Journal of Advances in Science and Technology

Vol. 10, Issue No. 21, February-2016, ISSN 2230-9659

A RESEARCH UPON PRESENT INSIGHTS OF MOLECULAR EPIDEMIOLOGY OF TUBERCULOSIS: ACHIEVEMENTS AND CHALLENGES

AN INTERNATIONALLY INDEXED PEER REVIEWED & REFEREED JOURNAL

www.ignited.in

Journal of Advances in Science and Technology
Vol. 10, Issue No. 21, February-2016, ISSN 2230-9659

A Research upon Present Insights of Molecular Epidemiology of Tuberculosis: Achievements and Challenges

Shashi Ranjan[1]* Dr. Surendra Sarsiya[2]

[1]Research Scholar, SSSUTMS, Sehore

[2]UTD, SSSUTMS, Sehore

Abstract – Over the past 10 years, molecular methods have become available with which to strain-type Mycobacterium tuberculosis. They have allowed researchers to study certain important but previously unresolved issues in the epidemiology of tuberculosis (TB). For example, some unsuspected micro epidemics have been revealed and it has been shown that the relative contribution of recently acquired disease to the TB burden inmany settings is far greater than had been thought. These findings have led to the strengthening of TB control. Other research has demonstrated the existence and described the frequency of exogenous reinfection in areas of high incidence. Much recent work has focused on the phenotypic variation among strains and has evaluated the relative transmissibility, virulence, and immunogenicity of different lineages of the organism. We summarize the recent achievements in TB epidemiology associated with the introduction of DNA fingerprinting techniques, and consider the implications of this technology for the design and analysis of epidemiological studies.

----------♦----------

INTRODUCTION

Consumption, King's Evil, lupus vulgaris, and phthisis are some of the more colorful names for tuberculosis (TB) that have been used in the last several centuries. Archeological findings from a number of Neolithic sites in Europe and sites from ancient Egypt to the Greek and Roman empires show evidence of a disease consistent with modern TB. TB was described by Hippocrates (400 B.C.) in *Of the Epidemics* and was clearly documented by Claudius Galen during the Roman Empire. Likewise, TB has been more recently immortalized by artists such as John Keats, D. H. Lawrence, Anton Chekhov, Emily Bronte, Charlotte Bronte, Franz Kafka, Amedeo Modigliani, and Frederick Chopin, all of whom were afflicted by the disease.

In 1882, Robert Koch made the landmark discovery that TB is caused by an infectious agent, *Mycobacterium tuberculosis*. Although demystifying, Koch's findings introduced the possibility that antimicrobial agents could be developed to combat this age-old scourge (144). Today, despite the availability of effective antituberculosis chemotherapy for over 50 years, TB remains a major global health problem. As the rates of TB infection have fallen dramatically in industrialized countries in the past century, resource-poor countries now bear over 90% of all cases globally. In fact, there are more cases of TB today than ever recorded. As such, there is a need for new therapeutics, diagnostics, and vaccines in conjunction with improved operational guidelines to enhance current TB control strategies.

While much is known about the epidemiology of TB, key questions have eluded classical epidemiologists for decades. These include the current rates of active transmission by differentiating disease due to recent or previous infection; the determination of whether recurrent tuberculosis is attributable to exogenous reinfection; whether all *M. tuberculosis* strains exert similar epidemiologic characteristics in populations; and an understanding of transmission dynamics on a population- or group-specific level, as well as in identifying extensive transmission or outbreaks from what appear to be sporadic, epidemiologically unrelated cases. Molecular epidemiologic methods have facilitated studies that address some of these very questions. In this review, we present the current approaches and issues surrounding the molecular epidemiology of *M. tuberculosis* and the insights that this relatively new field has contributed to our general understanding of TB epidemiology, pathogenesis, and evolution.

Styblo defined tuberculosis (TB) epidemiology as ''the study of the interactions between the tubercle bacillus and man in his environment (in a community)'' (1), and remarked that it was particularly important to

study them under natural conditions without any interference in the form of direct or indirect control measures. The data that Styblo relied on to assess the burden of clinical TB included case notifications based on the examination of sputum by microscopy, bacteriological cultures, and chest radiographs. Rates of TB infection were estimated from surveys involving serial tuberculin skin tests.

