ADRENOCORTICAL FUNCTION IN ACTIVE AND TREATED TUBERCULOSIS IN CHEST CLINIC IPOH HOSPITAL

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ABSTRAK

KAJIAN TENTANG FUNGSI ADRENOKORTIKAL KE ATAS PESAKIT-PESAKIT TUBERKULOSIS YANG AKTIF DAN TELAH DIRAWAT DI KLINIK DADA HOSPITAL IPOH

Kegagalan fungsi adrenal adalah salah satu komplikasi penyakit tuberkulosis. Di Hospital Ipoh, kita telah mengkaji fungsi adrenokortikal di dalam pesakit tuberkulosis yang datang untuk rawatan susulan di klinik dada, Hospital Ipoh.

Objektif

Objektif utama dalam kajian yang dijalankan ini adalah untuk mengenal pasti prevalens kegagalan fungsi adrenokortikal dalam dua kumpulan pesakit iaitu satu adalah golongan pesakit yang sedang dirawat untuk tuberkulosis dan lagi satu adalah golongan pesakit yang telah habis rawatan anti tuberculosis dalam tempoh 18 bulan yang lalu.

Tujuan dalam menjalankan kajian ini adalah:

1. Untuk mengenalpasti prevalens kegagalan fungsi adrenokortikal di antara pesakit tuberkulosis yang masih mengikuti rawatan susulan di klinik dada Hospital Ipoh.
2. Untuk membandingkan kegagalan fungsi adrenokortikal dengan tahap tuberkulosis.
3. Untuk mengenalpasti jika ada, hubungan antara kegagalan fungsi adrenokortikal dengan jangkamasa penyakit tuberkulosis

4. Untuk membandingkan kegagalan fungsi adrenokortikal dengan tempat jankitan tuberkulosis
5. Untuk mengenalpasti hubungan antara penyakit-penyakit lain seperti diabetes(kencing manis) dengan fungsi adrenokortikal
6. Untuk mencari hubungan antara penyakit kurang daya ketahanan badan (HIV) dan fungsi kegagalan adrenokortikal.
7. Untuk mengkaji serum sodium dan serum potassium dan mengenal pasti hubungan dengan kegagalan fungsi adrenokortikal yang didapati daripada ujian synacthen.

Rangka kajian

Pesakit tuberkulosis yang sedang dirawat dan pesakit yang telah dirawat dalam tempoh 18 bulan yang lalu telah diambil untuk kajian ini. Penglibatan kelenjar adrenal akibat penyakit tuberkulosis dikaji daripada ujian darah untuk tahap cortisol basal dan respons cortisol kepada suntikan intravena tetracosactrin (Synacthen,Novartis,USA) 1.0μg/1.73m^2 "body surface area".

Peserta

Setiap pesakit yang dirawat di klinik dada di Hospital Ipoh daripada bulan Januari hingga September 2001, dan didiagnosakan sebagai tuberkulosis daripada ujian "smear positive" acid fast bacilli, ujian histopatologi, radiology dan juga secara klinikal oleh pakar perubatan akan diterima untuk memasuki kajian ini. Kebenaran bertulis telah diambil daripada setiap pesakit setelah kajian diterangkan secara terperinci kepada kesemua peserta kajian.

Metodologi

Setiap peserta kajian telah disoalsiasat dan sejarah kesihatan mereka direkodkan. Rekod rawatan susulan setiap peserta kajian digunakan untuk menentukan tahap biasa beberapa keputusan darah seperti paras glukosa, paras sodium dan potassium dan juga status HIV setiap pesakit dan kesemua ini direkodkan.

Sampel darah vena diambil dari setiap peserta untuk menentukan tahap basal cortisol darah. Sampel darah vena kedua diambil selepas setengah jam selepas suntikan intravena synacthen pada dos 1μg/1.73m^2 "body surface area". Serum cortisol telah diperiksa menggunakan kaedah "Fluorescence Polarization Immunoassay".

