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ROLE OF CD 1B IN TUBERCULOSIS PATIENTS IN WASIT PROVINCE, IRAQ

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Abstract

Background and aims: Human leukocyte antigens (HLA), which are highly polymorphic (more than 26,000 alleles), present a variety of Mtb peptides to T-cells, which is considered a key element in the immune response against Mtb. On the other hand, lipids, glycolipids, and lipopeptides are presented to T cells by the non-polymorphic CD1 molecules. Several Mtb lipid antigens are presented by CD1 molecules such as CD1a, CD1b, CD1c, and CD1d, increasing the breadth of the T-cell responses to Mtb. **Aim of study**: The role of CD1b in the recognition and phagocytosis of Mycobacterium tuberculosis. **Materials and methods**: The current study has been conducted as a comparative case-control study in Wasit Province. The study consisted of 120 blood specimens collected from human individuals and divided into three groups, each consisting of 40 participants. **Result**: contains information about the diagnostic performance of different variables for treated and new untreated cases of tuberculosis (TB) groups. the CD1b appear diagnostic value in distinguishing between treated and (new) untreated cases of TB. The AUC values in the table indicate the ability of cd1b to discriminate between treated and new untreated TB cases. Higher AUC values (closer to 1) indicate better discriminatory power. 95% CI: a P-value less than a certain threshold (often 0. 05) suggest that the AUC is significantly different from a random classifier**. Conclusion**: The study showed that CD1B increased in newly diagnosed patients and decreased after two months of intensive treatment, while its percentage was normal in healthy people.

Keywords: CD1B, Tuberculosis, Flowcytometry, Mycobacterium

Introduction

CD1 molecules have a deep and hydrophobic antigen-binding groove that allows the presentation of large hydrophobic antigens [1]. Among the members of the human CD1 family, CD1b shows the largest binding groove, capable of accommodating hydrophobic chains of about 70 carbons [2]. After biosynthesis, the integrity of the CD1b hydrophobic channels is maintained by association with endogenous phosphatidylcholine and a long endogenous spacer (EnSpacer). Together, these two lipids stabilize the CD1b groove [1]. In consequence, the presentation of the native Ac2SGL antigen on CD1b most likely requires a repositioning of the EnSpacer. The discovery of new specific ligands capable of specifically detecting MTB-infected cells may contribute to the development of new diagnostic and therapeutic tools. On the other hand, the recognition of infected cells by ligands such as antibodies implies the recognition of MTB-specific antigens presented on surface molecules such as HLA and CD1. In this sense, ligands recognizing HLA-Mtb-epitopes and CD1b-Mtb-lipid complexes have been reported [3], but the high polymorphism of the HLA molecules is a drawback for their use as universal markers for infected

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cells, so, antigens bound to the non-polymorphic CD1 molecules, offer an interesting alternative as universal markers of Mtb-infected cells. Using phage display technology, our group obtained a single-chain T cell receptor (scTCR) construct composed of the variable alpha and beta domains from the Z4B27 clone. This recombinant scTCR recognizes both the CD1b-Ac2SGL and CD1b-SGL12 complexes, showing a higher reactivity for the complex with the natural antigen. The phage-displayed scTCR was also able to recognize Mtb-infected cells from a TB patient [4], showing the potential to become a diagnostic tool. Interestingly, a very similar behavior was found for a Vk (variable kappa) domain antibody fragment (dAbκ11) selected from a phage display library using CD1b-transfected cells loaded with Ac2SGL [5].

