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Vitamin D status among Pulmonary Tuberculosis Drug Induced Hepatotoxicity of Anti-tubercular Therapy

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Abstract
Background: *Serum 25-hydroxy vitamin D (S-25(OH) D), in pulmonary Tubercular patient enhances host protective immune response to mycobacterium tuberculosis (TB) and reduces disease associated inflammation. The aim of present study is to evaluate correlation between S-25(OH) D levels, Body mass index (BMI) and albumin in drug induced hepatotoxicity (DIH) under Directly Observed Therapy Short course (DOTS) treatment.*
Methods: *Total of 107 (70 male, 37 female) age-sex matched pulmonary TB control and 58 (38 male, 20 female) DIH patients, age grouped 20-70 years, were included in the study. Anthropometric measurements, Liver function test (LFT), S-25(OH)D, biochemical, radiological and microbial markers were computed. Biochemical marker assessment was performed on 1st, 4th, 8th and 16th week in all study patients and P-values by unpaired t test of various parameters obtained on 1st and 16th week were calculated.*
Results: *The comparative study of S-25(OH)D, Albumin and BMI in control TB and DIH patient under ATT on 1st and 16th week showed significant differences. DIH patients showed S-25(OH)D level deficient (less than 20 ng/ml) in comparison to non-DIH patient insufficient value (20-29 ng/ml) under chemotherapy ($P < 0.0001$). BMI was found less in DIH patients than control but the statistics were insignificant ($P < 0.118$). Hypoalbuminemia was seen in DIH patient where as control albumin levels were in baseline ($P < 0.039$).*
Conclusions: *The pulmonary TB patients having DIH should be supplied with higher doses of S-25(OH)D to accelerate the radiological and immunological recovery as deficient levels of vitamin D worsen the severity of infection.*
Keywords: *Tuberculosis, S-25(OH)D, Innate immunity, Immuno-modulatory activity, Drug Induced Hepatotoxicity.*

Introduction

Tuberculosis (TB) one of the deadliest bacterial killer, a major health concern in the South-East Asia Region (SEAR) of WHO. The study region accounts for 38% global burden of tuberculosis. Nearly 4, 40, 000 people died in 2013 due to TB

infection [1]. In spite of available antitubercular antibiotics, the incidence of TB has increased significantly. Long term treatment with antitubercular drug leads to severe side effects in TB patient which is the major cause of treatment failure and development of MDR-TB and XDR-TB [2]. Malnutrition and hypovitaminosis are the major predisposing factor of TB [3]. Most common side effect of the antitubercular therapy is hepatotoxicity [4]. It has been reported that the fat soluble hypovitaminosis of S-*25(OH)D* in TB patients leads to low immunity which makes the patient vulnerable to TB. Vitamins bio-molecules maintain normal/path-physiology which boosts immunity in humans. Among these, S-*25(OH)D* referred as 'sun-shine vitamin, has a major role in boosting immunity. The active form of S-*25(OH)D* is 1, 25-Dihydroxy cholecalciferol (DHCC) or calcitriol synthesized in mitochondria of proximal convoluted tubules of the kidney by 1-alpha hydroxylase [5-9]. Calcitriol provides innate immunity in humans against certain infectious diseases as it has immuno-modulatory activity which facilitates the proliferation of monocytes by activating them, where as suppresses immunoglobulin production, proliferation of lymphocytes and cytokine synthesis. This is crucial in the body's defense mechanism against TB, in which the attack of macrophages is a key step in pathogenesis [10-12]. S-*25(OH)D* carries out its action through binding to Vitamin D Receptor (VDR) [13]. The vitamin D supplementation may represent a new strategy for the shortening of TB treatment in the face of growing drug resistance. Vitamins play vital role in metabolism and detoxification of antitubercular drugs. S-*25(OH)D* decreases synthesis of inflammatory cytokines, interleukin-2 and interferon-c with increase production of anti-inflammatory cytokines [14, 15]. Hence in this regard, the aim of this study is to review the role of fat soluble S-*25(OH)D* in tuberculosis patients having hepatotoxicity during antitubercular therapy.

Materials and Methods

The present study was carried out in the department of Biochemistry in collaboration with Respiratory Medicine, Subharti Medical College, Chatrapati Shivaji Hospital Meerut from May 2014 to October 2016. A total of 165 pulmonary tubercular patients (107 control and 58 DIH), attending outpatient department (OPD), inpatient department (IPD) or admitted, having drug induced hepatotoxicity (DIH) during antitube-rcular chemotherapy. The criteria for diagnosis of DIH were: (a) Elevated levels of serum Aspartate aminotransferase (AST) and Alanine aminotran-sferase (ALT), five times the upper range of normal levels (45 IU/L) on at least one occasion or more than three times (>135 IU/L) on three consecutive occasions. (b) An elevation of serum total Bilirubin >1.1 mg/dl. (c) Any rise in serum AST or ALT above pre-treatment levels with symptoms of anorexia, nausea, vomiting and jaundice etc. (d) Absence of infection with viral hepatitis A, B, C, or E. (e) Improvement in liver functions (serum total Bilirubin <1.1 mg/dl, ALT and AST < 100 IU/L) after withdrawal of anti-TB drugs. The DIH was diagnosed positive if any one of above listed criteria a, b or c was observed along with d and e respectively.

