Circulating Monocytes to Lymphocytes Ratio and Vitamin D levels in patients with Active Tuberculosis and Close Family Members

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Abstract

Introduction: Relationship of ratio of monocytes to lymphocytes with vitamin D may predict the risk of development of tuberculosis in close family members of tuberculosis patients. Aims and objectives: A cross-sectional study was designed to find out the levels of circulating vitamin D levels and monocytes, lymphocyte, ratio of monocytes/lymphocytes ratio and in active tuberculosis patients and close family members. Material and Methods: Among 90 subjects, 45 were diagnosed tuberculosis patients while 45 subjects were adult family member residing with and taking care of diagnosed tuberculosis patients. 40 normal subjects were taken as a control group. Values of Circulating monocytes, lymphocytes, ratio of monocytes to lymphocytes were estimated by SWISS Max analyzer. Level of vitamin D was estimated by ELISA. Results: Most of the first degree relatives were male with mean age 26.65 years, residing in populous area with one close contact having tuberculosis. Prior history of TB was observed in four family members. This study exhibits significant difference in monocyte lymphocyte ratio and vitamin D levels in the three groups. A significant statistical correlation was observed in patient's monocyte, lymphocyte count but non-significant correlation was observed between Monocytes/Lymphocyte ratios (ML) and vitamin D. Conclusion: Increased values of circulating monocytes, lymphocyte and their ratio, along with decreased levels of vitamin D was observed in TB patients followed by family members residing with tuberculosis patients. However, no association of vitamin D with ML ratio was noted.

Keywords: Tuberculosis; Latent tuberculosis; family member; monocyte lymphocyte ratio.

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1. Introduction

Tuberculosis (TB) contributes to the causes of morbidity and mortality worldwide. About 1/3rd people of world are infected with *Mycobacterium tuberculosis*. Family members or first-degree relative residing with diagnosed tuberculosis patients may have a chance of getting infection, which is termed as latent tuberculosis infection (LTBI). This condition causes a constant immune response that ismotivated by antigens of *Mycobacterium tuberculosis* without any sign and symptoms of active form of TB. The risk of LTBI is found to be 5.0–10.0 % [1.2].

Relationship of number of circulating monocytes/lymphocytes or the ratio of monocytes to lymphocytes (ML ratio) was discussed by some studies and proposed that these parameters may predict the risk of development of TB in immune-compromised subjects/family members of TB patients [3,4]. It is highlighted that the high ratio of ML is related with variation in transcription of gene in monocytes that may alter the anti-microbial function of monocytes.The ratio of ML may therefore be an indicator of latent form of TB [5,6].Additionally, impairment in the function of monocytes may change crosstalk with circulating lymphocytes and adaptive immune responses [7].

Vitamin D deficiency was seen in TB patients. Vitamin D play an important role in immune system through the receptors present on macrophages and monocytes[8]. It is found that vitamin D endorses the process of autophagy in circulating monocytes as well as promoting antibacterial activity related with the environment via production of reactive oxygen species[2]. However, some reports showed no effect of vitamin D on CD4+ T-cells (the cells play a role in immune system)[9,10].

Tuberculosis is a major health problem in Pakistan with high prevalence and its rate increasing with time due to high drug resistant tuberculosis[11].According to a Pakistani study, the cut off value of circulating vitamin D is less than 30 ng/mL, whereas values between 10 to 30 ng/mL were taken as inadequate and deficient level of vitamin D[12]. Deficiency of vitamin D in patients with pulmonary TB is related with increased risk of developmening an active form of tuberculosis[13].However,the supplementation of vitamin D non-significantly decreased the count of lymphocytes in TB patients[14].

This disease has been eradicated in majority of developed countries, yet it is still prevalent in Pakistan to this day. Most of the people are not aware of their illness and spread it to others who are in close contact with them. Those who are diagnosed develop multidrug resistance due to patients not complying to the treatment.Therefore, there is a need to improve the investigative tools which are simple and cost effective. This may help in preventing the spread of this diseases and provide supplemental therapy to improve management.