These tools allowed him to describe the downward trend in the incidence of TB in Europe during the 20th century, to measure the mortality associated with untreated disease, to estimate infectiousness and to measure the contribution of exogenous reinfection to TB morbidity.

Notwithstanding the work of Styblo, Gryzbowski, Comstock, Stead, and others, many important questions remain unresolved, largely because the natural history of the disease makes it so difficult to study. The armamentarium has often been inadequate for studying patterns of occurrence of tuberculosis, especially in those areas of the developing world where its toll is highest. Surveys based on the tuberculin skin test are often difficult to interpret because of cross-reactivity with BCG vaccine and environmental mycobacteria. Case notification data continue to underestimate the disease burden in areas where the prevalence of TB is high but resources for diagnosis and record-keeping are limited. Until relatively recently it has not been possible to trace pathways of TB transmission within populations.

Tuberculosis (TB) continues to be one of the highest burdens and greatest challenges to public health. Annually, TB causes approximately 1.7 million deaths and 9.4 million incident cases worldwide. Although the incident rate of TB is slowly falling due to the expansion of the population, the absolute number of new TB cases is still increasing. It is estimated that two billion people (i.e., one-third of the global population) are infected with *Mycobacterium tuberculosis* (MTB), the causative agent of TB (World Health organization [WHO], 2009).

MTB is one of the most successful human pathogens. Many efforts and resources have been invested to conquer this disease. Despite continuous efforts to generate effective strategies and approaches for prevention, control and treatment of TB, there has been little progress on developing vaccinations and drugs and increasing our understanding of the disease compared to the progression of the adaptability and pathogenicity of the pathogen, which is, even now, full of ambiguity regarding virulence factors and pathogenicity.

Tuberculous meningitis (TBM), or TB meningitis, is the most devastating form of TB. The disease involves the infection of the meninges of the host, which is caused by MTB and other mycobacteria. This form of TB is of greatest concern due to its fatal outcome and neurological sequelae. The challenge is concentrated around rapid reliable diagnosis, treatment and understanding of its pathogenesis. The incidences of extrapulmonary TB and TBM are increasing (Kruijshaar *et al*., 2009). Drug resistance and HIV infection are the complications that make the treatment and management of TBM patients more difficult, and there are still doubts regarding many aspects of the disease. The lack of knowledge regarding TBM is challenging for us and other researchers.

EPIDEMIOLOGY OF TUBERCULOSIS

Global incidence and prevalence. The World Health Organization (WHO) estimates that approximately one-third of the global community is infected with *M. tuberculosis*. In 2000, an estimated 8 to 9 million incident cases and approximately 3 million deaths due to TB occurred worldwide. After human immunodeficiency virus (HIV)/AIDS, TB is the second most common cause of death due to an infectious disease, and current trends suggest that TB will still be among the 10 leading causes of global disease burden in the year 2020.

The global distribution of TB cases is skewed heavily toward low-income and emerging economies. The highest prevalence of cases is in Asia, where China, India, Bangladesh, Indonesia, and Pakistan collectively make up over 50% of the global burden. Africa, and more specifically sub-Saharan Africa, have the highest incidence rate of TB, with approximately 83 and 290 per 100,000, respectively. TB cases occur predominantly (approximately 6 million of the 8 million) in the economically most productive 15- to 49-year-old age group. Our understanding of TB epidemiology and the efficacy of control activities have been complicated by the emergence of drug-resistant bacilli and by the synergism of TB with HIV coinfection.

Drug resistance. No sooner were the first antituberculosis agents introduced in humans than the emergence of drugresistant isolates of *M. tuberculosis* was observed. In vitro studies showed that spontaneous mutations in *M. tuberculosis* can be associated with drug resistance, while selective (antibiotic) pressure can lead to enhanced accumulation of these drug-resistant mutants. The efficient selection of drug resistance in the presence of a single antibiotic led investigators to recommend combination therapy using more than one antibiotic to reduce the emergence of drug resistance during treatment. Indeed, when adequate drug supplies are available and combination treatment is properly managed, TB control has been effective.

