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Reactivation of Tuberculosis and Vitamin D Deficiency: the contribution of diet and exposure to sunlight.

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ABSTRACT:

As well as its role in the regulation of calcium metabolism, vitamin is an immunoregulatory hormone. Epidemiological evidence also suggests a link between vitamin D deficiency and TB. In this study we have examined serum vitamin D concentrations before treatment in patients with active TB and their contacts from the same ethnic and social background. We have further investigated the relative contributions of diet and sunlight exposure.

Methods: Serum vitamin D concentrations were measured pre-treatment in 178 patients with active TB and 130 healthy contacts. Prevalence of vitamin D deficiency and its relation to skin colour, month of estimation and TB diagnosis were determined. 35 patients and 35 frequency matched contacts completed dietary and sun exposure questionnaires to determine the relative contribution of these to serum vitamin D concentrations . **Results:** There was a statistically significant difference ($p<0.0001$; 95% CI 7.14 to 14.34) in serum vitamin D concentrations between patients (20.1nmol/l) and contacts (30.84 nmol/l) and significantly more patients (114/178, 64%) had severely deficient concentrations (<21 nmol/l) compared with controls (40/130, 31%, $p<0.0001$). There was no association between serum concentrations of vitamin D and skin pigmentation. The healthy contacts showed a predictable seasonal pattern, rising to peak concentrations in the summer months, but this response was absent in patients with TB. Dietary intake was the same in both TB patients and contacts matched for age, sex and skin colour, but TB patients displayed a stronger correlation between serum vitamin D concentrations and dietary intake ($r=0.42$, $p=0.016$) than controls ($r=0.13$, $p>0.1$). There was no difference in sunlight exposure between the groups. **Conclusions:** Patients with active TB have lower serum vitamin D concentrations than contacts from similar ethnic and social backgrounds and with comparable dietary intake and sun exposure, and do not show the expected seasonal variation. These observations indicate that other factors are contributing to vitamin D deficiency in the TB group, and suggests abnormal handling of this vitamin.

INTRODUCTION

Links have been made between tuberculosis (TB) and deficiency of vitamin D (25-hydroxycholecalciferol) following a number of observations. Serum concentrations of 25-hydroxycholecalciferol in patients presenting with TB are on average lower than in healthy matched controls (1) and the prevalence of TB is higher among those with low serum 25-hydroxycholecalciferol concentrations eg., the elderly and Asian immigrants to the UK (2). In one study, patients with pulmonary TB who had higher vitamin D levels were shown to have less extensive radiological disease (3). Furthermore, Wilkinson et al (2000) demonstrated an association between vitamin D deficiency and vitamin D receptor polymorphisms and TB in Gujerati Asians (4). In addition, notifications of tuberculosis (TB) in England and Wales are reported to peak in the summer months, suggesting a low post winter trough of vitamin D concentration contributing to reactivation of latent infection (5). Further work (6), however, suggests the association may not be so clear cut as, although a summer peak of TB was confirmed in patients of Indian Subcontinent (ISC) ethnic origin, seasonality was not present in the white population.

The above associations between vitamin D deficiency and TB may be explained by evidence for an immunoregulatory role for this vitamin. Impaired T-cell functions, including decreased production of the Th1 cytokines interleukin-2 (IL-2) and interferon-gamma (IFN-γ), have resulted from deficiencies of protein, zinc and the active metabolite of vitamin D, 1,25-dihydroxyvitamin D3 (calcitriol)(7). *In vitro* studies have shown that monocytes have receptors for calcitriol, and vitamin D metabolites can activate the anti-mycobacterial responses of human monocytes and macrophages, enhancing phagocytosis and granuloma formation (8-10). These metabolites can act synergistically with IFN-γ to contain intracellular replication of *M.avium* and *M.tuberculosis*, and can induce nitric oxide synthase to suppress growth of *M.tuberculosis* in human macrophages (11-13).
Many factors can influence serum vitamin D concentrations, but diet and sunlight exposure are particularly important. In this study we have measured serum vitamin D concentrations at presentation and throughout treatment in a mixed ethnic group attending an inner London chest clinic for possible TB to look for seasonality, ethnic differences and association with active disease. We have further investigated a group of patients with TB and their frequency matched controls to assess the influence of dietary intake and sunlight exposure on vitamin D concentrations.

