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Hypocalcaemia in HIV infection and AIDS

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Abstract. Kuehn EW, Anders HJ, Bogner JR, Obermaier J, Goebel FD, Schlöndorff D (Ludwig Maximilians University, München, Germany). Hypocalcaemia in HIV infection and AIDS. *J Intern Med* 1999; **245**: 69–73.

Objectives. To study the prevalence and possible mechanisms of hypocalcaemia in HIV infection and AIDS.

Subjects. 828 patients with HIV infection or AIDS and 549 controls.

Interventions. Measurements of total serum calcium and albumin levels. Parameters of calcium homeostatsis were determined in a subgroup of 21 hypocalcaemic AIDS patients.

Results. Mean serum calcium was 2.34 ± 0.13 mmol L^{-1} in the HIV group vs. 2.46 ± 0.10 mmol L^{-1} in controls ($P < 0.0001$). After adjusting for serum albumin, hypocalcaemia was present in 6.5% of the HIV group vs. 1.1% of controls. Mean serum calcium was declining according to CDC groups, and differed significantly from controls in each group. Regression coefficients of cacium vs. albumin were 0.147 amongst HIV-infected patients and 0.106 for controls. In the subgroup of hypocalcaemic patients with AIDS, 10/21 had vitamin D deficiency, six of these with low ionized calcium levels. Low serum PTH was found in 2/21 patients, Magnesium deficiency in 1/21. Of the remaining eight patients, only one had secondary hyperparathyroidism, while the other seven lacked an adequate PTH response, despite low ionized calcium levels in four subjects.

Conclusions. Mean serum calcium concentrations were lower through all CDC stages, irrespective of albumin, resulting in a higher prevalence of hypocalcaemia in HIV-positive patients compared with controls. In a considerable number, this seems to be caused by vitamin D deficiency and potentially a lack of adequate PTH secretion, but further studies are needed to confirm this.

Keywords: AIDS, calcium, electrolytes, HIV, hypocalcaemia and hypoparathyroidism.

Introduction

Multiple disturbances of electrolyte metabolism and endocrine regulation have been observed in patients infected with the human immunodeficiency virus (HIV). Most interest has focused on hyponatraemia as the most frequent disorder, followed by hypo- and hyperkalaemia [1]. Until now, only few data have been reported on calcium levels in these patients. Generally hypocalcaemia has not been considered to be a frequent phenomenon in HIV infection and reports of reduced calcium levels in AIDS have been mostly attributed to hypoalbuminaemia or to pharmacotherapy, e.g. treatment with foscarnet or ketoconazole [2–5]. In addition, renal failure, pancreatitis, malabsorption and sepsis have been discussed as possible causes of hypocalcaemia [4–8]. However, endocrine profiles in hypocalcaemic HIV-infected patients have not been investigated. The controversial reports in terms of prevalence, the paucity of information regarding the endocrine regulation in AIDS-related hypocalcemic states, and our clinical observation that hypocalcaemia frequently occurs in all stages of HIV infection prompted us to investigate the prevalence of hypocalcaemia in HIV infection in comparison with non-infected controls.

Patients and methods

Patients

The study group consisted of 828 patients with documented HIV infection, as confirmed by ELISA and Western blot, or AIDS with an average of 37.5 ± 8.3 years who attended our outpatient clinic between 1982 and 1994; 86% were male, 97% were Caucasian. The mode of transmission was homosexual encounters in 64%, intravenous drug use in 17% and heterosexual encounters in 8%. In 10% the route of transmission was not clarified. The degree of HIV-related immunosuppression was classified following the CDC classification as group 1, 2 or 3 if the patient's CD4 cell count was above 0.5×10^9 cells L^{-1}, between 0.2 and 0.5×10^9 cells L^{-1} or below 0.2×10^9 cells L^{-1}, respectively. The control group consisted of 549 patients who were seen in our hospital for reasons other than HIV infection. They were mostly outpatients who attended our general medical clinic and subspeciality clinics such as hypertension, rheumatology, diabetes, lipids and peripheral vascular disease. No history of a risk factor for acquisition of HIV infection was present. These patients did not present with an indication for HIV testing. None of the in-patients was in the intensive care or dialysis unit. The mean age of the control group was 48.5 ± 18.7 years; 54% were male and 98% were Caucasian. All patients were evaluated for serum calcium and albumin levels at the time of their first visit. For a sample of 21 consecutive patients with AIDS and decreased total serum calcium levels without any obvious cause such as chronic renal failure, obvious intestinal pathology or postoperative states after thyroid surgery, levels of intact PTH, 25-OH-vitamin D_3 and ionized calcium were obtained. None of these patients was taking vitamin supplements, aminoglycosides, pentamidine, ketokonazole or foscarnet.

