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Examining ANGPTL4 mRNA levels and IgG and IgM and Their Relationship Through Immunological Markers in Iraqi Tuberculosis Patients

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Abstract

New approaches are needed to control tuberculosis (TB) worldwide. In particular, new tools for detection and novel biomarkers are needed to assess key pathogen and host components in the response to infection. Angiopoietin-like 4 (Angptl4) is a protein that belongs to the angiopoietin-like family. Angptl4 promotes the recruitment of immune cells and the production of inflammatory cytokines, helping to modulate inflammatory processes. It can be induced by pro-inflammatory signals, such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β) and lipopolysaccharides (LPS). Due to its multifaceted role in metabolic disorders, angiogenesis and inflammation, Angptl4 has attracted attention as a potential therapeutic target. In this study, the expression levels of ANGPTL4, IgM, IgG, TNF-α, IL-6 and IL-1β were investigated in TB patients (n=50) and healthy individuals using western blot and ELIZA. were measured. The expression levels of ANGPTL4, IgM, TNF-α, IL-6 and IL-1β increased significantly in patients compared to the control group (healthy group) ($P < 0.05$) and IgG changes were not significant. The results of our study show that the identification of specific immunological markers and their association with ANGPTL4 expression can lead to the discovery of new diagnostic biomarkers for TB. These biomarkers can be used to improve the accuracy and efficiency of TB diagnosis, especially in resource-limited settings where traditional diagnostic methods may be limited.

Keywords: Tuberculosis; Angptl4; Biomarkers; miRNA; Immunoglobulin

1- Introduction

Tuberculosis is one of the most successful human pathogens that infects one third of the world's population every year and causes the death of more than 2 million people (1). Despite the treatment and various anti-inflammatory drugs, the incidence of tuberculosis has been increasing in recent years, and this is related to several factors, including the co-infection of tuberculosis with the human immunodeficiency virus and the emergence of drug-resistant strains of tuberculosis bacteria. There are ideal chemotherapy regimens for the treatment of tuberculosis, which require the use of several anti-tuberculosis drugs over long periods. Side effects of current drug regimens, combined with long-term treatment, often lead to poor patient compliance, treatment failures, and the emergence of drug resistance associated with major financial consequences (2). The close monitoring of treatment required to raise efficacy to acceptable levels, such as the World Health Organization's DOTS program, puts the cost beyond the reach of many of the world's most needy populations. The development of new and shorter treatments for TB is now an urgent need (3).

The possibilities of immunotherapy deserve more attention than in the past, especially because immunotherapy can circumvent the problems of drug resistance (4). However, caution should be taken because the disease itself is a consequence of the immune response, and one must stimulate the protective rather than the harmful aspects of the response (5). tuberculosis is a facultative intracellular pathogen and a cell-mediated Th1-type immunity involving cytokine-mediated monocyte activation and T-cell cytotoxicity toward infected macrophages is a major component of the protective immune response. The role of antibodies in protection is less clear, but has been reevaluated in light of a number of recent publications (6).

Patients with active tuberculosis usually have high titers of IgG and IgM lacking terminal galactose (Gal). In contrast to fully sialylated IgG, agalactosyl IgG and IgM has proinflammatory activity, which can contribute to immunopathology in advanced tuberculosis, and high doses of IVIg can replace this abnormal IgG, producing a protective effect (7).

ANGPTLs are a group of proteins involved in vascular remodeling and lipid metabolism and are found in the liver, small intestine, blood plasma, and adipose tissue (8). ANGPTL-4 is an important member of the special secreted protein family. ANGPTL-4 has been confirmed to be involved in angiogenesis-related disorders such as inflammation (9) and can be induced by pro-inflammatory signals such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β) and lipopolysaccharides (LPS) and acts as a mediator of the inflammatory response. Angptl4 promotes the recruitment of immune cells and the production of inflammatory cytokines, helping to modulate inflammatory processes. Consequently, it is an attractive and promising biomarker for the detection or diagnosis of various inflammations (10).

It has been shown that tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β) and interleukin-6 (IL-6) are all associated with the increase of IgG and ANGPTL4. In this study, using ELIZA and western blot, we will examine the relationship between IgG and ANGPTL4 through cytokines in Iraqi tuberculosis patients.

