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ASSESSMENT OF SERUM LEVEL OF VITAMIN D IN PATIENTS WITH PULMONARY TUBERCULOSIS BEFORE AND AFTER SPUTUM CONVERSION

By

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ABSTRACT

Background: Tuberculosis (TB) is a chronic disease caused by Mycobacterium tuberculosis, and the prevalence of tuberculosis in developing countries like Egypt still higher. Vitamin D is a fat-soluble vitamin that acting significant role against infectious diseases including tuberculosis, thus, human monocytes have receptors for vitamin D, which activates anti mycobacterial responses in human monocytes and macrophages through enhancing phagocytosis and granuloma creation.

Objective: To assess the serum level of vit. D in patients newly diagnosed as pulmonary tuberculosis, at time of diagnosis and after sputum conversion to detect the effect of Vit. D on tuberculosis patients.

Subjects and Methods: The current study designed to evaluate the level of serum VD on group of patients with active pulmonary TB, as well as on healthy control subjects, in addition to measuring the level of VD after sputum conversion from positive to negative. The study conducted on Abbassia chest Hospital from the start of January 2020 until the end of December 2020, and included 50 patients with PTB and 20 healthy controls.

Results: The level of TLC and PLT were significantly higher than in the control subjects, $P < 0.001$, while the HB level showed significant lower level, $P < 0.001$. The level of ESR was significantly higher in TB patients than in control groups, $P < 0.001$, it measured about (83.6± 30.7) mm/h, in TB group, opposite to (7.05± 3.03) mm/h, in control group. The level of VD in TB patients was significantly lower than in the control subjects; (30.3±10.2 versus 38.81± 3.48) ng/mL at the beginning of the study. The change of serum level of vitamin D was significant after sputum conversion, it elevated from 30.3±10.2 ng/L to 31.0.9±9.63 ng/L, $P = 0.02$.

Conclusion: The present work concluded that; vitamin D deficiency was common in patients with pulmonary TB and that level was significantly associated with healing and good response to treatment.

Keywords: Tuberculosis, vitamin D deficiency.

INTRODUCTION

Tuberculosis (TB) remains a major public health problem globally. It was estimated that there were 10 million reported new TB cases and that 1.2 million people died from TB in 2018 *(World Health Organization, 2018)*. Various factors that could possibly affect the incidence and progression of TB have been reported, one of them is vitamin D deficiency (VDD) *(Kim et al., 2014)*.

Cholecalciferol (vitamin D3) is synthesized upon exposure of the skin to (ultraviolet) UV radiation resulting in the conversion of endogensou 7-dehydrocholesterol to previtamin D3, which isomerizeds to vitamin D3. To a lesser extent, vitamin D can be sourced in the diet from foods such as fortified dairy products and cereals, oily fish and fish liver oils. Synthesized or dietary vitamin D, from the skin and the gut, respectively, are hydroxylated in the liver to form 25-hydroxyvitamin D [25-OHD] through the action of cytochrome P450 enzymes, which is dihydroxyvitamin D [1,25(OH)2D], in the kidney and other tissue by the 1□-hydroxylase *(Cristina Karohl 2010)*.

Deficiency of vitamin D (25-hydroxycholecalciferol) has long been implicated in activation of tuberculosis (TB). Serum levels of vitamin D in TB patients are lower than in healthy controls. *(Maceda et al. 2018)*.

AIM OF THE WORK

The aim of this work is to assess the serum level of vitamin D in patients newly diagnosed as pulmonary tuberculosis, at time of diagnosis and after sputum conversion to detect the effect of vitamin D on tuberculosis patients.

SUBJECTS AND METHODS

A cohort study that included two groups; control (n=20) and cases with pulmonary TB (n=50) was conducted on Abbassia Chest Hospital. The aim of the study was evaluating the level of serum VD at the time of diagnosis and after sputum conversion from positive to negative.

Inclusive criteria: All cases with positive pulmonary TB depending on direct smear analysis for acid-fast bacilli (AFB) or gene expert.

Exclusive criteria: Extra pulmonary TB. Smear negative for AFB. Pregnant female. Associated hepatic and renal comorbidity. TB with MDR or XDR.

The two groups were subjected to the following: History taking and through clinical examination, complete blood picture, ESR and serum VD level.

For patients' group: Sputum analysis under microscope for AFB detection with successive 3 sample evaluation, chest X ray and serum VD level at the time of diagnosis and after sputum conversion.