Across-sectional study was designed to find the levels of circulating monocytes and ratio of circulating monocytes/lymphocytes in diagnosed tuberculosis patients and family membersresidingwith and taking care of them. We also tried to compare and correlate the ratio of monocytes to lymphocytes with Vitamin D in diagnosed tuberculosis patients and their family members.

2. Objective of the study

The objective of the study was to

1. Evaluate the circulating Monocytes to Lymphocytes ratio and Vitamin D levels in active tuberculosis patients and in close relatives residing with them

2. To determine the correlation between M/L ratio and Vitamin D levels in them

3. Patients and Methods

A total of 90 subjects were enrolled in this study by the Department of Medicine of Lahore General Hospital. Diagnosed tuberculosis patients under treatment for three months (Group A) were told to bring one family member who is living with and taking care of them (Group B) for participation in the study which may help them screen for any possible latent tuberculosis. Adult patients of both genders (18 -75 years) were recruited in the study.

Among 90 subjects, 45 were diagnosed tuberculosis patients (Group A) with a duration of TB three months (confirmed by Tuberculin test and sputum for AFB) while 45 subjects were adult family member residing with and taking care of diagnosed tuberculosis patients(Group B). 40 subjects with no history of any disease or family history of tuberculosis were taken as controls (Group C). Sample size was calculated with Raosoft Inc. Study was carried out in Biochemistry Department of Shalamar Medical & Dental College, Lahore.

Diagnosed tuberculosis patients and their family members (One of each patient) residing and taking care of them were included in the study. Those with other respiratory disorders or inflammatory disease including cardiovascular disease, cerebrovascular disease, arthritis, malignancy were excluded from the study. Questionnaire based studyproforma was used to collect relevant information. Informed consent was recievedand the study was approved by Institutional Review Board Shalamar Medical and Dental College. This study was explained to them withthe benefits and risksdescribed in detail by the Principle Investigator.

4. Study Protocol

Three ml of fasting blood sample was drawn and collected in EDTA tubes for estimation of values of circulating monocytes, the ratio of monocytes to lymphocytes and vitamin D in both patients and family members. Values of circulating monocytes and the ratio of monocytes to lymphocytes were estimated by the SWISS Max analyzer. For estimation of levels of vitamin D, blood sampleswere centrifuged for 15 minutes at 1000 x g at 2 - 8°C andplasmawas stored at -20°C until use. Level of vitamin Dwas estimated by ELISA. The cutoff value vitamin D was taken as<20 ng/ml [15].

5. Statistical Analysis

Results were analyzed by SPSS 23. Qualitative data wasexpressed as a frequency. Quantitative variables were

expressed as mean ± SD. ANOVA and Post-hoc test was applied to determine the difference in values between active TB patients, their family members and healthy subjects (controls). P< 0.05 was taken as statistically significant difference.Correlation of vitamin D with ratio of M/L was carried out by Pearson correlation co-efficient.

6. Results

According to demographics of first degree relative of TB patients, most of the first degree relatives (FDR) were male with mean age 26.65 year. Most of the family members live in a populous area with one being in close contact with a person with active TB. There was no history of BCG and worms in them. Previous history of TB was observed in 04 family members who are in close contact. However Tuberculin test was positive but AFB was negative in family members (Table 1).

Significant difference was observed in serum vitamin D, mean lymphocyte count and M/L ratio amongst three groups (P<0.000 and P<0.005) (Table2).

Significant correlation was detected between monocytes, lymphocytes, and ML ratio in patients (group A) but non-significant correlation exists between them and vitamin D (Table 3& Fig 1a). Significant correlation was observed between monocyte count and lymphocyte count and monocyte count with ML ratio in family members (Group B)(Table 4& Fig 1b, 1c).

7. Discussion

Latent tuberculosis infection (LTBI) is a forerunner of the active form of tuberculosis, usually observed in a first degree relative or family member (caretaker). In latent TB infection,the *Mycobacterium tuberculosis* organism is present in its dormant form in host.Subjects with LTBI are usually non-infectious and asymptomatic, although these subjects may have risk of developing TB in the future [16,17]. The risk of developing infection with TB may be increased with deficiency of vitamin D as this vitamin may have a role in preventing the infection[18].