Materials and Methods

Specimen collection

This study control was conducted after due permission from the Institutional Ethics Committee. Collection 5 ml of the venous blood were punctured from a vein for each one of the participants in the three studied groups (controls, new diagnosis patients, and after two months of infection) in an EDTA tube. Flow cytometry is an optical-to-electronic coupling system device that helps in recording how a cell scatters incident light and emits fluorescence. It's a technique used to measure and detect the physical and chemical characteristics of a population of cells or particles that are suspended in a fluid and injected into the flow cytometer instrument. Flow cytometry is derived from two words: flow, meaning cells in motion, and cytometry, meaning measurement of cells. A flow cytometer helps quantify a set of parameters from a particle in suspension. A sample containing cells or articles is suspended in a fluid and injected into the flow cytometer instrument. A beam of light of a single wavelength is directed at a continuous flow of suspended particles. Cells are often labelled with fluorescent markers so that light is first absorbed and then emitted in a band of wavelengths. Each particle in suspension that passes by this light beam scatters light that is caught by detectors perpendicular to it. These complexes of fluorescent substances conjugated with microscopical particles, when excited, emit light of a lower frequency than that from the light source. This emitted light is then caught by detectors and analyzed according to the brightness fluctuations of each detector or fluorescence emission. Statistical differences were set at $P<0.05$ [6].

Results

The results of this study were based on the analysis of data obtained from 120 participants who were divided into three groups. Patients who newly diagnosed with TB and never received any treatment yet were represented 40 cases while 40 patients were with chronic TB and previously received medications. The remaining 40 samples were the control healthy people.

The minimum age for the whole sample was 23 years and the maximum was 54 years old. Patients were distributed almost evenly according to the age-interval scale in the form of three groups, reflecting that all age groups were susceptible to tuberculosis and most cases of infection occur with age less than 50 years (35%). This may be attributed to the fact that individuals under 50

years of age represent the majority of the population and are the most active stages of life making them more at risk of exposure to microorganisms than patients with an open form of the disease. The results agreement with other studies obtained by [7] who showed that the young and middle-aged groups showed higher frequencies of tuberculosis cases than other age groups, [8] showed an increase in tuberculosis cases between the age groups 41-50. The incidence was also matched with the [9] which reported that 75% of tuberculosis cases in the developing world fall in the most economically productive age group (15-54) years.
Another recent study reported that all age groups of the population are targeted for tuberculosis [10]. The American Thoracic Society (2000) showed that people of all ages, nationalities, and all incomes can develop TB, [11] demonstrated that the age group studies were different between studies and covered all ages.

Table (1): Difference of CD1B according to study group and sex

Sex	Study groups Mean ±SD			P-value
	Treated TB	New TB	Control	
Male	22. 7±3.5	31. 4±5.6	11.1±2. 9	Groups*sex=0.567 Sex=0.753 Groups<0. 001
Female	23. 7±3.6	31. 9±4.8	10.3±3. 0	

(Table 2): Difference of CD1b between all groups according to age groups

Age (years)	Genes	Study groups Mean ±SD			P-value
		Control healthy	Newly diagnosed TB	Chronic treated TB	
≤ 33	CD 1b	11. 97±2. 06	34. 11±3. 84	22. 85±4. 14	<0. 001
34 - 43	CD 1b	10. 18±3. 46	29. 92±3. 44	23. 15±2. 19	<0. 001
≥44	CD 1b	9. 77±2. 95	31. 22±6. 78	23. 60±4. 25	0. 003

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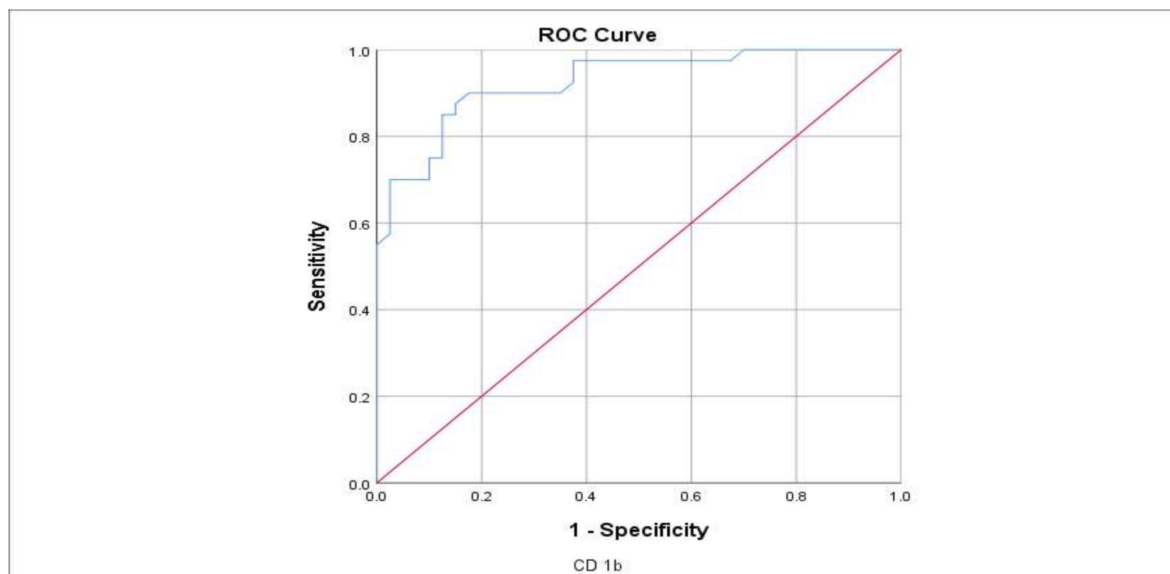

