

ORIGINAL ARTICLE

Correlation of Vitamin D with Monocytes and Lymphocytes in Care Takers of Tuberculosis Patients

KIRAN NAMOOS¹, ANILA JALEEL², SADAF SALEEM UPPAL³, SAMAR ASIM⁴, RUKHSHAN KHURSHEED⁵, ARSALAN SALEEM CHUGHTAI⁶

¹Assistant Professor Biochemistry, Shalamar Medical & Dental College, Lahore

²Professor Biochemistry, Shalamar Medical & Dental College, Lahore

³Associate professor Biochemistry, Shalamar Medical & Dental College, Lahore

⁴Senior Registrar, Pulmonology Department, Shalamar Medical and Dental college Lahore

⁵Assistant Professor Biochemistry, Shalamar Medical & Dental College, Lahore

⁶Senior Lecturer, Biostatistics, Shalamar Institute of allied Health Sciences

Corresponding author: Anila Jaleel, Email: aneelajaleel@hotmail.com, Cell: 03009205779

ABSTRACT

Background: Close relatives of tuberculosis patients may be at increased risk of developing the disease as they are in close contact with them

Objectives: A cross-sectional research was conducted to determine correlation of Vitamin D with monocytes and lymphocytes in individuals with active tuberculosis as well as their care takers.

Material and Methods: Total of 83 subjects was divided into three groups. Of the 83 participants, 31 had active tuberculosis, while 31 were adult family members who lived with and looked after the patients. 21 healthy age matched individuals were chosen to form the control group. SWISS Max analyzer was used to evaluate the levels of lymphocytes and monocytes in the blood and Vitamin D was measured by ELISA.

Results: Vitamin D levels and monocyte lymphocyte count in the three groups showed substantial variation. The patient's monocyte and lymphocyte count showed a statistically significant correlation. However there was non-significant correlation of vitamin D with both monocyte and lymphocyte.

Conclusion: Caretakers of active tuberculosis patients showed increased levels of circulating monocytes, lymphocytes as well as lower vitamin D levels with increased risk of developing tuberculosis.

Keywords: care taker, tuberculosis, vitamin D, monocyte and lymphocyte

INTRODUCTION

The global causes of morbidity and mortality include tuberculosis (TB). A third of the world's population is afflicted with Mycobacterium tuberculosis. A danger of contracting latent tuberculosis (LTBI) infection exists for family members and/or close friends living with diagnosed TB patients. Without any signs or symptoms of an active form of TB, this illness results in a continuous immunological response that is stimulated by antigens from Mycobacterium tuberculosis. LTBI risk is estimated to be between 5% and 10%. [1, 2].

The quantity of circulating monocytes and lymphocytes and the monocyte-to-lymphocyte ratio (ML ratio) have been discussed in some studies, and it has been proposed that these parameters may be used to predict the risk of developing tuberculosis (TB) in immune-compromised people or in the relatives of TB patients [3,4]. Additionally, altered monocyte function may alter the way that circulating lymphocytes and adaptive immune responses interact. [5, 6, 7]. It has been discovered that vitamin D supports the autophagy procedure in blood monocytes and promotes antimicrobial activity in the environment by causing the generation of reactive oxygen species. However, several studies indicated that vitamin D didn't affect CD4+ T-cells (the cells play a role in immune system) [8, 9, 10].

Due to a high prevalence and rising rate of drug-resistant tuberculosis, tuberculosis is a serious health concern in Pakistan [11]. A Pakistani study determined that readings between 10 and 30 mg/mL were considered to be insufficient and deficient levels of vitamin D, whereas less than 30 mg/mL was the cutoff threshold for circulating vitamin D [12].

A higher risk of developing an active type of tuberculosis is associated with vitamin D deficiency in individuals with pulmonary TB [13]. However, vitamin D treatment did not significantly lower the lymphocyte count in TB patients [14].

Even though the majority of modern nations have completely eradicated this disease, Pakistan continues to be plagued by it. Most people who have the disease are unaware of it and they spread it to those who come into contact with them frequently. Those with active tuberculosis experiences multidrug resistance, as a result of non-compliance to treatment. Therefore it is necessary to enhance easy and affordable investigation

instruments. This could aid in early detection of risk and prevent the spread of the illnesses..

