Assessment of Serum Concentrations of Vitamin D in Young Male Patients with Tuberculosis

Amani Ezz Al Arab*, Dina Abaza**, Azza Al Sebaye***
*Chest Disease, **Endocrinology Al Azhar University For Girls, ***Clinical Pathology Zagazig University

Abstract
Tuberculosis is highly prevalent worldwide, accounting for nearly two million deaths annually. Vitamin D influences the immune response to tuberculosis, and vitamin D deficiency has been associated with increased tuberculosis risk in different populations (Bedoya and Ronnenberg, 2009).

The aim of this study has been to determine the possibility of an association between tuberculosis and low serum vitamin D concentration in young male patients and to monitor the changes in vitamin D levels after TB treatment.

Material and Methods: Twenty five (25) Patients aged 20-40 with newly diagnosed TB were enrolled in this study. They were divided into eleven (11) cases on first line TB treatment for 2-3 months and fourteen (14) cases before starting TB treatment. Twenty five (25) age and sex matched healthy volunteers were enrolled as controls. For all groups body mass index (BMI) was calculated. Also serum calcium (Ca⁺), 25-hydroxyvitamin D (25-OHD) and 1-25-hydroxyvitamin D (1-25-OHD) levels were measured and compared.

Results: There was significant difference between groups as regard BMI, serum Ca⁺, 25-OHD and 1-25-OHD (p<0.0001 for all groups). In the TB group both 25-OHD and 1-25-OHD were lower in patients who were under TB treatment compared to patients who didn't received treatment (p<0.001).

Conclusion: Low serum vitamin D concentrations may be a consequence of TB disease. The possibility that low serum 25-OHD and 1-25-(OH)² D concentrations may predispose to tuberculosis infection cannot be excluded. Antituberculous treatment has been shown to reduce serum 25-OHD and 1-25-(OH)²vitD, which may increase the risk of vitamin D deficiency.

Key words: Vitamin D, Tuberculosis

Introduction
Tuberculosis remains a major public health problem worldwide. One-third of the world population is infected with Mycobacterium tuberculosis, (Bedoya and Ronnenberg, 2009). According to WHO reports, Tuberculosis (TB) is the second most common Egyptian health problem after Schistsomiasis. Both TB and pulmonary fungal infections are chronic diseases of immune compromised hosts (Meawed et al., 2012).
Vitamin D deficiency has been suggested to be a risk factor for activation of TB. Vitamin D appears to play a role as an immunomodulator of the innate immune response by inducing anti-mycobacterial activity (Koo et al., 2012).

Deficiency of vitamin D (25-hydroxycholecalciferol) has long been implicated in activation of tuberculosis (TB) (Talat et al., 2010). Serum levels of vitamin D in TB patients are lower than in healthy controls (Nnoahamand Clarke, 2008). Recent studies showed that 1,25-dihydroxyvitamin D induced the expression of the antimicrobial peptide, cathelicidin, which restricts the growth of mycobacterium tuberculosis in monocytes under in vitro culture conditions (Martineau, 2007).

The aim of this study: to determine the possibility of an association between tuberculosis and low serum vitamin D concentration in young male patients and to monitor the changes in vitamin D levels after TB treatment.

Subjects And Methods

Study design and participants

Twenty five patients aged 20-40 years who had been newly diagnosed with pulmonary or extra-pulmonary TB were enrolled in the study. TB was diagnosed if at least one of the following criteria were met: 1- Isolation of Mycobacterium tuberculosis from sputum or other clinical specimens. 2- A positive polymerase chain reaction test for TB in sputum or other clinical specimens. 3- Presence of caseation granulomas in tissue. 4- Lymphocyte predominant exudative effusion with an adenosine-deaminase level >40 IU/L. They were divided into eleven (11) cases on first line TB treatment for 2-3 months and fourteen (14) cases before starting TB treatment. Patients were compared to 25 sex and age matched controls with no previous history of TB and no radiographic lesions suggesting current or previous TB infection. Subjects, who had taken vitamin D supplements during the 6 months preceding enrolment, were excluded. The recruitment of cases and control was taken place at approximately the same time.

All participants were provided with full information about the study’s purpose and gave informed consent to participate in the study.

Measurement

Prior to commencing TB treatment, fasting serum was obtained for serum albumin, total calcium, 25-hydroxyvitamin D and 1-25-dihydroxyvitamin D. Blood samples were collected in serum separator tubes. The serum was separated by centrifugation and stored at -70°C. Total serum 25-hydroxyvitamin D and 1-25-dihydroxyvitamin D were measured for all study participants by radioimmunoassay (DiaSorin, Stillwater, MN, USA)
Serum concentrations of calcium and albumin were measured with a Beckman-Coulter Unicel DxC 880i Analyzer (Roche) using photometric techniques. Albumin corrected calcium concentrations were calculated as follows: calcium + 0.02 × (40 – [albumin]).

