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Novel and Emerging Approach in the Diagnosis and Prognosis of Tuberculosis by Using Biomarkers

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Abstract

Tuberculosis (TB) is the 9th global health hazard therefore new optimal approaches towards its rapid, accurate diagnosis and treatments are required to address the goal of global tuberculosis elimination. Biomarkers are the indicators of any infection and helps in the early diagnosis. A Biomarker can show functional, physiological and biochemical response due to the interaction of antigen with the host cell. There are some pathogen-specific biomarkers such as lipoarabinomannan (LAM), antigen 85 (Ag85), secretory antigenic target 6 (ESAT6), culture filtrate protein (CFP-10) and mycobacterial adhesins and some host-specific markers which up-regulates and down-regulates during infection and can be detected easily in blood and urine samples of patient. Consideration of the relationship for linking host immune system and pathogenesis of the bacterium may provide novel pathways in the diagnosis and treatment of this disease. Biomarkers can be used in risk prediction, provides a systematic approach for diagnosing a disease and evaluation of new drug therapies but there is a lack of specific validated biomarkers for tuberculosis up till now. Here in this manuscript, authors acknowledge various immunological biomarkers, their mode of action, there role in tuberculosis pathogenesis and their prospective exploitation in struggling with this deadly disease.

Keywords: Tuberculosis; Biomarkers; $H_{37}Rv$ pathogenesis; Mycobacterial genetics

Abbreviations:

M. tuberculosis $H_{37}Rv$: Mycobacterium tuberculosis $H_{37}Rv$; TB: Tuberculosis; SDG: Sustainable development goals; MDR: Multidrug resistance; XDR: Extensively drug resistance; TDR: Total drug resistance; HIV: Human immunodeficiency virus; IFN-γ: Interferon gamma; LAM: Lipoarabinomannan; Ag85: Antigen 85; ESAT6: Secretory antigenic target 6; Hbha: Heparin binding haemagglutinin adhesion; IL : Interleukins; IP-10: IFN-γ induced protein; TNF: Tumor necrosis factor; LPS: Lipopolysacharide; SAA1: Serum amyloid A1; PTX-3: Pentraxin; PCT: Propionate CoA transferase; CRP: C-reactive protein and MMP-8: Matrix metallopeptidase 8.

Introduction

Tuberculosis is one of the major causes of death globally caused by Mycobacterium tuberculosis $H_{37}Rv$ (*M. tuberculosis* $H_{37}Rv$). Infection transmits through aerosol particles by coughing and sneezing of an infected person. Generally after inhalation of any bacteria it reaches to lungs and get ingested by alveolar macrophages and gets eliminated but *M. tuberculosis* $H_{37}Rv$ can persist in macrophages due to its unusual survival strategies such as presence of high lipid content such as lipoarabinomannan (LAM) and mycolic acid, inhibition of phagosome acidification and phagosome-lysosome fusion (Figure 1) [1,2]. Smoking, infection of Human Immunodeficiency Virus (HIV) and poverty is influencing the epidemics of this disease. There were 10.4 million people who fall ill and 3.1 million who die because of tuberculosis (TB) in 2016. The Mortality rate of TB is falling ~3% every year because people are treated successfully if diagnosed earlier. But the rate of successful treatment is low due to the development of the drug-resistant TB like multidrug resistance (MDR), extensively drug resistance (XDR) and total drug resistance (TDR).

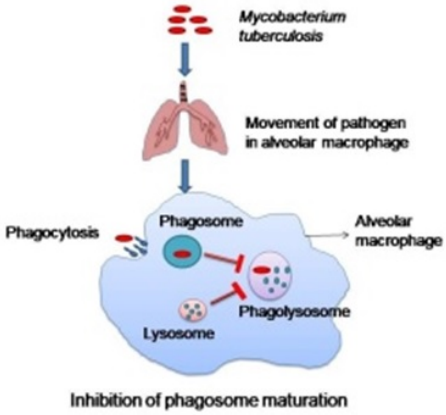

Figure 1: Mycobacterial infection mechanism and inhibition of phagolysosome formation.