According to our study the mean age of family members was 26.65 %. A study found majority of family membersunder 30 years old suspected with LTBI were four times more at risk of developing latent TB than those who had no family history of infection of TB[19]. It is proposed that risk of TB infection from close related subjects and community rose with age[20]. We also agreed with a study who found that prevalence rate of LTBI was higher in male as compared to female [21].

TB infection in developing country may be due to poor diet and unhygienic conditions may play important role in causing tuberculosis rather than family history in our settings. A study demonstrated that there was a higher risk of developing TB in family membersliving in populous areas compared with ones living in low populous areas [22].. Another study emphasized the role of genetic factors and spread of infection through coughing [23]. On the other hand, a study found that most of LTBI subject were those who were not in contact with TB patients. This study suggested that coverage ofscreening TB exposed subjectsmust be extended to individuals who are living in boarding houses and urban slums [24].

We also agreed with a study who observed increased values of T-lymphocytes, in both LTBI and active form TB. It is suggested that in LTBI subjects there is an increase activation of T-cells without an activation of monocyte related cells, while in active form of TB, there is markedly elevation of markers of both lymphocyte and monocyte. It is suggested that non-replicating and replicating bacteria may drawvaried immune responses[25].

We assented with a study who found significantly high ratio of monocytes to lymphocytes of TB patients as compared to LTBI subjects. This study proposed that the increased expression of CD64 on blood monocytes along with ratio of MLin active form of TB subjects may be an added predictor for diagnosis the infection of TB. MLR also help to improve the specificity of the asymptomatic features of LTBI and TB patients [6,26].

We observed that between groups and within groups, values of vitamin D displayed a significant difference. A number of studies reported that vitamin D deficiency is a risk factor for developing TB. It is proposed that vitamin D has immuno-modulatory characteristics and having it in alow level(< 30.0 nmol/l) may increase the risk of airway infection [27,28]. Besides, metabolites of Vitamin D can raise the innate antimicrobial response to T cell-mediated interferon-γ. Additionally,vitamin D blocks the secretion and expression of various inflammatory mediators and extracellular matrix, degrading enzymes driving immunopathology during the state of active TB. A study found that Adjuvant Therapy of vitamin D in TB patients may decrease lung infection and increase the cavitation and thereby reduce the risk of transmission and prevent relapse [29]. Another study found supplementation of vitamin D_3 also raised the ratio of ML (predictor of healing lesion)as well as reduce the values of acute phase markers, C-reactive protein, erythrocyte sedimentation rate, etc[30].

We observed a non-significant negative correlation between vitamin D and ML ratio. An associationbetween deficiency of vitamin D and a high risk of infections (increase monocyte, lymphocyte, and ratio of ML) with mycobacterium tuberculosis was also observed by a study[31]. However,a different study found a negative association of vitamin D with lymphocyte, and a positive association with lymphocyte:monocyte ratios in peripheral blood[32]. It is demonstrated that vitamin D exerted anti-inflammatory actions and enhanced the ratio of ML in peripheral blood[28,33] which may have a role in the prevention of *M. tuberculosis* infection due to the presence of its receptor on nearly all cells of the immune system[34].

8. Conclusion

Increased values of circulating monocytes, lymphocyte and their ratio along with decreased levels of vitamin D was observed in TB patients followed by family members residing with tuberculosis patients. However no association of vitamin D with monocyte, lymphocyte and ML ratio was observed.

9. Recommendations

Close contacts with TB patients were found to be risk of developing TB in family members especially care takers. It is suggested that subjects who are family member and a close contact with TB patients should be rapidly screened, diagnosed, and monitored. It may help to eliminate TB from country.

Acknowledgments

10. Limitations

In this study, family members with age < 18 year were not included. As the study plan was cross-sectional, the precise relationship between vitamin D, ML ratio into patients of TB and LTBI in large number of people was not determined.

11. Authors' Contributions

Rukhshan Khurshid: Manuscript writing

Anila Jaleel : Study Design and Data interpretation

Kiran Namoos :Data collection

Sadaf Saleem Uppal: Literature search

12. Conflict of Interest

None

13. Funding source

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