Selection for drug-resistant mutants in patients mainly occurs when patients are treated inappropriately or are exposed to, even transiently, subtherapeutic drug levels, conditions that may provide adequate positive selection pressure for the emergence and maintenance of drug-resistant organisms de novo. One of the contributing factors is the exceptional

length of chemotherapy required to treat and cure infection with *M. tuberculosis* (142). The need to maintain high drug levels over many months of treatment, combined with the inherent toxicity of the agents, results in reduced patient compliance and subsequently higher likelihood of acquisition of drug resistance. Therefore, in addition to identifying new antituberculosis agents, the need for shortening the length of chemotherapy is paramount, as it would greatly impact clinical management and the emergence of drug resistance. Since the early 1990s, an alarming trend and a growing source of public health concern has been the emergence of resistance to multiple drugs (MDRTB), defined as an isolate that is resistant to at least isoniazid (INH) and rifampin (RIF), the two most potent antituberculosis drugs (133, 269). Recent estimates suggest that in 2003 there were 458,000 incident cases (including new and retreatment cases) of MDR-TB globally (95% confidence interval, 321,000 to 689,000). These figures suggest that prevalent cases may be two or three times more numerous than incident cases and that a far greater number of individuals are latently infected. While treatment for MDR-TB has greatly improved (mainly in resource-rich settings), it is generally more difficult to treat and has been associated with very high morbidity and mortality, prolonged treatment to cure, and an increased risk of spreading drug-resistant isolates in the community.

HIV/AIDS. HIV infection exerts immense influence on the natural course of TB disease. Individuals with latent *M. tuberculosis* infection who contract HIV are at risk of developing active TB at a rate of 7 to 10% per year, compared to approximately 8% per lifetime for HIV-negative individuals. HIV-infected persons recently infected with *M. tuberculosis* may progress to active disease at a rate over 35% within the first 6 months, compared to 2 to 5% in the first 2 years among HIV-negative individuals (70). With the introduction of highly active antiretroviral therapy for HIV, the risk of progression to TB among those coinfected with *M. tuberculosis*, while higher than among HIV-negative cases, is considerably lower. The role for CD4_ T cells in protecting against disease progression is underscored by the marked susceptibility to TB in patients with advanced HIV-induced CD4_ T-cell depletion. The natural course of HIV disease may also be influenced by *M. tuberculosis* infection. *M. tuberculosis* infection results in macrophage activation, which can house resident HIV virions, resulting in active expression of HIV antigens rather than the prolonged latency without antigenic expression of HIV proteins. In support of this, Pape et al. observed more rapid progression to AIDS among tuberculin skin test (TST)-positive individuals not given treatment for latent TB infection (INH) than among those who were treated with INH. Thus, HIV infection tends to accelerate the progression of TB, while in turn, the host immune response to *M. tuberculosis* can enhance HIV replication and may accelerate the natural course of HIV/AIDS.

MOLECULAR EPIDEMIOLOGY

Molecular epidemiology is a field that has emerged largely from the integration of molecular biology, clinical medicine, statistics, and epidemiology. In essence, molecular epidemiology focuses on the role of genetic and environmental risk factors, at the molecular/cellular or biochemical level, in disease etiology and distribution among populations. More specifically to infectious diseases, molecular epidemiology attempts to utilize a multidisciplinary approach to identify factors that determine disease causation, propagation/dissemination, and distribution (in time and space). This is primarily achieved by associating epidemiologic characteristics with the biologic properties of clinical isolates recovered from symptomatic individuals.

The mid-1980s saw the first integration of molecular methods to discriminate between clinical isolates of *M. tuberculosis*. While previous methods, such as colony morphology, comparative growth rates, susceptibility to select antibiotics, and phage typing, were useful, they did not provide sufficient discrimination, thus limiting their utility in TB epidemiology.

That is, prior to molecular methods, understanding the spread of TB was imprecise and relied on observational data or anecdotal correlations. However, given the plethora of molecular tools available, it is critical to choose an appropriate method(s) to address a particular study question, e.g., transmission dynamics, outbreaks, or phylogenetics. In general, the key aspects in choosing an adequate molecular approach for studying TB epidemiology are the observed rate of polymorphism (stability of biomarker) and the genetic diversity of strains in the population.