Sustainable development goal (SDG) is to finish the TB infection by 2030 [3,4]. By focusing on the low specificity of diagnostic procedures like sputum smear microscopy and sputum cultures etc. we need to develop new strategies or some rapid, accessible and effective diagnostic approaches [5,6]. Interferon-gamma release assay (IGRA) was designed to overcome the low sensitivity of tuberculin skin test

(TST) and for better prediction of infection. IGRA measure T-cell responses to *M. tuberculosis* $H_{37}Rv$ specific antigen (Table 1). Interferon-gamma (type II interferon) is a key signaling molecule which belongs to cytokine proteins. IFN-γ induces the expression of class II major histocompatibility complex (MHCs) on antigen presenting cells (APCs) and promotes immune cells proliferation (T-cells and B-cells), cell adhesion, apoptosis of antigen affected cells and *M. tuberculosis* $H_{37}Rv$ growth control [7,8]. Observation and quantification of biomarkers are very helpful in the early diagnosis and treatment of infectious diseases [9]. Any characteristics like specific cells, molecule, gene, hormone or enzymes which indicates the emergence of an infection or a disease, can be detected and measured in blood or tissue can act as a biomarker for that disease. Host immunological biomarkers are needed for diagnosis, risk correlation and to generate novel methods for treatment. In this review, the authors explore various immunological biomarkers which may be used as diagnostic tools for *M. tuberculosis* $H_{37}Rv$ and can help in declining the global epidemic of this disease.

Mycobacterium invades inside the host and reaches into lungs and recruits alveolar macrophages. Phagocytosis takes place against Mycobacterium tuberculosis to eliminate it, but this bacterium can persist due to its unusual survival strategy, by inhibiting phagosome and lysosome fusion, therefore phagolysosome does not form to degrade the pathogen. So mycobacterium can easily get away from the hydrolytic environment of phagolysosome.

Mycobacterial Genetics and Mechanism of Pathogenesis

Whole genome sequencing of *M. tuberculosis* $H_{37}Rv$ reveals various gene of this bacterium that plays a significant role in the survival of this bacterium inside host cell phagosome, Later on, a portion of these qualities, the proteins they encode, and additionally newfound ones, ought to give new bacterial focuses on that can be utilized for making antibodies, develop medications and also more specific symptomatic reagents. Genome in the *M. tuberculosis* $H_{37}Rv$ complex, including the human and creature pathogens *M. africanum*, *M. microti*, and *M. canetti*, and additionally *M. tuberculosis* and *M. bovis* were portrayed by DNA sequencing and related strategies. These investigations have demonstrated a more prominent than 99.9% compatability of DNA sequence among the individuals from the *M. tuberculosis* $H_{37}Rv$ complex; however the presence of uncommon synonymous single-nucleotide polymorphisms (sSNP) permits separation between these firmly related microscopic organisms [10]. *M. tuberculosis* $H_{37}Rv$ principally remains in macrophages. As opposed to other bacterial pathogens that keep away from phagocytosis as a particular pathogenic procedure, *M. tuberculosis* $H_{37}Rv$ is unbridled in its utilization of numerous cell surface receptors to pick up passage into macrophages [11]. These receptors incorporate the mannose receptor, supplement receptors and Fc receptors. Once inside the host macrophage, *M. tuberculosis* $H_{37}Rv$ lives inside a film bound vacuole [12,13].

Test	Accuracy	Reference
Sputum smear microscopy	The sensitivity is grossly compromised when bacterial load is less than 10,000 organism/ml sample	37
	Also it is difficult to detect extra pulmonary tuberculosis, pediatric tuberculosis and TB co infection with HIV	
Sputum culture	Time consuming procedure takes almost 3-4 weeks to culture the bacteria	37
Tuberculin skin test (TST)	Time taking job and give accurate result (false positive results) for latent TB	40-41
Interferon Gamma Release Assays (IGRA)	Blood samples need quick sampling with precautions	38-39
	Only detects latent TB	
	Unable to identify TB-HIV co infection	

Table 1: Different tests used for diagnosis of TB with their accuracy.