Keputusan Kajian

49 pesakit telah menyertai kajian dan seramai 8.2% daripada mereka mempunyai keputusan darah cortisol selepas suntikan intravena synacthen yang kurang daripada 550nmol/l dan seramai 22.4% mempunyai kenaikan paras serum cortisol selepas stimulasi kurang daripada 300nmol/l. Tidak terdapat sebarang kaitan yang memuaskan antara kegagalan fungsi adrenokortikal dengan jangkamasa simtom, bahagian anatomi yang dijangkiti, paras basal plasma sodium dan paras basal plasma potassium.

Kesimpulan

Prevalens kegagalan fungsi adrenokortical diantara pesakit tuberkulosis di Malaysia adalah lebih tinggi daripada yang disangka dahulu. Jangkitan tuberkulosis yang serius dan kehadiran penyakit yang lain adalah factor risiko untuk kegagalan fungsi adrenokortikal. Ujian dos rendah synacthen adalah satu ujian yang sensitif dalam mengenalpasti kegagalan fungsi adrenokortikal primari. Bilangan pesakit dengan HIV dan jangkitan tuberkulosis luar paru-paru adalah amat sedikit untuk membuat satu kesimpulan yang memuaskan untuk menunjukkan sebarang pertalian dengan kegagalan fungsi adrenokortikal.

ABSTRACT

STUDY OF ADRENOCORTICAL FUNCTION IN PATIENTS WITH ACTIVE AND TREATED TUBERCULOSIS IN CHEST CLINIC HOSPITAL IPOH

Tuberculosis is a well known cause of adrenal dysfunction. We assessed the adrenocortical function in patients with tuberculosis on follow up with chest clinic in Hospital Ipoh.

Objectives

The primary objective of this study which was conducted in Hospital Ipoh was to diagnose the prevalence of adrenocorticoid dysfunction in two groups of patients namely those treated as tuberculosis and those who have completed antituberculosis treatment within the last 18 months.

The aims of this study include:

1. To find out the prevalence of adrenocortical dysfunction among patients with tuberculosis currently attending chest clinic follow up in Hospital Ipoh
2. To compare adrenocortical dysfunction with the severity of tuberculosis
3. To look for any correlation with the duration of the disease
4. To compare the site of disease in the context of adrenocortical dysfunction
5. To ascertain any relationship between co morbid factors such as diabetes mellitus and adrenocortical response
6. To correlate between Human Immunodeficiency Virus (HIV) and adrenocortical dysfunction
7. To look at serum sodium and potassium levels and to correlate with adrenal hypofunction as diagnosed with the synacthen test

Design

Patients with active and treated tuberculosis were studied as well as those who have completed treatment within the last 18 months. Involvement of the adrenal gland was assessed by basal cortisol level and cortisol response to intravenous dose of 1.0 μg/1.73 m^2 body surface area of tetracosactrin (Synacthen, Novartis, USA).

Subjects

Consecutive patients seen at the chest clinic in Hospital Ipoh from January to September 2001 diagnosed as tuberculosis based on acid fast bacilli smear positive, histopathological diagnosis, radiological as well as clinical diagnosis by a physician as tuberculosis were eligible for entry into the study. Written consent was obtained from all subjects after adequate explanation.

Methods

Every patient was interviewed and history was taken. The patient's follow up notes were also utilized to determine baseline investigation results which included serum sodium, serum potassium and HIV status.

Samples of venous blood were collected from patients for determination of basal plasma cortisol levels. Venous blood was collected again at exactly 30 minutes after intravenous injection of synacthen. The serum cortisol was assayed using the Fluorescence Polarization Immunoassay technology.

Results

49 patients participated in this study with 8.2 % of patients having abnormal post synacthen serum cortisol of less than 550nmol/l and 22.4% with a subnormal rise of serum cortisol following stimulation of less than 300nmol/l.

