyperferritinaemia has been cited by some authors as a highly specific marker for disseminated infection [13–15], although this view is not generally accepted [9].

The aetiology of varying degrees of hyperferritinaemia has not been described clearly within the HIV-1-positive patient population. The aim of this study was to provide an epidemiological description of causes of hyperferritinaemia within an HIV-1-positive population compared to an HIV-1-negative population.

MATERIALS AND METHODS

The University of Pretoria Research Ethics Committee approved the study protocol.

Baseline serum ferritin estimation

Serum ferritin is considered to be an acute-phase reactant. For this reason, a baseline serum ferritin level was established to exclude the possibility of a selection bias based on immune activation in HIV-1-infected patients. This was effected by obtaining serum ferritin values for HIV-1-positive and HIV-1-negative patients within the same clinical setting in patients with simultaneously confirmed HIV-1 tests, over a 1-year period.

Patient population

All patients admitted to the Steve Biko Academic Hospital from May 2005 to September 2010, with a serum ferritin measurement of $\geqslant 1500\,\mu g/l$ and who were tested for HIV-1 were included in the study. The serum ferritin levels were determined on venous blood samples using the UniCel® DxC 800i Synchron® DxCi Clinical System (Beckman Coulter, South Africa) based on a solid-phase two-site immunoenzymatic assay. This assay has been validated up to a concentration of $1500\,\mu g/l$ and final concentrations were therefore determined using serial dilutions. In cases where the serum ferritin levels were obtained on multiple occasions in a single patient, only one result was used. Cases therefore reflect patients and not laboratory results.

Demographic data, as well as HIV-1 status data, were collected retrospectively from the laboratory database. HIV-1 testing was performed by detecting HIV-1 antibodies with the HIV Combi Assay (Roche Diagnostics, Germany). All positive results were confirmed using the HIV Ag/Ab Combo Assay (Abbott, USA). Patients aged <18 months were further investigated using either the p24 antigen assay (Roche Diagnostics) or the HIV-1 DNA Amplicor assay version 1.5 (Roche Diagnostics).

Diagnostic criteria

Hereditary haemochromatosis diagnosis was based solely on histological or genotypical confirmation. Chronic renal failure was defined as being present in those patients receiving renal replacement therapy, most commonly by haemodialysis. Acute hepatitis was defined as aspartate aminotransferase (AST) and alanine transaminase (ALT) values exceeding 200 IU/l and 300 IU/l, respectively, in the absence of any biochemical features indicating biliary obstruction [16]. Acquired iron overload due to transfusion was accepted as being the cause of hyperferritinaemia in patients that had received at least four units of packed red cells within the preceding 6 months [3]. Haematological disease had to be confirmed by either bone marrow aspirate and/or biopsy or peripheral blood film [17]. Diagnosis of malignancy was only accepted when based on histological confirmation and haemophagocytosis based on bone marrow aspiration. Infection was accepted as the aetiology based on a single positive culture from a sterile site, or repeated positive cultures from non-sterile samples.

Finally, *M. tuberculosis* was accepted as the pathogen based on culture or histological positivity in patients with radiological and/or clinical findings in keeping with active infection.

In some cases, more than one clinical condition was identified which may have given rise to the hyperferritinaemia. In these settings, a chronological evaluation of the patient's clinical progression was performed to determine the predominant contributor to the hyperferritinaemia.

Statistical analysis

The two patient populations (HIV-1 positive and HIV-1 negative) were compared using bivariate analysis of the data with regards to odds ratios (OR) and proportions. Furthermore, logistic regression was utilized for the HIV-1-positive group to determine whether different strata of cluster of differentiation 4 (CD4) count would attenuate the effect of the OR. CD4 counts were sub-divided as >200 cells/ml, <100 cells/ml and values between these two parameters.

RESULTS

Baseline serum ferritin

The median serum ferritin estimation for HIV-1-positive and HIV-1-negative patients differed markedly being 304 μg/l and 1518 μg/l, respectively. However, the majority of HIV-1-positive patients had serum ferritin levels exceeding normal values compared to their HIV-1-negative counterparts (Fig. 1). It should be noted that the HIV-1-negative population in this instance consisted on the whole of patients who were evaluated for renal replacement therapy and that this probably caused a bias towards elevated serum ferritin values. Of note is the much lower mean value of serum ferritin in the HIV-1-positive population, which in itself has been cited as a cause of hyperferritinaemia [4].

Patient population

In total, 542 patients had serum ferritin measurements in excess of 1500 μg/l. Of these, 180 were HIV-1 positive and 140 were HIV-1 negative. In 222 of the cases, HIV-1 status was unfortunately not determined and these patients were excluded from the analysis. The age distribution for the entire study population varied from 3 months to 83 years, with a median of 36 years. Serum ferritin values varied from 1500 to 82732 μg/l (mean values of 4079 and 2348 μg/l for HIV-1-positive and HIV-1-negative patients, respectively).

