

CD4 and CD20 as important immune markers in patients with pulmonary tuberculosis

Firas Mohammad Hussein Kamoona¹ and Ahmed Abduljabbar Jaloob Aljanaby^{1*}

¹Department of Biology, Faculty of Science, University of Kufa, Kufa, Iraq.

Abstract. Tuberculosis (TB) is a serious lung infection caused by the bacterium *Mycobacterium tuberculosis* (*M. tuberculosis*) is highly contagious, and can be spread through the air from an infected person to others. The aim of this article was to evaluate the immunological role of CD4 and CD20 in patients infected with *M. tuberculosis* and multi-drug resistant tuberculosis (MDRTB). A total of 182 individuals suspected with *M. tuberculosis* admitted to the tuberculosis center in AL-Najaf City, Iraq. A sputum acid-fast stain was performed for each individual and GeneXpert[®] heminested real time PCR has been performed for MDR-*M. tuberculosis* detection. CD4 and CD20 have been measurement in serum of infected individual using ELISA technique. Serum CD4 and CD20 levels were significantly elevated ($P<0.05$) in patients with *M. tuberculosis* and MDR-*M. tuberculosis* groups as compared with control subjects. The MDRTB group showed higher serum CD4 and CD20 levels ($P<0.05$) than the *M. tuberculosis* group. This study showed that CD4 and CD20 had a relationship with *M. tuberculosis* and might be used to help diagnose TB.

Keywords. CD4, CD20, TB, MTB, MDRMTB, GeneXpert.

1 Introduction

Tuberculosis (TB) is an airborne disease caused by the bacterium *Mycobacterium tuberculosis* (*M. tuberculosis*). Symptoms can include a persistent cough, fever, and night sweats [1]. If left untreated, tuberculosis can lead to serious health problems, including lung cancer. Tuberculosis can be transmitted from person to person by airborne droplets from coughing or sneezing. In 2016, the World Health Organization reported that there were 10.4 million new cases of tuberculosis and 1.7 million deaths from the disease [2]. Tuberculosis is treatable with antibiotics, but is difficult to diagnose and cure, because it often presents with nonspecific symptoms such as fever, cough, fatigue, night sweats, and weight loss. This often leads to delays in diagnosis and inadequate treatment [3]. Today, most patients with pulmonary TB are diagnosed by detecting bacteria in the sputum or by a skin test to identify an immune response to *M. tuberculosis*. In 1997, researchers reported that reactivation of TB occurred in 20% of cases; they also showed that the presence of human CD8-positive T cells that target the antigen ESAT-6 correlated with high risk of developing active TB after respiratory exposure [4]. Since then, researchers have identified several

* Corresponding author: ahmedaj.aljanabi@uokufa.edu.iq

proteins from *M. tuberculosis* that are immunogenic, including mycolyl transferase (MPT), lipoarabinomannan (LAM), and the mycobacterial cell wall-associated protein ESAT-6. In 2011, researchers reported that *M. tuberculosis* impairs dendritic cell response by altering CD1d expression through a non-canonical signaling pathway that involves post-translational modification of a G-protein-coupled receptor called Toll-like receptor 2 (TLR2) [5]. CD8 is a cell surface protein found on the majority of T-cells. CD8 helps the T-cell to identify and destroy cells that are infected with a virus or cancer, is also important in the immune response against other infectious organisms. The CD20 antigen is a protein that is found on the surface of some cancer cells [6]. Cancer cells that overgrow or spread quickly can be identified by the presence of CD20. The antigen is also found on the surface of some normal cells. However, in a normal cell, CD20 is only found in low levels and does not play an important role in controlling cell growth [7]. The aim of this study is to evaluate the levels of CD4 and CD20 in serum of patients with TB and using as diagnostic markers.

2 Methods

2.1 Ethical Consideration

It has been approved by the Institutional Ethics Committees of Kufa University's College of Science and AL-Kufa General Hospital that a study concept for human studies has been approved. Additionally, each participant gave written, informed consent prior to taking part in the study. The World Medical Association's Ethics Code applies to studies involving humans in accordance with the Declaration of Helsinki.

