

Serum concentrations of vitamin D metabolites in untreated tuberculosis

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ABSTRACT A prospective study of 50 consecutive patients presenting with tuberculosis has shown that patients have on average lower serum concentrations of 25-hydroxycholecalciferol (25-OHD) than healthy matched controls. No difference was shown in serum 1,25-(OH)₂D, 24,25-(OH)₂D, or parathyroid hormone. Uncorrected serum calcium was lower in the patient group but identical when correction concentrations were made for albumin. Serum liver enzyme concentrations were significantly higher in the patient population. Low serum vitamin D concentrations may be a consequence of disease. The possibility that low serum 25-OHD concentrations may predispose to tuberculosis infection cannot, however, be excluded. Prolonged treatment with isoniazid or rifampicin, both of which have been shown to reduce serum 25-OHD, may increase the risk of vitamin D deficiency and consequent osteomalacia in groups of patients most at risk, such as those of Indian subcontinental ethnic origin.

The high incidence of vitamin D deficiency and resultant rickets or osteomalacia in the population of the UK whose ethnic origin is the Indian subcontinent (Indian, Pakistani, or Bangladeshi) is now well established.¹⁻³ It has also been apparent since the early 1960s that this population has very much higher rates of tuberculosis than the indigenous white population.⁴⁻⁶ As both tuberculosis and osteomalacia are common in the same population, there may be an association between low serum vitamin D concentrations and tuberculosis. The aim of this study has been to estimate the serum vitamin D and related parameters of patients initially diagnosed as having tuberculosis and compare these values with those of matched healthy controls to determine the possibility of an association between tuberculosis and low serum vitamin D concentration in the individual patient.

Method

Serum 25 hydroxycholecalciferol (25-OHD), calcium, albumin, and alkaline phosphate were measured and other liver functions tests were done in consecutive culture positive tuberculous patients,

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presenting either as inpatients or as outpatients to one of the Cardiff group hospitals. Serum 1,25-dihydroxycholecalciferol (1,25-(OH)₂D), 24,25-dihydroxycholecalciferol (24,25-(OH)₂D), and parathyroid hormone have also been measured in a random group of paired samples (see below). Blood specimens were taken before treatment with minimal stasis and spun down within two hours, and serum was extracted for analysis. A suitable control, either from the patient's family or from a healthy volunteer matched by age, sex, and ethnic origin, was selected and serum taken within one month of the patient's sample, for the above estimations, to eliminate as far as possible seasonal fluctuation in serum vitamin D.

Measurement of serum 25-OHD, 1,25-(OH)₂D, and 24,25-(OH)₂D was by a radioimmunoassay technique adapted from that of Clemens *et al.*⁷ Measurement of calcium, phosphate, albumin, total alkaline phosphatase, and liver enzymes was by autoanalyser. Samples of serum taken from 10 patients (and matched controls) showing abnormally raised serum alkaline phosphatase were analysed for alkaline phosphatase isoenzymes.⁸⁻¹⁰ Normally distributed results were analysed by Students T test and expressed as mean \pm one standard deviation. Non normally distributed results were analysed by the Mann Whitney U test.

Results of determinations of vitamin D metabolites, parathyroid hormone, and calcium and of liver function tests

| | No of patients | Patients | | Controls | | Patients as % of controls | p |
|-----------------------------------|----------------|-------------|-------------|-------------|--------------|---------------------------|--------|
| | | Median | Range | Median | Range | | |
| 25-OHD (ng/ml) | 40 | 6.4* | 0.9 – 29.7† | 10.9* | 3.6 – 53.0† | 59* | <0.005 |
| 1,25-OHD (pg/ml) | 15 | 35.7* | 7.3 – 49.2† | 28.7* | 18.7 – 57.7† | 124* | NS |
| 24,25-OHD (ng/ml) | 15 | 0.1* | 0.1 – 0.98† | 0.12* | 0.09 – 0.57† | 83* | NS |
| | | Mean (SD) | | Mean (SD) | | | |
| Parathyroid hormone (ng/ml) | 17 | 0.92 (0.87) | | 0.90 (0.6) | | 102 | NS |
| Calcium (mmol/l) (uncorrected) | 49 | 2.23 (0.17) | | 2.37 (0.11) | | 94 | <0.001 |
| Calcium (mmol/l) (corrected) | 49 | 2.31 | | 2.32 | | 100 | NS |
| Alkaline phosphatase (IU/l) | 46 | 145 (74) | | 94 (46) | | 154 | <0.001 |
| Liver alkaline phosphatase (IU/l) | 10 | 201 (73) | | 103 (63) | | 195 | <0.002 |
| Bone alkaline phosphatase (IU/l) | 10 | 72.4 (51) | | 76.6 (22) | | 95 | NS |
| Phosphate (mmol/l) | 48 | 1.12 (0.18) | | 1.20 (0.23) | | 93 | <0.05 |
| Albumin (g/l) | 47 | 38.8 (5.8) | | 45.2 (3.1) | | 86 | <0.001 |
| Total protein (g/l) | 49 | 72.3 (6.6) | | 73.3 (4.5) | | 99 | NS |
| Gamma glutamyl transferase (IU/l) | 44 | 45 (38) | | 26 (24) | | 173 | <0.005 |
| Aspartate aminotransferase (IU/l) | 49 | 42 (61) | | 23 (65) | | 182 | <0.05 |
| Bilirubin (μ mol/l) | 50 | 11.6 (11.6) | | 9.3 (5.6) | | 125 | <0.05 |

*Median.

