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Recombinant proteins: Their potential roles in the diagnosis of bovine tuberculosis: A review

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Abstract

Tuberculosis (TB) is a highly contagious, economically devastating and wasting disease of both man and animal and is primarily caused by *Mycobacterium tuberculosis* and *Mycobacterium bovis*. Apart from that, few other bacteria under the genus mycobacteria are also found to be responsible for TB disease. *Mycobacterium tuberculosis* secretes a number of proteins and they help the bacteria a virulence factor to continue in host body and also to evade the host immunity. *M. tuberculosis* culture filtrate is also considered as important source of antigens and responsible for inducing protective immunity and immune responses and therefore, they possess significant diagnostic potential. *M. bovis* and *M. tuberculosis* both are found to be genetically similar but both of them show different susceptibility towards host in order to establish the infection. The recombinant rMPT63-MPT83 fusion protein showed strong reaction when they come in contact with anti-rMPT63 and anti-rMPT83 in the serological assays. Delayed-type hypersensitivity (DTH) is being induced against the purified protein derivative (PPD) is used for the detection of TB in humans and animals and is assessed on the basis of the concentration of gamma interferon (IFN) produced in response to stimulation with PPD. The monoclonal antibodies raised against the heat shock protein (HSP) of 65-kDa can be used as a diagnostic with high specificity. The *M. tuberculosis* complex shows not as much of inter-strain genetic diversity and nucleotide changes like point mutation are infrequent and therefore, it is promising in terms of immunity and vaccine development. Few studies established that, few secretory protein antigens, and lipoprotein induce interleukin (IL)-12 at increased rate from the macrophages and therefore they act as immune-modulator and also take part in suppression of antigen-presentation signaling pathways. Taking in to account of above context, the present studies and overview are highlighted on the *M. tuberculosis* derived recombinant proteins and their diagnostic potentials as future diagnostic methods. The high sensitive and specificity diagnostic tests for early diagnosis are the considered as best possible way of eradication of TB from the population of man and animals.

Keywords: Bovine TB, MPT63, MTB complex, *Mycobacterium bovis*, *Mycobacterium tuberculosis* and recombinant mycobacterium proteins

Introduction

Tuberculosis is a contagious, infectious and terminal disease affecting man, animal and birds. Further, TB established itself a extremely zoonotic and economically devastating disease in the world (Tufariello *et al.*, 2004) ^[76] and accountable for two million deaths annually (Arentzand Hawn, 2007) ^[3]. As regard the etiology, it is caused by several mycobacteria like *Mycobacterium tuberculosis* in human, *M. bovis* in cattle, but other non-tuberculous mycobacteria also responsible for this disease such as *M. kansasii*, *M. ulcerans*, etc. The TB global burden estimates, 10.6 million in 2021(WHO) and India tops the list in the world. The TB cases for the year 2021 stood at 210 per lakh population and it has been predicted an occurrence of only 77 cases per lakh population by 2023.

Unfitting diagnosis and improper treatment may add to complications, and results in emergence of multidrug resistance-TB. *M. bovis* is the principal causative agent for bovine TB, and due to its zoonotic and infectious nature, it has been recognized as a major disease in terms of financially viable and down grade the agricultural sectors (Krebs, 1997) ^[40].

M. bovis infects wide range of living hosts like livestock, humans and ecosystem as it is one among the zoonotic diseases (Michel *et al.*, 2010) ^[51] in under developed countries. The TB disease has raised a concern and threat of extinction of few endangered animal species. (Lantos *et al.*, 2003) ^[42]. The inappropriate diagnostic tests in terms of early diagnosis, lack of regular monitoring and emergence of MDR strain of mycobacteria make the situation untenable for control and eradication of TB.