Classification of vitamin D deficiency:

Vitamin D deficiency was defined as 25(OH)D less than 30 ng/ML, the intensity of affection further categorized in to; mild (20-30) ng/mL, moderate (10-20) ng/mL and severe < 10 ng/mL *(Wendy et al., 2013)*.

Vitamin 25(OH)D3 assay was used (Roche Diagnostics) on a Cobas e411 by electrochemiluminescence (ECLIA). The Roche Diagnostics Vitamin D total assay is a Quantitative chemiluminescent immunoassay (CLIA) methods. *(Wendy et al., 2013)*.

Ethical considerations:

All subjects were giving a written consent prior to participation and the study protocol approved by the Institutional Research and Medical Ethics Committees of Al-Azher University.

Statistical analysis:

The data collected tabulated and statistically analyzed using Minitab 17.1.0.0 for windows (Minitab Inc., 2013, Pennsylvania, USA). By the following methods:

1. **Descriptive statistics;** Continues data was represented as mean and standered deviation (SD), while non numerical data as number and percentage (%).
2. **Analytical statistics including: Paired t-test:** used to compare between mean of one group before and after treatment. Independent t-test: used to compare between two mean of independent groups Chi square test: used to compare between frequency of two or more categories. All statistical tests were two-sided, P considered significant if < 0.05.

RESULTS

Table (1): Demographic characters of the studied groups

Factors	Control (n=20)		Patients (n=50)		P
	Mean	SD	Mean	SD	
Age	36.9	17.7	34.4	14.6	0.58$
Sex	N	%	N	%	
Female	11	55	15	30	0.05#
Male	9	45	35	70	
	Mean	SD	Mean	SD	
BMI	21	2.08	21.01	2.03	0.98$

N: number, SD: stander deviation, $: Independent t-test, #: chi square test, P considered significant if < 0.05.

The patients and control were matched as regarding age and BMI, P = 0.58 and 0.98 respectively. While considering sex, the TB patients were significantly common in male patients; 70% of patients were males opposite to 30% female, P = 0.05.

Table (1): Risk factors among the studied subjects

Factors	Control (n=20)		Patients (n=50)		P#
	N	%	N	%	
Smoking habits					
Non-Smoker	9	45	20	40	0.71
Smoker	11	55	30	60	
Comorbidity					
DM (Yes)	6	30	15	30	0.99
HTN (Yes)	4	20	11	22	0.85
HIV (Yes)	0	0	4	8	0.09
Drug addiction (Yes)	0	0	9	18	0.01

N: number, #: chi square test, P considered significant if < 0.05.

The patients and control group were matched as regarding smoking habits and comorbidities (DM and HTN), P = 0.71, 0.99 and 0.85 respectively. While regarding drug addiction habits and HIV

positive status, the control was free. The prevalence of HIV in TB cases was 8%.

Table (2): Symptoms of TB cases

Symptoms	No	%
Hemoptysis	9	18%
Toxic symptoms	39	78%
Cough	45	90%

Table (3): Laboratory data of the studied groups.

Factors	Control (n=20)		Patients (n=50)		P$
	Mean	SD	Mean	SD	
TLC	7.34	1.85	10.9	4.08	< 0.001
HB	14.03	1.61	10.47	1.84	< 0.001
PLT	268.8	50.8	414	151	< 0.001
ESR	7.05	3.03	83.6	30.7	< 0.001

N: number, SD: stander deviation, $: Independent t-test, #: P considered significant if < 0.05.

The patients with pulmonary TB showed significant elevation of TLC, PLT and ESR in comparison with control subjects, P < 0.001 for all. In addition, the HB was significantly lower in patients than in control subjects, P < 0.001.

Table (4): Serum vitamin D level in the studied groups at the start of the study

Factors	Control (n=20)		Patients (n=50)		P$
	Mean	SD	Mean	SD	
VD (ng /L) at the start of the study.	38.81	3.48	30.3	10.2	< 0.001

N: number, SD: stander deviation, $: Independent t-test, #: P considered significant if < 0.05.

The table showed that, the level of serum VD in patients was significantly lower than in control subjects, P < 0.001.

Table (6): VD status in both groups

VD status	Control (n=20)		Patients (n=50)		P#
	N	%	N	%	
Normal VD	7	35	21	42	0.47
Mild VD deficiency	11	55	20	40	
Moderate VD deficiency	2	10	9	18	

N: number, #: chi square test, P considered significant if < 0.05.

As shown in table 7; both patients and control had insignificant difference as regarding the frequency of normal VD status or Mild and moderate deficiency, P = 0.47. Hence, more than half of patient group and control subjects suffered from VD deficiency.