PATENTS AND METHODS

Patients

Serum 25-hydroxycholecalciferol concentrations were measured routinely in all those presenting to an inner London chest clinic with either a history of exposure to TB or a clinical history suggestive of active disease. Concentrations of parathyroid hormone were measured in 10 cases chosen at random to ensure that low vitamin D levels indicated true

vitamin D deficiency. Further measurements were taken during and at the completion of treatment for TB. Data was collected at presentation throughout the year (note being taken of the month of presentation) on 178 patients with culture positive pulmonary or extra pulmonary TB and 130 healthy contacts from similar ethnic and social backgrounds. Contacts were close household or social contacts with positive tuberculin skin tests or evidence of old primary disease on chest radiograph but no evidence of active TB.

Skin colour of patients and contacts was defined as "dark", ie black; (these were generally people of West Indian or African origin and included some South Indians and Sri Lankans); "mid", ie brown (generally those of Asian and South American origin); and "light", ie white (generally those of North European origin).

Their demographic data is shown in Table 1.

Table1.
Patient characteristics.

	TB (n=178)	**Contacts (n=130)**
Age (years)*	30 (2-75)	33.5 (3-75)
M:F	0.9:1	1.28:1
Skin Colour:		
Dark	118	97
Mid	40	13
Light	20	20

TB - tuberculosis
EM – environmental mycobacteria
*Data expressed as median (range)

As several factors are known to influence 25-hydroxycholecalciferol concentrations we excluded infants under 2 years, women over 55 years, significant smokers (>10/day), the clinically obese (BMI>30kg/m^2), and patients with concurrent disease e.g., carcinoma of prostate, any disseminated carcinoma, uncorrected thyroid disease or diabetes mellitus and renal disease. These factors can themselves either depress or elevate serum levels of vitamin D and would therefore add unnecessary variables to the results. These exclusion criteria were applied to both cases and contacts.

Measurement of serum 25OH-vitamin D3 and Parathyroid Hormone

Serum was extracted with acetonitrile (to precipitate protein) and 25OH-vitamin D3 measured by radioimmunoassay (Diosorin Ltd.). Inter assay coefficient of variation of this method was 9.1% and the recovery of added 25OH vitamin D3 to both normal sera and hypergammaglobulinaemic sera was >92%.
Intact parathyroid hormone was measured by a chemiluminescence assay on the Nicholas Advantage Specialty system. The intra assay and inter assay coefficients of variation were 5.4% and 9.2% respectively.

Definition of Vitamin D Deficiency

Severe 25-hydroxycholecalciferol deficiency is generally defined by a serum concentration of 20 nmol/l or less (1). Others define clinical vitamin D deficiency as a serum concentration of less than than 40 nmol/l as this level has been associated with severe skeletal effects (9). For the purposes of this study we defined severe deficiency as a serum concentration of 20 nmol/l or less, deficiency as 21-39 nmol/l and adequate concentrations as 40-195 nmol/l. All subjects with vitamin D levels <40nmol/l were given dietary advice and supplementation with Adcal D3 (calcium 600mg as carbonate plus colecalciferol 10 micrograms [400 units]) one or two tablets daily, depending on the degree of deficiency. Follow up measurements were taken after a minimum of three months on replacement therapy.

Seasonal Variation

Serum vitamin D levels were measured in patients and contacts at presentation. Patients and contacts presented at different times throughout the year and, because of the additional effects of UV radiation in the summer months, data is presented for each month of the year. Comparisons were made for patients and contacts for each month and for the six summer months compared with the six winter months.

Investigation of Diet

To asses the involvement of diet, 35 patients with TB and 35 controls matched for age, sex and skin colour, completed a validated food frequency questionnaire and a questionnaire to establish levels of sun exposure. Ages of patients and contacts in these groups ranged from 9-55, (median 30), 36 were male and 46 had dark skin. As there were no pre-existing food frequency questionnaires relating to vitamin D, one was developed using the USDA table of foods naturally containing vitamin D (mainly oily fish, lard, egg yolk and liver)(15), and information from manufacturers' packaging on supplemented foods. In the UK, margarine has been fortified since 1925. More recently, some breakfast cereals have been fortified and soya milk also contains 0.75µgm of added vitamin D/100mls. We are not aware of any other commonly available foods being fortified in the UK.
The International Unit (IU) is a measure of the activity of vitamin D and equates to 0.025µgm. Using this conversion, we calculated each individual's average daily intake in micrograms. In 1991, nutrients were given new dietary reference values by the

Department of Health (UK) and, from the Dietary Nutritional Survey of British Adults 1990, recommended nutrient intake (RNI) values were published for different age groups (16,17). There was no RNI for the 4-64 age group as it was concluded that most vitamin D was supplied by the sun in this group.

