BioScientific Review (BSR)

Volume 4 Issue 1, 2022

ISSN(P): 2663-4198 ISSN(E): 2663-4201

Journal DOI: https://doi.org/10.32350/bsr

Issue DOI: https://doi.org/10.32350/bsr.0401

Homepage: https://journals.umt.edu.pk/index.php/bsr

Article: **Monocyte-to-Lymphocyte Ratio (MLR) as a Possible Prognostic Marker of Latent Tuberculosis (LTBI) among Household Contacts of Active Tuberculosis (TB) Patients**

Author(s): Rukhshan Khurshid[1], Farwa Sijjeel[2], Samar Asim[3], Maira Mahmood[4], Huma Ashraf[5], Shazia Rashid[6], Muhammad Yousaf Khan[2], Safdar Abbas[7], Basharat Nawaz[2], Mashal Naeem[8], Noor Ul Ain Malik[8]

Affiliation:

[1]Shalamar Medical College, Lahore, Pakistan

[2]Department of Pathology (Haematology/Blood Bank) PIMS Islamabad, Pakistan

[3]Pulmonology Department, Shalamar Medical & Dental College, Lahore, Pakistan

[4]Fatima Memorial Medical College/Hospital, Lahore, Pakistan

[5]Combined Military Hospital Lahore, Pakistan

[6]Department of IBB, University of Lahore, Pakistan

[7]Department of Biochemistry, Faculty of Biological Sciences Quaid-i-Azam University Islamabad, Pakistan

[8]COMSATS University Islamabad, Pakistan

Article DOI: https://doi.org/10.32350/bsr.0401.i

Article History:

Received: October 20, 2021

Revised: February 1, 2022

Accepted: February 16, 2022

Citation: Khurshid R, Sijjeel F, Asim S, et al. Monocyte-to-Lymphocyte Ratio (MLR) as a Possible Prognostic Marker of Latent Tuberculosis (LTBI) among Household Contacts of Active Tuberculosis (TB) Patients. *BioSci Rev.* 2022;4(1):01–11. https://doi.org/10.32350/bsr.0401.i

Article QR Code

Rukhshan Khurshid

Indexing

Crossref

HEC

EuroPub

Internet Archive

HJRS HEC Journal Recognition System

J-Gate Indexed

LOCKSS

Estd. 1990

A publication of
The Department of Life Sciences, School of Science
University of Management and Technology, Lahore, Pakistan

Letter

Monocyte-to-Lymphocyte Ratio (MLR) as a Possible Prognostic Marker of Latent Tuberculosis (LTBI) among Household Contacts of Active Tuberculosis (TB) Patients

Rukhshan Khurshid[1*], Farwa Sijjeel[2*], Samar Asim[3], Maira Mahmood[4], Huma Ashraf[5], Shazia Rashid[6], Muhammad Yousaf Khan[2], Safdar Abbas[8], Basharat Nawaz[2], Mashal Naeem[8], Noor Ul Ain Malik[8]

[1]Shalamar Medical College, Lahore, Pakistan
[2]Department of Pathology (Haematology/Blood Bank) PIMS Islamabad, Pakistan
[3]Pulmonology Department, Shalamar Medical & Dental College, Lahore, Pakistan
[4]Fatima Memorial Medical College/Hospital, Lahore, Pakistan
[5]Combined Military Hospital Lahore, Pakistan
[6]Department of IBB, University of Lahore, Pakistan
[7]Department of Biochemistry, Faculty of Biological Sciences Quaid-i-Azam University Islamabad, Pakistan
[8]COMSATS University Islamabad, Pakistan
*Corresponding Author's email: rakhshan99@yahoo.com
doi: https://doi.org/10.32350/BSR.0401.i

Article Info

Received: 20-10-2021
Revised: 01-02-2022
Accepted: 16-02-2022

Keywords

active tuberculosis, latent tuberculosis (LTBI), monocyte-to-lymphocyte ratio (MLR)