Bovine TB is mainly caused by *Mycobacterium bovis*, and it infects a wide range of hosts including humans and animals, and studies revealed that it was indistinguishable from that caused by *M. tuberculosis*. Verma and Srivastava (2001) ^[78] isolated 8 numbers of *M. tuberculosis* strains out of 52 numbers of suspected TB samples of sputum. Shah and co-workers (2006) ^[66] reported about the mixed infection of TB and isolated both *M. tuberculosis* and *M. bovis* from CSF samples of infected TB patient. Thoen *et al.* (2009) ^[73] opined on the subject of the host susceptibility of animals to *M. tuberculosis*, *M. bovis* and also described about their public health concern as because, livestock, humans and ecosystem get affected especially in the developing countries (Michel *et al.*, 2010) ^[51]. Bovine TB disturbs animal productivity and it negatively impacts the global trade of animal products. There are no plans and policies adopted to control *M. bovis* infections among population in few under developed countries in the world (Cousins *et al.*, 2004) ^[15]. Skins testing with tuberculin derived from *M. bovis* were used and infected animals were culled or slaughtered in order to control the infection in cattle. Ziehl-Neelsen acid-fast bacilli smear microscopy was accepted as a common laboratory diagnosis of TB. *M. bovis* needs 6 to 8 weeks for proper growth in the culture media and therefore, the microscopic examination has lost its shine in terms of promising diagnostic method (Christie and Callihan, 1995) ^[12]. The molecular detection of TB based upon the nucleic acid amplification test (NAAT) and whole-genome sequencing and it was considered as faster and self-effacing current diagnostic method for TB (Fariz *et al.*, 2018) ^[21]. In an experimental studies, the indirect *in vitro* assay showed increase concentration of gamma interferon after inoculating the purified protein derivative (IFN) and exhibited delayed-type hypersensitivity and the study demonstrated the roll of gamma interferon in the diagnosis of TB (Monaghan *et al.*, 1994) ^[53] and though it was the best possible and suitable way of diagnosis, but it has lacked the cross-reactive immune response to T and B-cell epitopes present in *M. avium* subsp. *paratuberculosis* and in non-pathogenic mycobacterium and therefore both TB organisms show high sensitivity and specificity in terms of diagnosis (De la *et al.*, 2006) ^[17]. *Mycobacterium tuberculosis* secretes numerous proteins antigens into the extracellular environment and that provide assistance to organism for survival in host environment, colonize and virulence factor for pathogenesis and disease production. Earlier studies revealed that, the immune responses or protective immunity was detected in the culture filtrate of *M. tuberculosis* and hence it was having diagnostic value (Cooper and Flynn, 1995) ^[14]. Boesen *et al.* (1995) ^[6] described that, the proliferation and cytokine production from T cells in the laboratory animals like mice, guinea pigs *in vitro* after stimulated with purified protein derivative-positive antigens and further studies revealed that, DTH responses was observed in guinea pigs due to reactive response provided by secreted proteins of *M. tuberculosis* (Haslov *et al.*, 1995) ^[31]. Nagai *et al.* (1991) ^[55] established that, several proteins were purified from culture filtrates of *M.*

tuberculosis and *M. bovis* Bacillus Calmette-Guerin (BCG) and MPT and MPB terms was derived from *M. tuberculosis* and *M. bovis* respectively and also based upon their relative mobility at the time of non-denaturing polyacrylamide gel-electrophoresis. Many secreted antigens of MPT and MPB provoke immune responses and that were found to be specific for the *M. tuberculosis* complex.

Studies revealed that, the polyclonal antibodies against MPT63 do not induce any cross-reaction with proteins of a common environmental *Mycobacterium species* as well as of *M. avium* (Manca *et al.*, 1997) ^[48]. The guinea pigs infected with virulent *M. tuberculosis* by the aerosol route induced humoral immune responses against MPT63 antigen. The MPT-63 is an immunodominant protein as indicated by its T-cell epitopes mapping (Lee and Horwitz, 1999) ^[43] and MPT63 is a novel secretory antigen found only in species of the *M. tuberculosis* complex. But in other studies, the antigenic properties were present in the cell envelope of recombinant MPT63-MPT83 and both were used as fusion protein in order to get a better immune response in terms of diagnosis (Braunstein *et al.*, 2000) ^[7]. Redchuk *et al.* (2012) ^[62] demonstrated that, the immunogenic properties of the recombinant proteins were similar to their native analogues. Apart from that, few studies uncovered that, the anti-recombinant-MPT-63 and anti-recombinant-MPT-83 in the sera were found to be highly reactive against the MPT63-MPT83 fusion protein, and that described the antigenic properties of the parent proteins and were retained by fusion protein.

The delayed type hypersensitivity test was prescribed tuberculin skin test (TST) of OIE for international trade (Anon, 2008) ^[2]. TST is recognized as a excellent herd test but it was not enough test for detecting TB animals in a large scale with proper precision (Dawson and Trapp, 2004) ^[16]. The several methods were adopted in PPD production and that resulted into diverse antigenic properties in terms of antigenic differences between PPDs (Tameni *et al.*, 1998) ^[72]. The presence of non-tuberculous mycobacteria showed less or rare sensitive reaction or response due to cross-reactive responses to PPD-B. Hence, the studies have revealed that the intra-dermal skin test give up to 5-30% false negatives and 1–5% false positives (De la Rua-Domenech *et al.*, 2006) ^[17]. Therefore, TST method gives a severe dilemma in terms of sensitivity and also this hampers the positive progress in the TB eradication programs.