Table (7): The VD level changes in patients before and after sputum conversion

Factors	Before conversion (n=50)		After conversion (n=50)		P$*
	Mean	SD	Mean	SD	
VD (ng /L)	30.3	10.2	31.09	9.63	0.02

$*: Paired t-test, SD: stander deviation, P considered significant if < 0.05.

The level of serum VD in patients after sputum conversion was significantly elevated from 30.3±10.2 ng/L in to 31.0.9±9.63 ng/L, P = 0.02.

Table (8): Correlation between VD status and time of sputum conversion in TB patients

VD status	No	Duration of conversion (week)	
		Mean	SD
Normal VD	21	3.43	0.51
Mild VDD	20	4.20	0.83
Moderate VDD	9	5.33	0.50
P^		< 0.001	

N: number, SD: stander deviation, ^: One Way ANOVA test, P considered significant if < 0.05

DISCUSSION

As a one of major public health problem, the TB disease considered as a major dilemma, especially in developing countries. In Egypt, the burden of TB disease still higher, as the prevalence of TB in Egypt was 26 for every 100,000 people *(WHO, 2014)*.

Many factors determine the cause of developing TB disease, and the variety of response in different individuals as genetic, immunological and environmental factors *(Marlo Möller, 2018)*.

However, vitamin D deficiency could be considered as one of responsible risk factors, hence the immunological role of VD through regulating the macrophage function, which stimulating the human anti-mycobacterium function empower that hypothesis of causality between VD deficiency and TB disease *(Nouri-Vaskeh et al., 2019)*.

The correlation between VD deficiency and developing TB disease was supported by many coincidences; the first was tendency of disease to occur in cold seasons while the VD synthesis in the skin is minimized by the effect of sunlight. The second; the untreated TB patients had lower level of VD in serum than healthy individuals and finally, the prevalence of TB infection was higher in elder patients as well as in uremic patients whom suffered from VD deficiency *(Kearns and Tangpricha, 2014; Nouri-Vaskeh et al., 2019)*.

The current study was designed to evaluate the level of VD in TB patients in comparing with healthy control group, in addition to estimating the changes of VD level in the patient's group after initiation of treatment by anti-tubercles drugs and sputum conversion.

The present work included 50 TB patients and 20 subjects as healthy

control, all of them were enrolled from Abbassia Chest hospital. The both groups were matched as regarding age and BMI, P 0.58 and 0.98 respectively. While considering sex, the TB patients were significantly common in male patients;70% of patients were males opposite to 30% female, P= 0.05 **(table 1)** that came in concordance with many studies while the TB infection was common in male *(Pednekar et al., 2012; Przybylski et al., 2014; Radović et al, 2019)*.

The epidemiological picture of tuberculosis showed that male predominant, hence, they more exposed to infection in the community than females because of occupational and mental stress or other social factors which prevent females from seeking medical advice, which may cause a false lowering of the incidence rate in females. In addition, these results came in accordance with World Health Organization reports included more males than females as men account for a higher proportion of notified TB cases *(WHO, 2014)*.

In the present study, both groups were matched as regarding many risk factors (smoking habits, DM and HTN), P = 0.71, 0.99 and 0.85 respectively **(table 2)**. Even though, the smoking habits were the common risks in pulmonary TB. The commonest comorbidity was DM (30%) then HTN (22%). Drug addiction and HIV positive were reported in 9 and 4 cases of 50 respectively. That came in agreement with recent study by *Sedky et al. (2018)*; hence, he reported that 26.7% of TB patients had DM, in the same line, *Dayem et al. (2019)* study who found that 30% of TB patients had DM.

The frequency of comorbidity among TB patients was varied from study to another; most of them recorded DM as a commonest associated disease, hence DM leaded to decrease immunity of the patients, caused reactivation of the primary TB infection, and may lead to multidrug resistance against TB medication *(Safwat et al., 2009)*.

Considering the clinical presentation of TB patients, the present work reported that cough and toxic symptoms were the commonest symptoms; it was founded in 45 and 39 cases of 50, while hemoptysis was founded in 9 cases only (table 3). In harmony with Smiljić et al. (2019) study, where night sweating presented in 60% of cases, and loss of weight in 50 % of them. In the same line, *Babamahmoodi et al. (2015)* stated that toxic symptoms were recorded in more than 70% of TB patients. Furthermore, recent meta-analysis conducted by *Patra et al. (2014)*, which analyzed 14 national studies of higher TB burden, the results concluded that loss of weight and hemoptysis were significantly associated with TB infection.

