Original Article

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Microbiology Section

Serum Vitamin D Level as a Risk Factor for Female Genital Tuberculosis (FGTB)

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ABSTRACT

Introduction: Vitamin D is now known to be essential to *Mycobacterium tuberculosis* containment and killing through activation of 25-hydroxyvitamin-D receptors (VDRs) present on all immune cells or obtained from dietary food stuffs as either vitamin D3 or vegetable vitamin D2 (also known as ergocalciferol).

Aim: To evaluate the association of serum vitamin D level between the Female Genital Tuberculosis (FGTB) cases and healthy controls.

Materials and Methods: Total 120 cases and 120 controls enrolled for the study following inclusion and exclusion criteria. Detailed clinical history was taken from each subjects. Total of 3 ml of the blood was collected in EDTA vial from each subject (case and control). Quantification of serum vitamin D level was measured by active human vitamin D ELISA kit using an ELISA reader. Statistical analysis was done using Statistical Package for Social Science (SPSS) version 21.0. A p-value <0.05 was considered as significant.

Results: A total of 120 confirmed FGTB cases and 120 healthy control enrolled for study. Out of 120 women 97.5%, 10.0%, 3.3%, 3.3% were detected positive for *M. tuberculosis* respectively. Comparing the mean demographic value of age and BMI were (29.03±3.127, 28.03±3.00) and (22.92±3.33, 24.15±3.97) respectively with the p=0.012* and p=0.010* found to be significant among cases and controls. The mean serum vitamin D level was 14.96±8.81 in cases and 23.00±8.83 in controls with p-value<0.001. There was a significant positive association found in low serum vitamin D level among FGTB cases than controls.

Conclusion: Vitamin D is important for normal immune cell function, as well as regression of FGTB disease. FGTB may be controlled by regulating the serum vitamin D level concentration. This study suggests that, vitamin D deficiency and BMI is strongly associated with the progression of active FGTB disease which alters the expression of antimicrobial peptide and lead to the persistence of TB infection. Therefore, serum vitamin D level may play an important role in treatment of FGTB.

Keywords: Body mass index, Cathelicidin, Infertility, Lowenstein-Jensen, Mycobacteria growth indicator tube

INTRODUCTION

Tuberculosis (TB) remains a great burden and major global health problem and the concerned mechanism of pathogenesis involved in the progression of disease needs to be explored [1]. TB exists in two forms: pulmonary tuberculosis and Extra-Pulmonary Tuberculosis (EPTB). Genital tuberculosis is one form of EPTB and it represents 12% of patients with pulmonary tuberculosis and 15%-20% of EPTB [2]. Transmission of disease occurs via the respiratory tract, and approximately 80% of those with active TB develop pulmonary disease. Some patients develop concurrent (secondary) EPTB, while in others the infection develops primarily in an extra-pulmonary organ. Symptoms of EPTB are non-specific and depend on the affected sites [3]. The term 'vitamin D' specifically refers to the parental vitamin D produced endogenously by the action of sunlight on 7-dehydrocholesterol in skin (also known as vitamin D3, or cholecalciferol) [4]. Antimicrobial activity of vitamin D has been accomplished by inhibiting the growth of *M. tuberculosis* and up-regulating innate host responses [5]. Intracellular *M. tuberculosis* bacilli killed through the production of Nitric Oxide (NO) as well as antimicrobial peptide human cathelicidin (LL-37). Serum level of 25-hydroxyvitamin D up to or more than 30 ng/ml is sufficient for the conversion of its inactive form to bioactive form, which enhances the expression of cathelicidin (antimicrobial peptide). Simultaneously, if levels of 25(OH)D found to be less than 20 ng/ml, patient is immunocompressed [6]. Addition of vitamin D to standard Anti Tubercular Therapy (ATT) drug regimen results in faster clearance of the infection [7]. Previous evidence suggests that hypovitaminosis D is associated with the susceptibility for cancer, autoimmune disease, diabetes and cardiovascular disease, which indicates the importance of sufficient vitamin D level [8]. A therapeutic value of the immunomodulatory effect of vitamin D on tuberculosis patients has already been proven in randomized, controlled clinical trials [9]. Vitamin D is supposed to be functionally important factor in susceptibility to bacterial as well as viral infection. It shows the significance of vitamin D, in the treatment of TB [10]. Low serum Vitamin D is premeditated to be correlated with tuberculosis while the "dangerous" level was still unclear [11]. Perceptivity to TB is also influenced by environmental and genetic factors or by gene-environment interactions [12]. The aim of this study was to identify the association between female genital tuberculosis and serum Vitamin D levels via synthesis of available evidence.

MATERIALS AND METHODS

This case-control study was done in Department of Obstetrics and Gynaecology at Department of Microbiology, King George's Medical University, Lucknow, India, from July 2015 to September 2016. Subjects were recruited with previous ethical approval and informed consent form was taken from each outdoor patient. Total 240 subjects (n=120 cases and n=120 controls) between age group 20-35 years were included in the study on the basis of inclusion and exclusion criteria. The sample size was calculated with 80% of power.