Figure (1): **Receiver operator characteristic (ROC) curve and area under the curve (AUC) for sensitivity and specificity of a cut-off value of CD1b between treated and untreated new TB groups**

Table (3): **Area under the curve, sensitivity, and specificity of cut-off values of treated from new untreated cases TB groups**

Variable	AUC	95% CI	P-value	Cutoff	Sensitivity	Specificity
CD 1b	0. 926	(0. 871-0. 981)	<0. 001	≤29. 9	98%	62%

Discussion

Cd1b is measured as the mean value (± standard deviation, SD) in each study group, categorized by sex. The P-value indicates the statistical significance of the differences observed.

the table provides the P-values for the comparisons made. The "Groups*sex=0. 567" indicates the interaction effect between study groups and sex, suggesting that there is no significant interaction effect between the two factors. The "Sex=0. 753" value represents the P-value for the comparison of Cd1b levels between males and females, indicating no significant difference in Cd1b levels based on sex. Finally, the Groups were at a significant difference in Cd1b levels among the study groups.

Based on the information provided in the table 1 there is a significant difference in Cd1b levels among the study groups but no significant difference based on sex. In table 2 appear the CD1b is a type of protein involved in presenting lipid antigens to immune cells (Dheda et al., 2010). The table shows the mean ± SD (standard deviation) values for CD1b in each study group according to age groups. The values represent the average levels of CD1b, with the SD indicating the variability around the mean. The data reveals that the mean CD1b levels are higher in the new TB group compared to the treated TB group in all age groups. The differences between the groups are statistically significant (P < 0. 001 for age ≤ 33, P < 0. 001 for age 34-43, and P = 0. 003 for age ≥

44). In table 3 appear the variable CD1b being evaluated for their diagnostic value in distinguishing between treated and new untreated cases of TB. AUC stands for Area under the Curve. It is a measure of the overall performance of a diagnostic test. The AUC values in the table indicate the ability of each variable to discriminate between treated and new untreated TB cases. Higher AUC values (closer to 1) indicate better discriminatory power. This column represents the 95% confidence interval for the AUC. It provides a range within which the true value of the AUC is likely to fall. The P-value indicates the statistical significance of the AUC. In this context, a P-value less than a certain threshold (often 0. 05) suggest that the AUC is significantly different from a random classifier. The cutoff value is a threshold used to classify individuals as either belonging to the treated or new untreated TB groups based on the variable being measured. For example, for the variable CD1b, the cutoff value is ≤29.9.

Sensitivity is a measure of how well a diagnostic test identifies true positive cases. It represents the proportion of individuals with the disease (new untreated TB cases) who are correctly identified as positive by the test. For CD1b, the sensitivity is 98%. Specificity: Specificity is a measure of how well a diagnostic test identifies true negative cases. It represents the proportion of individuals without the disease (treated TB cases) who are correctly identified as negative by the test. For CD1b, the specificity is 62% as shown in table (3) and figures (1).

Conclusions

The study provides information about the diagnostic performance of different variable (CD1b) for distinguishing between treated and new untreated cases of TB. The AUC values indicate the overall discriminatory power of each variable, while the sensitivity and specificity values provide information about their ability to correctly identify positive and negative cases, respectively.

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