A novel vaccine against TB is in advance impetus based upon the antigenic architecture of *M. tuberculosis* with respect to protective immune responses of T-cell epitopes (Sable *et al.*, 2007) ^[64]. *M. tuberculosis* complete genome sequence paved the way for the development of this vaccine (Cole *et al.*, 1998) ^[13]. Earlier studies ensured that, the induction of efficient protective T-cell responses only possible through immunization with live *M. tuberculosis* (Orme *et al.* 1993) ^[57].

Genus and genome of mycobacterium: The bacteria under Mycobacteriaceae family are Gram positive rods, non-motile and biochemically catalase positive organism. TB organism requires six or eight weeks of incubation and further 12–18 hours for growth and proliferation in the culture media in order to attain the size for microscopic observation. It was found to be resistant to decolorization with aryl methane dye stain (Ehrlich, 1882) ^[19]. The cell wall of mycobacteria contain mycolic acid and it was found to be reason behind the resistance to decolorize (Minnikin, 1982) ^[52]. The molecular

weight and G+C content of DNA were the basis to be taken into the account for their pathogenesis of two major mycobacteria like *M. tuberculosis* and *M. leprae* (Baess and Mansa, 1978) [4] but *M. leprae* was found to be more than 50% in terms of G+C content than the *M. tuberculosis* (Imaeda *et al.*, 1982) [35].

The complete genome sequence of *M. tuberculosis* and *M. bovis* were recorded by Cole *et al.* (1998) [13] and Granier *et al.* (2003) [24] respectively. There was no evidence of translocations or inversions or alterations with respect to *M. leprae* in the genome or in the nucleotide base pairs but further study revealed that, the genome was showing >99.95% similarity at the nucleotide level to that of *M. tuberculosis* (Garnier *et al.*, 2003) [24]. The secreted proteins of *M. tuberculosis* response showed that more than 350 secreted proteins share no homology with known proteins other mycobacteria and therefore it is one of the excellent characters in terms of diagnostic tool. Apart from that, few studies also described that, more than 50% of the genes showed gene duplication and the genome was composed of prophases and insertion sequences (IS) (Garnier *et al.*, 2003). Frothingham *et al.*, (1998) [24] recorded more than 50 copies of insertion sequences such as IS3, IS5, IS21, IS30, IS110, IS256 etc. and they appeared to be used for the production of transposes. Sreevatsan *et al.*, (1997) [70] observed 99.9% similarity at the nucleotide level and indistinguishable 16S rRNA sequences of mycobacteria group of bacteria and therefore, all have been included in the *Mycobacterium tuberculosis* complex but all the mycobacterial in the complex differ widely in terms of their host affinities and pathogenicity. The earlier research studies proposed that, the cell wall components as well as other quite a few secreted proteins exhibited significant difference, and take significant part the in between mycobacteria and host-interactions and besides that, it also possessed characters to overpower the host immune system. Few studies also opined that, there were no genes present exclusive to *M. bovis* and this types of variance in gene expression of *M. bovis* contribute in the affinity towards settle down in the human host.

***Mycobacterium tuberculosis* complex (MTC):** *M. tuberculosis* complex comprises seven numbers of species and subspecies such as *M. tuberculosis*, *M. canettii*, *M. africanum*, *M. pinnipedii*, *M. microti*, *M. caprae* and *M. bovis* (Smith *et al.*, 2006a) [67] and apart from that, the separate species status to *M. bovis* and *M. tuberculosis* based upon the taxonomic studies. In spite of their close genetic similarities of bacteria under MTC but differ in their host range and pathogenicity. Further, the *M. tuberculosis* complex showed diverse host choices due to their steady molecular differences (Smith *et al.*, 2006b) [68]. *M. tuberculosis* and *M. bovis* harbour few antigenic properties for different host range of animal species, including domesticated and wild (De Lisle *et al.*, 2001 [18] and O'Reilly and Daborn, 1995) [56]. Humans and voles (small rodents) were primarily infected by *M. microti* (Van Soolingen *et al.*, 1998) [77]. The origin of *M. tuberculosis* complex was from a soil but earlier studies established that, the mycobacteria infection in human was transmitted from the bovine origin. The genetic diversity is found to be limited in between the mycobacteria under this MTB complex but their nucleotide alterations were also found to be very inadequate (Sreevatsan *et al.*, 1997) [70]. The majority of the proteins were found to be antigenically and genetically indistinguishable in all the strains of bacteria in this MTB-complex. Therefore possibility of getting antigenic drift is less and hence the

diagnostic potential of secreted proteins could be unparallel and also could be used as a suitable candidate for vaccine production.