**Statistical analysis**

Data of tests were analyzed using the arithmetic mean, standard deviation (SD), unpaired students t-test. The data were then analyzed statistically using SPSS statistical package version (*Hmama et al.*, 2004).

P > 0.05 insignificant (NS)

P < 0.05 significant (Sig.)

P < 0.001 highly significant (H.S)

**Results**

The summary data for laboratory investigations among studied groups are shown in (Table 1). There was no significant difference between both groups as regard age. There was highly significant difference between both groups regarding BMI, S calcium, 25-OHD and 1-25-OHD.

In (Table 2) correlations was done in TB group between 25-OHD and 1-25-OHD and age, BMI and calcium. Significant correlation was found only between BMI and 1-25-OHD. When patients under TB treatment were compared to patients not under treatment, 25-OHD and 1-25-OH were significantly lower in patients under TB treatment (Table 3).

**Table (1): Comparison between TB group and control group as regard Age, BMI, S calcium, 25-OHD and 1-25-OHD.**

<table>
<thead>
<tr>
<th></th>
<th>TB patient group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=25 mean ±SD</td>
<td>n=25 mean±SD</td>
</tr>
<tr>
<td>Age(years)</td>
<td>30.76±7.16</td>
<td>32.5±6.42</td>
</tr>
<tr>
<td>BMI(Kg/m²)</td>
<td>20.38±2.81</td>
<td>26.32±1.25</td>
</tr>
<tr>
<td>Serum calcium(mg/dl)</td>
<td>8.71±0.89</td>
<td>10.1±0.7</td>
</tr>
<tr>
<td>25-OHD(ng/ml)</td>
<td>19.57±6.99</td>
<td>39.34±9.78</td>
</tr>
<tr>
<td>1-25OHD(pg/ml)</td>
<td>37.34±5.48</td>
<td>40.61±5.01</td>
</tr>
</tbody>
</table>

**Table (2): Correlation between both 25-OHD and 1-25-OHD and other studied parameters in TB group**

<table>
<thead>
<tr>
<th></th>
<th>25-OHD(ng/ml)</th>
<th>1-25-OHD(pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>P</td>
</tr>
<tr>
<td>Age(years)</td>
<td>0.15</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>BMI(Kg/m²)</td>
<td>0.016</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>calcium(mg/dl)</td>
<td>0.15</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>
Table (3): Comparison of both 25-OHD and 1-25-OHD between TB patients under treatment and before treatment.

<table>
<thead>
<tr>
<th></th>
<th>Patients under treatment</th>
<th>Patients before treatment</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=11 mean±SD</td>
<td>n=14 mean±SD</td>
<td></td>
</tr>
<tr>
<td>25-OHD/ng/ml</td>
<td>16.46±2.1</td>
<td>19.48±2.9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>1-25-OHD/pg/ml</td>
<td>22.21±3.03</td>
<td>31.9±3.31</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Discussion

Tuberculosis is probably the oldest disease known to human (Bedoya and Ronnenberg, 2009). Although the adjunctive role of vitamin D supplementation during TB treatment has been controversial, hypovitaminosis among TB patients has been reported by many studies (Bedoya and Ronnenberg, 2009). In this study BMI was significantly lower in TB patient group compared to control group. In agreement to these results, Leung (2007) demonstrated that Low body weight is associated with risk of tuberculosis, and that BMI below 18.5 increases the risk by 2 to 3 times.

As regard serum calcium, 25-OHD and 1-25-OHD they were highly significant lower values in TB patient group when compared to control group. Several recent studies in different populations have associated a deficiency in vitamin D with increased risk of tuberculosis (Nansera et al., 2011).

In a recent meta-analysis by Nnoaham and Clarke, 2008, vitamin D levels were lower in persons with tuberculosis than in controls. However, these findings cannot be considered conclusive since the association may be confounded by important variables, such as smoking and sunlight exposure, which were not accounted in the analysis. It is well-established that immune cells can produce the hormonally active metabolite of vitamin D (Bedoya and Ronnenberg, 2009). Macrophages and other immune cells can express 1α-hydroxylase, the enzyme that converts circulating 25-hydroxyvitamin D3 into 1, 25-dihydroxyvitamin D3, the active form of vitamin D (Martineau et al., 2007, Houben et al., 2006).