Unmistakably *M. tuberculosis* $H_{37}Rv$ alters the development of this phagosomal compartment keeping in mind the end goal to upgrade its own particular intracellular survival [14,15]. This modified phagosomal development is related with modifications in the protein substance of the vacuole including adjusted Rab GTPase structure rejection of the vacuolar proton ATPase with resulting absence of acidification and maintenance of a protein assigned TACO [16]. Uptake of Mycobacteria by macrophages and ensuing maintenance of TACO on the phagosome seems, by all accounts, to be reliant upon the gathering of host cell–inferred cholesterol in the plasma surface of bacterial part [17,19].

Types of Biomarkers and their Application

The biomarker is "a characteristic that is evaluated as a display of any biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. WHO states that "any physical, chemical or biological characteristic which reflects an interaction between a biological system and a potential hazard" known as a biomarker. The observed response could be functional, physiological, and biochemical at the cellular level [20]. The two major types of biomarkers are "biomarkers of exposure," helps in risk prediction, and "biomarkers of disease", used for screening, diagnosis and monitoring the disease [21]. There are some pathogen-specific biomarkers and some host-specific biomarkers. The diagnostic utility of biomarkers revolutionized the field of genomics, proteomics, metabolomics, imaging modalities and neurophysiology [22]. Biomarkers also provide a systemic approach to understand the pathogenesis of disease and its associated risk factors. Serological tests based on biomarkers might be helpful in the diagnosis and prognosis of any disease. An ultimate tuberculosis biomarker should contain the following properties: they must be capable of distinguishing among patients with active tuberculosis and patients having dormant *M. tuberculosis* $H_{37}Rv$ infection, resuming the standard levels of treatment; expecting clinical

effects (for instance, treatment, declining hazard or elimination of *M. tuberculosis* $H_{37}Rv$ infection) in varied patient populations; and are able to forecast effectiveness of vaccines. There were various exercises have been propelled to target biomarkers in case of tuberculosis. Through increasing dose of drugs, for instance, biomarkers might support for intentional assortment, direct recognition, optimization, representing evidence of perception and selecting drug amalgamation for stabilizing synergistic communication between drugs and immune cells. Even though several immunological biomarkers might be a further proper précising phase of improvement, the sustained use for a solitary marker from preclinical studies during dose selection stage examinations would be an imperative improvement.

Interrelation between Host Immunological Biomarkers and *M. Tuberculosis* $H_{37}Rv$ Pathogenesis

Various pathogen-specific biomarkers were identified for TB but only the antigen 85 complex (Ag85), secretory antigenic target 6 (ESAT6) and the lipoglycan lipoarabinomannan (LAM) are useful [23]. In the pathology of tuberculosis infection, one key virulence factor is LAM present in the cell wall of *M. tuberculosis* $H_{37}Rv$ is amphiphilic in nature and associated with host lipid carrier molecules such as high-density lipid (HDL) and is detected in low concentrations in serum and in higher concentration in urine of TB infected person [24,25]. Presence of LAM in urine sample confirmed the infection of TB in HIV-positive population also [26, 27]. LAM is also a potential indicator of bovine tuberculosis (bTB) infection [28]. Ag85 is a major secretory protein with fibronectin binding capacity and mycolyltransferase activity and this antigen involved in receptor-mediated diagnosis of *M. tuberculosis* $H_{37}Rv$ [29]. Tuberculosis-associated meningitis or extrapulmonary TB can be diagnosed by the detection of Ag85 in the blood or urine sample [30]. ESAT6 helps *M. tuberculosis* $H_{37}Rv$ in translocation from phagolysosomes to host cytosol, so it can be considered as a virulent factor. Intracellular presence of ESAT6 can be used as a biomarker because it is related to metabolically active *M. tuberculosis* $H_{37}Rv$ [31]. Culture filtrate protein (CFP-10) forms a heterodimeric complex with ESAT6. ESAT6/CFP10 complex is secreted by the ESX-1 secretion system which is used by *M. tuberculosis* $H_{37}Rv$ to deliver virulence factors into host macrophages. This complex is a basis of infection, and can be detected by the interferon-γ release assays [32]. The microbial adhesions may also act as determinants at the time of host-pathogen interactions because their interaction leads to an induce immune response which is required for host defense such as 19 kDa lipoprotein antigen (Rv3763), malate synthase, *M. tuberculosis* $H_{37}Rv$ pili, and heparin binding haemagglutinin adhesion (HbhA) [33].