2- Methods

2-1 Data Collection

This research was mainly done in Tabriz Immunology Research Center. Sampling was done from 2 groups of healthy and TB patients (50 people in each group). Blood samples of more than 50 patients with tuberculosis were obtained from Imam Reza Hospital (AS) and Tabriz International Hospital in Tabriz with the explicit written consent of the participants. Specialist doctors identified patients with tuberculosis (TB).

2-2 Isolation of blood cells with ficoll

PBMCs include lymphocytes (i.e. T cells, B cells, and NK cells), monocytes, and dendritic cells, and are defined as white blood cells with round nuclei. Preparation of a PBMC fraction from whole blood is a common step prior to the isolation of specific immune cell subsets. The most common PBMC isolation method involves using a density gradient medium (e.g. Ficoll™ or Lymphoprep™) and centrifugation. This method takes advantage of the differences in density between the cells in blood and the density gradient medium. Whole blood is first diluted with phosphate buffered saline (PBS) and then carefully layered over the density gradient medium. During centrifugation, the cells with higher densities (i.e. granulocytes and erythrocytes) sediment through the density gradient medium. The peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood. Briefly, 25 ml of supernatant was carefully layered over 15 ml of Ficoll after blending with PBS and centrifuged at 3200 ×g for 20 min without brake and acceleration. After centrifugation, the interphase was collected and centrifuged again at 2500 ×g for 6 min. Finally, they seeded in RPMI medium supplemented with 10% fetal bovine serum (FBS) (11).

2-3 Eliza assay

Using prepared ELISA plates coated with specific IgG and IgM Mab, tested plasma samples were dispensed in 100 µL per well. Plates were incubated for 1 h at 37°C, washed 3 times with PBS-tween 20 wash buffer (0.05%) and dried. After that, 100µl/well of 1/1000 dilution anti-IgM and anti-IgG peroxidase conjugate (obtained from Harbin-Weike Research Institute, China) was added to each well. The plates were incubated for 1 hour at 37°C and then washed 3 times using washing buffer. The plates were dried and ABTS 100 substrate solution was added to the well (Substrate PeroxidaseÒABTS (1 Component) Sigma) and the plates were incubated at 37°C for 30 minutes. Stop solution (1M H_2SO_4) was added to all wells (100µl/well) and OD was determined using a spectrophotometer (12).

2-4 Western Blot

Cells after washing twice with PBS, the cells were placed in a lysis buffer for 30 min and suspended. The suspended cells were centrifuged at 14,000 rpm for 20 min. The supernatant was collected, and the protein content was measured through the Bradford method. Protein lysates were separated by 12% SDS gel electrophoresis and then transferred to a PVDF membrane. The

membrane was then placed in a blocking solution (2% skim milk in TBS buffer) for 1 day. After that, IL-6, TNF-α and IL-1β antibodies were mixed with the blocking solution, and the membrane was further incubated for 16 to 18 h. After initial staining of the membrane with secondary rabbit antibody with concentration (1: 1000) for all primary antibodies for one hour and 15 min at room temperature. The GAPDH and U6 antibody was used as a loading control (13).

2-5 Statistical analysis

Statistical analyses were performed using one-way ANOVA followed by Tukey HSD test or by Student's t-test. Data are presented as mean ± SEM (animal experiments) or mean ± SD (cell culture studies). $P < 0.05$ was considered statistically significant.

3 Result

The expression level of proteins related to inflammation and immune response was measured. After separating the blood cells of people with tuberculosis and healthy people, the expression level of ANGPTL4, IL-6, TNF-α and IL-1β was measured and it was found that the amount of pro-inflammatory factors and miRNA in people with tuberculosis was significantly ($P < 0.05$) has increased (Figure 1).