Conclusion

The prevalence of adrenocortical dysfunction in patients with tuberculosis in Malaysia is higher than previously suspected. Severe tuberculosis and presence of co-morbid factors are risk factors for adrenocortical dysfunction. The low dose synacthen test is a sensitive method to assess primary adrenocortical failure There was no definite association with the duration of symptoms, site of disease, baseline serum sodium and serum potassium. As the numbers of patients with HIV and extra pulmonary tuberculosis were small it was difficult to draw a conclusion regarding any association with adrenal hypofunction.

INTRODUCTION

The prevalence of adrenal tuberculosis in Malaysia still remains a mystery in spite of numerous studies and various diagnostic tests used. To date few studies have been published on the frequency of adrenal disorder during active and treated tuberculosis[1,2,3,4,5]. A study in Asian subjects by Prasad et al on ninety seven HIV negative patients with various forms of tuberculosis revealed that forty five of the 91 patients (49.5%) who underwent the ACTH (Adrenocorticotrophic Hormone) stimulation test had compromised adrenal reserve[1].

The prevalence of Addison's disease in Western countries may be as high as 120 per million population [6,7,8,] but the clinical spectrum has changed dramatically of which autoimmunity has become the most common cause[9] in the Western population. But current data shows that the disseminated tuberculosis is still a major cause of adrenal insufficiency in developing countries where the prevalence of tuberculosis is still high[10]. In fact as a result of hematogenous dissemination in HIV-infected individuals, extrapulmonary tuberculosis is seen more commonly today than in the past[12].

Unfortunately we still do not know the exact frequency and extent of adrenal involvement in tuberculosis in Malaysia. That is why we have decided to embark upon this study to determine the prevalence of the problem in Malaysia and to determine whether we are able to predict those patients with tuberculosis who are more likely to develop adrenal dysfunction

LITERATURE REVIEW

3.1 HISTORY

The term tuberculosis was first used in 1839, and it is derived from the Latin word ***tubercula***, meaning small lump, which referred to the small scars seen in tissues of infected individuals. ***Hippocrates*** had clearly described tuberculosis and even associated pulmonary nodules with spinal disease. ***Franciscus Sylvius***, in the mid-17th century, was the first to describe the relation of consumption and pulmonary nodules and he thought that scrofula might also be related to this disease. It was in 1882, that the tubercle bacillus was isolated by ***Robert Koch***, a German physician and it was subsequently identified as the single cause of these several diseases[13]. By this time, ***Mycobacterium tuberculosis*** (*M. tuberculosis*) was responsible for almost one-seventh of all the deaths in Europe[12]. At the time when ***Thomas Addison*** described **Addison's disease** 150 years ago, the commonest cause of bilateral adrenal destruction was tuberculosis[14].

It remains the most common infectious cause of death in adults worldwide. The human host serves as the only natural reservoir for M. tuberculosis, but the ability of the organism to efficiently establish latent infections has enabled it to spread to nearly one-third of the world's population[15, 16].

3.2 EPIDEMIOLOGY

Tuberculosis in the world

The rate of tuberculosis has been declining steadily for decades until the 1980's and 1990's when a resurgence of the disease was noted[88]. This was due to many contributory factors including waning interest in tuberculosis, deteriorating public health infrastructure, inadequate control of infection, urban crowding, emergence of multi drug-resistant (MDR) tuberculosis due to strains resistant at least to isoniazid and rifampicin, social problems such as poverty, homelessness, drug abuse, HIV epidemic and immigration[88,89,90]. This in turn has lead to a rise in tuberculosis in most urban centers and the persistence of disease in developing countries[88, 89, 90].

Currently more than one third of the world's population are infected with tuberculosis with 9 million new cases in 1995 reported with 3 million deaths [89,90]. Initially, in the United States the number of cases of tuberculosis had decreased dramatically from 84,304 in 1953 to 22,201 in 1985[92]. During this time, cases were due to reactivation of old infection, and tuberculosis was progressively becoming a disease of the elderly. During that time, transmission of new infection to infants and children was decreasing progressively. However since 1985, there has been a dramatic increase in the number of cases. In 1992, an 18 percent increase in tuberculosis cases occurred compared to 1985[91,92].