Abstract

Tuberculosis (TB) is endemic in many developing countries including Pakistan. It is a leading cause of death from a single infectious agent worldwide. Identification and early treatment of latent conditions help reduce the complications associated with TB. However, the identification of individuals with latent infection is a time taking and expensive process. According to previous studies, a promising and cheap biomarker of TB may be the monocyte-to-lymphocyte ratio (MLR). It may indicate a body's immune response to *Mycobacterium tuberculosis*. Since household contacts of tuberculosis (TB) patients have an increased risk of latent tuberculosis (LTBI), using the established diagnostic procedures as well as checking their MLR might help determine if they contracted LTBI or not. We conducted across-sectional study to determine if MLR could be used to identify LTBI among household contacts of patients with active tuberculosis. Out of the 100 subjects selected for this study, about 40 patients were recently diagnosed with active tuberculosis, 40 were close contacts of these patients, while20 were chosen to be controls. The mean was 0.165, 0.06 (range 0.03–0.08), and 0.04 (0.02–0.04) inpatients with active tuberculosis, close contacts of patients, and control subjects, respectively. Hence, it was determined that MLR ($\geq$ 0.6 %)is a significant predictor for LTBI

and can be used to diagnose it in close contacts of TB patients. It was additionally observed that patients over the age of 50 with pulmonary tuberculosis have higher MLR.

1. Introduction

Tuberculosis (TB) is a highly contagious infection. It claims about 4000 lives a day, making it the worst life-threatening infection in the world. In 2019, 1.4 billion individuals died because of TB, while roughly 1.8 billion people were infected latently worldwide with chances of developing TB [1]. According to previous studies, about 10% of people infected with latent *Mycobacterium tuberculosis* (LTBI) develop an active form of TB, whereas 90% of people manage to repress the invasion of bacteria [2, 3].

It is important to understand how *M. tuberculosis* (Mtb) manifests in individuals to further comprehend its underlying pathogenesis. It is known that host lymphoid cells. The myeloid-specific cells are responsible for survival and multiplication of Mtb. These cells may be the main effector for hiding and multiplication of Mtb in TB related immunity. Monocytes are different from macrophages and dendritic cells since they provide Mtb antigen to T-cells. Therefore, raised numbers of monocytes may activate more T-cells. It was determined that the interaction of T-cells with dendritic cells and macrophages via the presentation of antigen is important for the development of an effective immune response [4, 5].

identify the ability of MLR to track the

Monocytes and lymphocytes play a central role in triggering the immune response. The monocyte-to-lymphocyte ratio (MLR)in the blood may indicate an individual's state of immunity against an MTB infection[4].It was determined that high values of MLR are related to the alteration of gene transcription in monocytes, which may affect their anti-mycobacterial ability [6].

The diagnosis of LTBI remains a challenge for medical professionals. The tuberculin skin test (TST) and Interferon Gamma Release Assay (IGRA) test, used to diagnose LTBI, are based on the response of memory T-cells. This T-cell response may still be positive after the infection has cleared [7]. Moreover, the IGRA test is expensive and needs special apparatus. In a like manner, TST is not an ideal tool to diagnose LTBI because it has a high rate of false positive and false negative results. These false results are due to prior vaccination with BCG (Bacille Calmette-Guerin vaccine for TB),cross-reaction with non-tuberculosis mycobacteria, immuno-compromised state, malnutrition, and improper administration. This test is also not as popular because the patient has to re-visit for the result [8].

During the period of anti-tuberculosis therapy, a change in MLR may be used to

response to treatment. Presently, due to

low reversion rates of IGRA and TST, it is difficult to determine which patient has cleared and which needs further treatment at the end of therapy. It was also determined that MLR reduces with the therapy of active TB and could be valuable in deciding when to end treatment. It is therefore hypothesized that MLR may be a marker for LTBI as it is a cheap, readily available test. It can also help in diagnosing LTBI, especially in high-risk individuals, such as household contacts of active TB patients [9].

In this paper, across-sectional study was conducted to find the efficacy of altered monocyte-to-lymphocyte ratio (MLR) in identifying latent tuberculosis (LTBI) among close contacts of patients having active tuberculosis.