Mycobacterial antigens and their potential role in the disease diagnosis: On the basis of physical and chemical properties, site of presence in the cell, types of secreted and constituent proteins or antigens and lipoproteins, the mycobacteria are being classified into different groups and these antigens are responsible for virulence, pathogenesis and disease production. These antigens are secreted proteins, membrane-bound proteins, lipoproteins and heat shock proteins. But out of these, the secreted proteins are being considered as agents primarily accountable for the host immune response and are being served as candidates for pathogenesis, clinical signs, and other discernible pathological lesions of the disease.

Secreted proteins: The actively secreted proteins of *M. tuberculosis* are the principal antigens for establishment infection and disease production. These secreted proteins were having DNA binding domain that are translocated to the host nucleus and bind with the gene promoters and responsible for eukaryotic nuclear localization signal. Apart from that, secretory proteins also regulate transcription by interacting with the host chromatin materials. Recent studies showed that, the *M. tuberculosis* bacteria harbor proteins with kinase and phosphatase activity that interferes with phosphorylation/dephosphorylation of the host cell and helps in the process of host cell damage and self-survival (Pal *et al.*, 2022) [58]. Besides that, the proteins secreted from the pathogenic *Mycobacterium* spp. into the culture medium display a major role in the development of immunity and these crude Culture Filtrate were rich source of antigens in various experimental animals (Andersen, 1994) [1]. The components of crude filtrate proteins of live attenuated mycobacteria are being used in the immunization rather than that of vaccination using killed organisms for getting better results in terms of getting immune responses (Sonnenberg and Belisle, 1997) [69]. The term MPT was designated as because the protein purified from *M. tuberculosis* but the later numbers were based upon their relative mobility in Poly Acrylamide Gel Electrophoresis and likewise the MPB denotes the protein isolated from *M. bovis* BCG (Nagai *et al.*, 1991). Wiker *et al.* (1991) [89] reported that the these two mycobacterial species also secreted proteins with N-terminal amino acid sequence and these proteins helped in the differentiating between extracellular and intracellular antigens and secreted to the culture fluid as soluble intracellular antigens such as MPT32, MPT53, MPT63, MPB70, MPT64, MPT46, MPT51, 85A/MPT44, antigen 85B, antigen 78/ antigen 5, antigen 84, antigen 63/hsp70 etc. The role of MPT-63 and MPT-64 to detect or recognize the binding site of immune cells for expression of IFN- β and production of reactive oxygen species during the use of TBK1 and p47 peptide in MTB-infected macrophages (Jae-Sung *et al.*, 2021) [36]. The diagnostic potential of recombinant MPT-63 has been already established in the earlier studies (Samal *et al.*, 2015) [65]. The detection of MPT63-specific IFN- γ -secreting T cells could be useful for the diagnosis of tuberculosis (TB) diseases (Zhiliang *et al.*, 2015) [92]. The polyvalent immunoglobulin as anti-BCG and anti-*M. tuberculosis* are directed towards intracellular antigens and those antigens with high LI values were found to be presented by macrophages harboring live mycobacteria. The molecular characterization of