†Range.

OHD—hydroxycholecalciferol.

Conversion: SI to traditional units—Calcium: 1 mmol/l = 4.0 mg/100 ml; phosphate: 1 mmol/l = 3.1 mg/100 ml; bilirubin: 1 μ mol/l = 58.5 μ g/100 ml.**The patients**

Fifty patients with culture positive tuberculosis were available for analysis. Forty eight patients had respiratory tuberculosis only and two had lymph node tuberculosis only. Of the 50 patients, 37 were male (72%) 42 were white and four were of Indian subcontinent and four of other ethnic origin (one West Indian, one African, one Malasian, and one Chinese). A total of five (10%) of the patients had had respiratory tuberculosis previously: two white men, one white woman, and two Indian subcontinental women. The average age of patients was 43.1 (SD 18.8) years and of controls 42.2 (17.9) years (range 20–82 years for patients, 19–82 years for controls). On average patients and controls smoked similar amounts (between 0–10 cigarettes a day) and drank similar quantities (nil to half a pint of beer or equivalent alcohol measure a day). Two patients and two controls were vegetarians.

Results

Serum concentrations for patients and controls are shown in the table. A distribution of serum 25-OHD in patients and controls (fig 1) shows a considerable skew towards the lower values. According to the Mann Whitney U test, the difference between patients (range 0.9–29.7 median 6.4

ng/ml) and matched controls (range 3.6–53.0, median 10.9 ng/ml) was highly significant ($p < 0.005$). The uncorrected serum calcium concentration was significantly lower in the patient group (fig 2), but when correction was made for albumin (to 42.5 g/l) serum concentrations were virtually identical. No differences in circulating immunoreactive parathyroid hormone was observed, but mean phosphate concentrations were significantly lower in the patients and mean alkaline phosphatase activities significantly higher than in the controls. Liver functions tests indicated significantly increased hepatic enzyme activity in patients (table).

Serum from a sample of 15 patients and controls showed no significant difference between the two groups for 1,25-(OHD)₂D (range 7.3–49.2, median 35.7 pg/ml, compared with 18.7–57.7, median 28.7 pg/ml) or for 24,25-(OHD)₂D (0.1–0.98, median 0.1 ng/ml, compared with 0.09–0.57, median 0.12 ng/ml). No correlation was found between 1,25-(OHD)₂D and 24,25-(OHD)₂D or between 1,25-(OHD)₂D and 25-OHD in either patient or control groups.

In the sample of 10 of the patients found to have raised serum alkaline phosphatase activity, isoenzyme analysis showed that the increase in total alkaline phosphatase was entirely due to raised liver alkaline phosphatase (201 (SD 73) IU/l compared with 103 (63) IU/l ($p < 0.002$), there being no

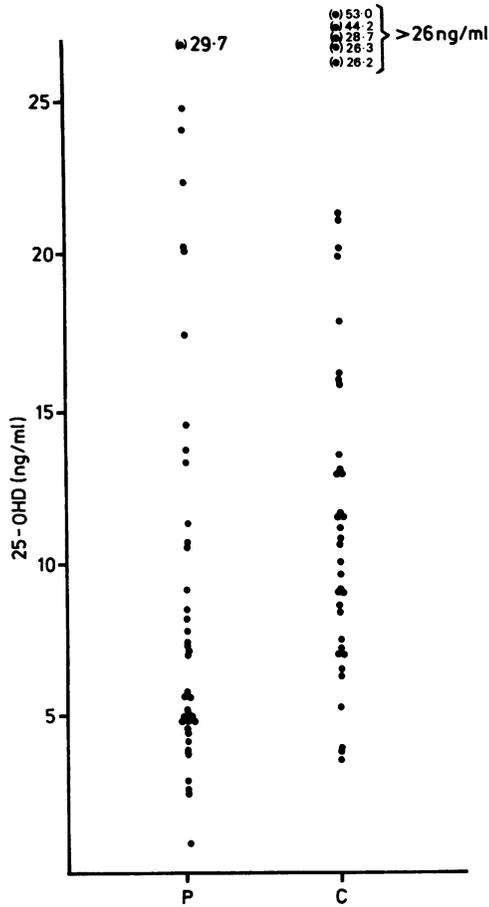


Fig 1 Serum 25-hydroxycholecalciferol (25-OHD) concentrations in 40 patients (P) (range 0.9–29.7, median 6.4 ng/ml) and controls (C) (range 3.6–53.0, median 10.9 ng/ml): $p < 0.005$.

significant difference between the two groups for bone alkaline phosphatase (72.4 (51) IU/l compared with 76.6 (22) IU/l).