2.2 Individuals

In the period of June to December 2022, 182 males and females suspected of *M. tuberculosis* were admitted to the tuberculosis center in Al-Najaf City, Iraq. GeneXpert® heminested real-time PCR was performed for MDRTB detection and ELISA was used to measure CD4 and CD20 in serum samples from infected and healthy individuals [8,9].

2.3 Statistically Analysis

A statistical package (Statistical Package for Social Sciences, version 20, IBM, and Armonk, New York) was used to collect and analyze the data. A mean and standard deviation were used to summarize the quantitative data; numbers and percentages were used to summarize qualitative data, correlation was used to determine correlations, and a P-value<0.05 was considered significant [10, 11].

3 Results

3.1 Total Patients and Age Groups

Thirty-five of 182 individuals were infected with *M. tuberculosis*, 20 were male (57.1%) and 15 were female (42.9%). The most infected age group was 41-50 with 18 cases (51.5%) (10 male and 8 female) followed by age group 20-30 with 8 cases (22.8%) and 31-40 with 7 cases (20%), the age group 51-60 was the lowest infected with 2 cases (5.7%) (Table 1). On

the other hand, Out of 35 patients infected with *M. tuberculosis* there were 15 infected with MDRTB, 9 male (60%) and 6 female (40%). Age group 51-60 was the most infected with 8 cases (53.4%) (5 male and 3 female) followed by age group 41-50 with 5 cases (33.4%) while, the age groups 20-30 and 31-40 were the lowest in infected with one case for each age group (6.6%) (Table 2, Figure 1).

Table 1. Total patients infected with *mycobacterium tuberculosis*.

Age group	Male No.(100%)	Female No.(100%)	Total No.(100%)
20-30	5	3	8(22.8)
31-40	4	3	7(20)
41-50	10	8	18(51.5)
51-60	1	1	2(5.7)
Total No.(100%)	20(57.1)	15(42.9)	35(100)

Table 2. Total patients infected with multi-drug resistant *mycobacterium tuberculosis*.

Age group	Male No.(100%)	Female No.(100%)	Total No.(100%)
20-30	1	0	1(6.6)
31-40	1	0	1(6.6)
41-50	2	3	5(33.4)
51-60	5	3	8(53.4)
Total No.(100%)	9(60)	6(40)	15(100)

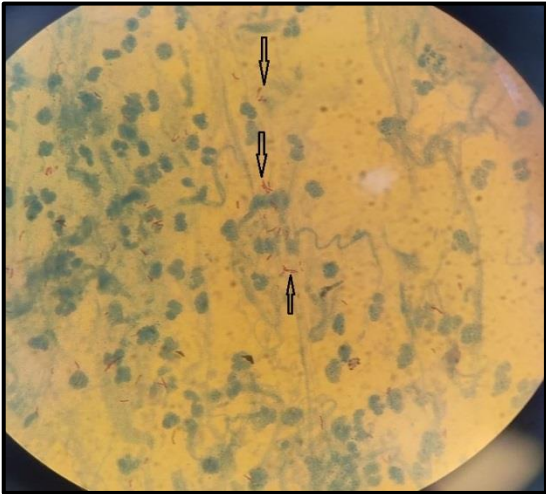


Fig. 1. Multi-drug resistant *Mycobacterium tuberculosis* cells isolated from the sputum of a 45-year-old male stained with acid-fast stain. Magnification force 1000X.

3.2 Cluster of Differentiation 4 (CD4)

Figure 2 proved there was significant increase (P-Value 0.0123) in CD4 level (0.3250 ± 0.0522) in patients infected with *M. tuberculosis* as compared with control (0.1733 ± 0.0406). Also, the results indicated that there was significant increase (P-Value< 0.0001) in CD4 level (1.086 ± 0.1017) in patients infected with MDRTB as compared with control (Figure 3). In the other hand, the results demonstrated there was significant increase (P-Value< 0.0001) in CD4 level in patients infected with MDRTB as compared with patients infected with *M. tuberculosis* (Figure 4).

