

Physiology and Anatomy

Elixir Physio. & Anatomy 95 (2016) 41159-41162

Elixir
ISSN: 2229-712X

25-hydroxy cholecalciferol in newly diagnosed pulmonary tuberculosis patients

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ARTICLE INFO

Article history:

Received: 13 May 2016;

Received in revised form:

17 June 2016;

Accepted: 22 June 2016;

Keywords

Pulmonary tuberculosis,
25-hydroxycholecalciferol
Sindh.

ABSTRACT

The present study was conducted to evaluate 25 hydroxy cholecalciferol levels in newly diagnosed pulmonary tuberculosis patients. Comparative case control study. Department of Medicine, Liaquat University of Medical and Health Sciences Hospital Jamshoro/Hyderabad, from February to October 2015. 100 diagnosed cases of pulmonary tuberculosis and 100 age and gender matched controls were selected according to criteria. 5 ml of venous blood sample was collected and centrifuged at 4000rpm for 10 minutes. The serum obtained was frozen at -20°C and used for estimation of 25-hydroxycholecalciferol on ARCHITECT I 1000 system. The data was analyzed on SPSS version 22.0 at 95% confidence interval ($p \leq 0.05$). Total mean \pm S.D of 25-hydroxycholecalciferol was noted as 26.1 ± 9.76 and 38.8 ± 7.14 (ng/dl) in cases and controls respectively ($p=0.0001$). 25-hydroxycholecalciferol <5.4 ng/dl was noted in the tuberculosis cases. Normal, insufficiency and deficiency of 25-hydroxycholecalciferol was noted in 17%, 19% and 64% & 56%, 18% and 26% of cases and controls respectively ($p < 0.01$). The present study reports significantly low 25-hydroxycholecalciferol in pulmonary tuberculosis cases. However further studies are warranted. Pulmonary tuberculosis patients may be supplemented with vitamin D.

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Introduction

The 25-hydroxycholecalciferol, commonly called vitamin D₃, is a fat soluble vitamin of secosteroids family. It is also known as sunlight vitamin. Its deficiency prevalence is reported as 50% in developing countries and 30% in the developed European adult population.¹ Serum 25-hydroxycholecalciferol is a sensitive indicator of vitamin D ordered in routine clinical practice² and the prevalence of 25-hydroxycholecalciferol insufficiency is 30% - 50% in the general adult population.³ 25-hydroxycholecalciferol is unique as being vitamin, it functions as a hormone in its activated form. It is involved in the physiology of calcium homeostasis and helps to mineralize the bones. It counteracts the bone wear and tear of parathyroid hormone. It stimulates cell differentiation, proliferation and growth. 25-hydroxycholecalciferol plays crucial role in the immune reactions.⁴⁻⁷ It augments the physiological functioning of macrophage, thus helps in killing of pathogenic organisms. Killing of intracellular pathogens like Mycobacterium tuberculosis by macrophage is augmented by 25-hydroxycholecalciferol.⁶⁻¹⁰ Reported recently is the role of 25-hydroxycholecalciferol in preventing neoplastic growth. Its deficiency has been speculated in systemic hypertension, cancers, infections, autoimmunity and diabetes mellitus.^{10,11} 25-hydroxycholecalciferol alters gene expression in the cells and tissues through Vitamin D specific receptors (VDR).⁶⁻⁸ 25-hydroxycholecalciferol deficiency has been suggested a risk factor for the pathogenesis of tuberculosis.^{12,13} Previous

studies had reported association of 25-hydroxycholecalciferol deficiency and pulmonary tuberculosis.¹⁴ Asymptomatic deficiency of 25-hydroxycholecalciferol is very common in Pakistan,^{15,16} and similarly the pulmonary tuberculosis. It is included in first five countries of high prevalence of tuberculosis.¹⁷ Instead of high prevalence of 25-hydroxycholecalciferol deficiency and high prevalence of tuberculosis, literature is lacking on the association of both. The present prospective study was planned to evaluate the 25-hydroxy cholecalciferol levels in newly diagnosed smear positive pulmonary tuberculosis patients.

