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Review Article

Expression of Alpha Crystallin Protein in Osteoarticular Tuberculosis in Latent Phase: A Review of Evidences

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Abstract

Due to scarcity of bacilli, an accurate diagnosis of osteoarticular tuberculosis is always being a challenge. Though conventional methods lack sensitivity and are time consuming, molecular diagnostic modalities are very effective for early diagnosis with high sensitivity and specificity. Timely diagnosis even in latent period will improve the efficacy of the treatment of osteoarticular tuberculosis. Under some stress conditions like hypoxia, nitric oxide stress, long term viability of *M. tuberculosis* is favoured by alpha crystallin protein (acr), which is adominant protein of latent phase. In this article we focus on protein that shows significant expression in the latent phase of *M.tuberculosis*. It may be further used as a prognostic biomarker for differentiating latent and active tuberculosis.

Keywords: Tuberculosis, Extra-pulmonary tuberculosis, Osteoarticular tuberculosis, Latent phase tuberculosis, Active phase.

1.Introduction

Tuberculosis (TB) has been a scourge of mankind since earliest times. Tuberculosis is a chronic infectious disease and the morbidity associated with this condition has major health implications. The disease has a worldwide distribution, and the incidence is high in developing countries [1]. The disease is divided into two categories – Pulmonary TB (PTB) i.e. infection affects the lungs and Extra pulmonary (EPTB) form of tuberculosis (TB) affects the organ outside the lungs parenchyma(eg-pleura, lymph nodes, abdomen, genitourinary tract, skin, joints and bones, or meninges). The causative agent is *M. tuberculosis*, which is an oxygen loving microbe. The spread of the bacilli is haematogenous and through lymphatic. Out of all EPTB cases approximately 10-50% has associated pulmonary involvement. Consequently, an assessment of suspects should be done, to check whether it is infectious [2].

Pulmonary TB (in the lungs) is contagious. It spreads when a person who has active TB breathes out air that has the TB bacteria in it and then another person breathes in the bacteria from the air. An infected person releases even more bacteria when he or she does things like cough or laugh. If TB is only in other parts of the body (extra-pulmonary TB), it does not spread easily to others. Due to wide variety of clinical manifestations, EPTB diagnosis become difficult and is delayed as compare to PTB [3,4]. Obtaining material for culture confirmation of EPTB is much more difficult than obtaining material for culture confirmation of PTB [3]. Studies suggest that the ratio of PTB was significantly higher in men, while the ratio of EPTB was significantly higher in women. Pulmonary involvement was more prominent in cases with BCG vaccinations. For the diagnosis of PTB, chest radiography, Mantoux, and BCG tests were done while EPTB was diagnosed using various diagnostic procedures [5].

Osteoarticular tuberculosis is a rare form of EPTB comprises of 10-15% in all EPTB cases and nearly 1-6% in total TB cases[6-9] The load bearing joint (i.e. spine, hip and knee) are the recurrent sites of the disease [10]. After the pleural and lymphatic