Ethical Statement

Study was approved by from the Institutional Ethical Committee (No.6139/Ethics/R.Cell-15) of KGMU, Lucknow, India.

Subject Selection Criteria

All subjects met the confirmed inclusion criterion for selecting FGTB suspected cases indicating clinical symptoms such as infertility

among reproductive age group (20-35 years), infertility primary or secondary for a period greater than one year, subjects positive for any one of these test on Acid-Fast Bacilli (AFB)/TB-PCR/ Lowenstein-Jensen (LJ)-culture/Mycobacteria Growth Indicator Tube (MGIT) culture in Endometrial Aspiration (EA), subjects were willing to follow protocol or who gave consents to participate. Controls were recruited with age group 20-35 years, no history of ATT and all test negative for TB.

Exclusion criteria: for cases and controls were following: subjects above 35 years of age, with endometriosis, Polycystic Ovarian Syndrome (PCOS), and polycystic ovaries, having chlamydia, gonorrhoea, subjects already on ATT or diagnosed to have active pulmonary tuberculosis or active extra pulmonary tuberculosis in regions other than female genital tract. Subjects having any genetic disease were also excluded.

Clinical Investigation

Detailed clinical history as well as family history and socioeconomic status were collected from each participant. A detailed physical examination (including general and gynaecological examination), biochemical investigations {such as T3, T4, TSH, complete Haemogram, Mantoux test (PPD), chest X-ray, Ultrasonography of abdomen, Hysterosalpingography (HSG)} and pathological examination as TB-PCR, AFB, LJ-culture, MGIT-culture was conducted for all included subjects.

Serum Vitamin D level Estimation

A total of 3ml of the blood was collected in EDTA vial from each participant and stored at -20°C. One milliliter of blood sample was used quantification for serum vitamin D level in cases as well as in control. Vitamin D level was measured by active human vitamin D ELISA kit (Bi0-Detect cat#071) using an ELISA reader.

STATISTICAL ANALYSIS

Categorical variables were presented in number and percentage (%) and continuous variables were to be presented as mean±SD. Quantitative variables was compared using Unpaired t-test. Qualitative variables were compared using Chi-square test/Fisher's-exact test as appropriate. A p-value of <0.05 was considered statistically significant. The data was entered in MS EXCEL spreadsheet and analysis was done using SPSS version 21.0.

RESULTS

During the study, 120 confirmed FGTB cases and healthy control enrolled for study following inclusion criteria. For the confirmation of *M. tuberculosis* in enrolled women, number of test was done as TB-PCR, AFB, LJ Culture, MGIT Culture. Out of 120 women 97.5%, 10.0%, 3.3%, 3.3% were detected positive for *M. tuberculosis* respectively. All these 120 FGTB Cases were further confirmed by Diagnostic Laparoscopy (DL). Of 120 cases 61 were normal i.e., Level-1 and 59 were suspected i.e., Level-2.

The demographic variables (Age and BMI) and biochemical parameters (haemoglobin, thyroid, prolactin) between cases and controls are summarized in [Table/Fig-1]. Comparing the mean demographic value of age and BMI were (29.03±3.127, 28.03±3.00) and (22.92±3.33, 24.15±3.97) respectively with the p=0.012 and p=0.010 found to be significant among cases and controls. However, in biochemical investigation (haemoglobin, thyroid, prolactin) there was no significant correlation found between cases and controls [Table/Fig-1,2]. The mean serum vitamin D level was 14.96±8.81 in cases and 23.00±8.83 in controls with p-value <0.001. Statistical analysis showed a significant association between FGTB cases and controls.

Levels of serum 25(OH)D observed in different sociodemographic parameter are shown in [Table/Fig-1]. There was positive correlation found between age, BMI, and serum vitamin D level with p=0.012 and p=0.010 respectively [Table/Fig-1]. The prevalence of low 25(OH)D3 level in FGTB patients than in controls. To demonstrate the role of Thyroid, serum prolactin, PPD, and tubercular test like TB-PCR, AFB, LJ-Culture and reduction of serum vitamin D level among FGTB cases, it was found that therewas no obvious correlation between above parameters [Table/Fig-2].

Variables	Cases (n=120)	Controls (n=120)	p-value
Age	29.03 ± 3.127	28.03 ± 3.00	0.012*
Height (cm)	155.50 ± 6.17	154.28 ± 6.78	0.147
Weight (kg)	55.37 ± 7.97	58.66 ± 18.71	0.078
BMI (kg/m^2)	22.92 ± 3.33	24.15 ± 3.97	0.010*
Haemoglobin	11.49 ±1.63	11.29 ± 1.30	0.297
Vitamin-D Level (ng/ml)	14.96 ± 8.81	23.00 ± 8.83	<0.001*

[Table/Fig-1]: Demographic distribution of FGTB cases and healthy controls serum vitamin D level (Mean±SD) of two groups.
Unpaired t-test applied for significance. *p<0.05 was significant.