A cross sectional study was designed to measure circulating levels of monocytes and lymphocytes in tuberculosis patients, caretakers of tuberculosis patients and controls and to correlate them with vitamin D levels.

Objective of the Study

- To estimate the levels of vitamin D, monocytes and lymphocytes in individuals with active tuberculosis as well as their care takers.
- To determine the correlation of vitamin D with monocytes and lymphocytes in individuals with active tuberculosis as well as their care takers.

PATIENTS AND METHODS

After receiving institutional review board approval, the study was carried out at the biochemistry department of SMDC. Each subject gave their informed consent.

A total of 83 individuals were gathered for the study. Diagnosed tuberculosis patients (Group A) receiving treatment for three months were instructed to bring one relative who is living with and caring for them in order to participate in the study that may help them screen for any possible latent tuberculosis (Group B). The study included adult volunteers of both sexes, aged 18 to 75.

A total of 83 individuals were gathered for the study. Diagnosed tuberculosis patients (Group A) receiving treatment for three months were instructed to bring one relative who is living with and caring for them in order to participate in the study that may help them screen for any possible latent tuberculosis (Group B). The study included adult volunteers of both sexes, aged 18 to 75. The study included patients with tuberculosis who had been diagnosed as well as one family member who resided with and looked after each patient. The study eliminated participants who also had cancer, cardiovascular disease, or cerebrovascular disease, as well as additional inflammatory disorders or respiratory illnesses. Data pertinent to the investigation were gathered using a study methodology that relied on questionnaires.

Study Protocol: In order to measure the quantities of circulating monocytes, lymphocytes, and vitamin D in patients and family

members, three ml of fasting blood was taken and collected in EDTA tubes.

The SWISS Max analyzer was used to measure the amounts of monocytes and lymphocytes. Blood samples were centrifuged for 15 minutes at 1000 x g at 2-8°C to assess the levels of vitamin D. Plasma was then kept at -20°C until use. ELISA was used to estimate vitamin D levels. The threshold for vitamin D was set at 20ng/ml [15].

Statistical Analysis: The data was analysed using SPSS 23. Quantitative data was expressed using frequency. Mean standard deviation was used to present quantitative variables. An ANOVA and Post-hoc test were employed to determine whether active TB patients, their family members, and healthy volunteers had different values. P0.05 was used to indicate that a difference was statistically significant. The link between vitamin D and monocytes and lymphocytes was determined using the Pearson correlation coefficient, respectively.

RESULTS

Out of 83 subjects, 60% were males and 40% were females. Frequency of clinical demographics in percentage is shown in figure 1A and 1B. The majority of the patients and their care takers were residing in crowded areas with poor hygiene conditions.