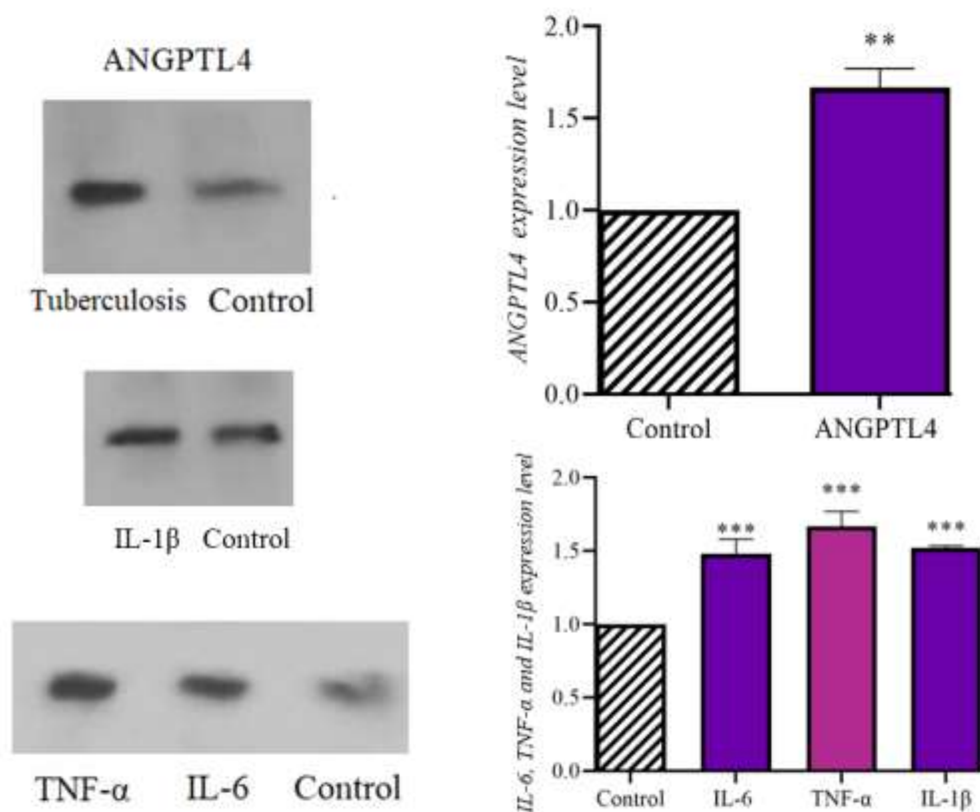

Figure 1. The effect of tuberculosis on the expression levels of ANGPTL4, IL-6, TNF-α and IL-1β proteins (P<0.05).

We first examined IgG and IgM levels to determine whether IgG and IgM increase during lung inflammation. Analysis of published transcriptional data from the blood of patients with tuberculosis revealed a significant enrichment of IgG and IgM heavy chain transcripts, indicative of humoral responses in this disease. Regarding the increase of commensal specific IgM, it was significantly higher in sick people than in healthy people. Meanwhile, IgG did not show a significant change.

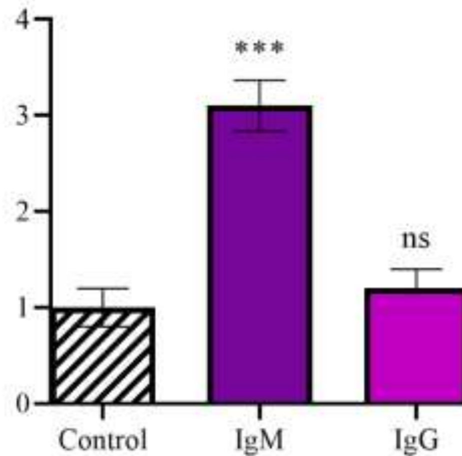

Fig 2. We investigated IgG and IgM levels in TB patients and healthy subjects. Considering the average absorption of IgG, the group of healthy people did not show significant absorption compared to patients with active pulmonary tuberculosis ($p<0.001$). Also, the chart shows a high concentration of IgM in the active TB group compared to the healthy group ($p<0.001$).

Discussion

The role of antibodies in the pathogenesis and control of tuberculosis has been discussed for a long time (Glatman-Freedman, 2010). From a pathogenic point of view, the inflammatory response of the participants is significant. This latter factor is very important because excessive inflammation causes structural and functional lung damage (Surkova & Dius'mikeeva, 2003). In this study, we showed that tuberculosis infection increased the expression of IL-6, TNF-α and IL-1β proteins and further increased ANGPTL4 protein expression through direct transcriptional regulation by IL-6, TNF-α and IL-1β. increased. Our analysis of pneumonia samples associated with human clinical infection revealed higher levels of ANGPTL4 compared to healthy subjects. These results obtained by studying A.J. Schuerwegh et al. were in line, they showed an increase in these cytokines in their study. Also, a high concentration of IgM was measured in people with tuberculosis. In the study of Alma Arce Mendoza et al., similar results were obtained from IgG and IgM.