That is, the rate of change of a biomarker must be adequate to distinguish nonepidemiologically related strains and yet sufficiently “slow” to reliably link related cases. This issue, coupled with general background TB prevalence, should be taken into consideration when choosing molecular epidemiologic methods or in evaluating data.

EPIDEMIOLOGY OF TBM

Information on the epidemiology of TBM is fundamental for the prevention, treatment and control of the disease. Nevertheless, due to the difficulty of the collection of specimens from patients and its low incidence rate, TBM is a relatively rare form of TB for study. A large collection of patient samples (e.g., more than 100 samples) is even less affordable for

study. Therefore, gathering study information is an easy way to obtain an overview of the available knowledge of TBM epidemiology.

Incidence-

Human migration and the availability of air travel are the major factors that have distorted the human population structure, geographic distribution and spread of MTB. Nevertheless, associations between the human race in certain regions and specific lineages of MTB have still been observed (Gagneux *et al.,* 2006). In 1997, TBM was the fifth most common form of extrapulmonary TB. From all TB cases, 90-95% of infected cases only have asymptomatic latent TB, whereas 5-10% of individuals (8-9 million people) have developed active disease, accounting for approximately 2 million deaths annually. The most common form of the disease is pulmonary TB, which has been estimated to represent 80% of all TB cases. Apart from the 20% of extrapulmonary TB cases, TBM has been calculated to represent 5.2% of extrapulmonary TB (WHO, 2009). The incidence of TBM from all reported TB cases is 0.7%. The incidence of central nervous system (CNS) TB is related to the prevalence of TB in the community. In countries with a high burden of pulmonary TB, the incidence of extrapulmonary TB and TBM are expected to be proportionately high. The incidence of TBM in the United States has been calculated to represent approximately 4.5% and 4.7% of total extrapulmonary TB cases in 1969-1973 and 1975-1990, respectively. From all samples that were sent to Siriraj Hospital in Bangkok, Thailand, for TB identification during 2000-2007, approximately 15% were CSF samples, 10% of which contained MTB and were identified as TBM.

Mortality rate-

TBM is the most severe form of TB that involves the central nervous system. The mortality rate ranges from 20-60%, with an average of approximately 35% . Neurological sequelae can be found in approximately 25% of surviving patients (Hosoglu *et al*., 2002). It is not clear whether HIV infection is associated with TBM outcome. The summarized data indicate that the high mortality rate of TBM in South Africa from both studies is associated with a high proportion of HIV-infected cases, whereas no such associations were found in Thailand or France. Conflicts of such associations have been found in previous studies. However, HIV infection seems to be the strongest factor that predisposes an individual to development of TBM. The mortality rate of TBM is different among countries, which may involve differences in medical personnel, management and facilities.

Predisposing risk factors-

The strongest risk factors for developing TBM are immunological status (e.g., HIV infection) and pre-diagnosed TB. Ages and genders of TBM patients also affect predisposition for TBM development. Prior to the predomination of HIV, the age of the patient was the most important factor leading to the development of TBM. The ages of TBM patients are different from pulmonary and other extrapulmonary TB diseases; TBM is commonly found in children ages 0-5 years (Farer and Meador, 1979). In populations with a low prevalence of TB, most cases of TBM occur in adults. In general, TBM is more common in children than in adults. Notably, the young ages of children are not always the most prevalent risk factors for TBM in all populations with high TB burdens, which might result from worldwide BCG vaccination. For instance, in the Thai population, the most predominant ages of TBM patients are 31-45 years (35.3%), and only 12% of TBM patients are less than 15 years old (Yorsangsukkamol *et al.,* 2009). With BCG vaccination, a significantly lower incidence of disseminated TB, such as TBM, was found in patients less than 12 years old compared to pulmonary TB (Chavalittamrong *et al*., 1986). The average ages of TBM patients in particular regions, even on different continents, are around 30 years higher. This evidence supports the idea that the HIV epidemic has increased the risk for adult TBM in the last three decades. In general, young children are more likely to develop meningeal or disseminated TB, whereas adolescents more frequently present with pleural or peritoneal TB compared to adults.