The age group of the study patients comprising from 20-70 years and mean age was 47±20 years. The pulmonary TB diagnosed by one or two positive sputum samples for acid-fast bacilli (AFB) by direct microscopy method and chest radiograph on those patients having evaluated sputum smear negative for AFB but suspected of TB to refrain the delay in diagnosis. Laboratory investigations like complete haemogram, blood chemistry with detailed liver functions (total and direct Bilirubin, Alanine amino transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP), Gamma-glutamyl transferase (GGT), Total serum protein (TP), serum Albumin) were performed in all patients using standard laboratory procedures. All patients involved in the study, interviewed complete history & physical examination was conducted & patient's

demographic characteristics, history of smoking, alcohol consumption, drug abuse as concomitant diseases and drugs, status of viral infections and other treatment information were collected and entered in pre-tested Performa. The anthropo-metric measurement was taken to calculate body mass index [BMI (kg/m^2)]. All subjects were kept on antitubercular regimen for short course composed of four drugs daily combination with minimum of two months with, Isoniazid (INH), Rifampicin (RIF), Pyrazinamide (PZY) and Ethambutol (EMB); followed by daily INH, RIF and EMB continued for four months. A total of 107 active pulmonary tubercular patients (70 male and 37 female), who were undergoing chemoth-erapy from study area but did not develop DIH, were taken as control group. Among the study subjects, a total of 5 DIH patients (2 female and 3 male) were dropped out because of Irregular follow up. Total of 58 DIH (38 male and 20 female) subjects, who were followed till completion of chemotherapy, i.e. for 6 months, were categorized under five different groups on basis of their age. The serum *25(OH)D* levels were assayed in the study subject twice, i.e., before initiation of chemotherapy and after completion of 16th week chemotherapy. The S-*25(OH)D* levels were quantitated using reagents, ready to use kits supplied by M/S BIOMERIEUX, Netherland on VIDAS auto-analyzer by Enzyme Linked Fluorescent Assay (ELFA) technique[16]. All patients were followed up in an interval of 2nd, 4th, 8th and 16th week and LFT along with other investigations were carried out during each visit. The details of controls were recorded in patients profile, like: name, age, sex, profession, location, ethnic group, literacy, socio-economic status, life style, diet pattern, medication if any other than antitubercular chemotherapy or any concurrent disease, were recorded. Ethical clearance obtained from Institute Ethical Committee (ICE) of Swami Vivekanand Subharti University, Subharti Medical College Meerut.

Inclusion criteria*:* Adult Patients between 20-70 years of age, with smear positive for Acid Fast Bacilli (AFB), diagnosed for the first time with active pulmonary TB, willing to co-operate and were not under any antibiotic treatment, included in the study.

Exclusion criteria: The patients found positive for HIV, Hepatitis B surface antigen, HCV, MDR-TB, XDR-TB, Diabetes mellitus, chronic alcoholics, the total serum Bilirubin above 1.1 mg/dl, GGT (>64 IU/L), ALT & AST above normal range(>45 IU/L), extrapulmonary TB, hepatic disease, renal failure, malignancy, pregnancy, sarcoidosis, hyperparathyroidism or those taking any corticosteroids, immunosup-pressive agents, thiazide diuretics or drugs known to interfere with vitamin D levels (Phenytoin, Phenobarbital, Carbamazepine, Theophylline) and patient who didn't give their written consent to co-operate, were excluded from the present study group.

Data analysis: The association of S-*25(OH)D* levels during pulmonary tuberculosis treatment with hypoalbuminemia and BMI patients and controls were compared. We assessed each parameter relative risks by calculating the average mean value difference during follow up from 1st to 16th week. The results were found statistically significant at 95% confidence intervals using Paired t-test significant, where $p< 0.05$ and highly significant when $P <0.0001$. The statistical analysis was performed by using Graph-pad Prism, Quick Calculations.