Analytical procedures

Albumin was determined by Ponceau S staining after protein electrophoresis and absolute protein concentrations were measured by the Biuret method in an Hitachi 717 analyser. Calcium was measured using emission flame photometry (Eppendorf 5051), with a reference range of 2.20–2.62 mmol L^{-1}. Calcium was adjusted for albumin, if it was below the lower reference value using the following equation as published by Payne *et al.* [9]:

$$Ca_{corr} = Ca - (albumin/4) + 1$$

with albumin in g L^{-1} and calcium in mmol L^{-1}. CD4 cells were counted using standard flow cytometry following erythrocyte lysis and staining with Leu 3a monoclonal antibodies as delivered by Becton Dickinson (Heidelberg, Germany) in a FACScan flow cytometer. Intact PTH (iPTH) was measured by immunoluminometric assay (ILMA, Ciba Corning; reference interval, 10–55 pg L^{-1}) and 25-OH-vitamin D_3 by a competitive protein binding assay (courtesy Professor Horn, Medizinische Klinik Innenstadt, München, Germany; reference interval, 50–300 nmol L^{-1}). For ionized calcium, the samples were handled anaerobically and analysed with an ion-sensitive electrode (Kone microlight analyser) with a reference interval of 1.12–1.32 mmol L^{-1}. Ionized calcium values were corrected for plasma pH to a pH of 7.40.

Statistical methods

Descriptive analysis consisted of determination of mean levels and distribution curves. Comparison of means for total serum calcium and albumin was done for independent groups by two tailed *t*-test. Because of multiple testing procedures, a conservative adjustment according to Bonferoni was used. Significant differences for nominal $\alpha = 0.05$ are marked in the Table 1. In addition, a one-way anova was applied which gave analogous results. Regression analysis was carried out for albumin as the independant variable. Comparison of hypocalcaemia fractions were calculated by χ^2-test with $\alpha = 0.05$.

Results

Mean serum calcium levels were within the normal range for HIV-infected patients and controls (Table 1). However, mean serum calcium was significantly lower in HIV-positive patients. This was also true for serum albumin. The percentage of hypocalcaemic patients was 12.2% in the HIV group and 2.2% for controls. After correction for albumin, the prevalence was 6.5 and 1.1%, respectively ($P < 0.001$). The histogram of the distribution for calcium values shows a 0.1 mmol L^{-1} shift towards lower calcium levels in the HIV-positive group, which is reflected by the fact that only 0.5% of the HIV-infected patients vs. 7.1% of the controls were hypercalcaemic (Fig.

Table 1 Serum calcium levels in HIV patients according to CDC groups vs. controls. Calcium was adjusted for albumin, if it was below the lower reference of 2.20 mmol L^{-1} using Payne's equation (see 'Methods'). Values are means ± SD.

	Controls	HIV	CDC1	CDC2	CDC3
n	549	828	209	282	337
Calcium (mmol L^{-1})	2.46 ± 0.10	2.34 ± 0.13	2.40 ± 0.10	2.38 ± 0.10	2.26 ± 0.14
P vs. controls	—	<0.0001	<0.0001*	<0.0001*	<0.0001*
P vs. CDC1	<0.0001*	—	—	0.0277	<0.0001*
P vs. CDC2	<0.0001*	—	0.027	—	<0.0001*
Albumin (gL^{-1})	45.8 ± 5.0	44.9 ± 5.9	47.0 ± 4.1	46.8 ± 4.5	42.0 ± 6.5
p vs. controls	—	0.0044	<0.0001*	<0.005	<0.0001*
P vs. CDC1	<0.0001*	—	—	NS	<0.0001*
P vs. CDC2	<0.005	—	NS	—	<0.0001*

*$P < 0.05$ after adjustment according to Bonferoni.
CDC1, CD4 cell count $> 0.5 \times 10^9$ cells L^{-1}; CDC2, CD4 cell count $= 0.2 \times 10^9$ cells L^{-1}; CDC3, CD4 cell count $< 0.2 \times 10^9$ cells L^{-1}; NS not significant.