ANTI-TUBERCULOUS DRUG RESISTANCE IN TBM

Many decades ago, TB was considered an incurable, deadly disease. With the availability of effective treatments, TB can now be cured. However, the complete treatment requires several months to stabilize the patient's condition and to prevent the reoccurrence of disease. Pandrug-resistant (PDR) TB strains are now a new emerging and serious public health problem.

Although the incidence of PDR-TB is not yet high, we cannot underestimate the adaptability of MTB, a highly successful human pathogen. In addition, the TB burden dominates in low- and middle-income countries (LMIC) where the drug susceptibility test is not fully available. The real prevalence of drug-resistant TB may therefore be markedly higher than the estimation.

Furthermore, extensively drug-resistant (XDR) TB, which is more expensive and more difficult to treat than multidrug-resistant (MDR) TB, has more serious adverse effects, and the outcomes for patients are much worse. Despite the fact that there are several novel antituberculous drugs that have been developed and discovered recently, the levels of drugresistant TB, especially MDR- and XDR-TB (and PDR-TB), are still increasing. It is very threatening to consider the imbalance between the effective anti-tuberculous drugs and drugresistant TB, especially with the outbreak of MDR-TB in large areas. The

most effective way to eradicate TB is with effective vaccination and control of latent TB. However, we are still struggling to find ways to beat this pathogen. For now, treatments for drug-resistant TB are still where we must focus. Yet, PDR-TB and TB with resistance to new drugs should be surveyed and monitored worldwide. The real hope lies with continuing to develop novel, effective anti-tuberculous drugs and vaccines.

In TBM, drug resistance and HIV infection constitute additional conditions that make the treatment of patients become more complex and difficult. The diagnosis, treatment and management of these patients are major challenges. The delay of diagnosis and treatment of

TBM can cause the subsequent deaths of patients. Therefore, drug-resistant TBM is a major issue of concern for the treatment and management of TBM. From data collected on TB cases from 81 countries between 2002 and 2006, the WHO reported that the incidence of pulmonary MDR-TB was approximately 4.8%. The highest

numbers of MDR-TB were found in China, India, and the Russian Federation.

In 2009, 3.3% of all new TB cases had MDR-TB. Unfortunately, information on the drug resistance of TBM from these countries was not available for this review. Studies on the drug resistance of TBM are relatively rare. From the data available, the drugresistance patterns for TBM were obtained from four countries: Thailand, Egypt, Vietnam and South Africa. Information gathered from previous studies found that the average incidence of TBM that was resistant to any drug was approximately 21.5% (with a range of 12-31%). MDR-TBM was found in 2.2 to 8.6% of cases, with an average of approximately 3.3% . The incidence of MDR-TBM was up to 18% in pediatric cases, with a mortality rate of 100%. Nevertheless, the sample size from this study was only 20 cases (Chander, 2008). Resistance to particular anti-tuberculous drugs, such as isoniazid, represented the most resistant MTB strains in four out of five studies in the available data. Isoniazid resistance in TBM has been suggested to correlate with HIV co-infection. The second most-resistant drug is streptomycin. TBM showed minimal resistance to rifampicin (which can partially penetrate an intact BBB) and ethambutol. There was no evidence to support an increase in penetration capacity through the BBB for rifampicin after inflammation. The information in this review may support the idea that rifampicin is a good drug for effective treatment of TBM, with a lower chance of development of drug-resistant TBM, compared to isoniazid and streptomycin. Ethambutol has been called a poor choice for a first-line treatment for TBM due to its poor penetration capacity and the adverse effect of optic neuritis. The summarized information shows that ethambutol resistance is less likely to occur when it is used as a first-line agent for TBM treatment. However, the low resistance level may be a result of poor exposure between the drug and the pathogen.

CONCLUSION

M. tuberculosis is an obligate pathogen that does not naturally replicate outside of its host environment. As such, *M. tuberculosis* complex members are believed to have coevolved with hominids for millions of years. Consequently, it is very possible that, unlike other opportunistic pathogens, viable tubercle bacilli encode the minimum ensemble of virulence genes required for successful infection, replication, and dissemination.