Mycobacterium bovis antigens were performed through western blot technique by Cataldi *et al.* (1994) [10]. Whole cell extract antigens were more frequently documented and *M. bovis* whole cell extracts and culture supernatant antigens were secreted from *M. bovis*-infected and healthy cattle. Serological assay recognized the molecular masses of 17 kDa, 23 kDa, 28 kDa, 42 kDa, 66 kDa, 71 kDa, 80 kDa, in cellular extracts and as 23 kDa and 33 kDa in supernatants. Hewinson *et al.* (1996) [32] described that, the MPB70 was a secreted protein and that of 10% of the *M. bovis* culture filtrate proteins. In case of MPB70, the intracellular matrix proteins have got the major role in the interaction between the cell membrane and the extracellular environment (Carr *et al.*, 2003) [9]. A delayed hypersensitivity (DTH) was recorded in cattle and guinea pig and the least cross-reactivity displayed when applied in the guinea pigs sensitized with *M. bovis* BCG and *M. phlei* and it was observed that, several preparations of Culture Filtrate Protein were showed protection when used as vaccines in animal models (Andersen, 1994) [1]. The Early Secretory Antigenic Target (ESAT-6) of 6-kDa as molecular weight antigen secreted from *Mycobacterium tuberculosis* and was found to be a principal target for cell-mediated immunity in the initial stage of tuberculosis. But ESAT-6 showed strong antibody responses and delayed type hypersensitivity skin reactions in guinea pigs infected with tuberculosis (Wang *et al.*, 2005b) [80]. The earlier studies on ESAT-6 protein secreted from the other mycobacteria such as CFP-10 and CFP-7 aggravated the T-cell as antigens (Brodin *et al.*, 2004) [8]. Culture Filtrate Protein-10 ESAT-6 stimulate Th-1 (T-helper) host immune response against *M. tuberculosis* infection (Meher *et al.*, 2006) [50] and further studies showed that, both the mycobacteria capable of producing gamma interferon (IFN γ) antigens in a massive scale but other environmental non tuberculous mycobacteria lack these proteins and therefore these proteins are being considered as suitable antigens for diagnostic purposes. The secretory proteins like CFP-10 and ESAT-6 are widely evaluated as antigens in the diagnosis of tuberculosis and potent immune reactions after 2 weeks of post challenge were observed even in the absence of adjuvant. Therefore, in the above stated context, it has been observed that, the inoculation recombinant antigens were recognized as major breakthrough in the research of diagnostic potentials among secretory antigen from MTB complex.

Kulshrestha *et al.* (2005) [41] expressed and purified few recombinant antigen such as ESAT6, 14-kDa, MTC28 and CFP10 and these purified proteins were proved to be helpful in detecting antibodies in the serum of TB infected person. Further, in combination with other immunodominant antigens, they turn displayed the increase sensitivity in detecting *M. tuberculosis* specific antibodies. Farshadzadeh *et al.* (2010) [22] reported the cloning and expression of ESAT-6 and further studies revealed that, the detection of ESAT-6 from the CSF of TB meningitis patients by indirect ELISA and hence proved its potential as a serological diagnostic and can be used to develop an immunodiagnostic assay (Kashyap *et al.*, 2009) [39]. Use of synthetic lipopeptide in combination with lower concentrations of ESAT-6 showed antigen-specific cell mediated immunity but the high dose of the recombinant protein would be costly and in order to make it convenient further addition of adjuvant may result in risk of sensitization (Whelan *et al.*, 2004) [86]. In cattle, the PPD-based TST showed comparable sensitivity due to use of ESAT-6, MPB83, and CFP-10 in a combination with low protein doses of recombinant proteins (Whelan *et al.*, 2009) [85]. The

peptides of ESAT-6 and CFP-10 are proficient of stimulating peripheral blood mono nuclear cells responses to IFN- γ in the MTB infected cases, but it did not show any immune reactions in the persons vaccinated with BCG vaccine (Hill *et al.*, 2005) [33]. Waters *et al.* (2004) [82] reported that, in cattle with pre-existing sensitization to *M. avium* or *M. avium* subsp. paratuberculosis was detected sensibly by the use of the recombinant ESAT-6: CFP-10 fusion protein. Nitric oxide and IFN γ like cytokines were released by blood leukocytes from the *M. bovis* infected calves but it exceeded alike responses of non-infected *M. avium* and *M. avium* subsp. *Paratuberculosis* infected calves due to immunization with recombinant-ESAT-6: CFP-10. The molecular masses of 28 kDa and 30 kDa were two major proteins identified as components of the BCG 85 complex of *Mycobacterium bovis* BCG from culture filtrates and further they were purified and used in enzyme-linked immunosorbent assays for the fortitude of specific immunoglobulin G (IgG) levels in tuberculosis or leprosy patients (Pessolani *et al.*, 1990) [60]. Further studies elaborated that, a low molecular weight antigen of MTB-12 constitutes a major component of the *M. tuberculosis* culture and gives the imprint as several other culture filtrate proteins, like that of 85B complex (Webb *et al.*, 1998) [83]. The single-copy gene with low molecular weight such as MTB-12 was secreted from infectious and non-infectious mycobacteria of the *M. tuberculosis* complex, BCG strain of *M. bovis* and *M. leprae* but the recombinant MTB-12 containing N-terminal-six-histidine label was expressed in *Escherichia coli* and further purified by chromatography and was responsible for inducing vitro proliferative responses from the peripheral blood mononuclear cells of human sensitized with PPD positive. Besides that, the MTB-12 in combination with 38-kDa antigen, was employed for the diagnosis of TB in human especially in the pulmonary origin (Lee *et al.*, 2008) [44]. The sensitivity and specificity of MTB-12 antigens was observed to be more than 50% and more than 90% respectively in patients infected with TB but few studies revealed that, the sensitivity was increased up to 73% when the combination of MTB-12 and 38-kDa antigen was inoculated. After anti-tuberculosis treatment, the ELISA test showed that, the mean IgG levels against MTB12 alone or MTB12 in combination with 38-kDa antigen were suggestively increased, but MTB-12 in combination with 30-kDa antigen did not show significant level of IgG. The assays like southern transfer and hybridization revealed that, MPT-53 was found to be conserved secreted protein of *M. tuberculosis* complex shared similarity of DNA from *Mycobacterium avium* and other non-tuberculous mycobacteria. The molecular weight of MPT-53 is of 15-kDa (Nagai *et al.*, 1991) [55] and that induces antibody responses in tuberculous infected cattle (Wiker *et al.*, 1991) [87]. MPT-53 of *M. tuberculosis* induced strong, tuberculosis-specific antibody responses in guinea pigs but delayed-type hypersensitivity was found to be nil (Johnson *et al.*, 2001) [38]. MPT-64 protein is one of the principal secreted antigens from tuberculosis bacteria and Haga *et al.* (1995) [27] expressed and purified recombinant maltose binding protein with MPT64 from *E. coli* as a competent bacterial host and was found to be effective for inducing DTH in sensitized guinea pigs. Roche *et al.* (1996) [63] expressed and purified recombinant MPT-64 but it did not efficiently provoke DTH responses in the sensitized animals. Wang *et al.* (2007) [81] studied the MPT-64 and reported that, the antigen can replace tuberculin, or purified protein derivative, as a rapid diagnostic of choice for uncovering of active TB infection. ESAT-6 and MPT-64 fusion protein immune-prophylactic potential was reported by