Two possible mechanisms have emerged as the most likely biological mechanisms through which vitamin D modulates the immune system to fight Mycobacterium infection (Liu et al., 2006). First, 1, 25-dihydroxyvitamin D3 appears to reduce the viability of M. tuberculosis by enhancing the fusion of the phagosome and lysosome in infected macrophages (Martineau et al., 2007). The capacity of Mycobacterium infection to prevent macrophage maturation and formation of the phagolysosome is completely reversed in the presence of 1, 25-dihydroxyvitamin D3.
In addition, 1, 25-dihydroxyvitamin D may enhance the production of an antimicrobial peptide of the cathelicidin family (Liu et al., 2006). Antimicrobial peptides, such as defensins and cathelicidins, are involved as a first line of defense in the prevention of infections, including tuberculosis (Bedoya and Ronnenberg, 2009, Selvaraj et al., 2004).

In the present study correlation was done in TB patient group between 25-OHD and 1-25(OH)D and other studied parameters. Only BMI was positively correlated with both. The finding of decreasing levels of serum 25-OH-vit D with increasing BMI is in accordance with previous reports, and it is today established that obese individuals as a group have decreased levels of 25-OH-vit D (Pham et al., 2010).

Several CYP450 are involved in vitamin D metabolism. Two of the standard first-line anti-tuberculosis drugs, isoniazid (INH) and rifampicin (RMP), are known for inhibiting and inducing CYP450 activity, respectively, and can affect vitamin D metabolism (Martineau et al., 2007).

Isoniazid (INH) reduces 25(OH)D and 1,25(OH)2D concentrations by the inhibition of 25-hydroxylase, as has been shown in vitro studies, animal studies and human volunteers (Bengoa et al., 1984, Desta et al., 2001 & Gupta et al., 2005). Rifampicin (RMP) is a strong inducer of CYP3A4 (Goodwin et al., 1999). Induction of these enzymes increases the enzymatic conversion of 25(OH)D to the inactive metabolite 24,25(OH)2D and results in decreased 25(OH)D and 1,25(OH)2D concentrations, as shown in studies in human volunteers (Goodwin et al., 1999, Brodie et al., 1980). Combined use of Isoniazid (INH), and rifampicin (RMP) reduces 25(OH) D and 1,25(OH)2D concentrations in both human volunteers and TB patients (Wejse et al., 2009).

In the present study, 25-OHD levels decreased significantly with TB treatment. Previous reports have shown a paradoxical decline in vitamin D levels after prolonged TB treatment (Hughes, 2008) which is in agreement with the present findings. The decline in vitamin D levels with treatment may be explained by enhanced vitamin D metabolism due to the influence of isoniazid and rifampin on cytochrome P450 activity (Desta and Flockhart, 2001). However, a recent study from Tanzania reported an increase in vitamin D levels during TB treatment. Improved dietary intake and increased sunlight exposure may have contributed to the increased 25(OH)D concentrations (Tostmann et al., 2010).

**Conclusion**

Low serum vitamin D concentrations may be a consequence of TB disease. The possibility that low serum 25-OHD and 1-25(OH)2vit D concentrations may predispose to tuberculosis infection cannot be excluded. Antituberculosis treatment has been shown to reduce serum 25-OHD and 1-25(OH)2vit D,
which may increase the risk of vitamin D deficiency.

Reference


Assessment of Serum Concentrations of Vitamin D...

**Assessment of Serum Concentrations of Vitamin D in Male Adolescents with Tuberculosis**

**Amani Al-Abare**, **Dina Abozeid**, **Zein El-saghi**

**Department of Pulmonary Diseases**, **Endocrinology Section**, **Pediatric Section**, **Zagazig Medical School, Al-Azhar University**

Tuberculosis is a widespread disease with millions of cases and deaths annually worldwide. Vitamin D affects the immune response of patients with tuberculosis and is associated with an increased risk of tuberculosis in different population groups.

The objective of this study was to determine the possibility of a relationship between tuberculosis and low serum vitamin D levels in male adolescents and to follow changes in serum vitamin D levels after tuberculosis treatment.

**Materials and Methods:**

- A total of 25 patients aged 20-40 years were recruited. Tuberculosis patients were included in this study.
- The patients were divided into two groups: 11 patients at the beginning of tuberculosis treatment after 2-3 months, and 14 patients before starting tuberculosis treatment. 25 healthy volunteers matched for age and gender were enrolled as controls.
- Body mass index (BMI), serum calcium, 25-hydroxyvitamin D (25OHD) and 1,25-dihydroxyvitamin D (1,25(OH)2D) were measured.

**Conclusion:**

A decrease in vitamin D levels may be a result of tuberculosis. It is not possible to exclude the possibility that a decrease in 25OHD and 25(OH)D may lead to tuberculosis. It has been shown that tuberculosis treatment leads to a decrease in 25OHD and 25(OH)D, which may increase the risk of vitamin D deficiency.