Aetiology of hyperferritinaemia

Aetiological epidemiology differed significantly between the HIV-1-negative and HIV-1-positive groups (Table 1). The most striking finding within the HIV-1-positive patient group was the prominence of

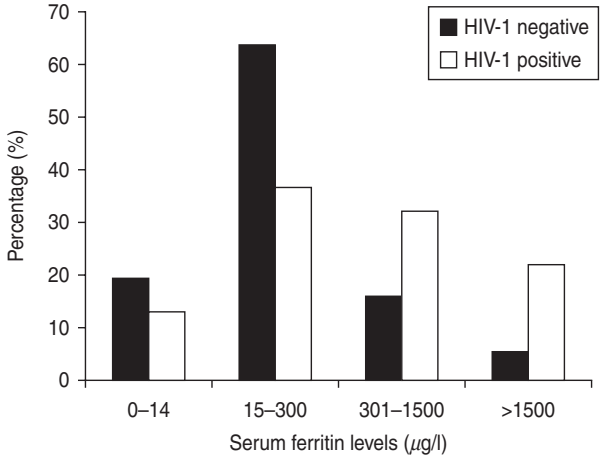

Fig. 1. Breakdown of serum ferritin levels within HIV-1-positive ($n=860$) and HIV-1-negative ($n=3500$) patients expressed as percentage of total subpopulation.

infectious causes as the aetiology of hyperferritinaemia. Of these, infection with *M. tuberculosis* seemed to be the most common, accounting for more than half of all cases (Fig. 1). Despite various publications associating *H. capsulatum* infection with severe hyperferritinaemia [8, 9, 14, 18], not a single case was diagnosed within the patient group of this study. Moreover, despite the clear association between HIV-1 infection and haemophagocytosis [15], no cases were identified within the HIV-1-positive patient group, and only two cases were identified among the HIV-1-negative patients. In total, 93 cases of active infection with *M. tuberculosis* infection were identified within the HIV-1-positive patient group. In two-thirds of cases, the organisms were isolated from the lungs, but various other sites of infection were also identified, suggesting disseminated disease.

The HIV-1-negative patient population group had a much wider spectrum of aetiologies, with chronic renal failure being the most predominant. Haemochromatosis was diagnosed in two patients within this group. Infection in general contributed to 8% of HIV-1-negative patients, with *M. tuberculosis* infection being confirmed in only 8/140 patients. Combined malignancy and haematological disease accounted for 21% of HIV-1-negative patients compared to 5% of HIV-1-positive patients.

Statistical analysis

The prevalence of infection with *M. tuberculosis* within the HIV-1-positive patient group was 51·96% compared to 5·67% in the HIV-1-negative group ($P<0{\cdot}001$) with an OR of 17·98 (95% confidence

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Table 1. *Comparison of clinical conditions associated with hyperferritinaemia in HIV-1-positive and HIV-1-negative populations* (*serum ferritin levels expressed in micrograms per litre*)

	HIV-1 positive ($n=140$)		HIV-1 negative ($n=180$)	
	Absolute number (%)	Mean (μg/l)	Absolute number (%)	Mean (μg/l)
Acute hepatitis	4 (2)	15 632	6 (4)	8436
Chronic renal failure	4 (2)	2236	54 (39)	2653
Haematological disease	3 (2)	7407	20 (114)	5198
Haematological malignancy	1 (0·5)	7500	6 (4)	3226
Haematophagocytosis	2 (1)	7317	0 (0)	n.a.
Haemachromatosis	0 (0)	n.a.	2 (1)	4260
Infection	103 (57)	8632	11 (8)	4238
Mycobacterium tuberculosis	93 (52)	8769	8 (6)	10 280
Malignancy	4 (2)	4946	9 (6)	3716
Transfusion	2 (1)	13 253	11 (8)	3872
Unknown	57 (32)	6391	26 (19)	6233
Other	0 (0)	n.a.	1 (1)	5377

n.a., Not applicable.

interval 8·31–38·88). Logistic regression using CD4 count differentiation as described above did not attenuate the OR significantly.

DISCUSSION

A markedly elevated serum ferritin value typically prompts investigation for hereditary haemochromatosis [2]. This involves relatively invasive investigations in the form of liver biopsies and expensive investigations including molecular testing and imaging for confirmation [19]. Therefore, it is essential to evaluate the benefit of this approach considering implications both on expenditure and patient morbidity. Within the HIV-1-negative patient population of this study, less than 2% of patients had confirmed hereditary haemochromatosis. Similar findings have also been described in other clinical settings [2, 3], although not to the same extent. For this reason, investigation of hereditary haemochromatosis in this clinical setting may be best delayed until more common causes have been excluded. Other accepted causes of hyperferritinaemia include iron overload (including haemochromatosis, excess supplementation and repeated blood transfusions [5]), parenchymal liver damage [6], renal disease [2], acute-phase response (due to infection or inflammation) and malignant disease [3, 6].

HIV-1 infection in isolation has been implicated as a cause of hyperferritinaemia [4], probably as part of an acute-phase response. Infectious causes represent a major proportion of cases identified within the HIV-1-positive population group, with *M. tuberculosis* being the most predominant pathogen isolated in this study. The significant association between severe hyperferritinaemia and mycobacterial infection has only recently been described [12], and traditionally this association has been largely with fungal infections, in the form of *H. capsulatum* [8, 14, 18]. In an HIV-1-positive patient population, the laboratory finding of hyperferritinaemia should prompt an aggressive search for infection with *M. tuberculosis*.

Despite a thorough clinical work-up of patients, in total 32% of HIV-1-positive and 19% of HIV-1-negative patients remained without definitive diagnosis. Although this this may be seen as a shortcoming of the current study, it may also be indicative of possible further causes of hyperferritinaemia not yet described in this context.

Understanding the local patient epidemiology is an essential part in interpreting the finding of a markedly elevated serum ferritin level [2]. This will not only guide interpretation, but also prompt appropriate further investigation to determine underlying aetiology and clinical significance.

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DECLARATION OF INTEREST

None.

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