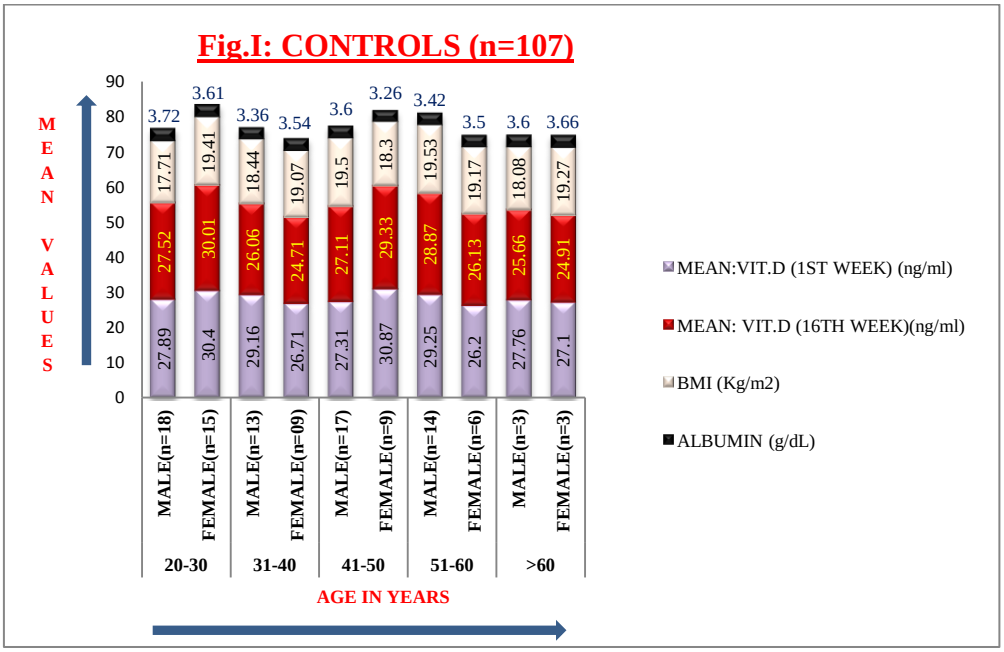
Results

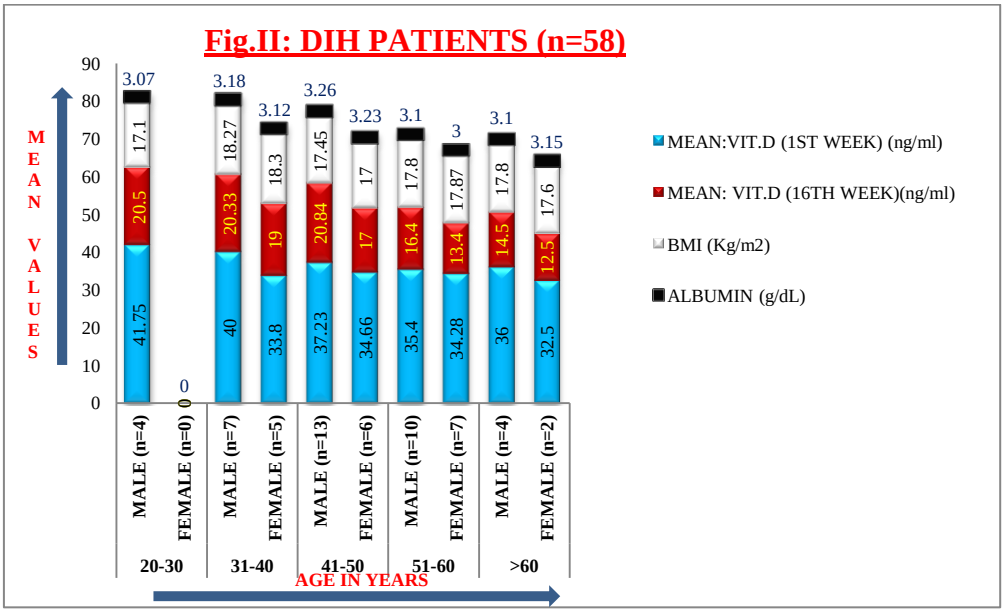
A total of 165 (65.45% male and 34.54% female) pulmonary TB patients were included in the study. All the TB patients under antitubercular therapy were followed and their clinical status was recorded on 2nd, 4th, 8th and 16th week the blood sample collected for the evaluation of LFT. Patients who showed elevated serum AST/ALT levels five time of baseline data and signs, symptoms of DIH after 16th week of treatment were included as patients whereas, patients having baseline LFT without any other symptoms of DIH after 4th week of treatment were considered as

control. The S-*25(OH)D* levels were evaluated in 1st and 16th week of treatment for control and DIH patients compared (Fig. I and II).