Bai *et al.*, (2008) ^[5] in mouse model and the purified ESAT-6 and MPT-64 fusion protein prompted stronger humoral response, better splenic lymphocyte stimulated index and higher levels of IFN- β and IL-12 production than that of the single MPT-64 inoculation in the experimental group of mice. Yang and his collaborators, (2011) ^[90] studied the antigenic peptide of MPT-64 protein by using rabbit antibodies raised against MPT64. *M. tuberculosis* as well as *M. bovis* BCG strains secrete MPT63 and is being considered as one of the most abundant secreted proteins of pathogenic mycobacteria (Nagai *et al.*, 1991) ^[55]. MPT-63 is a credible candidate as well as exclusively specific to the *M. tuberculosis* complex, and hence could be used as diagnostics purpose. Horwitz *et al.* (1995) ^[34] reported MPT-63 protein acts as an important antigen behind the pathogenesis of TB and also responsible for initiating the immune response in the infected host therefore, could be a target molecule in terms of diagnostic. Besides that, Manca *et al.* (1997) ^[48] described about the *M. tuberculosis* cultures filtrates fractionation and that produced MPT-63 protein of 18 kDa. The recombinant MPT-63 protein was purified through *E. coli*, and native MPT-63 was also purified from *M. tuberculosis* culture filtrates and both were found to be indistinguishable in serological assays and responsible for inducing delayed hypersensitivity reactions and similar type of studies in terms of diagnostic potential of recombinant MPT-63 has already been reported by Samal *et al.* (2015) ^[65]. The structural similarity of MPT-63 with host immunoglobulin as well as with the cell surface binding proteins was found to be a major reason for the host-cell interactions and that encouraged phagocytosis of mycobacteria by the phagocytic cells. Other than that, the MPT-63 produce humoral immune responses in guinea pigs infected with virulent form of *M. tuberculosis* (Goulding *et al.*, 2002) ^[25]. In the earlier studies it has already been established that, the immunogenic properties of MPT-63 could be provisional and the hyper degree of immune dominance was evidenced by presence of high density of T-epitopes in its N-terminal region of MPT-63 (Lee and Horwitz, 1999) ^[43]. Due to the above stated reasons MPT63 was projected as suitable antigen in terms of diagnosis of this disease and also for vaccine development (Horwitz *et al.*, 1995) ^[34]. In the antibody assays it has been observed that, MPT-63 protein showed immunoreactive in humans and MPT63 and ESAT-6 antigens as a combine DNA vaccine was found to be accountable for a better immune response in animals and in mice (McShane *et al.*, 2001) ^[49]. The reasonable Th1 cell reactivity is being induced by MPT-63 and that it has been observed parallel types of responses also induced by other secreted antigens of *M. tuberculosis* such as MT-24 and MPB-70. Few earlier studies on MPT-63 also described that, it was the reason for proliferation of mononuclear cells and helped in secretion of Interferon- γ from peripheral blood mononuclear cells of TB infected patient but these types of reactions were not observed in the patient infected with *M. avium* (Werninghaus *et al.*, 2003) ^[84]. The T-helper-1 (Th1) cell reactive epitopes are being distributed throughout the sequence of MPT63 (Mustafa, 2009) ^[54]. In continuation to the above stated studies with regard to the diagnostic potentials of recombinant protein complex, Wang *et al.* (2005a) ^[79] developed, a multi-antigen complex constituted of CFP-21, ESAT-6, MPT-63 and MPT-64 and the multi antigen complex was found to be exhibited promising results in terms of diagnosis of TB. Wu *et al.* (2010) ^[88] evaluated the diagnostic potentials of several secretory and soluble protein and also described their sero-

