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

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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 patientsand 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 and1-25-OHD ($p<0.0001$ for all groups). In the TB group both25-OHD and1-25-OHD were lower in patients who were underTB 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 and1-25-$(OH)^2$ D concentrations may predispose to tuberculosis infection cannot, be excluded. Antituberculous treatment has been shown to reduce serum 25-OHD and1-25-$(OH)^2$vitD, 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 and1- 25-dihydroxyvitamin D. Blood samples were collected in serum separator tubes. The serum was separated by centrifugation andstored at -70°C. Total serum 25-hydroxyvitamin D and1-25-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 and1-25-OHD.

In (Table 2) correlations was done in TB group between 25-OHD and1-25-OHD and age, BMIand calcium. Significant correlation was found only between BMI and1-25-OHD. When patients under TB treatment were compared to patients not under treatment , 25-OHD and1-25-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 and1-25-OHD.

	TB patient group n=25 mean ±SD	Control group n=25 mean±SD	P value
Age(years)	30.76 ±7.16	32.5 ±6.42	>0.05
BMI(Kg/m²)	20.38± 2.81	26.32±1.25	<0.001
Serum calcium(mg/dl)	8.71±0.89	10.1±0.7	<0.001
25-OHD(ng/ml)	19.57± 6.99	39.34±9.78	<0.001
1-25OHD(pg/ml)	37.34± 5.48	40.61±5.01	<0.001

Table (2): Correlation between both 25-OHD and1-25-OHD and other studied parameters in TB group

	25-OHD(ng/ml)		**1-25-OHD(pg/ml)**	
	R	**P**	**R**	**P**
Age(years)	0.15	>0.05	0.17	>0.05
BMI(Kg/m²)	0.016	<0.05	0.02	<0.05
calcium(mg/dl)	0.15	>0.05	0.16	>0.05

Table (3): Comparison of both 25-OHD and1-25-OHD between TB patients under treatment and before treatment.

	Patients under treatment n=11 mean ±SD	Patients before treatment n=14 mean±SD	P value
25-OHD(ng/ml)	16.46±2.1	19.48±2.9	<0.05
1-25-OHD(pg/ml)	22.21±3.03	31.9±3.31	<0.001

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 and1-25-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 Dwith increased risk of tuberculosis *(Nansera et al., 2011)*.

In a recent meta-analysis by *Nnoaham and Clarke, 2008*, vitamin D levels were lower inpersons with tuberculosis than in controls. However, these findings cannot be considered conclusive since theassociation may be confounded by important variables,such as smoking and sunlight exposure, which were notaccounted in the analysis.It is well-established that immune cells can producethe hormonally active metabolite of vitamin D *(Bedoya and Ronnenberg, 2009)*. Macrophages and other immune cells can express 1a-hydoxylase,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 theviability 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 phagolysosomeis completely reversed in the presence of 1, 25-dihydroxyvitamin D3

(Hmama et al., 2004).In addition, 1, 25-dihydroxyvitamin D may enhancethe 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 ofdefense 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-OHD and other studied parameters. Only BMI was positively correlated with both. The finding of decreasing levels of serum25-OH-vitD with increasing BMI is in accordance with previousreports, and it is today established that obese individualsas a group have decreased levels of 25-OH-vitD *(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 withthe present findings. The decline in vitamin D levelswith treatment may be explained by enhancedvitamin D metabolism due to the influence of isoniazidand rifampin on cytochrome P450 activity *(Desta and Flockhart, 2001)*. However, a recent study from Tanzania reported anincrease in vitamin D levels during TB treatment. Improved dietary intake and increased sunlight exposure may have contributedto 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 and1-25-$(OH)^2$vitD concentrations may predispose to tuberculosis infection cannot be excluded. Antituberculosis treatment have been shown to reduce serum 25-OHD and1-25-$(OH)^2$vitD,

which may increase the risk of vitamin D deficiency.

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تقييم تركيزات مصل فيتامين د في المرضى الذكور الشباب مع السل

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السل في جميع أنحاء العالم واسع الانتشار، وهو يمثل ما يقرب من مليوني حالة وفاة سنويا. فيتامين (د) يؤثر على الاستجابة المناعية لمرضي السل، وارتبط نقص فيتامين (د) بزيادة خطر مرض السل في مختلف قطاعات السكان.

وكان الهدف من هذه الدراسة هو تحديد إمكانية وجود علاقة بين مرض السل وانخفاض تركيز فيتامين (د) في الدم في المرضى من الذكورالشباب، ورصد التغيرات في مستويات فيتامين (د) بعدعلاج السل.

مواد وطرق:- تم تسجيل خمسة وعشرون (25) مريضا تتراوح أعمارهم بين20-40. المرضى المصابين بالسلتم تشخيصها حديث افي هذه الدراسة. تم تقسيمها إلى أحد عشر (11) مريضفيبداية علاج الس للمدة 2-3 أشهر, وأربعة عشر(14) مريض قبل بدء العلاج لمرض السل.تم تسجيل خمسة وعشرين (25) فى نفس العمر والجنس كمتطوعين أصحاء.تم قياس مستويات ومقارنة حساب كتلة الجسم جميعا لجماعات (BMI). الكالسيوم في الدم ، 25 هيدروكسيفيتامينD (25 OHD) و 1-25- هيدروكسي فيتامين D(1-25-OHD).

الخلاصة: ان انخفاض مستوي فيتامين **(د/ D)** قد يكون نتيجة لمرض السل. لا يمكن استبعاد احتمال أن انخفاض مستوي 25 OHDو 25-(OH) D ² قد يؤدي للإصابة بالسل. وقد تبين أن علاج السل يؤدي الي انخفاض مستوي 25 OHD و 25- (OH) D، والتي قد تزيد من خطر نقص فيتامين (د).