2. Methodology

2.1. Features of Patients Studied

In this study,40 patients with active TB were included. These patients visited Lahore General Hospital from October 2020 to November 2020. Additionally, patients who experienced treatment failure, re-infection during the treatment, any type of viral hepatitis, acquired immune deficiency syndrome, or suffered from diseases affecting the complete blood count (CBC), such as measles, lung cancer, syphilis, leukopenia, or rheumatoid arthritis, were not included in the study. Furthermore, to accurately evaluate the effect of anti-tuberculosis treatment, patients who did not properly use the prescribed drugs, that is, intermittent/irregular therapy, were also excluded from the study (Figure 1). Household contacts of the patients included parents, siblings, offspring, or any other persons living in the same house [10].

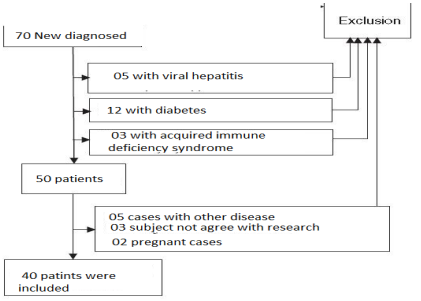

Figure 1.Criteria of Patient selection

2.2. Patient Selection Criteria

CBC data from 100 subjects including 40 patients with TB, 40 household contacts, and 20 normal subjects (controls) were included in the research. A total of 40 household contacts of patients (age range 18 to 40 years) were taken as suspected cases of latent tuberculosis (LTB), while 20 healthy age matched donors with no history of any diseases were taken as controls. The selected patients were those individuals whose anti-TB treatment was near to completion.

Subjects with no symptoms of active TB, namely the absence of fever, cough, night sweating, and weight loss in the past 30 days, were selected to be a part of the control group. The diagnosis of TB was based on clinical/imaging characteristics, the presence of acid-fast bacilli in sputum, and biopsy proved TB.

To get the CBC of blood samples (of

patients with active TB, household contacts and controls), blood was collected in ethylene-diamine tetra-acetic acid or EDTA tubes. The count was done manually (Leishman stain) by Sysmexhematology analyzer (Model XS, Hamburg, Germany).

2.3. Statistical Methods

Statistical analysis was carried out using SPSS 20. Parameters including mean difference, standard deviation, and the value of significance were used for data analysis using ANOVA (one-way analysis of variance).

Table 1: Demographics of TB patients and household contacts

Characteristics	Frequency	Relative Frequency
Gender		
Male	25	62.5 %
Female	15	37.5 %
Residence		
Crowded area	30	75 %
Un-crowded area	10	25 %
Family member suffering from TB		
One member	32	80 %
Two members	08	20 %
History of Worms		
Yes	15	62.5%
No	25	37.5 %
BCG Scar		
Yes	12	30 %
No	28	70 %

Table 2: Comparison of monocyte count, lymphocyte count and M/L ratio between groups and within groups using ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Monocyte count	Between Groups	2611.852	2	1305.926	2.249	.112
	Within Groups	46454.111	80	580.676		
	Total	49065.963	82			
Lymphocyte count	Between Groups	1978.266	2	989.133	28.434	.000
	Within Groups	2782.960	80	34.787		
	Total	4761.226	82			
Mono/Lympho ratio	Between Groups	.973	2	.486	5.568	.005
	Within Groups	6.987	80	.087		
	Total	7.960	82			

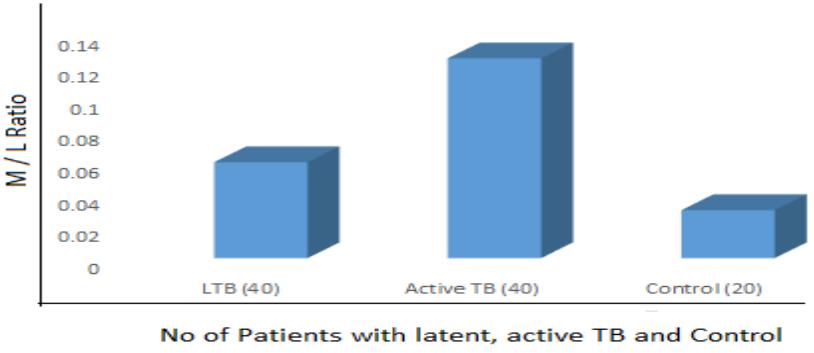

Figure 2.Monocyte / Lymphocyte ratio of study subject

3.Results

According to demographics of household contacts, most TB patients were male with a mean age of 27.56 years. Additionally, most TB patients lived in a crowded area with only one family member having TB. They also had no history of worms and BCG scar (Table 1).