diagnostic sensitivities for detecting antibodies against individual recombinant antigens such as 38-kDa, MTB-48, and CFP-10/ESAT-6 of *M. tuberculosis* and the response in terms of sensitivity were found to be 73.6%, 73.2%, and 60.4% respectively and the specificities were found to be 85.4%, 77.7%, and 73.8%, respectively. The sensitivity and specificity of antigens of LAM, 38kD, kat-G, 16kD, and MPT-63 for detecting antibodies reached up to 70% and more than 90% respectively, where as in a separate studies, the sensitivity and specificity against other antigens like LAM, kat-G, 16kD, MTB-39 and MTB-81 were found to be up to 67.1% and 92.7% respectively. The diagnostic tests for tuberculosis in cattle are based on the detection of antigen-specific cell-mediated immune responses through the intradermal tuberculin test (Monaghan *et al.*, 1994) ^[53]. This test was being used as the primary diagnostic or for the surveillance test for bovine TB. It measures delayed-type hypersensitivity (DTH) responses induced following intradermal inoculation of purified protein derivative (PPD) of *M. avium* and *M. bovis*. The purified protein derivative is derived from the antigens of all pathogenic, environmental and vaccine strain of *Mycobacterium bovis* (BCG) mycobacteria and therefore, the imprecision tuberculin skin test often reflects a low diagnostic specificity due to the presence in tuberculin of antigens shared by many mycobacterial species. Lyashchenko *et al.* (1998) ^[47] demonstrated the combination of *M. tuberculosis* complex specific antigen for a skin test and multi-antigen combinations were evaluated by skin testing in guinea pigs sensitized with *M. bovis* BCG. Besides that, the four purified antigens such as MPB70, MPT63, Ag85B, and MPT51 specific for the *M. tuberculosis* complex induced skin test responses only in BCG-immunized guinea pigs, but similar skin test responses are not observed in control animals immunized with *M. avium*. In view of the above context, a inference could be drawn that, the purified protein derivative consists of immunodominant proteins like MTB-99, MPT-63 and MPT-64 found to be more sensitive than standard PPD (Stella *et al.*, 2007) ^[71]. Further study also revealed that, the ESAT-6 and MPT64, elicited delayed-type hypersensitivity (DTH) skin responses in out bred guinea pigs infected with *M. tuberculosis* by aerosol and intravenous routes but alike DTH responses were not observed in guinea pigs sensitized with *M. bovis* BCG or *M. avium* (Elhay *et al.*, 1998) ^[20].

Lipoproteins: Lipoproteins are being actively secreted from Mycobacteria with different molecular weights of 38, 27, 26 and 19 kDa. The little binding of the protein to the lipid-rich mycobacterial cell wall was possible due to the N-terminal cysteine and also due to acylated with a lipid tail (Young and Garbe, 1991) ^[91]. Out of these lipoproteins, the 38-kDa protein was a principal constituent of culture fluid of both *M. tuberculosis* and *M. bovis* BCG but it is present in far lower concentrations and besides that the antibodies against this protein were being induced at higher level in TB patients. The further studies showed high specificity for tuberculosis was observed in enzyme linked immunosorbent assays and by employing monoclonal antibodies (Harboe and Wiker, 1992) ^[29]. *M. tuberculosis* secreted a lipoprotein of 19 kDa antigen and that induced enhanced level of interleukin (IL)-12 from macrophages and therefore this lipoprotein was acknowledged or recognized as an immune-modulator leading to inhibition of antigen-presentation signaling pathways and apart from that, studies showed that, the mice produced elevated levels of the 19 kDa antigen after immunization with recombinant BCG strain and that responsible for production of high level