Thus, the relative success of one clonal *M. tuberculosis* family over another might rely on the interplay between levels of gene expression and environmental factors (e.g., host). The differential gene expression may also be influenced by variations resulting from chromosomal rearrangements, such as recombinations/deletions, transposition of IS elements, or SNP, aspects which may be studied through the multidisciplinary nature of molecular epidemiological studies. Such genomic events may lead to adaptive advantages and hence determine a successful clonal lineage, as indicated by some studies.

REFERENCES

Alland, D., T. S. Whittam, M. B. Murray, M. D. Cave, M. H. Hazbon, K. Dix, M. Kokoris, A. Duesterhoeft, J. A. Eisen, C. M. Fraser, and R. D. Fleischmann. 2003. Modeling bacterial evolution with comparative-genome- based marker systems: application to *Mycobacterium tuberculosis* evolution and pathogenesis. J. Bacteriol. 185:3392–3399.

Aranaz, A., E. Liebana, E. Gomez-Mampaso, J. C. Galan, D. Cousins, A. Ortega, J. Blazquez, F. Baquero, A. Mateos, G. Suarez, and L. Dominguez. 1999. *Mycobacterium tuberculosis* subsp. *Caprae* subsp. nov.: a taxonomic study of a new member of the *Mycobacterium tuberculosis* complex isolated from goats in Spain. Int. J. Syst. Bacteriol. 49:1263–1273.

Beggs, M. L., K. D. Eisenach, and M. D. Cave. 2000. Mapping of IS*6110* insertion sites in two epidemic strains of *Mycobacterium tuberculosis*. J. Clin. Microbiol. 38:2923–2928.

Chander, PB. (2008). Multi Drug Resistant Tuberculous Meningitis in Pediatric Age Group. *Iran J Pediatr*, Vol. 18, No. 4, pp 309-14.

Gagneux, S.; DeRiemer, K.; Van, T.; Kato-Maeda, M.; de Jong, BC.; Narayanan, S.; Nicol, M.; Niemann, S.; Kremer, K.;' Gutierrez, MC.; Hilty, M.; Hopewell, PC. & Small, PM. (2006). Variable host-pathogen compatibility in Mycobacterium tuberculosis. *Proc Natl Acad Sci U S A*, Vol. 103, No. 8, pp. 2869-73.

Hosoglu, S.; Geyik, MF.; Balik, I.; Aygen, B.; Erol, S.; Aygencel, SG.: Mert, A.; Saltoglu, N.; Dokmetas, I.; Felek, S.; Sunbul, M.; Irmak, H.; Aydin, K.; Kokoglu, OF.; Ucmak, H.; Altindis, M. & Loeb, M. (2002). Predictors of outcome in patients with tuberculous meningitis. *Int J Tuberc Lung Dis*, Vol. 6, No. 1, pp. 64-70.

Kruijshaar, ME. & Abubakar I. (2009). Increase in extrapulmonary tuberculosis in England and Wales 1999-2006. *Thorax*, Vol. 64, pp. 1090-5.

van Soolingen D, Hermans PW, de Haas PE, Soll DR, van Embden JD. Occurrence and stability of insertion sequences in Mycobacterium tuberculosis complex strains: evaluation of an insertion sequence-dependent DNA polymorphism as a tool in the epidemiology of tuberculosis. Journal of Clinical Microbiology 1991;29:2578-86.

Warren R, Richardson M, van der Spuy G, Victor T, Sampson S, Beyers N, et al. DNA fingerprinting and molecular epidemiology of tuberculosis: use and interpretation in an epidemic setting. Electrophoresis 1999;20:1807-12.

World Health Organization. (2009).Global tuberculosis control report 2009. Epidemiology, strategy, financing. Accessed 2011, Available from http:// whqlibdoc.who.int /publications /2009 / 9789241563802_eng. df.

Yorsangsukkamol, J.; Chaiprasert, A.; Prammananan, T.; Palittapongarnpim, P.; Limsoontarakul, S. & Prayoonwiwat, N. (2009). Molecular analysis of *Mycobacterium tuberculosis* from tuberculous meningitis patients in Thailand. *Tuberculosis (Edinb)*, Vol. 89, No. 4, pp. 304-9.

Corresponding Author

Shashi Ranjan*

Research Scholar, SSSUTMS, Sehore

E-Mail – chairman.iab@gmail.com