A comparison of monocyte count, lymphocyte count, and M/L ratio between groups and within groups using ANOVA is tabulated (Table 2). It was observed that between groups and within groups mean lymphocyte count and lymphocyte-to-monocyte ratio (MLR) showed significant differences ($P<0.000$ and $P<0.005$). On the other hand, an insignificant difference was observed in mean monocyte count between groups and within groups

4. Discussion

Monocytes area vital part of the innate immune response that links the adaptive immune system to lymphocytes via the presentation of antigen. Therefore, any factor that disturbs the action of these cells or MLR may affect a person's response to infection [11].

According to our study, majority of the household contacts were male with having a mean age of 27.56 years and an age range of 18-40 years (data not shown). The majority of the subjects lived in crowded areas and had only one family member diagnosed with TB. Our results were in agreement with the results of a prior study, reporting that males within the age bracket of 19-35 years were more at risk of developing TB as compared to females [6]. It was also determined that the age group of 18-40 years is the age of earning. Individuals falling within this age group interact with others in the workplace and are at great risk of contracting MTB. [12]. Another study proposed that men smoke more cigarettes as compared to women, which may cause lung injury and reduced immune cell function, making them susceptible to infection [13]. A study found that younger household contacts of TB patients, especially those with ageless than16 years of age, are more susceptible to latent TB, which at a later age, may progress to active TB [14].

According to ANOVA results, we observed that between groups and within groups mean lymphocyte count, monocyte count, and lymphocyte-to-monocyte ratio (MLR) showed significant differences. On the other hand, there was an insignificant difference between groups and within groups in the mean monocyte count.

Various studies agree with our study's findings. Al Hajoj [2] reported that any alteration of monocytes-to-lymphocytes ratio (MLR) may depict a patient's ability to counter mycobacterial infections. A study reported that absolute counts of lymphocytes and monocytes in the blood may be another biomarker for active TB since it has the ability to predict an individual's risk of developing an active form of TB [15].The reason behind this ability may be that monocyte cells are the target cells of *M. tuberculosis*, and lymphocytes are the key effector cells of immunity against TB. The values of monocytes and lymphocytes might exhibit the condition of an individual's immunity against the infection. The interplay

between the immune system of host and *Mycobacterium* decides if the infection leads to the containment of MTB or not [16,17]. Hence, it is established that the immune system has a significant role in the development of TB[18]. It was determined that the ability to identify pathogens and provide enhanced protection against any re-infection are the characteristics of adaptive immunity. This was determined based on the selection of lymphocytes having receptors which are antigen specific and may remove the infection [19].

It is known that *M. tuberculosis* can block the response of the immune system using different mechanisms, especially those that inhibit the process of phagocytosis. The phagocytes are used as environmental niches and replicate there. A study reported that a partial infection with an incomplete adaptive immune reaction against *M.tuberculosis* may increase the percentage of circulating monocytes in household contacts of TB patients; it may be the reason behind the development of latent TB [20].

Several studies found high MLR in patients with TB as compared to the control group. One study reported that the MLR in peripheral blood may reveal an individual's ability to produce an active immune response, which may determine their ability to inhibit the growth of *Mycobacterium* [21]. The results of De Martino's study proved that MLR may be an earlier pathophysiologic modification of TB. The study further stated that latent TB transition to active TB is based on the complex interaction between host and bacterial factors [22].