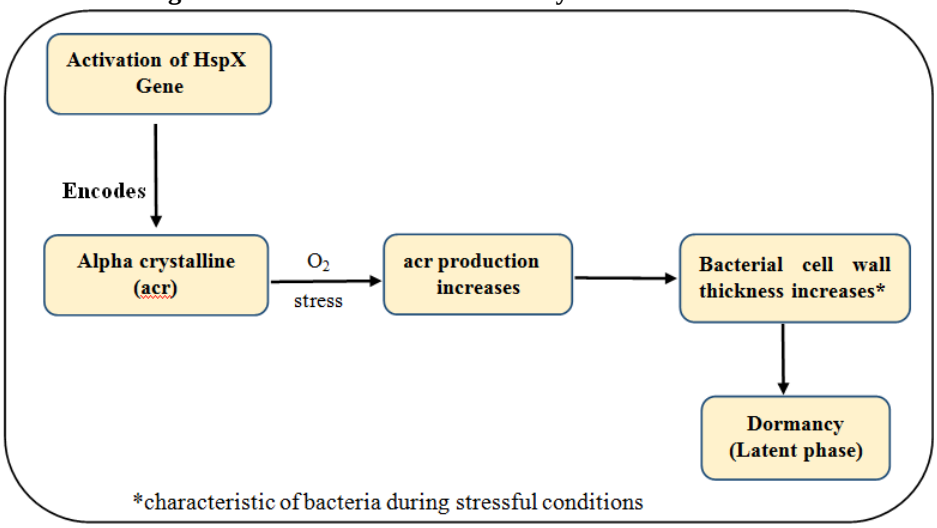
TB, osteoarticular TB is the commonest infection. Initially the infection is insidious, hence it takes several months and sometimes years from the appearance of first symptom to the diagnosis [7,11-13]. It is differentially diagnosed with other skeleton infections mainly because of its paucibacillary nature and less disease related symptoms. Illiteracy and poverty leads the affected people to restrain from consulting to doctors at the time of initial infection, unless any functional damage occurs [11]. Risk factors involved in the development of disease are mainly age, female gender, concurrent HIV infection and co-morbidities such as diabetes mellitus or immunosuppression [14].

The dosR gene responses to hypoxic and nitric oxide stresses are associates with *M. tuberculosis* dormancy [15] and others have demonstrated that acr protein expression was strongly upregulated in *M. tuberculosis* on entry into and remains at a high level during latent phase. This indicates that acris implicated in *M. tuberculosis* persistence or latent phase [16,17]. In the present review, the function of alpha crystallin protein is highlighted and suggesting it as a diagnostic marker for the latent infection of *M. tuberculosis* in osteoarticular tuberculosis.

2. Latent Tuberculosis

Latent TB means a patient is infected with *M. tuberculosis*, but the patient does not have active TB. Active TB can be transmissible while latent TB is not, and it is therefore not possible to get TB from a person who is latently infected with *M. tuberculosis*. During stress conditions bacteria go into latent phase **(Figure 1)**. The one-third of the total human population may have the latent infection of *M. tuberculosis* [18]. Presence of *M. tuberculosis* in dormant form in the tissues, forced the patients to undergo prolonged chemotherapeutic schedules. It is probably main barrier in TB eradication programme [19]. The majority of the viable bacilli are wiped outby the host's immune system. But some are able to survive within the host and lying there in inactive form for decades before it reactivates and causes the disease [20]. A long term antibiotic therapy is required to reduce the disease relapse rate and this hampers the disease control globally. At least 6 months multi-drug therapy is required for drug-susceptible infections which is quiet inconvenient for patients. While 18 months therapy is needed for multi drug-resistant tuberculosis (MDR-TB). In developing countries implementation of MDR-TB control programmeis challenging. Hence, novel therapeutic targets, especially those associated with *M. tuberculosis* dormancy that can potentially shorten the duration of chemotherapy and reduce relapse rates would be of great value [21].

Figure 1: Establishment of dormancy in *M. tuberculosis*.



3. Alpha Crystallin Protein

Under the oxygen and nitric oxide stress the hyper-virulent gene dosR results in activating the transcription of nearly 50 genes. Among these genes hspX(acr, Rv2031c) is most dramatically activated (~80 folds) [17]. This hspX gene (acr) in *M. tuberculosis* encodes a 16kilodalton alpha-crystallin-like protein which is abundantly present in the bacterial cell wall [22]. dosR upregulation is required for *M. tuberculosis* to survive within macrophages [23]. However, the 16-kDa alpha-crystallin homologue of *M. tuberculosis* is the dominant protein produced by stationary phase cultures *in vitro*, it is unnoticeable in exponentially growing cultures [17]. Hypoxia is one factor frequently associated with the establishment and maintenance of latent TB [24]. *In*

