Association of Vitamin D level with Tuberculosis in Iraqi patients

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Abstract

Back ground: Tuberculosis (TB) has troubled mankind for millennia, Deficiency of vitamin D has long been concerned in activation of Tuberculosis, vitamin D influences the immune response to tuberculosis and vitamin D deficiency has been associated with increased risk of tuberculosis in different populations.

Objectives: This study was designed to show the relationship between vitamin D level (deficiency) and risk of tuberculosis.

Methods: 140 blood samples of tuberculosis patients and 50 from control group (25 healthy control and 25 of others respiratory disease) were taken from Specialized Chest and Respiratory Diseases Center \ Baghdad to detect vitamin D level using CMIA technology.

Results: Vitamin **D** was low in all tuberculosis patients and more occur in middle age than old age .

Conclusions: The current study had shown an association between serum levels of vitamin D and tuberculosis. Vitamin D deficiency is probably a risk factor for tuberculosis.

Keywords: *tuberculosis; vitamin D; ZN; Mycobacterium tuberculosis; IRAQ.*

علاقة مستوى فيتامين (د) لدى مرضى السل العراقيين

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الخلاصة

استعراض المراجع: يعد السل (التدرن) هو من الامراض المعدية التي تصيب البشرمنذ آلاف السنين ، وان نقص فيتامين (د) منذ له دورمهم في تفعيل الاصابة بالمرض حيث يؤثر على الاستجابة المناعية لمرضى السلز وارتبط نقص فيتامين (د) مع زيادة خطر الإصابة بالسل في مختلف المجتمعات.

الأهداف: تم تصميم هذه الدراسة لإظهار العلاقة بين مستوى فيتامين (د) وخطر الإصابة بالسل.

طريقة العمل: تم أخذ 140 عينة دم لمرضى السل و 50 من مجموعة سيطرة (25 شخص يبدون بحالة صحية جيدة سريريا و 25 آخرين مصابين بأمراض الجهاز التنفسي غير تدرن) الوافدين الى مركز أمراض الصدر والجهاز التنفسي التخصصي/ بغداد للكشف عن مستوى فيتامين (د) باستخدام تقنيةCMIA

النتائج: كان فيتامين (د) منخفضًا في جميع مرضى السل ويحدث في الاشخاص بمنتصف العمر أكثر من كبار السن.
الاستنتاجات: أظهرت الدراسة الحالية وجود علاقة بين مستويات مصل فيتامين (د) والسل. فقد يكون نقص فيتامين (د) أحد عوامل الخطر لمرض السل

الكلمات المفتاحية: *التدرن ; فيتامين د;* Zn ; المتفطرة السلية ; العراق .

Introduction

Tuberculosis (TB) is an old infectious disease and one of the top 10 causes of death, the causative agent is the bacillus *Mycobacterium tuberculosis* (Mtb) [1]. The clinical features of Vitamin D (Vit.D) deficiency associated with signs and symptoms suggestive of tuberculosis (TB) were first described during the nineteenth century, cod liver oil, rich in Vit.D, was largely distributed in TB patients[2] .Vit.D is a fat soluble vitamin [3] it is either consumed in the diet or produced in the skin following sunlight exposure , Vit.D is inactive and is hydroxylated twice; once in the liver and once in the kidney to make the active form of the vitamin, 1,25 dihydroxy Vit.D [4] Vit.D metabolism involves both endocrine and paracrine systems [5] Vit.D modulates the immune system to fight Mtb by promoting phagosome maturation and enhancing the production of anti- microbial peptides [6] .

Vit.D exerts its effects via activation of its cellular receptor Vit.D receptor [VDR] which in turn alters the transcription rates of target genes responsible for various biological responses, it has a definitive role in suppression of proliferation of Mtb and generalized inflammatory response produced secondary to it. Similarly, on triggering of toll-like receptors by molecules of the tubercle bacillus, the production of microbe-killing cathelicidin is impaired in the absence of adequate serum Vit.D[7], So TB patients have an elevated rate of Vit.D deficiency [8].

Material and Methods

Clinical samples

In this study, the blood specimen and sputum was obtained from 140 pulmonary TB patients aged from (12-70) years old with cough for more than two weeks were investigated during the study period in Specialized Chest and Respiratory Diseases Center \ Baghdad from October 2018 to March 2019. In addition,50 specimens were collected from apparently healthy patients or with other bacterial chest infection than TB.

Measurement of Vitamin D levels in blood

The architect 25-OH Vitamin D assay is a quantitative delayed one –step competitive immunoassay to determine the presence of Vit.D in human serum and plasma using CMIA technology with flexible assay protocols referred to as chemiflex.

The assay was done according to manufacturer's instructions. Briefly: mixing the micro particle bottle to re-suspend micro particles. Then Invert the micro particle bottle 30 times. Loaded the reagent kit on the architect system and Loaded samples (60 ul). The results were calculated automatically and printed on computer screen. The normal values of D levels in blood 30-40 ng/mL was compared with our results.