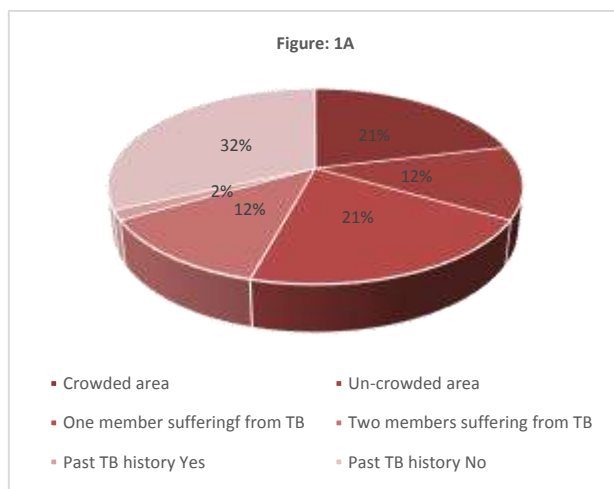


Figure 1: FREQUENCY OF CLINICAL DEMOGRAPHICS

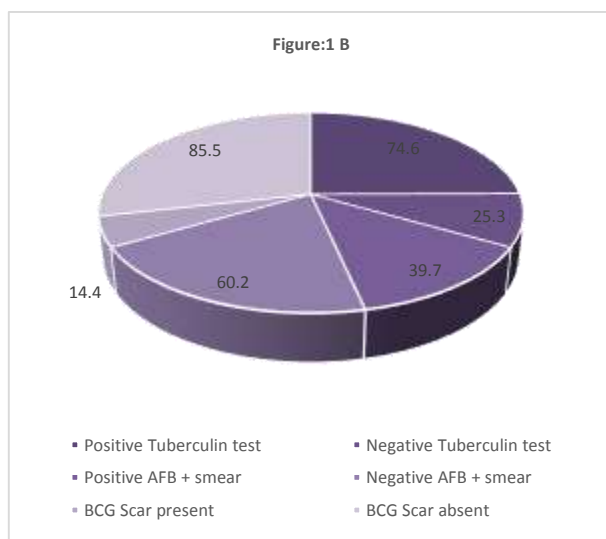
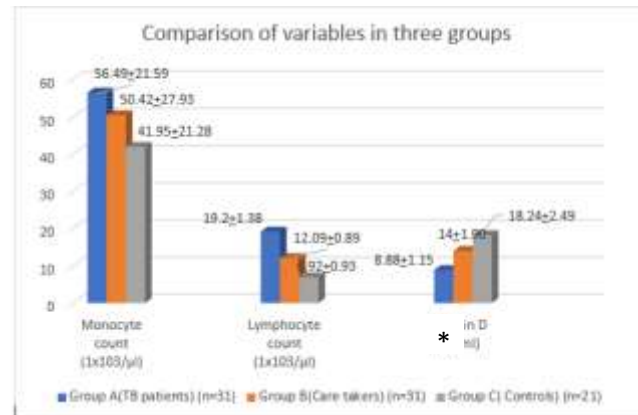


Figure 2: Comparison of Vitamin D, Monocytes and Lymphocytes Levels in Three Groups



The values are expressed as mean and standard deviation, Significant is <0.05 (*)

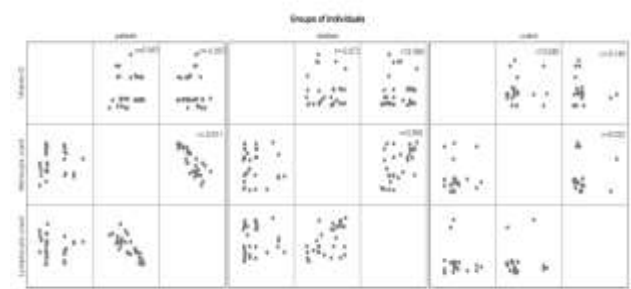


Figure 3: Correlation of Vitamin D with Levels of Monocytes and Lymphocytes

Out of 83 research subjects 75% were tuberculin test positive while 25% had negative test results. Of them 40% were AFB + smear positive while 60% were negative. BCG scar was present in 14.4% and absent in 85.6% (figure 1B).

Figure 2 shows significant differences between the three groups in the mean lymphocyte count and serum vitamin D levels (P < 0.05). Vitamin D levels were significantly raised in healthy subjects as compared to patients and care takers while lymphocyte count was higher in patients and care takers as compared to the healthy subjects. However circulating monocyte levels were non-significant amongst the three groups.

Figure 3 shows correlation between vitamin D, monocytes and lymphocytes in three groups. Significant positive correlation exists between monocyte and lymphocyte in patients and care takers while non-significant correlation was found between monocytes, lymphocytes with vitamin D in three groups. Non-significant correlation also exists between monocytes and lymphocytes in healthy control.

DISCUSSION

The study included 83 subjects with 31 active TB patients, 31 caretakers of them and 21 healthy subjects. The study shows significantly increased levels of lymphocytes in active TB patients followed by care takers as compared to healthy subjects. Significantly decreased levels of vitamin D were observed in active TB patients as compared to controls. Similarly vitamin D level was significantly decreased in caretakers as compared to healthy subjects but was significantly increased compared to active TB patients. Circulating monocyte levels were non significantly raised in both groups as compared to healthy subjects. Correlation of Vitamin D with monocytes and lymphocytes was non-significant in active TB and caretakers of them however significant positive

correlation was observed between monocytes and lymphocytes in both groups.