Sub-Saharan Africa, India, China, the islands of southeast Asia and Micronesia have the highest incidence of tuberculosis. This incidence of tuberculosis is expected to increase worldwide during the next 10 years because of the interaction between the tuberculosis and HIV epidemics[93].

25% of all avoidable deaths in developing countries are due to tuberculosis[89, 90]. 95% of tuberculosis new cases and 98% of tuberculosis deaths occur in developing countries[90]. 75% of tuberculosis cases in developing countries are in the economically productive age group (15-50 years) [90]. The serious global threat of tuberculosis resulted in the WHO declaring this disease a global emergency in 1993. The World Health Organisation has calculated that, unless urgent action is taken, the annual number of deaths could rise from 3 million to 4 million by the year 2004[94].

Tuberculosis in Malaysia

In Malaysia, the number of cases detected per year has not declined substantially. Since 1989, around 11 500 – 12 000 cases were detected per year. In 1998, 14 115 cases were detected of which 55% were infectious. In 1985, the incidence of tuberculosis was 68.6/100 000 population and in 1998 was 63.8/100 000 population and the mortality rate for tuberculosis in 1994 was 1.7/100 000 population. This has accounted for 384 certified tuberculosis deaths in 1994. Tuberculosis cases with HIV has also been on an increasing trend. Between 1990 to 1994, there were 137 cases of tuberculosis with HIV/AIDS with 23 deaths. The cumulative number of cases since 1990 was 288 (2.5% of total tuberculosis cases) of which 42 have since died. 96.9% of these cases were among the 15 - 49 years age group.[156,157]

Apart from HIV, immigration has also been a factor in the rising trend of tuberculosis cases in the country. In 1998, the total number of tuberculosis cases reported among foreigners residing in Malaysia were 1626 cases. Of these, 1019 cases were detected in Sabah. Thus 10.5% of all tuberculosis cases detected in Malaysia were among the immigrants especially from Indonesia, Phillipines and Bangladesh[142].

Factors that affect resurgence of tuberculosis

1. Tuberculosis and the HIV epidemic

One of the main causes of the resurgence of tuberculosis is the HIV epidemic. This is because HIV is a risk factor for the reactivation of tuberculosis. The estimated risk for developing tuberculosis in a tuberculin positive individual who develops HIV infection is 7 to 10 percent per year[95]. Therefore tuberculosis is prevalent in populations infected with both HIV and tuberculosis, such as intravenous drug users. This increase was higher in persons aged 25 to 44 and in areas with a high incidence of HIV infection.

Apart from this, HIV infection also markedly increases the susceptibility for new tuberculous infection to develop into active disease. Tuberculosis epidemics have occurred in crowded facilities such as in prisons, hospitals, HIV outpatient clinics, homeless shelters, and community living quarters[96, 97, 98].

Usually epidemics in HIV infected persons have involved multiple drug resistant strains of tuberculosis [99, 100] which can be epidemiologically identified through DNA fingerprinting techniques[101, 102]. In a study of patients with multi drug resistant tuberculosis in New York the incidence of HIV infection increased from 16 percent in 1987 to 58 percent in 1990[102]. Resistance to antituberculous drugs can be attributed to either re-infection with a new strain which is drug resistant or development of resistance in the initial strain[101].

A survey of drug resistance by *Granich* et al[103] among tuberculosis isolates in Mexico which showed a rate of primary resistance of 12.9 and 2.4 percent to one drug and multiple drugs, respectively, which rose to 50.5 and 22.4 percent, respectively, for re-treatment cases. Another study has found that patients with drug resistance had a higher rate of treatment failure and mortality[104].

The highest incidence of tuberculosis occurs in sub-Saharan Africa, India, China, and the islands of Southeast Asia and Micronesia. The HIV epidemic is expected to dramatically increase the number of cases of tuberculosis over the next decade[105].