In 2019, Wang et. al conducted a study to conclude that MLR may be used to identify the active form of TB. CBC is a commonly used test in clinical practice; however, MLR as a simple marker of TB or LTBI is not commonly recognized [17]. A study conducted by Liana et. al reported that the predicted ratio of monocytes to lymphocytes in TB patients is 0.28. It may forecast the occurrence of TB with the sensitivity and specificity of 91.04% and 93.55 %, respectively [22]. In contrast, we found the median value of patients with active TB to be 0.125 with IQR 0.075-2.0.

In 2018, Reece conducted a study and reported that the normal ratio of ML is altered by infection of *Mycobacterium* since it may alter subsections of stem cells of the hematopoietic system or directly infect the mesenchymal stem cells of bone marrow [23]. The ML ratio of patients may become normal with anti-TB treatment. These changes in ML ratio may also show the effectiveness and response of treatment. Our findings were in agreement with the study [24] which reported that a high MLR may help to distinguish people with an active form of TB and latent form of TB from persons who may have close contact with TB patients. It was also determined that a very low MLR is also a risk factor for developing TB [25]. Therefore, MLR may be a good indicator of determining the risk of developing active TB and a promising biomarker of progression of TB[5].

According to our study, MLR is disturbed

in individuals within the age bracket of 45-50 years (data not shown). In contrast, some studies found that MLR of patients having extra-pulmonary tuberculosis with age greater than 60 years is altered significantly as compared to others [23]. Multivariate analysis showed that people who have an active form of TB or extra pulmonary TB had different ML ratios [2].

Study Limitations

The information was collected from patient data collected by other experts and hence may be inaccurate. One limitation is that the sample size was small. For more valuable results CBC data was collected at the late stage of patient discharge and MLR is best recorded in multiple stages of treatment for a more valuable study

5. Conclusion

Despite the limitations, the results of our study are in agreement with the hypothesis that MLR is significantly altered in both active and latent TB patients as compared to the control group. It has the potential to be a reliable inexpensive predictor of LTBI, especially in household contacts of TB patients. It may be used as an alternative biomarker that can screen asymptomatic relatives of TB patients to determine if they are infected or not.

Further studies are needed to confirm these findings.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Zaidi SM, Waseem HF, Ansari FA, Irfan M, Fahim S, Ahmad M. Sample size estimation of diagnostic test studies in health sciences. In*14th Int Conference* on 2016 (p. 239).

2. Al Hajoj S, Varghese B, Datijan A, et al. Interferon gamma release assay versus tuberculin skin testing among healthcare workers of highly diverse origin in a moderate tuberculosis burden country. *PloSOne*. 2016;11(5):0154803.https://doi.org/10.1371/journal.pone.0154803

3. Sadaf R, Munir T, Farrukh S, Abbasi S. Prevalence of latent tuberculosis infection in healthcare workers in tertiary care hospitals of Pakistan.*Pak J Med Sci*. 2020;36(2):198-202. https://doi.org/10.12669/pjms.36.2.936

4. Wang J, Yin Y, Wang X, et al. Ratio of monocytes to lymphocytes in peripheral blood in patients diagnosed with active tuberculosis.*Braz J Infect Dis* 2015;19(2):125-31.https://doi.org/10.1016/j.bjid.2014.10.008

5. Sibley L, Gooch K, Wareham A, et al. Differences in monocyte: lymphocyte ratio and Tuberculosis disease progression in genetically distinct populations of macaques. *Sci Rep*. 2019;9(1):1-9.

6. Yang J, Zhang L, Yu C, Yang XF, Wang H. Monocyte and macrophage differentiation: circulation inflammatory monocyte as biomarker for inflammatory diseases. *Biomark Res.* 2014;7(2):1-2.https://doi.org/10.1186/2050-7771-2-1

7. Esmail H, Barry III CE, Wilkinson RJ. Understanding latent tuberculosis: The key to improved diagnostic and novel treatment strategies. *Drug*

Discov Today. 2012;17(9-10):514-21.https://doi.org/10.1016/j.drudis.2011.12.013

8. Ahmad S. Pathogenesis, Immunology, and Diagnosis of Latent Mycobacterium Tuberculosis Infection. *Clin& Develop Immunol*. 2010;2011:1–2.