Measures of Exposure to Sunlight

Ultraviolet-B (UV-B) rays from the sun trigger the synthesis of vitamin D3 in the skin. A questionnaire was developed based on studies that have determined the variables affecting cutaneous vitamin D production from sun exposure i.e., season, latitude, time of day, cloud cover, smog and sunscreens. Sunscreens with a sun protection factor of 8 or more will block UV-B rays (18). At London's latitude of 52^{o}N, there is no radiation of the appropriate wavelength (290-310nm) from the end of October to the end of March and 1-2 hours daily of sun exposure to the face, arms and legs during the summer months would be required to obtain sufficient vitamin D to maintain normal concentrations throughout the year (18). This estimate formed the basis for defining adequate levels of sun exposure.
Ethical approval was obtained for the study and subjects provided written consent to participate.

Statistical Analysis

Data are reported as mean (standard error of the mean) unless otherwise stated. Unpaired student's t-tests were used to compare serum 25-hydroxycholicalciferol concentration, adequacy of sun exposure and dietary vitamin D intake between patients and their contacts. Seasonal variations in vitamin D levels at presentation in patients and contacts were also compared using the unpaired t-test. Chi^2 was used to compare numbers of patients and contacts with severe, deficient or adequate concentrations of serum vitamin D. Pearson rank correlation coefficients were calculated separately for patients and controls to assess the relationship between vitamin D intake and serum 25-hydroxycholecalciferol concentration. Statistical significance was defined as $p<0.05$ and 95% confidence intervals (CI) are given for comparisons between means.

RESULTS

Serum Concentrations of 25-hydroxycholecalciferol

Mean 25-hydroxycholecalciferol concentrations were significantly lower in the patients with TB compared with healthy contacts (20.1(0.95) nmol/l compared with 30.8(1.71) nmol/L; $p<0.0001$; 95% CI 7.1 to 14.3). Only 11 of the 178 TB patients (6%) had adequate serum concentrations and 114 (64%) had concentrations <21 nmol/L. Of the 130 contacts tested, 35 (27%) had adequate concentrations while 40 (31%) had severe deficiency with concentrations below 21 nmol/L (Table 2). The vitamin D status indicated by the serum level was confirmed in 10 cases chosen at random by elevated concentrations of parathyroid hormone (92(2.9) ug/L; reference range 10-65). Calcium

levels corrected for albumin were within the normal range and there was no significant difference in albumin levels between patients and contacts.

Table 2

Serum 25-hydroxy vitamin D concentrations (nmol/l) in patients with active TB and their contacts.

	TB	Contacts	Mean Difference	95% CI	p
	(n=178)	(n=130)			
Mean(SE)	20.1(0.95)	30.84(1.71)	10.74	7.14 to 14.34	<0.0001
Dark skinned	19.0(1.15)	27.8(1.45)	8.8	5.19 to 12.41	<0.0001
Mid coloured	20.9(1.73)	43.8(8.54)	22.9	11.53 to 34.27	=0.00026
Light skinned	24.4(3.63)	36.7(4.89)	12.3	-6.69 to 17.97	=0.053
Numbers with Vitamin D concentrations				χ^2 (2df)	p
<20 nmol/l	106(60%)	36(28%)			
20-39 nmol/l	61(34%)	57(45%)		39.99	<0.0001
40+ nmol/l	11(6%)	35(27%)			

All patients and contacts found to have deficient concentrations were given supplements. To date concentrations in only 10/48 (21%) patients have risen above 40nmol/l on replacement therapy but 38 continued to have deficient levels despite treatment for several months (Figure 1), although 13 of these showed some improvement, i.e., 25/48 (52%) failed to show any improvement in 25-OH-cholecalciferol concentrations after a minimum of three months on replacement therapy. Of 10 contacts with deficient concentrations (where follow up data is available), 5 returned to adequate levels after 3 months replacement therapy, 3 increased significantly (at least doubled) and 2 did not improve.