Detection for tuberculosis

all sputum specimens were cultured on LJ medium after doing slide smear staining by acid fast ZN stain [9].

Results and Discussion

Study subjects groups: Patients were grouped according to their microbiological, radiological, clinical findings at enrollment, and if a new diagnosis without any TB drug treatment or old TB Patients under treatment.

A total of 140 blood specimens from tuberculosis patients (122 males and 68 females) (80 new and 60 old) The mean age was 38.63 ± 14.27 and 38.03 ± 10.99 for new and old patients respectively. In addition to 50 samples as control from adults without TB. The new and old Patients were divided according Smear ZN stain results as shown in table 1

i. TB smear positive (S+) with microbiologically confirmed pulmonary TB those with smear (+) culture (+)

ii. TB smear positive (S−) Patients with microbiologically confirmed pulmonary TB those with smear (-), culture (+).

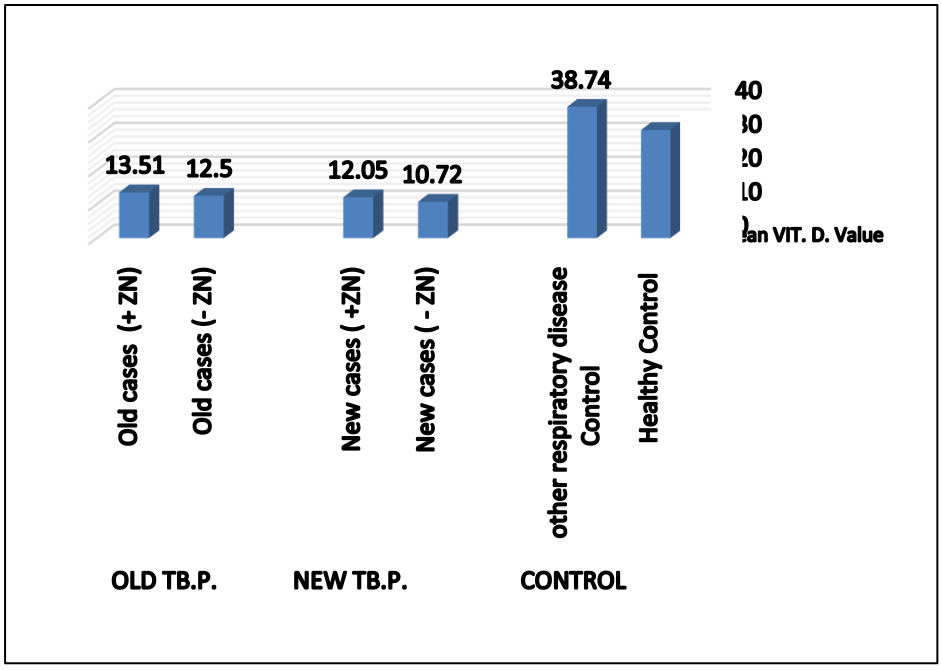
iii. No TB as control: 50 Patients with bacterial chest infection or apparently healthy who were sputum smear negative and culture negative no bacteriological, clinical, pathological, or radiological evidence of tuberculosis and responded to antibiotic treatment with amoxicillin, co-trimoxazole, or cefpodoxime with full recovery. No anti-TB treatment was given to these patients.

Table (1) and fig. (1) clarify that Mean ±Std of Vit.D were 35.31±4. ng/mL 70 in healthy control with mean 11.80±5.04 ng/mL and 13.20±4.97 ng/mL In new cases and old TB patients respectively.

The Vit.D level in smear positive was 12.05±5.09 ng/mL and 13.51±5.34 ng/mL in new and old TB patients respectively as showed it was higher Vit.D level in smear negative TB patients which were 10.72±4.79 ng/mL and 12.50±4.04 ng/mL in new and old TB patients respectively Data illustrated by table (2) show that there was no significant difference in the level of Vit.D between between smear positive and smear negative New cases P>0.05 Similarly comparison among Old cases showed a non-significant between smear positive and smear negative patients.