vivo, the bacilli count in a lesion generally correlates well with the degree of oxygenation [25], this indicates that oxygen supply may limit *M. tuberculosis* growth during infections. The formation of granulomas *in vivo* restricts the growth of *M. tuberculosis* [26]. Oxygen is necessary for *M. tuberculosis*, yetit shows a remarkable potential to survive for decades in the absence of oxygen *in vitro* [25]. *In vitro*, *M. tuberculosis* unable to maintained their acid-fast character under oxygen stress[27], and some human studies [28] have associated latent TB with tubercle bacilli that were no longer acid fast.The *M. tuberculosis* acr is a dominant antigen *in vivo*, recognized by most *M. tuberculosis* patient sera [29]. The acr is a member of the small heat shock protein family that forms high-molecular-weight aggregates and has chaperone activity *in vitro*. The acr is the abundantly present proteins in latent phase bacteria. It has been proposed that the 16-kDa protein is important for the survival of latentphase bacteria [30].

4. Diagnosis

The diagnosis of osteoarticular tuberculosis is often compromised by the paucibacillary nature of the disease, newer diagnostic tools and policies have been eagerly awaited [31]. Since the conventional methods like staining for acid-fast bacilli (AFB) and culturing of *M. tuberculosis* have low sensitivity and specificity [32-34]. Furthermore, the appearance of growth takes time in culturing of *M. tuberculosis*[35]. Symptoms and signs can be relativelyvague and sometimes occur in normal chest x-rays and smear-negative patients, therefore hampering the consideration of the disease in the initial approach.So, mostly, the diagnosis of tuberculosis depends on histological evidence. Due to the paucibacillary nature of osteoarticular TB, diagnosis by mycobacterial culture and Histopathological examination has limitations and low detection rate [36]. Hence, the traditional techniques have limitations in timely and specific diagnosis **(Table 1)**.

The sensitivity and specificity of Mantoux test (PPD) in extra-pulmonary tuberculosis has been reported as 47% and 86% respectively [43]. While various studies have reported sensitivity of polymerase chain reaction ranging from 61 to 90% and a specificity of 80–90% [44-48]. The clinical specimens has been monitored on Real time PCR for rapid and specific detection of *M. tuberculosis*[49]. RT-PCR has high sensitivity and specificity in comparison to traditional techniques. This technique can be used for confirming the diagnosis and also monitoring the progress.

Table 1: Limitations of various diagnostic methods

Diagnostic methods	Limitations
Acid fast bacilli (AFB)	Low specific results [32-33].
Purified Protein Derivative (PPD)	Due to its highly cross reactive nature do not givereliable results, in areas where highenvironmental load of non-tuberculousmycobacteria [37,38].
Culturing of MTB	Time consuming nearly 6-8weeks to show growth, less sensitive [35].
X-rays	By the time disease is apparent on x-rays patient is already reached at the advanced stage.[39]
MRI	There is no pathognomonic finding that reliably distinguishes tuberculosis from otherinfections [40].
CSP-Ag's ELISA	Could not recognise latent infection[41].
DNA-PCR	The DNA-PCR is unable to differentiateviable and non-viable organisms [42].

A WHO (2011) research revealed that the commercial blood tests shows low sensitivity results in the increase of number of patients diagnosed with false negative. Wrong diagnosis leads in spreading the disease to others or causes death from untreated tuberculosis. Low specificity of these tests increases the number of patients with false positive which leads to unnecessary treatment, while the real cause of their illness remains undiagnosed, which may then also result in premature death. Across the globe aroundone million people worldwide are misdiagnosed with tuberculosis when in reality they have an incurable disease with a similar outlook to many cancers. More than a million of these inaccurate blood tests are carried out annually to diagnose active TB, often at great financial cost to patients [50]. In order to evaluate new anti-tuberculosis vaccines, diagnostic tools are needed for distinguishing non-infected persons, non-symptomatic persons infected with *M. tuberculosis*, and persons with active tuberculosis [51].