Effect of skin pigmentation

Patients and contacts were grouped for skin colour into dark, mid and light skin pigment groups. All three groups of TB patients had deficient concentrations of serum 25-hydroxycholecalciferol and there was no difference between the different ethnic groups. A similar pattern was seen in the contacts but mean concentrations were all consistently higher than patients from the same racial groups with TB (Table 2).

Seasonal variation

Patients and contacts presented at different times throughout the year and measurements at presentation are shown in Figure 2. Serum 25-hydroxycholecalciferol concentrations in the healthy contacts displayed a predictable seasonal pattern, rising to a peak in July, August and September following the increase in UV-B in the summer months, with a statistically significant difference between patients and contacts in July (p=0.013; 95%CI 1.9 to 31.0 nmol/L) and September (p=0.027; 95%CI 5.7 to 35.8 nmol/L). This seasonal variation was, however, strikingly absent in the TB patients (Figure 2). Concentrations at presentation in this group were consistently low throughout the year although there was a very small increase in mean concentrations for those who presented between the months of May to October compared with those who presented from November to April and May to October of 3.36(1.48)nmol/L. The difference in mean concentrations for contacts for the same periods was 14.82(3.0)nmol/L (p<0.0001; 95%CI 5.13 to 18.51).

Role of Diet and Exposure to Sunlight

Of the 35 patients with TB who completed the dietary and sun exposure questionnaires, only 6 had adequate serum concentrations of vitamin D and 20 were severely deficient. Of the 35 healthy contacts, 10 had adequate concentrations, 10 had concentrations just below the reference range and 5 had severely deficient concentrations. Patients with TB had significantly lower mean serum 25-OH-cholecalciferol concentrations than healthy matched contacts (21.1(2.52) compared with 33.8(3.04) nmol/L, p=0.002; 95% CI 4.8 to 20.6). There was, however, no difference in mean daily dietary intake of vitamin D or sunlight exposure (Table 3). Interestingly, the correlation between daily dietary vitamin D intake and serum 25-hydroxycholecalciferol concentrations was stronger in TB patients than healthy contacts (r=0.42, p=0.016; r=0.13, p>0.1 respectively)(Figure 3).

Table 3
Summer sunlight exposure, daily dietary vitamin D intake and serum 25-OH-cholecalciferol concentrations in patients with TB and their contacts frequency matched for age, sex and skin colour.

	TB (n=35)	Contacts (n=35)	Mean Difference	95% CI	p
Sun exposure:					
Adequate	12	12	0		
Inadequate	23	23	0		
Dietary vitamin D					
micrograms	6.09(5.39)	6.06(8.2)	0.03	-3.28 to 3.34	
Serum vitamin D					
nmol/l	21.1(2.52)	33.77(3.04)	12.67	4.79 to 20.55	=0.002

DISCUSSION

This study confirms previous studies (1,19) that patients presenting with active tuberculosis have significantly lower mean concentrations of serum 25-hydroxycholecalciferol than their contacts from the same ethnic and social backgrounds. There was no evidence that the vitamin D assay used was affected by hypergammaglobulinaemia which can sometimes be associated with active TB.

Our study also suggests that this association is independent of skin colour. A large study looking specifically at skin type, sun exposure and serum vitamin D concentrations concluded that vitamin D concentrations were not linked with phototype but with sun exposure behaviour (20). Many of our patients failed to increase their serum 25-hydroxycholecalciferol concentrations to adequate levels even after successful treatment for TB combined with at least three months and usually six months or more of vitamin D replacement therapy. Given that all these patients successfully completed treatment for TB with varying degrees of supervision, it is unlikely that this observation can be explained simply by non-compliance. Furthermore, patients generally received higher doses of vitamin D replacement than contacts. Anti-tuberculous chemotherapy can itself lower 25-hydroxycholecalciferol concentrations (21) and this may have been a contributing factor in the failure to raise concentrations to normal on treatment, but ten patients still failed to increase their vitamin D concentrations despite continuing with replacement therapy after anti-TB treatment ceased, thus raising the intriguing possibility of a group of patients with deficient handling of ingested and cutaneous vitamin D. Clinical disease only develops in around 10% of immunocompetent people infected with *Mycobacterium tuberculosis* suggesting host genetic factors influence the outcome of infection. Vitamin D acts via the vitamin D receptor (VDR) that is present on activated T and B lymphocytes. Genetic variation in the VDR has been associated with bone mineral density aswell as circulating concentrations of 25(OH)-vitamin D3 (22). Among TB patients in a large Gambian case controlled study, those with the genotype associated with high circulating concentrations of 25(OH)-vitamin D3 were under-represented (23). Polymorphisms in the vitamin D receptor gene, especially when assessed in combination with 25-hydroxycholecalciferol deficiency, have been associated with an increased risk of tuberculosis in Gujerati Asians (4).