2. Migration

Immigration has played a major part in the resurgence of tuberculosis. For instance in the United States tuberculosis in immigrants consisted of 7 591 cases in 1998 compared to 7 402 in 1993, an increase of 2.6 percent. In fact foreigners consisted of 41.6 percent of all TB diagnoses in the United States, up from 29.8 percent in 1993[106, 107]. Nearly 50% of the cases of tuberculosis among foreign-born persons occur more than five years after arrival in the United States[108]. Age >5 years upon migration confers a 2 to 6 fold greater risk for tuberculosis versus individuals of a similar age who arrive before their fifth birthday.

In Canada, one study by *Cowie et al*[109] revealed that foreigners, the majority whom were from Asian countries, accounted for 70 percent of the tuberculosis cases in Alberta over a five year period. A study in the Montreal region by *Rivest et al* reported similar findings[110].

In addition transmission of tuberculosis has also been on the rising trend in young children. From 1985 through 1990, tuberculosis cases increased 19 percent in children less than four years of age and 40 percent in children ages 5 through 14.

This pattern of resurgence has also been seen in western European countries of which tuberculosis in foreigners accounts for 40 to 50 percent of all cases of tuberculosis[111].

3. Socioeconomic Status

Low socioeconomic status itself is a significant risk factor for tuberculosis. In the United States elevated risk for tuberculosis has been noted among African-, Hispanic-, Asian-, and Native Americans which is probably attributed to low income, crowded living conditions, unemployment, and lower education[109]. Further more this is compounded by the decreased funding of public health services which has caused a deterioration of the public health infrastructure.

Rieder et al stated that the numbers of extrapulmonary cases were 17.5% of all cases of tuberculosis and mainly occurred among racial ethnic minorities and the foreign-born[33]. He found that the proportion of extra pulmonary tuberculosis among all patients with tuberculosis by age was found to be largest in children, among African Americans , Asians, and Native Americans than among non-Hispanic white patients, females compared to male patients.

Before the advent of effective antituberculosis therapy, untreated tuberculosis was often fatal. About one-third of patients died within 1 year after diagnosis, and one-half within 5 years. Five-year mortality among sputum smear-positive cases was 65%[147].

In clinical practice we have observed that even patients on effective antituberculous chemotherapy died while on treatment. This could be due to several factors including severe advanced pulmonary disease, disseminated tuberculosis with adrenal crisis and tuberculous myocarditis[154].

Tan et al looked at mortality of patients on treatment for active tuberculosis and found that 20 % of patients that died had disseminated tuberculosis[143]. This would suggest that disseminated tuberculosis was more prevalent than previously thought and therefore the incidence of adrenal dysfunction higher. *Lam et al* had performed a retrospective analysis of autopsies and adrenalectomies of 13 762 patients and found that extra-pulmonary tuberculosis was seen in 30% of patients with active tuberculosis. Even though adrenal tuberculosis was present in 6% of patients with active tuberculosis, only 12 % of these patients presented overt signs and symptoms of Addison's disease due to bilateral adrenal involvement[155]. Therefore we would infer that extra pulmonary tuberculosis such as adrenal tuberculosis is a common under-diagnosed problem. A high index of suspicion as well as sensitive and specific screening tests is needed for the diagnosis of adrenal insufficiency.

With the rising number HIV infection in Malaysia it would be expected that the prevalence of disseminated and adrenal tuberculosis would also increase due to the unchecked haematogenous dissemination of tuberculosis.

3.3 MICROBIOLOGY

Tuberculosis is defined as a disease caused by members of the *M. tuberculosis complex*, which includes *M. tuberculosis, M. bovis, M. africanum* and *M. microti.* The cell envelope for Mycobacterium consist of peptidoglycan, arabinogalactan and mycolic acids as well as lipoarabinomannan (LAM)[37].

The organism stains positive with Gram stain. There is resistance to destaining by acid alcohol after being stained by aniline dyes leading to the term acid fast bacillus (AFB). Ziehl-Neelsen stain is the most commonly used procedure to diagnose tuberculosis. However, a specimen must contain at least 10^4 colony forming units (CFU)/mL to yield a positive smear[38].