1). When stratified for the degree of immunosuppression, mean calcium and albumin levels were lowest in CDC group 3 (significant vs. 1 and 2; Table 1), and the values in each group differed significantly from controls. Mean albumin concentrations were significantly higher in the CDC groups 1 and 2 than in controls. Regression analysis for calcium vs. albumin showed a regression coefficient of 0.147 in the HIV group (95% confidence interval, 0.136–0.159; independent variable, albumin) and 0.106 for the control group (95% CI, 0.089–0.124; independent variable, albumin). The steeper regression line in the HIV group indicates that total serum calcium per gram albumin is higher in HIV patients than in controls.

In a group of 21 consecutive patients with AIDS and decreased total serum calcium without any obvious cause, low 25-OH-vitamin D_3 levels were found in 10/21 patients, six of whom also had low ionized calcium levels; 1/21 patients had a decreased magnesium level. Suppressed levels of iPTH were found in 2/21 patients. Out of the remaining eight patients only one had an elevated iPTH level (indicating secondary hyperparathyroidism), while the remaining seven had normal levels (mean 22.7 pg L^{-3})

Discussion

Evaluation of serum calcium in a population of mostly Caucasian HIV-infected homosexual patients yielded lower levels compared with a control patient group, and resulted in a shift of the mean towards lower calcium levels in patients with HIV. Since total

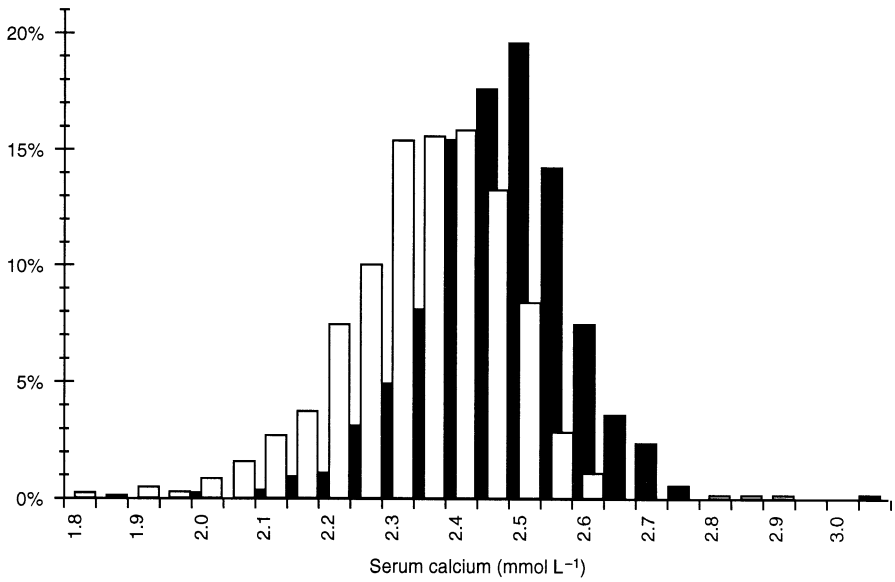

Fig. 1 Serum calcium was measured in 828 HIV-positive patients and 549 controls. The histogram distributions for both groups were calculated from the number of patients for each calcium value on a 0.05 mmol L^{-1} interval basis. Open bars, HIV-positive patients; black bars, HIV-negative controls. Means were 2.34 ± 0.13 vs 2.46 ± 0.10 mmol L^{-1} ($P < 0.001$). (Serum calcium results are shown without albumin correction.)