We did not find any seasonal variation in new cases of tuberculosis in this study group, the majority of whom were from sub-Saharan Africa. There was a striking seasonal increase in 25-hydroxycholecalciferol concentrations in the contacts but only a very small increase in the summer months in the disease group. This does not support the idea that reduced sunlight in winter is responsible for the seasonal peak found in some populations (5) but suggests possible abnormal uptake, metabolism and storage of vitamin D, thus increasing the risk of reactivation of disease. Furthermore, there have been recent reports of low 25-hydoxycholecalciferol concentrations in healthy subjects resident in South Asia and elsewhere in the Tropics where there is abundant sunshine (24,25). Concentrations in women in these countries may well be low due to dress but this is less likely to be the case in men. An alternative explanation could be that, rather than low vitamin D concentrations resulting in reactivation of TB, low concentrations of 25(OH) vitamin D3

could be the result of tuberculosis-induced nutritional deficiencies. A large prospective study would be needed to find out whether vitamin D deficiency precedes the development of active tuberculosis.

There was a weak association between dietary intake and serum 25-hyroxycholecalciferol concentrations in the TB group but not in contacts, suggesting consumption of vitamin D may be less important for maintaining normal concentrations in healthy individuals. Only six patients with active tuberculosis had normal serum concentrations. All were Black African and three came from the same family and had a diet rich in oily fish and in addition took supplements. This did not, however, protect them from developing tuberculosis and it is likely that other nutrients may be involved in maintaining the integrity of the immune system. Protein malnutrition, for example, potentiates *M. tuberculosis* H37Rv-infected monocyte-macrophages to produce higher concentrations of TGF-beta (7), a cytokine implicated as a likely mediator of immunosuppression in tuberculosis. Copper, zinc and selenium are also essential elements for a healthy immune system, and zinc concentrations increase during TB treatment (26). Despite generally higher concentrations of serum 25-hydroxycholecalciferol in the control (contact) group, there was no difference in dietary intake. Both TB patients and contacts had marginally higher dietary intake of vitamin D than the average daily intake reported in the last National Dietary Survey in the UK (17). In 1986-7, the average daily intake in 16-64 year olds was 3.78 micrograms for men and 3.09 micrograms for women.

There were comparable numbers in both contact and TB groups who had adequate levels of sun exposure. This was based on results of a questionnaire and it is possible that this form of self reporting did not reflect true exposure to sunlight. More accurate results could be found in groups who spend no time in the sun or cover up for religious reasons. This observation, however, is supported by a small study from India which concluded that sunlight exposure was adequate in TB patients with vitamin D deficiency but dietary intake was reduced (27).

From our data it appears that patients with active tuberculosis from similar ethnic and social backgrounds and with comparable vitamin D intake and sun exposure have lower serum 25-hydroxycholecalciferol concentrations than their healthy contacts. This indicates that other factors contribute to vitamin D deficiency in the TB group. Furthermore, the lack of seasonal variation together with failure of a significant number of patients to increase 25-(OH) vitamin D3 concentrations to normal despite replacement therapy, suggests abnormal handling of this vitamin. A further study is required to investigate vitamin D intake, metabolism and storage to unravel the relationship between vitamin D and tuberculosis.

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CONFLICT OF INTEREST:

None.

CONTRIBUTIONS:

AS-L devised and validated the questionnaires and collected data. GL collected and analysed data. RS collected data and provided biochemical advice. HM devised and co-ordinated the study and wrote the paper. All authors contributed to the final manuscript.

FIGURE LEGENDS:

Figure 1:
Serum 25-OH-cholecalciferol concentrations (nmol/l) in patients with TB before and after a minimum of 3 months vitamin D replacement therapy. Concentrations in 10/48 patients recovered but 38/48 (79%) failed to return to normal although 13 of these showed some improvement.