Regarding the hematological parameters of TB patients, the current work reported that, the level of TLC and PLT were significantly higher than the control subjects, P < 0.001, while the HB level showed significant lower level, P < 0.001 **(table 4)**. This supported by many studies; in *Jeon et al. (2019)* study, the level of TLC and PLT were significantly higher in TB subjects than control one, P = 0.001 for both. Moreover, *Iliaz et al. (2014)* reported that, the level of TLC was significantly higher in TB patients than in control subjects.

The ESR is a surrogate marker of the acute phase reaction. The test mainly measures the plasma viscosity by assessing the tendency for red blood cells to aggregate and 'fall' through the variably viscous plasma *(Harrison, 2015)*.

In the present study, the level of ESR was significantly higher in TB patients than in control groups, P < 0.001. Hence, it measured about (83.6± 30.7) mm/h, in TB group, opposite to (7.05± 3.03) mm/h, in control group **(table 4)**. That came in accord with *Dayem et al. (2019)*, thus; the level of ESR was (115.92± 38.41) mm/h, however, in *Khalil et al. (2020)* the level of ESR was lower than the current results (49.41± 29.62) mm/h, and that may be duo to early measuring of ESR level at the beginning of disease.

Levels of vitamin D show a discrepancy among populations and affected by a mixture of racial, cultural and geographical causes *(Lips et al., 2001; Mansoor et al., 2010)*.

In previous studies, the vitamin D levels in patients with TB have been controversial, hence, *Wilkinson et al. (2000)*, and *Friis et al. (2008)*, found that the level of VD was lower in patient with TB compared to the healthy individuals while *Koo et al. (2012)* and *Ho-Pham et al. (2010)* did not detect these findings. However, that may be duo to different study design, inclusion and exclusion criteria, and variety in socio-demographic characters of participant.

The current study agreed with *Wilkinson et al. (2000)*, and *Friis et al. (2008)*, hence, the present data showed that, the level of VD in TB patients was significantly lower than in the control subjects; 30.3±10.2 versus 38.81± 3.48 ng/mL, P < 0.001 **(Table 5)**.

In harmony with *Nouri-Vaskeh et al. (2019)*, he studied the prevalence of VD deficiency among Iranian TB patients. The study included 60 subjects; 30 patients with TB and 30 control, the VD level in serum was evaluated at the diagnosis and after treatment, he found that the mean serum vitamin D levels in the control subjects were higher than TB patients (73.03±25.6 vs. 22.66±15.17 ng/mL), P < 0.001. Moreover, the mean serum vitamin D level in patients with TB after treatment was significantly lower than that of the control subjects, P < 0.001.

A recent supportive Brazelian study conducted by *Maceda et al. (2018)*, and included 24 cases with pulmonary TB and 48 control without TB empower the present data, thus, the mean serum 25(OH)D3 level was significantly lower among the cases (27.7±7.85 ng/mL) than controls (37.1±8.94 ng/mL, p<0.01.

In the current study the vitamin D status among the control group showed normal VD level in 7 (35%), mild VD deficiency in 11(55%) and moderate VD deficiency in 2 (10%) while in TB patients the vitamin D status revealed normal VD in 21(42%), mild VD deficiency in 20 (40%) and moderate VD deficiency in 9(18%) of the studied patients, P=0.47 **(table 6)**. Hence more than half of patient group and control subjects suffered from VD deficiency.

Tessema et al. (2017), found that vitamin D deficiency and insufficiency (level less than 20 ng/mL and 20-29 ng/mL respectively) was reported in TB and non-TB subjects, hence, of the total

study participants, 134 (46.2%) were vitamin D deficient, and only 56 (19.3%) had sufficient vitamin D level. A total of 59 (61.5%) TB patients and 75 (38.7%) non-TB controls were vitamin D deficient.

The current study reported that, the change of serum level of vitamin D was significant after sputum conversion, it elevated from 30.3±10.2 ng/L to 31.0.9±9.63 ng/L, P = 0.02 **(table 7)**. That came in agreement with Afzal et al. (2018); hence, the level of serum VD in placebo group (TB patients that did not received VD supplementation) was changed from 17.07±1.44 to 21.77± 2.23 ng/mL.

In different study, anti-tuberculous therapy (ATT) drugs especially isoniazid and rifampicin influence Vitamin D metabolism causing low levels of vitamin D during course of the treatment *(Sato et al., 2012)*.

Regarding the duration of conversion in TB groups, the more the severity of VDD the longer the time need for conversion. As shown in **(Table 8)** the mean duration in patients with normal VD status was 3 weeks opposite to 4 and 5 weeks in patients with mild and moderate level of VD deficiency, with significant statistical difference, P < 0.001.