The *Mycobacterium tuberculosis* organism is present in the host during a latent TB infection in its dormant state. Although people with LTBI are typically asymptomatic and non-infectious, they are still at risk of later getting tuberculosis (TB) [15,16,17]. Lack of vitamin D may raise the chance of contracting TB because this vitamin may have a function in preventing the illness [18]. This study is in agreement with a study that found elevated T-lymphocyte counts in both LTBI and active TB. In LTBI participants, it is hypothesized that there is an increase in T-cell activation without an increase in cells associated to monocytes, whereas in active TB, there is a noticeably elevated level of markers for both lymphocytes and monocytes. It has been hypothesized that replicating and non-replicating microorganisms may elicit different immunological reactions [19]. This study observes significant variation in vitamin D levels both across and within groups. Vitamin D insufficiency has been linked in several studies to an increased risk of TB development. According to theories, vitamin D modulates the immune system, and having levels over the safe limit (>30.0 mmol/l) may make airway infections more likely [20, 21]. Innate antimicrobial defenses against T cell-mediated interferon also may be increased by vitamin D metabolites.

Vitamin D also inhibits the release and production of a number of mediators of inflammation, destroying the enzymes responsible for Immunopathology while the TB is in active stage. According to a study, vitamin D adjuvant therapy may minimize lung infection and increase cavitation in TB patients, lowering their risk of transmission and preventing relapse [22].

Study by Zdrenghea et al., 2017 [23] found a link between vitamin D deficiency and a significant probability of infections (increased monocyte, lymphocyte) with *Mycobacterium TB*. However, a different study discovered both a positive and a negative correlation between vitamin D and lymphocyte: proportion of monocytes in peripheral blood [24].

The study also found increased frequency of males suffered from active TB and LTBI, mean age was 26 years and most of them resides in overcrowded areas with poor hygiene and poor diet. According to a study, family members living in high-population areas had a higher risk of contracting tuberculosis than those living in low-population areas [25].

CONCLUSION

Caretakers of active tuberculosis patients showed increased levels of circulating monocytes, lymphocytes as well as lower vitamin D levels with increased risk of developing tuberculosis. However non-significant correlation exists between Vitamin D and monocyte and lymphocytes but significant positive correlation was observed between levels of monocytes and lymphocytes.

Recommendations: Screening, diagnosis, and monitoring of persons who are caretakers of active TB patients is advised. It might aid in the country's TB eradication.

Acknowledgments: We are grateful to Ms. Ayla Siddiqi, a junior college student in her third year at Texas Christian University in the United States, who proofread the text. We appreciate the technical assistance provided by Mr. Nouman and Mr. Fiaz.

Limitations: Family members under the age of 18 were not included in this study. The sample size was small due to reluctance by the subjects to participate