Figure 2:
Seasonal variation in serum 25-OH-cholecalciferol concentrations in TB patients and controls.
Controls show an expected increase to normal concentrations in the summer months but this response was absent in patients with TB.
*$p<0.05$
**$p<0.01$

Figure 3:
Relationship between dietary vitamin D intake and serum vitamin D level (nmol/l)
Patients with TB $r=0.42$, $p<0.02$
Controls $r=0.13$, $p>0.1$

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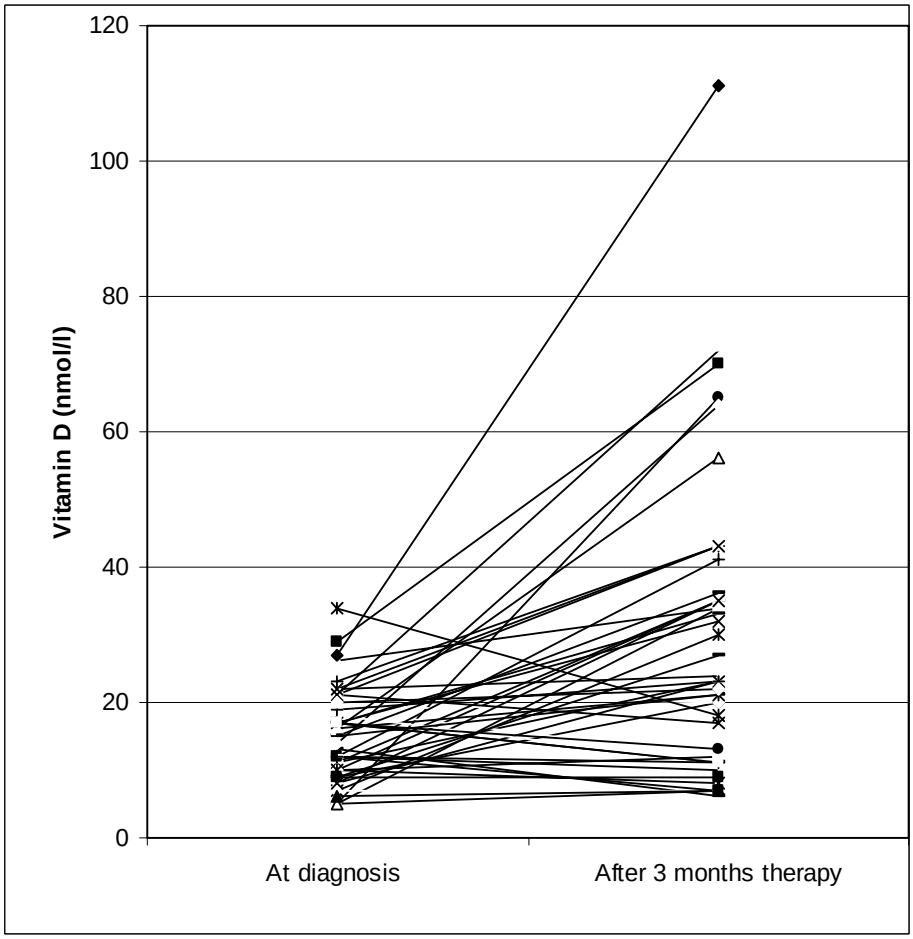
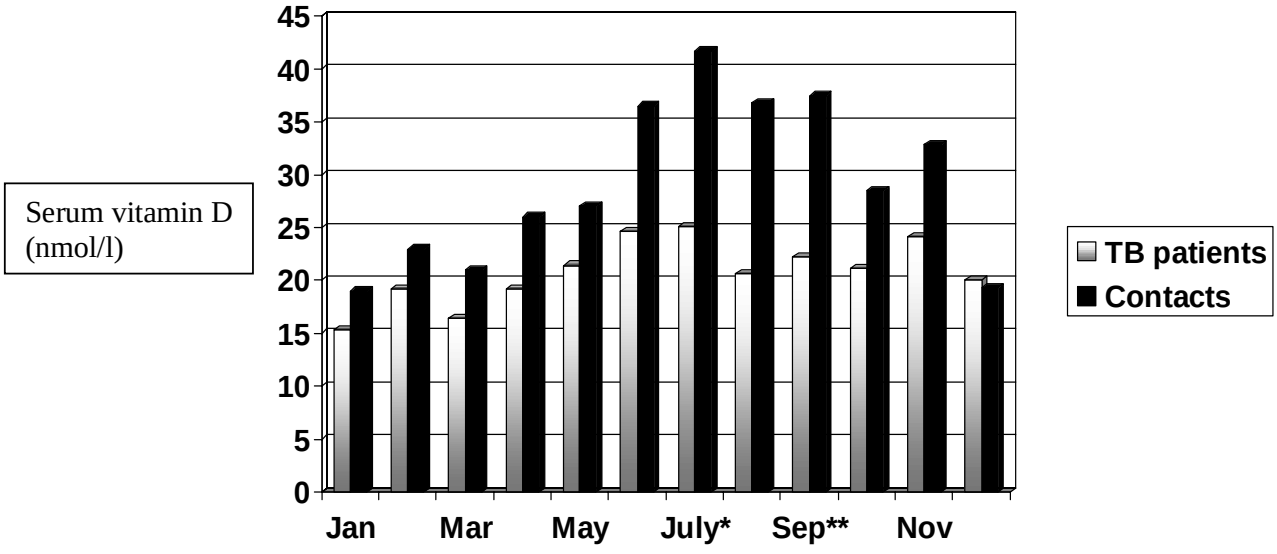