Subjects and Methods

The present comparative case control study was conducted prospectively at Department of Medicine, Liaquat University of Medical and Health Sciences Hospital Jamshoro/Hyderabad, from February to October 2015. A sample of 100 diagnosed cases of pulmonary tuberculosis and 100 age and gender matched controls were selected according to criteria. Inclusion criteria observed were; a typical clinical history of weight loss, evening pyrexia, night sweating, sputum positive for AFB (acid fast bacilli), and raised erythrocyte sedimentation rate (ESR) and pulmonary cavitation's. Willing participants was also the inclusion criteria. Patients with history of anti-tuberculosis drug therapy for > 3 months, extra pulmonary tuberculosis anti tuberculosis drug Defaulters, patient taken vitamin D supplements

during last three months and smokers were excluded from the study. All subjects were screened by a medical officer and were diagnosed by a consultant physician or a chest specialist. 5 ml of venous blood sample was collected and centrifuged at 4000rpm for 10 minutes. The serum obtained was frozen at -20°C and used for estimation of 25-hydroxycholecalciferol ARCHITECT I 1000 system. Institutional ethical approval was taken. Informed consent form was signed by each willing participant. Patient's data was noted on a pre-structured proforma. Confidentiality of patients information was ensured. The data was analyzed on SPSS version 22.0. Student's t test and chi square test was used for continuous and categorical data respectively at 95% confidence interval ($p \leq 0.05$).

Results

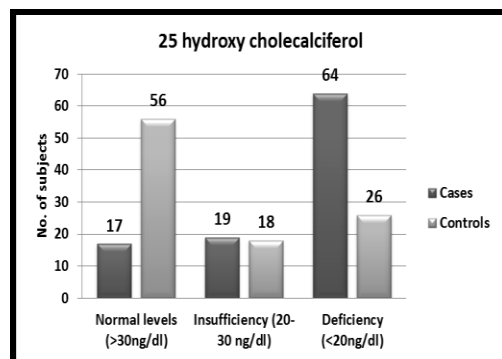
Cases and controls showed mean \pm S.D age of 49 ± 6.7 and 48.1 ± 5.6 years respectively. The baseline characteristics of cases and controls are shown in table 1. Age and gender distribution showed non-significant ($p > 0.05$) differences. BMI revealed low body weight of cases. Tuberculosis cavity was noted in 69 (69%) of cases. Complete blood counts showed low hemoglobin and red blood cell counts in the cases compared to controls ($p < 0.05$). Alkaline phosphatase, serum calcium & phosphate, blood urea nitrogen and serum creatinine were evaluated at baseline as shown in table 1. Total mean \pm S.D 25-hydroxycholecalciferol was noted as 26.1 ± 9.76 and 38.8 ± 7.14 (ng/dl) in cases and controls respectively ($p = 0.0001$). 25-hydroxycholecalciferol less than 5.4 ng/dl was noted in the cases. Table 2 shows the normal, insufficiency and deficiency of 25-hydroxycholecalciferol in cases and controls. Graph 1 shows the normal, insufficiency and deficiency of 25-hydroxycholecalciferol in 17%, 19% and 64% & 56%, 18% and 26% of cases and controls respectively ($p < 0.01$).

Table 1. Characteristics of cases and control subjects.

	Case (n=100)	Control (n=100)	p-value
Age (years)	49 ± 6.7	48.1 ± 5.6	0.06
Male	56 (56%)	44 (52%)	0.09
Female	46 (46%)	54 (48%)	0.07
BMI (kg/m^2)	25 ± 5.9	29 ± 3.5	0.03
Hemoglobin (g/dl)	10 ± 5.9	13 ± 2.9	0.021
RBC counts ($\times 10^9/\mu\text{l}$)	2.49 ± 5.1	4.1 ± 3.7	0.012
WBC counts ($/\mu\text{l}$)	7283 ± 78	70178 ± 56	0.07
Platelets ($\times 10^9/\mu\text{l}$)	3.57 ± 2.1	3.73 ± 1.4	0.06
Erythrocyte sedimentation rate (mmFHR)	57 ± 19	5 ± 3.5	0.001
Alkaline phosphatase (IU)	107.3 ± 5.6	103.5 ± 11.2	0.01
Serum calcium (mg/dl)	7.90 ± 1.9	9.3 ± 0.7	0.002
Serum phosphate (mg/dl)	2.13 ± 0.4	2.15 ± 0.6	0.05
Blood urea nitrogen (mg/dl)	7.90 ± 1.9	9.3 ± 0.7	0.002
Serum creatinine (mg/dl)	2.13 ± 0.4	2.15 ± 0.6	0.05

Table 2. 25-hydroxycholecalciferol in cases and controls (n=200).