In the study of Miao-Miao Tian et al., it was shown that the increase in the production of

anti-inflammatory cytokines and pro-inflammatory cytokines, including IL-6, significantly increases ANGPTL4. (17). In the study of Yuyue Zuo et al., it has been shown that ANGPTL4 is involved in the inflammatory processes of acute lung diseases, including influenza pneumonia and LPS-induced acute lung injury, as well as chronic lung diseases such as chronic obstructive pulmonary disease. The IL6-STAT3 signaling cascade directly regulates ANGPTL4 expression (18). Both in lung tissue from a mouse acute injury model and in human alveolar epithelial cells treated with LPS, ANGPTL4 expression is significantly increased and positively correlated with inflammation in lung tissue (IL-6 and neutrophil infiltration). These studies are consistent with our study and confirm the possible increase of IL-6 in association with ANGPTL4. In Kenichiro Maeda et al.'s study (14), the role of IL-6 in a mouse model of lung damage caused by intra-alveolar deposition of IgG immune complexes was studied and it was determined that IL-6 acts as an intrinsic regulator of inflammatory lung damage after the deposition of IgG immune complexes. And the protective effects of exogenously administered IL-6 may be partly related to suppressed TNF-alpha production, which was in line with our results. Based on these results, it can be said that the increase of ANGPTL4 by IL-6 and TNF-α is related to the decrease of IgG. In the study of Beatriz Abós et al. (15), it was determined that IL-6 is a differentiation factor for B cells that stimulates IgM responses in the absence of follicular structures, and this result is in line with the results of our study, and the increase of ANGPTL4 by IL-6 with increasing IgM is related.

The proinflammatory cytokine IL-1β is a key mediator of inflammation and plays an important role in host resistance to TB infections. To date, most studies have investigated the mechanisms of IL-1β secretion using in vitro TB strains, and the findings may not be widely applicable to contemporary clinical strains (16). In a study by Ryan Kolb, IL-1β was shown to directly regulate ANGPTL4 in adipocytes (17). And J M Noh's study showed that IL-1β increased the expression of Angptl4 through a mechanism dependent on the JNK-MAPK signaling pathway in MC3T3-E1 cells (18). In our study, an increase in both IL-1β and ANGPTL4 was seen, which is in line with the above studies. Also, in the study of Laura del Barrio (19), it was found that in lung infection, IL-1β activates non-redundant protective responses against tularemia and defines the essential role of IL-1β in the rapid production of pathogen-specific IgM by B1a cells. that the increase of both IgM and ANGPTL4 in our study can be related.

In the study of J. P. H. Drenth (20), it was found that IgG has no effect on the synthesis of IL-1β, and in the study of A. B. Lentsch (21), it was also found that in vivo blockade of TNF-α or IL-1β suppressed the activation of lung NF-kappaB during lung damage caused by IgG immune complex, while ANGPTL4 increases NF-kappaB. The results obtained in our study are in line with the mentioned studies.

Conclusion

The results of our study show that investigating specific immunological markers and their association with ANGPTL4 expression can lead to the discovery of new diagnostic biomarkers for tuberculosis. These biomarkers can be used to increase the accuracy and efficiency of TB diagnosis, and can be suitable for resource-limited settings. This study showed that by assessing

ANGPTL4 mRNA levels and infection-related immune markers, clinicians could potentially track treatment efficacy and make informed decisions about treatment settings. Understanding the role of ANGPTL4 in pathogenesis can help identify subsets of patients that are most likely to respond to therapeutic approaches. The results of this research can clarify potential therapeutic targets related to ANGPTL4 and immunological markers. Research findings can help develop new drugs or treatment strategies for TB. By understanding the immunopathogenesis of TB and the role of ANGPTL4, potential drug targets or pathways involved in the immune response can be identified.

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