calcium is closely related to albumin levels, we determined serum albumin in all study members and found that severely immunocompromised patients had significantly lower albumin levels than HIV-negative controls. Hypoalbuminaemia in AIDS is caused by recurrent catabolic states, malabsorption, hypermetabolism and decreased oral intake, with the clinical appearance of the wasting syndrome [10,11]. After correction for low albumin, the prevalence of serum calcium below the reference level was 6.5% for HIV-infected patients, compared with 1.1% for controls. The only previous study that focused on the prevalence of hypocalcaemia in this population determined serum calcium corrected for albumin in 66 AIDS patients and found hypocalcaemia in 18% [8]. Since this may indicate that the prevalence of low calcium levels is correlated with immunosuppression, we looked at calcium levels at different stages of HIV infection and found that they were significantly lower in all stages of HIV infection compared with controls. The finding that severely immunocompromised patients with AIDS had the lowest calcium levels compared with milder degrees of immunosuppression indicates a relationship between hypocalcaemia and the progression of AIDS.

The causes of hypocalcaemia have not been well defined. Textbooks and major review articles basically relate AIDS-associated hypocalcaemia to drugs such as foscarnet, ketoconazole, aminoglycosides and pentamidine [4–7]. However, none of these drugs were given to our study members within 2 months of laboratory testing. Other causes of hypocalcaemia, such as acute pancreatitis, burns, Gram-negative sepsis and osteobastic metastatic disease, were absent in our patients and are unlikely to cause reduced calcium levels as a widespread phenomenon in HIV infection [12–15].

Having established the finding of an increased prevalence of hypocalcaemia in HIV-infected individuals, we tried to get some insight into its pathophysiology by measuring determinants of calcium homeostasis in a small group of patients, in whom no obvious cause for hypocalcaemia could be identified. We found vitamin D deficiency to be present in almost half of these patients, which is consistent with previous findings in small numbers of patients [16]. Malnutrition is common in patients with AIDS and may contribute to hypovitaminosis D as another cause for hypocalcaemia in this population, but the underlying mechanisms have not been established [16,17]. Only 2/21 patients had suppressed serum PTH levels, but none of them had low ionized calcium levels or clinical signs of tetany. High PTH levels suggestive of secondary hyperparathyroidism were only found in one patient, which is explained by the fact that patients with chronic renal insufficiency were excluded; 7/21 patients had normal PTH levels, four of them despite low ionized calcium levels. This finding raises the question of an inadequate response of parathyroid cells in the setting of hypocalcaemia in AIDS. In a previous report a case–control study found lower iPTH levels in 44 AIDS patients, compared with HIV-positive patients without relevant immunosuppression [18]. In another study, maximal secretion of iPTH after EDTA-induced hypocalcaemia was reduced in patients with AIDS compared with HIV-negative controls [19]. Symptomatic hypoparathyroidism has also been reported in HIV infection [20]. However, nothing is known about the underlying mechanisms of a decreased parathyroid reserve in this population. Causes of acquired latent hypothyroidism, such as iron storage or metastatic disease, autoimmune disease, drug toxicity of antracyclines or polyglandular insufficiency syndrome [21–25], are not suitable to explain hypocalcaemia as a widespread phenomenon. Despite the fact that various kinds of endocrine glandular insufficiency, such as panhypopituitarism, hypogonadism and impaired adrenal function [4,5,26–31], have been linked to lymphoma, pneumocystis, CMV or the HIV antigen itself [31–33], postmortem pathology of the parathyroid in AIDS has only rarely been reported. To our knowledge only one report found CMV inclusion bodies in the parathyroid of a patient [32]. One study showed experimental data which suggested that parathyroid cells express CD4 molecules and might therefore be infected by HIV [34]. Histopathological examination in patients whose parathyroid function has been evaluated during their lifetime may answer the question as to whether inflammatory destruction of the parathyroid might be responsible for a decreased PTH response in AIDS.

In conclusion, mean serum calcium levels were lower in HIV-infected patients than in controls, resulting in a higher percentage of hypocalcaemic individuals. This was also true in the early stages CDC1 and CDC2. Severely immunocompromised patients have the lowest mean calcium levels. In a subgroup of hypocalcaemic patients with AIDS, hypovitaminosis D was established as the most com-

mon cause for hypocalcaemia. There seems to be an element of inadequate PTH secretion in a considerable number of patients, in whom hypocalcaemia cannot otherwise be explained. Further studies are needed to confirm this finding.

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