Culture of tuberculosis is needed to assess drug sensitivity of the isolate. Culture is also much more sensitive than microscopy, being able to detect as few as 10 bacteria/mL of material[130]. In general, the sensitivity of culture is 80 to 85 percent with a specificity of approximately 98 percent[131, 132]. Artificial media such as the Lowenstein-Jensen (LJ) medium[39] and the BACTEC (BBL) system are used to culture the organism.

M. tuberculosis has non pigmented corded colonies on albumin-based agars. It tests positive in the niacin test, has a weak catalase activity, which is inactivated at 68°C, and reduces nitrate[46]. Therefore niacin, nitrate reductase, and catalase can be used to identify *M. tuberculosis* from other mycobacterial species as well as tests for pyrazinamidase and susceptibility to thiophen-2-carboxylic acid hydrazide (TCH)[46,47]. Apart from the traditional method of obtaining smears and cultures, newer techniques such as the nucleic acid amplification techniques can be used to detect and identify *M. tuberculosis* directly[158, 159].

3.4 TRANSMISSION AND PATHOGENESIS

Tuberculosis is spread from person to person through the air by droplet nuclei, particles 1 to 5 mm in diameter that contain *M. tuberculosis complex* [76]. Droplet nuclei which contain two to three *M. tuberculosis* organisms[77] remain airborne for long periods of time[78]. These droplet nuclei are small enough to reach the pulmonary alveoli, where the organisms replicate.

The transmission of *M. tuberculosis* depends on the number of organisms being expelled into the air, the concentration of organisms in the air determined by the volume of the space and its ventilation, the length of time an exposed person breathes the contaminated air, and the immune status of the exposed individual.

After inhalation, the droplet nucleus is carried down the bronchial tree and implants in a respiratory bronchiole or alveolus. Further establishment an infection in the lung depends on both the bacterial virulence and the inherent microbicidal ability of the alveolar macrophage that ingests it[76, 80]. There are usually four possible outcomes i.e. either immediate clearance of the organism, chronic or latent infection, rapidly progressive disease or active disease many years after the infection (reactivation disease).

Only 5 to 10 percent of patients with no underlying medical problems who become infected develop active disease in their lifetime[48], but the risk increases markedly in patients with AIDS[49]. When host defense system fails the tubercle bacilli will proliferate and destroy the alveolar macrophages.

The invasion of macrophages by mycobacteria is due to the association of C2a with the bacterial cell wall and opsonization by C3 which is recognized by the macrophage complement receptor CR3 (Mac-1 integrin) [160, 162]. After invasion, it will multiply within the alveolar macrophage and divide slowly approximately every 25 to 32 hours within the macrophage. Eventually the macrophage will lyse under the burden of the multiplying bacilli and release cytokines and chemokines which are chemotactic factors. This will cause nonactivated monocytes from the bloodstream to converge at the site and ingest the bacilli that had been released from the lysed macrophages[160].

If the bacterial replication is not controlled the bacilli will enter the local draining lymph nodes. This leads to lymphadenopathy, a characteristic manifestation of primary tuberculosis. The lesion in the lung parenchyma with lymph node involvement is called the Ghon complex[160]. The tuberculosis bacilli will grow and divide until specific cellular immunity develops and limits multiplication. This cellular immune response[81, 82] will occur after 2 to 12 weeks once the bacilli reach 10 000 to 100 000 in number and this can be detected by a reaction to the tuberculin skin test. Success of the host cell mediated immunity will result in the resolution of infection[162].

The host response to the organism is reduced by certain diseases such as silicosis, diabetes mellitus, HIV infection, corticosteroids, liver disease and malnutrition[161]. If there is no containment of infection the tuberculosis bacilli will spread haemotogenously to more distant sites to involve multiple organs including the adrenal glands[162].

Adrenal tuberculosis is a manifestation of disseminated disease usually presenting with adrenal insufficiency. Pathological changes that occurs in the adrenal gland is similar to the changes that occurs in the in the lung[160].