9. Mayito J, Meya DB, Rhein J, Sekaggya-Wiltshire C. Utility of the monocyte to lymphocyte ratio in diagnosing latent tuberculosis among HIV-infected individuals with a negative tuberculosis symptom screen. *PLoS One*. 2020;15(11):e0241786.https://doi.org/10.1371/journal.pone.0241786

10. Yang J, Lee S, Oh S, et al. The risk of active tuberculosis among individuals living in tuberculosis-affected households in the Republic of Korea, 2015. *PLoS ONE* 2019; 14(12):e0225744. https://doi.org/10.1371

11. Simon D, Simon HU, Yousefi S. Extracellular DNA traps in allergic, infectious, and autoimmune diseases. *Allergy*. 2013;68(4):409–16.

12. Marahatta SB, Yadav RK, Giri D, et al. Barriers in the access, diagnosis and treatment completion for tuberculosis patients in central and western Nepal: A qualitative study among patients, community members and health care workers. *PLoS ONE* 2020;15(1):e0227293.https://doi.org/10.1371/journal.pone.0227293

13. Watkins RE, Plant AJ. Does smoking explain sex differences in the global tuberculosis epidemic? *Epidemiol Infect*. 2006;134:333–9.

14. Lee H, Kim J, Ae Kang Y, et al. In vitro Mycobacterial Growth Inhibition in South Korean Adults with Latent TB Infection.*Front Immunol.* 2019;10:896https://doi.org/10.3389/fimmu.2019.00896

15. 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. https://doi.org/10.1371/journal.pone.0171358

16. Philips JA, Ernst JD. Tuberculosis pathogenesis and immunity, Annual Review of Pathology: *Mech Dis*. 2012;7(1):353–384

17. Wang W, Wang L, Liu YY, et al. Value of the Ratio of Monocytes to Lymphocytes for Monitoring Tuberculosis Therapy. *Canadian J Infectious Dis Med Microbiol* 2019;2019:1-5.

18. Naranbhai V, Fletcher HA, Tanner R, et al. Distinct transcriptional and anti-mycobacterial profiles of peripheral blood monocytes dependent on the ratio of monocytes: lymphocytes. *EBioMed*. 2015;2(11):1619-26. https://doi.org/10.1016/j.ebiom.2015.09.027

19. Liu C, Liu H, Ge B. Innate immunity in tuberculosis: host defense vs pathogen evasion. *Cell MolImmunol* 2017;14:963–975. https://doi.org/10.1038/cmi.2017.88

20. Rakotosamimanana N, Richard V, Raharimanga V, Gicquel B, Doherty M, Zumla A. Biomarkers for risk of

developing active tuberculosis in contacts of TB patients. *Eur Respir J* 2015;46(4):1095-103.

21. Liana P, Brestilova B, Rahadiyanto KY. The ratio of monocytes to lymphocytes accuracy as tuberculosis predictor. InJournal of Physics: Conference Series 2019 Jul 1 (Vol. 1246, No. 1, p. 012024). IOP Publishing.

22. De Martino M, lodi L, Galli L, Chiapinni E. Immune Response to Mycobacterium tuberculosis: A Narrative Review. *FrontPediatr*. 2019;7:1-8. https://doi.org/10.3389/fped.2019.00350

23. Reece ST, Vogelzang A, Tornack J, et al. Mycobacterium Tuberculosis-Infected Hematopoietic Stem and Progenitor Cells Unable to Express Inducible Nitric Oxide Synthase Propagate Tuberculosis in Mice. *J Infect Dis*. 2018;217(10):1667-1671. https://doi.org/10.1093/infdis/jiy041

24. Iqbal S, Ahmad U, Zaidi SBH. Monocyte Lymphocyte Ratio as a Possible Prognostic Marker in Ant tuberculous Therapy. *J Rawal Med College*. 2014;18(2):178-181.

25. Tabassum MN, Muhammad AK, Afzal S, Gilani A, Gureja AW, Tabassum S. Demographic Characteristics of Tuberculosis Patients at Public Sector Health Facilities in Lahore, Pakistan. *Ann of King Edward Med Univ.* 2018;24:1-6.https://doi.org/10.21649/akemu.v24i1.2309