The main role of vitamin D is to maintain the function of monocytes and macrophages which are linked to human innate immunity to certain infectious agents, this role is very important in body's natural defense against infection in which macrophages plays a vital role in pathogenesis. Vitamin D act by combining the nuclei receptor on affected cells so both abnormality in receptor function and structure or low vitamin D level alter the immunity against tubercle bacillus *(Huang et al., 2017; Harishankar et al., 2017)*.

In spite of controversial results of the previous studies about the level of VD in TB patients and its changes along with the course of treatment, a significant data suggested that the application of VD supplementation beside the first line treatment therapy of TB might augment the effect of treatment, and enhance the clinical improvement of patients *(Hassanein et al., 2016; Wu et al., 2018; Afzal et al., 2018)*.

CONCLUSION

The present work concluded that; vitamin D deficiency was common in patients with pulmonary TB and that level was significantly associated with healing and good response to treatment.

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تقييم مستوى فيتامين (د) في الدم لدى مرضى الدرن الرئوي قبل وبعد التحول السلبي للبصاق

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خلفية البحث: يعد مرض الدرن الرئوي مشكلة صحية رئيسية علي مستوي العالم حيث تشير التقديرات إلي أن هناك عشرة ملايين حالة إصابة جديدة سنويا وأن مليون ومائة ألف شخص قد ماتوا بسبب الدرن عام 2018 كما أن معدل انتشاره يزداد في الدول النامية التي من بينها مصر، ويعد فيتامين (د) من محفزات المقاومة لمرض الدرن حيث أنه ينشط استجابة الخلايا المناعية ضد البكتريا المسببة للدرن، وقد أشارت الدراسات إلي وجود صلة وثيقة بين انخفاض مستوي فيتامين (د) والاصابة بمرض الدرن.

الهدف من الدراسة: تقييم مستوي فيتامين (د) في الدم لدى مرضى الدرن الرئوي وتأثيره علي التحول السلبي للبصاق.

المرضي وطريقة البحث: أجريت هذه الدراسة في مستشفي صدر العباسية علي سبعين شخصا تم تقسيمهم إلى مجموعتين؛ المجموعة الأولى وتشتمل على خمسين مريضا مصابون بالدرن الرئوي وتم حجزهم بقسم الدرن بالمستشفي والمجموعة الثانية وتشتمل على عشرين شخصا سليما من العاملين بالمستشفي أواقارب المرضى بعد الموافقة من المريض أو أهليته علي الاشتراك في البحث الذي تم طبقا لتوصيات لجنة الأخلاقيات الطبية بجامعة الأزهر وذلك في الفترة الزمنية من يناير 2020 حتي ديسمبر 2020 وبعد استبعاد الحالات التي لا تتماشى مع معايير هذه الدراسة تم أخذ التاريخ المرضي كاملا لكل المرضى مع إجراء فحص إكلينيكي شامل، صورة دم كاملة، عمل أشعة عادية علي الصدر خلفي أمامي، فحص البصاق للكشف عن البكتريا المسببة للدرن باستخدام الصبغة الخاصة بالميكروب وتم سحب عينات لقياس مستوى فيتامين (د) في الدم لدى المرضى الذين ثبت إيجابيتهم لميكروب الدرن قبل وبعد التحول السلبي للبصاق

وحساب المدة المستغرقة للتحول السلبي للبصاق وعلاقة ذلك بمستوى فيتامين (د) في الدم لدى هؤلاء المرضى كما تم سحب عينات لقياس مستوى فيتامين د في المجموعة الثانية من الأشخاص الغير مصابين بالدرن.

نتائج البحث: أظهرت الدراسة ان هناك نقصا ذو دلالة إحصائيه في مستوى فيتامين (د) في الدم لدى مرضى الدرن الرئوي مقارنة بمستواه في الأصحاء (30.3±10.2) ng/ml، (38.81±3.48) ng/ml على التوالي في بداية الدراسة، و قد استغرق المرضى الذين ثبت لديهم نقص في مستوى فيتامين (د) في الدم فترة أطول في التحول لسلبية البصاق.

الإستنتاج: أن مستوى فيتامين (د) في الدم لدى مرضى الدرن الرئوي أقل من الأشخاص الأصحاء وأن له علاقة وثيقة بفترة التحول السلبي للبصاق فكلما كان مستوى فيتامين (د) في الدم أقل كلما استغرق المريض فترة أطول للتحول السلبي للبصاق.

الكلمات الدالة: الدرن الرئوي، مستوى فيتامين (د) فى الدم.