Table (1): Level of Vitamin D for groups study

Study group			No.	Vit. D –Value(ng/ml)		
				Mean ±Std.	Min	Max
No TB as control			50	38.31±4.70	29.4	45.1
TB 140	**New cases**	New cases (- ZN)	15	10.72±4.79	5.8	20.7
		New cases (+ZN)	65	12.05±5.09	4.3	24.1
		Vit. D of Total New cases	80	11.80±5.04	4.3	24.1
	Old cases	Old cases (- ZN)	18	12.50±4.04	6	22.2
		Old cases (+ ZN)	42	13.51±5.34	5.7	24.1
		Vit. D of Total Old cases	60	13.20±4.97	5.7	24.1



Figure(2): Mean of vitamin D value

Table (2): Comparison in vitamin D level between studied groups

Vitamin D Value (ng/ml)	t-test	P-Value	C.S
New cases (- ZN) *Ve* New cases (+ ZN)	0.920	.361	P>0.05(NS)
Old cases (- ZN) *Ve* Old cases (+ ZN)	0.715	.478	P>0.05(NS)
Vit.D of Control *Ve* Vit.D of New cases	26.556	.000	P<0.01(HS)
Vit.D of Control *Ve* Vit.D of Old cases	23.800	.000	P<0.01(HS)
Vit.D Value\Healthy Control *Ve* Vit.D –Value\ New cases	19.682	.000	P<0.01(HS)
Vit.D Value \Healthy Control *Ve* Vit.D -Value \Old cases	18.479	.000	P<0.01(HS)
Control *Ve* New cases (- ZN)	17.687	.000	P<0.01(HS)
Control *Ve* New cases (+ZN)	25.092	.000	P<0.01(HS)
Control *Ve* Old cases (- ZN)	18.274	.000	P<0.01(HS)
Control *Ve* Old cases (+ ZN)	20.828	.000	P<0.01(HS)

(NS): Non significant ;**(HS):** highly significant

While a highly significant difference was found between control and smear positive and negative in both old and new cases and the association between level of Vit.D of healthy control and level of Vit.D in old and new cases were with highly significant P<0.01.

This results is in line with results In Brazil which showed that Mean Vit. D level was significantly lower among the TB cases (27.7 ± 7.85 ng/mL) than Healthy controls (37.1 ± 8.94 ng/mL by [10], similarly results from Korea agree with the result of the present study showed that serum concentrations of Vit.D, was significantly lower in patients with tuberculosis than in control subjects (10.6 vs. 19.3 ng/mL, $P < 0.001$) [11].

Such situation explained that low levels of Vit.D have been shown to be associated with an enhanced susceptibility to develop active TB [12]. Low levels of Vit.D varies depending on the population studied, adherence to food fortification policies, demographic features, geographic location to [13]. Table(3) showed that from the 50 samples control the level of Vit.D were within normal rang in males more in females which were 24 in males while in females 17 ,and also in males 6 samples showed level above 40 while in femals were only one ,in females no samples were low 30 while in males 2 samples were below 30 this relation was statistically non significant difference P>0.05 **,** in TB patients all males (90) were below 30 and all females (50)were below 30 and this relation also was non- significant P>0.05 .

The relation between Vit.D level and genders of both control and patients showed a highly significant difference p<0.01. Several studies by Tostmann *et al* [14] from Tanzania and Ho-Pham *et al*. [15] from Vietnam have reported a significant association between Vit.D deficiency and TB.

Table (3): Comparison in Level of Vitamin D of TB Patients and Control Groups according to the Gender

Groups		Vitamin D Value (ng/ml)					
		Normal (30-40)	**High >40**	**Low <30**	**Total**	**P.value**	**C.S**
Control (n=50)	Male	24	6	2	32	0.000	P<0.001 (HS)
	Female	17	1	0	18		
	p-value	0.213					
	C.S	P>0.05 (NS)					
patients (n=140)	Male	0	0	90	90		
	Female	0	0	50	50		
	P-Value	0.319					
	C.S	P>0.05 (NS)					

In the present study, it was noted that a higher level of Vit.D deficiency was observed among females compared with males and this agree with Tessema *et al.* [16] from Ethiopia who showed that 134 patients who had low levels of Vit.D 78(52.3%) were females and 56(39.7%) were males. Another study from Pakistan which included 109 samples showed that Vit.D levels was significantly lower in 74 female patients and in 54 male patients by [17] , while in United Kingdom Vit.D levels in the males were significantly lower Male/Female (24.5 ± 3.65 ng/ml) showed by [18] .

Possible reasons for this female preponderance might be due to poorer nutritional status than their male counterparts, inadequate exposure to sunlight because of the culture of most females to stay at home, and pregnancy experiences.

Table (4) show comparison the level of Vit.D in TB patients groups according to the Age, so there are no significant differences P>0.005 between level of Vit.D and all age groups of TB patients all of which had low level of Vit.D.

Table (4): Comparison the Level of Vit. D in TB patients according to the age.

Groups		Vitamin D Value ng/ml			Total
		Normal (30-40)	**High >40**	**Low <40**	
Patients (n=140)	15-24 y	0	0	22	22
	25-34 y	0	0	38	38
	35-44 y	0	0	32	32
	45-54 y	0	0	34	34
	55-64	0	0	10	10
	65 &more	0	0	4	4
P- Value		0.626			
C.S		P>0.005 (NS)			

Conclusions

The current study had shown an association between serum levels of Vitamin D and tuberculosis. Vitamin D deficiency is probably a risk factor for tuberculosis

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