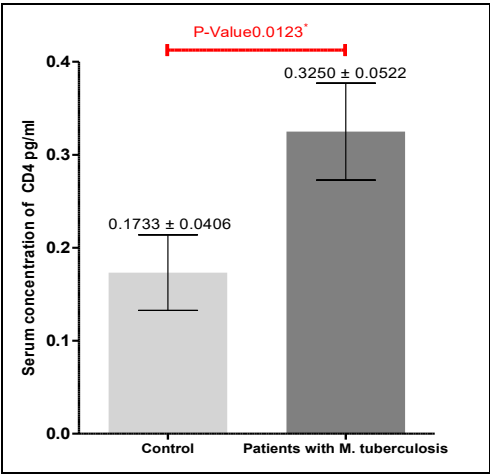


Fig. 2. Statistical analysis of CD4 levels in patient's serum infected with *M. tuberculosis* and control.

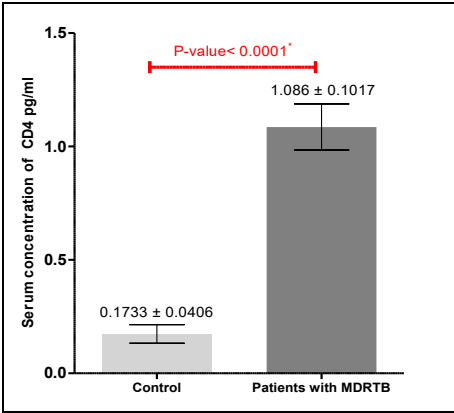


Fig. 3. Statistical analysis of CD4 levels in patient's serum infected with MDRTB and control.

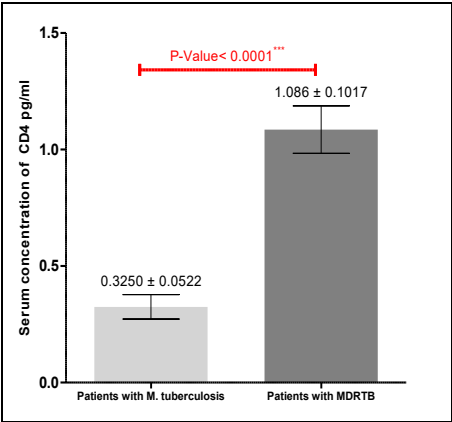


Fig. 4. Statistical analysis of CD4 levels in patient's serum infected with *M. tuberculosis* and MDRTB.

3.3 Cluster of Differentiation 20 (CD20)

Figure 5 showed the results of statistical analysis of CD20 levels in patient's serum and control. The results demonstrated there was significant increase (P-Value 0.0121) in CD20 level (169.7 ± 11.98) in patients infected with *M. tuberculosis* as compared with control (139.8 ± 6.246). Also, there was significant increase (P-Value< 0.0001) in CD20 level (243.9 ± 17.59) in patients infected with MDRTB as compared with control (Figure 6). On the other hand, the results showed there was significant increase (P-Value0.0006) in CD20 level in patients infected with MDRTB as compared with patients infected with *M. tuberculosis* (Figure 7).

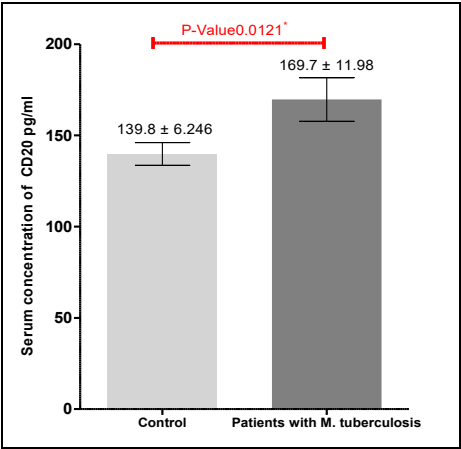


Fig. 5. Statistical analysis of CD20 levels in patient's serum infected with *M. tuberculosis* and control.