REFERENCES

1. Kiazky S, Ball TB. Latent tuberculosis infection: An overview. *Can Commune Dis Rep*. 2017;43(3-4):62-66.

2. Paik S, Kim JK, Chung C, Jo EK. Autophagy: A new strategy for host-directed therapy of tuberculosis. *Virulence*. 2019;10(1):448-459.
3. Agarwal A, Bhat MS, Kumar A, Shaharyar A, Mishra M, Yadav R. Lymphocyte/monocyte ratio in osteoarticular tuberculosis in children: ahaematological biomarker revisited. *Trop Doctor* 2016;46(2):73-7
4. Petruccioli E, Navarra A, Petrone L, Vanini V, Cuzzi G, Gualano G et al. Use of several immunological markers to model the probability of active tuberculosis. *DiagnMicrobiol Infect Dis*. 2016; 86(2).
5. Lastrucci C, Bénard A, Balboa L, Pingris K, Souriant S, Poincloux R, et al. Tuberculosis is associated with expansion of a motile, permissive and immunomodulatory CD16⁺ monocyte population via the IL-10/STAT3 axis. *C. Cell Res*. 2015;25(12):1333-51.
6. La Manna MP, Orlando V, Dieli F, et al. Quantitative and qualitative profiles of circulating monocytes may help identifying tuberculosis infection and disease stages. *PLoS One*. 2017;12(2):e0171358.
7. Naranbhai V, Fletcher HA, Tanner R, O'Shea MK, McShane H, Fairfax BP, et al. Distinct transcriptional and anti-mycobacterial profiles of peripheral blood monocytes dependent on the ratio of monocytes: lymphocytes. *EBioMedicine*. 2015;2(11):1619-26.
8. Nikanfar S, Rashedi J, Mahdavi B and Mohammad A. "Vitamin D and Tuberculosis Patients". *EC Pulmonol&Resp Med* 2018;7: 466-476.
9. Sudfeld CR, Wang M, Aboud S, Giovannucci EL, Mugusi FM, Fawzi WW. Vitamin D and HIV progression among Tanzanian adults initiating antiretroviral therapy. *PLoS One* 2012;7(6):e40036
10. Giacomet V, Vigano A, Manfredini V, Cerini C, Bedogni G, Mora S, et al. Cholecalciferol supplementation in HIV-infected youth with vitamin D insufficiency: effects on vitamin D status and T-cell phenotype: a randomized controlled trial. *HIV Clin Trials* 2013; 14(2):51-60.
11. WHO Global tuberculosis report 2017 Geneva, Switzerland
12. Azam F, Shaheen A, Arshad R. Frequency of hypovitaminosis D and its associated risk factors in newly diagnosed pulmonary tuberculosis patients. *Pak J Med Sci*. 2016;32(2):480-484
13. Junaid K and Rehman A. Impact of vitamin D on infectious disease-tuberculosis-a review. *Clin Nut Exp* 2019; 25: 1-10
14. Afzal A, Rathore R, Butt NF, Randhawa FA. Efficacy of Vitamin D supplementation in achieving an early Sputum Conversion in Smear positive Pulmonary Tuberculosis. *Pak J Med Sci*. 2018;34(4):849-854. DOI: 10.12669/pjms.344.14397
15. Roomi MA, Lone KP, Madassar A. Vitamin D and cardiometabolic risk factors in adult non-diabetic offspring of type 2 diabetic parents. *JPMMA* 2014; 64(11):1229
16. Collin SM, Wurie F, Muzyamba MC, et al. Effectiveness of interventions for reducing TB incidence in countries with low TB incidence: a systematic review of reviews. *Eur Respir Rev*. 2019;28 (152):180107.
17. Furin J, Cox H, Pai M. Tuberculosis. *Lancet*. 2019 ;393(10181):1642-1656.
18. Brighenti S, Bergman P, Martineau AR. Vitamin D and tuberculosis: where next? *J Int Med* 2018; 284 (2):145-162
19. Sullivan ZA, Wong EB, Ndung'u T, Kasproicz VO, Bishai WR. Latent and Active Tuberculosis Infection Increase Immune Activation in Individuals Co-Infected with HIV. *E Bio Medicine* 2015; 2: 334-340
20. Zittermann A, Pilz S, Hoffmann H, März W. Vitamin D and airway infections: a European perspective. *Eur J Med Res*. 2016; 24;21:14.
21. Huang SJ, Wang XH, Liu ZD, Wen-Li Cao³, Yi Han¹, Ai-GuoMa et al. Vitamin D deficiency and the risk of tuberculosis: a meta-analysis. *Drug Des DevelTher*. 2017;11:91-102.
22. Coussens AK, Martineau AR, Wilkinson RJ. Anti-Inflammatory and Antimicrobial Actions of Vitamin D in Combating TB/HIV. *Scientifica (Cairo)*. 2014;2014:903680.
23. Zdrenghea MT, Makrinioti H, Bagacean C, Bush A, Johnston SL, Stanciu LA. Vitamin D modulation of innate immune responses to respiratory viral infections. *Rev. Med. Virol*. 2017; 27: e1909
24. Ganmaa D, Munkhzul B, Fawzi W, Spiegelman D, Willett WC, Bayasgalan P et al. High-Dose Vitamin D₃ during Tuberculosis Treatment in Mongolia. A Randomized Controlled Trial. *Am J RespirCrit Care Med*. 2017 Sep 1;196(5):628-637.
25. deVries G, van Hest NA, Baars HW, Sebek MM, Richardus JH. Factors associated with the high tuberculosis case rate in an urban area. *Int J Tuberc Lung Dis*. 2010;14(7):859-865.