interferon-gamma (IFN-gamma) and relatively little IL-10 against the purified 19 kDa antigen (Rao *et al.*, 2005) [61]. The MPT-83 was also considered as a potent immunogenic mycobacterium lipoprotein, which was obtained as a cell surface-associated antigen by flow-cytometry and also established via electron microscopy (Harboe *et al.*, 1998) [30]. The monoclonal antibodies raised against the MPB-83 surface antigen of *M. bovis* enhanced the survival time of infected mice and it altered the size, shape and orientation of granuloma lesion and upturned the distribution of acid-fast bacilli in the lungs tissues (Chambers *et al.*, 2004) [11]. *M. tuberculosis* MPT83 antigen has got the potential of inducing both humoral and cell mediated immunity and hence could be used as a potential candidate for vaccine (Green *et al.*, 2009 and Xue *et al.*, 2004) [26, 89].

Heat shock protein: Heat shock proteins were first described and named as heat shock protein because of their production due to sudden rise of temperature in the cell (Tissieres *et al.*, 1974) [75]. Further studies revealed that, they are cytoplasm proteins in *M. tuberculosis* and the production of heat shock proteins (HSP) is being increased during cellular stress and several heat shock proteins do take part or play an essential role in normal cell function. HSP have been classified into different families such as Hsp90, Hsp70, Hsp60, and small Hsps based upon the their molecular weight (Lindqvist and Craig, 1988) [46]. HSP exhibits itself as a conserved nature of protein, with widespread homology in the genome sequence between different species of mycobacteria (Jindal *et al.*, 1989) [37]. During the broad study of the 65-kDa protein of *M. tuberculosis* and related Mycobacteria, it revealed 12 different antibodies complimentary epitopes along with other surface molecules by using a series of monoclonal antibodies (Hajeer *et al.*, 1992) [28]. 65-kDa protein of *M. tuberculosis* derived monoclonal antibodies were showing high specificity as well as enhanced level of reaction to the protein in the other species of the *M. tuberculosis* complex. Thole *et al.* (1990) [74] recorded that, the heat-shock proteins of 12 kDa and 65 kDa did not display any code for any secretory proteins of high immunogenic values and in this connection, it was ascertained that, the 65 kDa antigen were found to be a soluble antigen without any hydrophobic regions and making it implausible as an integral membrane protein of mycobacterium.

Conclusion

TB has established itself as a global threat to both man and animals. Its zoonotic potential for spreading among human population is rapid and thereby it is being considered as an ever increasing contagious mycobacteria disease of the world. Therefore, the earliest possible diagnosis of the patients will break the spread of TB. Several attempts were made by all stake holders in order to develop a multifunctional protein derived diagnostic methods and also to develop novel therapeutic agents for the complete cure or recovery of patient. In this connection, the detection of the role of recombinant MPT against MTB could be a ground breaking innovation towards vaccine development or curative drugs. Several proteins include secretory, conserved, constituent and derived from pathogenic and non tuberculosis mycobacteria organism, their actions, response, reactions and sensitivities and specificities were elaborately discussed in order to evaluate the diagnostic potentials. Out of these, MPT-63 and MPT-64 were found to be given promising results in terms of expression of IFN- β and production of antioxidants from both MTB-infected and stimulated macrophages. Few studies

highlighted about the potential of MPT-64 and can be included in the regimen of TB diagnosis in place of tuberculin. MPT63 is one of the most abundant secreted proteins as well as a highly specific and credible candidate for TB diagnosis. Besides that, ESAT-6 and Culture Filtrate Protein-10 produce significant level of specific T- helper-1 cell (Th-1) for the host immune reaction. Therefore, the use of ESAT-6, CFP-10, and MPB83 in a combination of recombinant proteins and even in low protein doses showed better response in the purified protein derivatives based tuberculin skin test in naturally infected cattle.

Future prospects of this study: The TB not only has emerged as a health hazard of both human and animals but also a devastating disease for the economy of the country. The rapid and proper TB detection with best possible accuracy are the vital aspects for the unbeaten approach towards control and containment of this killer zoonotic disease. The proper and efficient treatment leads to reduced hospitalization and other disease complications. The molecular diagnostic testing to be adopted for high sensitive and accurate diagnosis is the need of the hour and should be encouraged in order to build public health awareness and sensitization to take positive and affirmative action towards control the disease both in human and animal population.

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Declarations

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