Figure 1

Serum 25-OH-cholecalciferol concentrations (nmol/l) in patients with TB before and after a minimum of 3 months vitamin D replacement therapy. Concentrations in 10/48 patients recovered but 38/48 (79%) failed to return to normal although 13 of these showed some improvement.

Figure 2

Seasonal variation in serum 25-OH-cholecalciferol concentrations in TB patients and contacts.
Contacts show an expected increase to normal concentrations in the summer months but this response was absent in patients with TB.
*p=0.013
**p=0.027



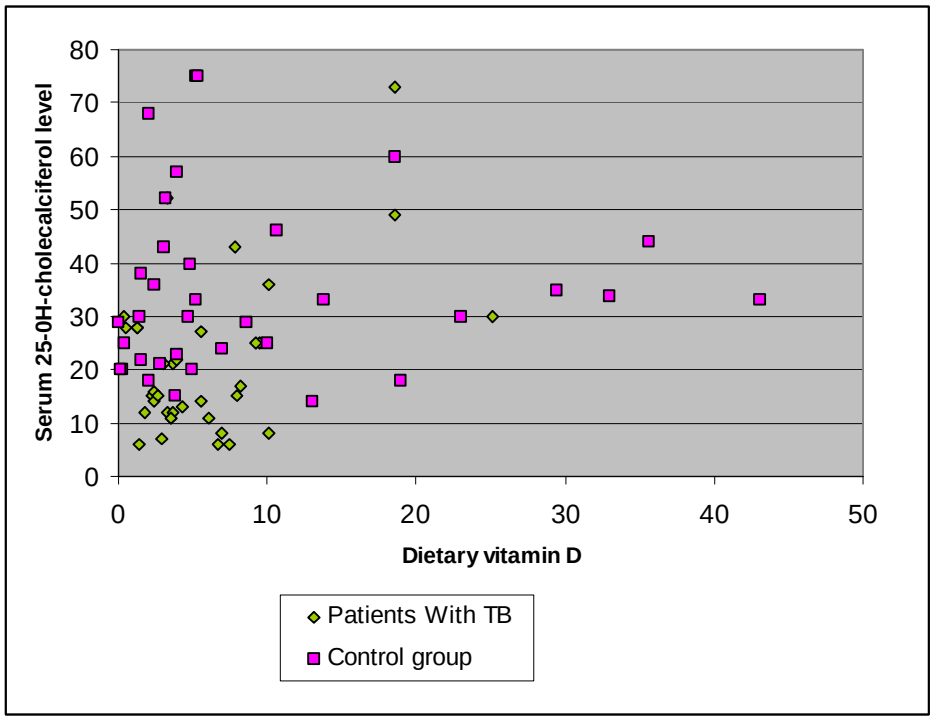


Figure 3.

Relationship between dietary vitamin D intake and serum vitamin D level (nmol/l)
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