There are some host regulatory factors which up and down-regulates during exposure of TB infection. There is an enhancement in the secretion of *M. tuberculosis* $H_{37}Rv$ specific antibodies and binding of any antigen with T-cell receptor stimulates secretion of certain proinflammatory cytokines and chemokines such as IL-2, IL-1β, IL-6 and tumour necrosis factor (TNF) and influence T cell response and TH cell balance [34]. IP-10 IFN-γ inducible protein, a chemokine-induced in various cells in response to IFN-γ and lipopolysaccharide (LPS) is an alternative marker to IFN-γ (Figure 2) [7]. Some acute phase proteins like serum amyloid A1 (SAA1); pentraxin (PTX-3); propionate CoA transferase (PCT); C-reactive protein (CRP) produced by the liver and promotes phagocytosis may also act as marker and factor associated with tissue reorganization matrix metallopeptidase 8 (MMP-8) can also provide blood-based targets to check the severity of infection and to monitor the disease during treatment [35]. Use of Biomarkers might be a basic mechanism for treatment of any disease. These are fundamentally based on epidemiological, therapeutic, pathophysiological shreds of evidences, therefore they are a primary candidate in drug discovery accelerate dose selection in an early phase of clinical research, and shortening the time to licensing of new drugs and vaccines [36-41].

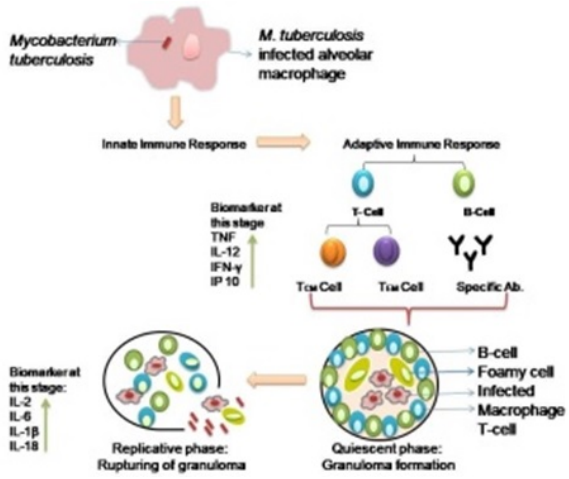

Figure 2: Immune Response and Host-specific biomarker for *Mycobacterium tuberculosis* H37Rv infection.

Host Alveolar macrophages upon activation by *M. tuberculosis* $H_{37}Rv$ generate an innate immune response which on further activation by secreted innate immunity compounds convert into an adaptive immune response. In adaptive immune response T cells and B cells are generated. Upon activation, T cell divided into TEM (effectors memory) which help in generating B cell and TCM (central memory cell) provide antigen recognizing property to host immune system. These cells also release various chemokine like TNF-α, IL-12, IFN-γ and IP-10 etc. which help in the elimination of foreign particle. In case of *M. tuberculosis* $H_{37}Rv$, this bacterium is capable of persisting in the macrophage which is termed as the quiescent phase. In this phase, a bacterium is surrounded by B cell, T cell, NK cell, dendritic cell and foamy macrophages etc. which forms granuloma-like structure, which provides bacterial cell with a protected environment to replicate itself. In the immune compromise stage, granuloma ruptures and releases bacilli in the surrounding environment and secretes various chemokine-like IL-1β, IL-2, IL-6 and IL-18 have been secreted which is a sign of an active disease [28,29].

Conclusion

Tuberculosis is a global health hazard so there is a need to develop new strategies for early diagnosis and successful treatment. Any specific characteristic present in tissue, cell and fluid of body, which gives the specific response to any infection and help in risk prediction, diagnosis and prognosis of the disease is known as a biomarker. Biomarkers provide a systematic approach for diagnosing a disease and evaluation of new drug therapies but there is lack of specific validated biomarkers for tuberculosis up till now. There are some pathogen-specific and some host-specific markers secreting during infection which can be detected in blood and urine sample of the patient which

might be useful and require further studies. Treatment and diagnosis of tuberculosis by using appropriate biomarker may be helpful for future perspective.

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Conflict of Interest

There is no conflict of interest.

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