Variables	FGTB Case (n=120)	Mean ±SD	p-value
Menstrual history			
Irregular	52	13.66±8.12	0.16
Regular	68	15.95±9.24	
PPD test			
Negative	84	15.12±8.71	0.75
Positive	36	14.58±9.16	
Infertility			
Primary	78	14.63±8.54	0.57
Secondary	42	15.58±9.36	
Thyroid profile			
Hyperthyroidism	26	13.21± 7.43	0.25
Normal	94	15.44±9.13	
Prolactin			
Normal	107	15.09±9.02	0.63
hyperprolactemia	13	13.86±7.09	
HSG			
Normal	58	15.22±8.46	0.75
Bilateral block	62	14.72±9.19	
TB-PCR			
Negative	3	8.83±1.00	0.22
Positive	117	15.12±8.8	
AFB			
Negative	108	15.12±8.93	0.54
Positive	12	13.5±7.83	
LJ- culture			
Negative	116	14.82±8.72	0.34
Positive	4	19.10±11.74	

[Table/Fig-2]: Risk factors associated with serum vitamin D level deficiency among FGTB* cases.
*Female Genital Tuberculosis (FGTB).
Unpaired t-test applied for significance.* p< 0.05 was significant.

DISCUSSION

The current study was carried out to evaluate the role of vitamin D cases in North Indian population. In our study we found a significant association between FGTB cases and controls. The low serum vitamin D level significantly associated to FGTB. The first report about the possibility of relationship between vitamin D and tuberculosis surfaced twenty years ago [13], but since then there have been conflicting reports about any such association in the subsequent studies [5]. A meta-analysis supported to our results showed that low serum vitamin D level was a risk for active TB and further verified the precise range of low serum vitamin D posing high risk of TB [11]. Another meta-analysis reported by Nnoaham et al., found that serum vitamin D levels were lower in TB patients compared to controls [14]. Vitamin D also plays an important role in reducing the

no. of risk chronic diseases as cancers autoimmune and infectious disease [15]. Vitamin D modulates the immune system to fight against *M. tuberculosis* by promoting phagosome maturation and enhancing the production of antimicrobial peptides [16,17]. Many other studies have investigated the association between vitamin D deficiency and TB. Similarly a number of studies that supported our results in Vietnam [15], Tanzania [18], West Africa [19], Gujarati Asians [20], Sub-Saharan Africa [21] had reported higher levels of vitamin D deficiency in patients with TB. Again, study conducted in Dow University of Health Sciences, Pakistan population have reported very low levels of 25(OH)D and higher prevalence of vitamin D deficiency in TB patients than non-TB individuals [5]. Thus, our results were consistent with the results of most previous studies. On the other hand, there was contrasting evidence in studies conducted in Tanzania and Vietnam, which showed no considerable difference in 25(OH)D levels between TB cases and matched controls [16,22] Demographic and biochemical factors like (thyroid, serum prolactin, haemoglobin) were found to have no association between FGTB and low serum vitamin D level. However, our study declared a positive correlation between BMI of cases and vitamin D level and this positive correlation is an indicator of major health nutritional condition of the subjects, which is supported by the study conducted by Mrinal et al., [23]. In addition, the presence of TB and a history of TB were independently associated with vitamin D deficiency as well as low BMI. Vitamin D is known to have numerous functions in response to infection, involving the innate and acquired immune systems. All of these functions are involved in the antimicrobial response to TB. Vitamin D insufficiency/deficiency is a worldwide, public health problem in both developed and developing countries [24]. Vitamin D deficiency is associated with many chronic diseases, such as cardiovascular disease, autoimmune disease, cancer and chronic infections, PCOS and this has been depicted in several previous studies [23]. Overall, studies suggested that supplementation of vitamin D through diet improves the immune response to TB. To the best of our knowledge, it is the first study that shows the effects of vitamin D level on FGTB cases in North Indian females.

LIMITATION

The limitations of our study is that, we could not found any significant association between 25(OH)D3 vitamin D deficiency and biochemical investigations (Hb, Thyroid and Serum Prolactin). It may be due to small sample size. Effect of seasonal variability upon vitamin D level could not be explained.

CONCLUSION

In conclusion, vitamin D deficiency is highly prevalent among FGTB cases. Serum vitamin D level is low as compare to healthy control, and also shows a strong association between some of the demographical parameters, which indicates that vitamin D is essential in routine diet. Thus, serum vitamin D level can be used as a risk factor for FGTB. Further studies with large population group needed to establish a strong association between FGTB, serum vitamin D level and other demographic parameters.

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