Discussion

We have shown that before treatment tuberculosis patients have significantly lower serum concentrations of 25-OHD than do their healthy matched controls, although there is no difference in serum 1,25 and 24,25-(OH)₂D.

The significantly lower serum vitamin D concentrations, as measured by 25-OHD, in tuberculosis patients may be as a result of poor diet and decreased exposure to sunlight brought about by malaise due to the infecting organism. It has been shown, however, that the macrophage has receptors

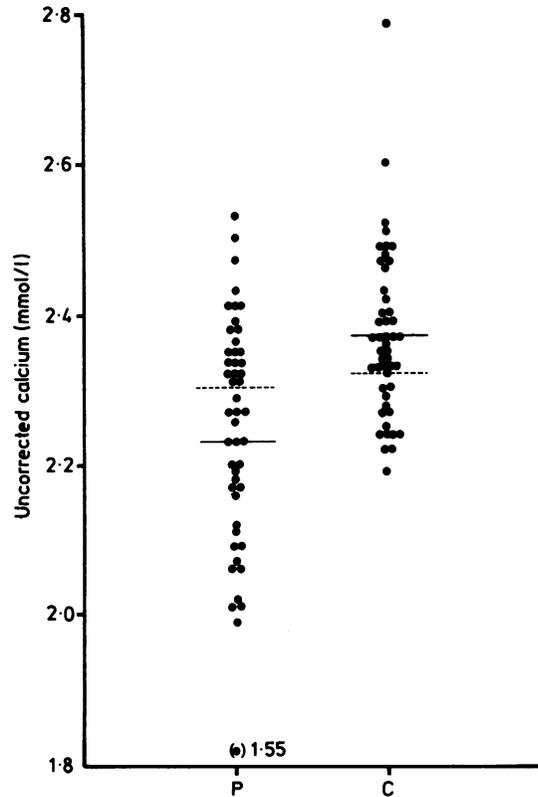


Fig 2 Serum calcium concentrations in 49 patients (P) and controls (C) uncorrected for albumin. — Mean uncorrected for albumin ($p < 0.001$); --- mean corrected for albumin (NS).

for 1,25-(OH)₂D, which increases macrophage formation and phagocytic activity.¹¹ Individuals with reduced serum 1,25-(OH)₂D may therefore be more susceptible to infections, such as tuberculosis, which are dealt with through type IV cellular immunity by the host defences.

This may offer an explanation for the observation that immigrants of Indian subcontinental ethnic origin arriving in the UK with a positive tuberculin response but normal chest radiograph may present with active disease only a few months after their arrival.¹² The time interval could represent the decline in serum 1,25-(OH)₂D and its precursor 25-(OH)D resulting from the move to a country with relatively little sunlight. The consequent reduction in cellular immunity may then allow a previously quiescent tuberculous focus to break down, resulting in overt disease.

The low albumin and high liver alkaline phosphatase, GGT, and AST levels suggest that,

although nearly all these patients were found to have pulmonary tuberculosis only, some might well have had disease affecting the liver, as well, with resultant disorganised liver function. This is substantiated by the observation that the increase in serum alkaline phosphatase applies to the liver and not the bone enzyme. Other workers have also shown that a proportion of patients with tuberculosis apparently confined to the lung were found both histologically and biochemically¹³⁻¹⁷ to have the liver affected also. No correlation, however, between histological evidence of tuberculous liver disease and abnormality in biochemical liver function tests was found.

The evidence suggests that tuberculosis, though only apparent at one site, commonly the lungs, may have disseminated, particularly to the liver.

The low serum vitamin D concentrations in the patients is an important observation for two reasons. Firstly, studies on the effects of rifampicin and isoniazid on both healthy controls and tuberculous patients show that these antituberculous drugs cause a fall in serum vitamin D.^{18,19} Patients with tuberculosis, who already have low serum vitamin D concentrations may therefore be put at risk of vitamin D deficiency and consequent bone disease as a result of antituberculous chemotherapy. As a third of all cases of pulmonary tuberculosis and a half of all cases of non-pulmonary tuberculosis notified to England and Wales⁶ are patients of Indian subcontinental ethnic origin, a group known to be at risk of vitamin D deficiency, there is a strong possibility that during the nine months of chemotherapy (longer for non-pulmonary tuberculosis) a proportion of these patients may suffer from osteomalacia as a result of prolonged low serum vitamin D concentrations. This iatrogenic disease could easily be prevented by supplementation with 1,25-(OH)₂D.

Secondly, the possibility that low vitamin D concentrations might render a population more susceptible to infection by the tubercle bacillus suggests that vitamin D supplementation may have a role in prophylaxis in subjects at risk of contracting the disease—for example, close contacts of an index patient.

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