Cellular immunity is responsible for both of the resulting tissue damage as well as conferring protection against the tuberculosis disease. The tissue damage is due to a delayed type hypersensitivity reaction to various bacillary antigens which destroys non activated macrophages that contain multiplying bacilli whereas the cellular immunity activates macrophages which kill and digest the tuberculous bacilli.

In the adrenal gland granulomatous tubercles are formed from lymphocytes and activated macrophages, such as epithelioid cells and giant cells[161]. These activated macrophages and T lymphocytes aggregate around the lesion's center and neutralize the tuberculous bacilli and form a central caseating necrosis[160]. The organisms tend to be localized in the necrotic center of the granuloma [84] and small numbers of viable bacilli remain within even after development of immunity[161].

With severe delayed type hypersensitivity reaction extensive caseation and tissue damage can occur in the adrenal glands. This may lead to progressive adrenal insufficiency or Addisonian crisis if the damage is bilateral.

When cell-mediated immunity is intact there is protection against re-infection. The chance of re-infection depends on the risk of re-exposure, the intensity of such exposure, and the integrity of the host's immune system[161]. Therefore in immunocompetent individuals tuberculosis due to inhalation of a second infecting strain is uncommon[161]. Once infected the complete eradication of the bacilli without adequate treatment is virtually impossible [80, 81, 82, 161].

M. tuberculosis virulence factors include mycolic acid glycolipids and trehalose dimycolate which can elicit granuloma formation in animal tissue, catalase-peroxidase and lipoarabinomannan which resist the host cell oxidative response, sulfatides and trehalose dimycolate which can trigger toxicity in animal models as well as lipoarabinomannan which can induce cytokines[50, 51, 52,53,54].

Other effector molecules specific to *M. tuberculosis* which are possibly associated with pathogenicity include mycobacterium cell entry protein (Mcep) encoded by mce1 which is located in an operon containing genes that encode integral membrane proteins[55].

Other genes that have been identified to be possibly associated with pathogenicity include among others a sigma factor sigA[56] and erp, a gene that encodes an exported repetitive protein[57].

Intracellular survival is essential to maintain latent infection which can then give rise to reactivation disease. An in vitro model by *Wayne et al* under microaerophilic conditions, a state called nonreplicating, persistent (NRP1) is produced and glycine dehydrogenase activity is induced. In contrast, growth under anaerobic conditions produces a state called NRP2 in which glycine dehydrogenase activity decreases, but the organism still survives as long as the loss of oxygen occurs slowly and is passed through the NRP1 stage for a period of time[58]. When oxygen is reintroduced to organisms grown anaerobically, the pathogen proliferates out of the NRP2 state.

A sigma factor gene sigF has also been identified in M. tuberculosis[59] which is expressed during stationary phase, nitrogen depletion, and cold shock, but not during exponential phase growth.

During the stationary phase growth of *M. tuberculosis* an alpha-crystallin-like heat shock protein (acr) [60] which has been found to enhance the long term survival of *M. tuberculosis*. Acr transcript was also induced in *M. tuberculosis* inside macrophages; acr gene replacement by homologous recombination in *M. tuberculosis* H37Rv led to impaired growth of the organism inside mouse bone marrow-derived macrophages[61].

Once the *M. tuberculosis* has entered the macrophages, the bacillus avoids intracellular killing by resistance to reactive oxygen intermediates (ROIs). Lipoarabinomannan (LAM) serves as a scavenger for oxygen intermediates[63]. Entry of the organism into macrophages via complement receptors (CR1 and CR3) does not stimulate ROI production[64, 65]. Cyclopropanated mycolic acids of the cell wall may help the organism resist hydrogen peroxide[66]. The mycobacteria also inhibits the phagosome-lysosome fusion which normally leads to the release of microbicidal lysosomal contents[67, 68, 69].

Phagosome acidification is also inhibited by *M. tuberculosis* by selectively excluding the proton-ATPase from the phagosome[70, 71].

In addition to resistance to ROI, *M. tuberculosis* may exhibit resistance to reactive nitrogen intermediates (RNIs) which are the only macrophage effector molecules associated with the killing of *M. tuberculosis*[72, 73].