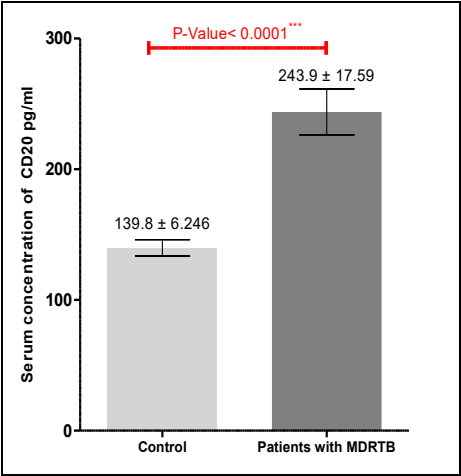


Fig. 6. Statistical analysis of CD20 levels in patient's serum infected with MDRTB and control.

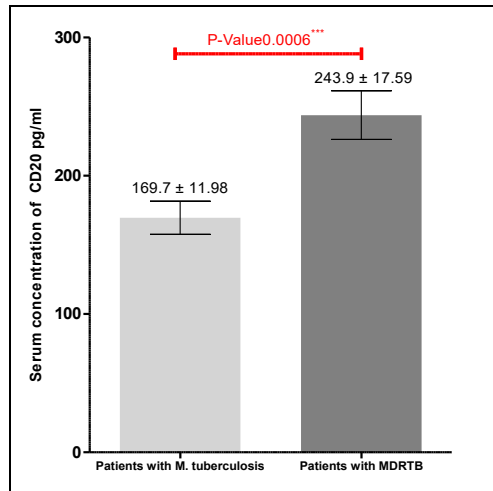


Fig. 7. Statistical analysis of CD20 levels in patient's serum infected with *M. tuberculosis* and MDRTB.

4 Discussion

Tuberculosis is a contagious disease that commonly affects the lungs but can also affect other parts of the body [12]. If left untreated, it can spread throughout the body and cause death. In this study, *M.tuberculosis* and MDR-*M.tuberculosis* patients had significantly higher serum CD4 and CD20 levels than controls ($P=0.05$) and it was found that the CD4 and CD20 levels in the MDRTB group were higher than those in the *M. tuberculosis* group ($P=0.04$). A person's immune system is responsible for defending them from infection. The immune system is designed to detect foreign objects or proteins that are not supposed to be in the body, and then remove them. It is made up of many different parts, including white blood cells (lymphocytes), antibodies, and proteins called cytokines [13]. CD4 cells are a type of white blood cell that plays an important role in protecting the body from infection [14]. When the body's natural defenses are overwhelmed by an infection, CD4 cells help to clear the area by stimulating other white blood cells called macrophages, and by activating other cells of the immune system, including T cells [15]. They also produce cytokines and other immune molecules that help to boost the immune system so that it can fight the infection more effectively. CD20 is a protein that helps to control the immune system. It can help to protect the body from infection [16]. It is found in the blood and other tissues. CD20 helps to create immunity against certain diseases found on the surface of B cells, a type of white blood cell. The CD20 molecule is a protein that helps to keep the immune system functioning properly. It helps to identify and destroy infected cells [17]. The CD20 molecule is found on the surface of some cells in the body. These cells include B lymphocytes (B cells), a type of white blood cell that helps make antibodies, and T cells, another type of white blood cell that fights infection. In a healthy person, CD20 molecules are usually present on B cells and T cells but not on other cells in the body [18]. However, the CD20 molecule can become present on the surface of other cells in the body under certain conditions. When this happens, the CD20 molecule can bind to molecules called antigens. An antigen is a substance that triggers an immune response. When CD20 molecules bind to antigens, they can trigger an immune response [19]. The immune response can lead to inflammation and tissue damage. This can cause the symptoms of rheumatoid arthritis, such as inflammation of the joints, stiffness and pain [20, 21].

Therefore, Patients with *M.tuberculosis* and multidrug-resistant *M.tuberculosis* had substantially higher serum CD4 and CD20 levels compared to healthy controls and serum CD4 and CD20 levels were significantly higher in the MDRTB group compared to the *M. tuberculosis* group [22].

5 Conclusion

This study showed that *M. tuberculosis* and MDRTB patients had higher serum CD4 and CD20 levels than controls. CD4 and CD20 had a relationship with *M. tuberculosis* and might be used to help diagnose TB.

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