Vitamin D, BMI and Albumin levels: The comparison of these parameters among control and DIH patients found significant. The study subjects (control and DIH patients) male and female were categorized under five groups based on their age to evaluate the risk factors for severity of TB and hepatotoxicity. Study subjects further classified according to circulatory levels of S-*25(OH)D* in to three groups as: Group – I: <20 ng/ml, which is referred as deficient. Group – II: 20-29 ng/ml which is referred as insufficient. Group – III: 30-100 ng/ml which is sufficient.

Basal levels of S-*25(OH)D* were assessed and thereafter initiation of anti-tubercular treatment with INH, RIF, PZY and EMB for two months followed by biochemical parameters along with microscopic and radiological findings. Patients were given ATT and followed for further four (04) months, thereafter evaluated for biochemical, microbiological, serological as well as S-*25(OH)D* levels. The detailed results obtained for 1st week and 16th week were follow by the control group and patients.





Figures highlight that immunological response of control and DIH ATT administered study subjects, the levels of biochemical, S-*25(OH)D*, BMI and Albumin levels showed slight variation, S-*25(OH)D* basal 1st week in the range of insufficient 20-29 ng/ml and four months level falls in the range of deficiency <20 ng/ml. The two-tailed P < 0.0001. By conventional criteria, this difference is considered highly statistically significant.

Albumin levels were observed as normal but at lower basal in all pulmonary TB cases at 1st week but DIH patients after 16 weeks of treatment developed hypoalbuminemia (albumin < 3.5 g/dl), which by conventional criteria is considered as statistically significant (P < 0.03). The BMI for all pulmonary tubercular patients were found below normal at 1st week, compared with control and DIH patients. The anthropometric measurements for BMI using unpaired *t* test results, P < 0.11, which was statistically insignificant.

Discussion

Present research was carried out to study an association between circulatory S-*25(OH)D* levels in non DIH pulmonary TB patients with that of DIH patients, during antitubercular therapy. However, the progression of disease from active pulmonary TB to DIH the levels of S-*25(OH)D* decline significantly to worsen the condition of TB patients. Present study suggests that S-*25(OH)D* deficiency is common in TB patients of older aged male and female, where as geriatric aged female more susceptible. The predisposing factors likely to be low socioeconomic status, poor nutrition, traditional or cultural traits and less exposure to sun light. This study supported by 107 control and 58 DIH patients, each receiving antitubercular treatment. Patients in age group 20-30 years and 31-40 years least reported to contribute S-*25(OH)D* deficiency where as age group 51 – 60 years and >60 years are susceptible to develop S-*25(OH)D* deficiency during treatment.

The S-*25(OH)D* play an important role in activation of 1α-hydroxylase to convert 25-hydroxy cholecalciferol to active 1, 25- DHCC that leads to expression of cathelicidin, a microbial peptide for Mycobacterium tuberculosis [17]. Circulatory S-*25(OH)D* levels <20 ng/ml likely to impair the macrophage-initiated innate immune response to tuberculous bacilli, which explains the possible susceptibility to TB based on geographical and ethnic variations [18, 19]. Researcher have demonstrated that 1,25-dihydroxy vitamin D is a patent modulator of the T-cell phenotype; it inhibits the T-helper (Th) 1Tcells associated with cellular immune response while conversely enhancing humeral Th2 cells response [20]. Recent report indicated that a balance between pro-(Th-1) and anti (Th2) inflammatory responses is optimal for control of TB thus suggesting that the role of 1, 25-dihydroxy vitamin D_3 may have relevant importance [21]. Recently, S-*25(OH)D* found to accelerates host inflammatory responses and this may contribute to vitamin D supplemented TB therapy [22]. It has been observed in present study that S-*25(OH)D* levels deficient in DIH patients doesn't show any significant improvement after vitamin D supplementation.

Source of Funding: self finance.

Conclusion

Present study focus on the significant role of vitamin D and Albumin levels in DIH pulmonary tuberculosis patients under Anti-Tubercular Therapy. Hypovitaminosis D levels strongly associated with tuberculosis patients contributed DIH during treatment. Malnutrition is predisposing factor in pulmonary TB which worsens the treatment outcome. In this study we conclude that the frequency of hypovitaminosis D in pulmonary TB patients recorded within insufficient range but deteriorates further to reach deficient values in DIH patients of all age group under treatment. Whereas, hypoalbuminemia proves to be a risk factor to worsen the outcome

of the disease. All the pulmonary TB patients showed derangements in S-25(OH)D levels which signifies that vitamin D should be administered as ancillary therapy with standard anti tubercular therapy. The validity of S-25(OH)D as marker of vitamin D status may be affected by infections. There is need for prospective studies to assess the relationship between vitamin D and risk of TB and other infectious diseases. Public health education should stress the need for adequate dietary intake of vitamin D in vulnerable groups of people all over the world. This may help to reduce the complication and severity of the disease.

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