Osteoarticular TB is mainly responsible for osteomyelitis [52]. Most prone sites of infections are spine, knee and hip, representing 70–80% of the infections [10,44,53]. Misdiagnosed and inadequately treated spinal tuberculosis may develop kyphosis and/or neurological complication [54]. The accurate diagnosis of osteoarticular TB poses difficulty owing to deep inaccessible lesions and initiation of empirical anti-tubercular therapy (ATT) in majority of the cases [55]. Mostly, the diagnosis of skeletal TB is based on clinical suspicion and imaging findings, particularly in the endemic regions [53].

A reduced metabolism of *M. tuberculosis* in the latent phase may play a role in stretching the duration of effective chemotherapy. The host immune

response and bacterial genetic make-up is primarily responsible for its long-term survival. The failure of bacteria to increase in numbers during latency, the lack of clinical sequelae and the enhanced resistance of latent TB to chemotherapy argue that the bacilli may be metabolically dormant [20,56-59]. If a person follows a proper chemotherapeutic schedule, relapse rate of the disease can be decreased in case of latent TB [60-61].

The nucleic acid amplification tests such as PCR emerges as a major breakthrough in the diagnosis of extra-pulmonary tuberculosis (EPTB) especially in health settings with a high prevalence of HIV associated EPTB. Specific *M. tuberculosis* nucleotide sequences are detected by the PCR directly in extra-pulmonary specimens which give results within few hours, offering better accuracy than AFB (acid fast bacilli) smear microscopy and greater speed than culture [62-65].

In a previous study, an increased production of 16 kDa a crystallin protein (acr) was observed after the exposure of *M. tuberculosis* to nitric oxide donors, thus resembling the conditions induced by the immune response [66]. Thickening of the bacterial cell wall is the characteristic feature in stressful conditions and presence of acr is associated with the increase in the cell wall thickening [67], and to other specific stressful conditions for *M. tuberculosis*, such as treatment with antibiotics (e.g. rifampicin or streptomycin) [68]. The higher expression of the sigma factor F (sigF), a ϭ- subunit of the RNA polymerase that is stimulated under stress, was also linked with elevated production of acr. The analysis of sigma factors plays a powerful role in understanding the mechanisms associated with the metabolic changes that may lead to the induction of latent bacilli [69]. With Real Time-PCR system, expression of alpha-crystallin was studied *in vitro* unstirred culture, permitting a restricted oxygen concentration; stressed bacilli was induced lowering pH and using two types of bacilli (i.e. from cultures in an exponential and a stationary phase); to establish a parallelism between bacilli in the acute and chronic phases in a murine model of TB was also studied. Results of the study suggest that the expression of acr has been closely related to latency, and thus could be a valuable marker [59]. Interestingly, the expression of acr was maintained even when the levels of 16S r RNA were undetectable following a long period of chemotherapy. Therefore, this was the best value for detecting the acr expression of latent bacilli with the Real-Time PCR may be useful as a diagnostic marker monitoring latent bacilli of TB[70].

5. Future Prospective

For correct and sensitive diagnosis we need to cope up with the flaws of the TB diagnostic techniques. In disease management, early and accurate diagnosis is very essential and plays a vital role in overall impact of giving treatment. The fallacious diagnosis of a disease leads to incorrect treatment which may badly affect patient's health and wealth or may also leads to death. The expressions of α-crystallin of MTB prove to be helpful in distinguishing between the latent as well as active infection of MTB among different individuals. Hence, the target population can more specifically be identified and may provide relevant medication. This may also helpful in prevention of antibiotic resistant strains and consequent development of MDR TB which in itself is a bigger challenge to dealt with. Accurately diagnosing these patients of latent TB may open new horizon in the management and may also help to formulate new specific treatment protocol.

Conflicts Of Interest: None

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