	Case (n=100)	Control (n=100)	p-value
Normal levels ($>30\text{ng/dl}$)	37.5 ± 5.67	39.5 ± 10.7	0.001
Insufficiency (20-30 ng/dl)	23.2 ± 2.7	29.1 ± 3.13	0.001
Deficiency ($<20\text{ng/dl}$)	12.78 ± 3.6	16.6 ± 1.5	0.001
Total	26.1 ± 9.76	38.8 ± 7.14	0.001



Graph 1. Frequency of 25-hydroxycholecalciferol in cases and controls.

Discussion

The 25-hydroxycholecalciferol has been reported to augment the killing of bacterial pathogens by the macrophages.^{18,19} As macrophages play pivotal role in immune reactions against mycobacteria this shows the essentiality of 25-hydroxycholecalciferol because it stimulates the gene expression in immune cells. Previous studies have also reported that the 25-hydroxycholecalciferol suppresses the growth of mycobacterium tuberculosis.^{18, 19} It is reported that the production of microbe killing cathelicidin protein is defective in 25-hydroxycholecalciferol deficient subjects, which is essential for killing of tubercle bacilli.²⁰ However, an *in-vivo* evidence of this association is debatable. In the present study, 25-hydroxycholecalciferol was noted as 26.1 ± 9.76 and 38.8 ± 7.14 (ng/dl) in cases and controls respectively ($p = 0.0001$) which shows serious vitamin D deficiency in pulmonary tuberculosis patients. In present study, the tuberculosis patients were having low BMI which may be considered as a confounding factor.

It is reported that the anti-tuberculosis drug therapy may induce vitamin D deficiency,²¹ hence drug naïve cases were selected in the present study.

A previous study reported association of 25-hydroxycholecalciferol deficiency in tuberculosis about 2 decades before, but later studies^{21, 22} showed controversial results. However, previous studies²³⁻²⁵ reported vitamin 25-hydroxycholecalciferol deficiency in tuberculosis patients. A study from Australia reported low 25-hydroxycholecalciferol levels in latent or active pulmonary tuberculosis patients. There is evidence that the low 25-hydroxycholecalciferol compromises cell immunity and activation of even latent tuberculosis.²⁶⁻²⁸

Additional information of role of 25-hydroxycholecalciferol against mycobacteria comes from the increased incidence of tuberculosis in chronic kidney disease patients in whom the vitamin D is deficient and not activated by the kidneys. Hence, this is in argument that the 25-hydroxycholecalciferol plays crucial role in immune reactions against mycobacterium tuberculosis.^{27, 28}

Moreover, in the present study 25-hydroxycholecalciferol deficiency was prevalent in both male and female population in both controls and cases; this suggests vitamin D supplementation may be served for the normal as well as tuberculosis patients. Frequency of 25-hydroxycholecalciferol in present study is in agreement with previous studies.^{16,29} A previous study³⁰ reported very high frequency of vitamin D deficiency in Pakistani population, which is in agreement to present study.

The most probable reason could be the different sample size, vitamin D criteria, and darkly pigmented skin people, chewing and food habits, may explain the controversial results. We postulate that the vitamin D supplements may be effective as add-on therapy in pulmonary tuberculosis patients, and our postulation is supported by the previous studies.^{31,32} The limitations of the present study include poor social class of pulmonary tuberculosis patients was studied with low BMI which is a risk factor of vitamin D deficiency; however, the study strength lies in its inclusion and exclusion criteria. Patients taking anti tuberculosis drug therapy and vitamin D supplements and smokers were excluded; this shows the strength of the present study. Further studies with large sample size are recommended.

Conclusion

The present study reports significantly low 25-hydroxycholecalciferol in pulmonary tuberculosis patients. However further studies are warranted. Pulmonary tuberculosis patients may be supplemented with vitamin D.

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