INTRODUCTION
CHAPTER I

INTRODUCTION

The problem of Tuberculosis disease has been an enigma to mankind from times immemorial. Tuberculosis is prevalent in all countries of the world, be it tropical, sub-tropical or the colder regions. Though it declined in all the developed countries, it still continues to be a major health problem in developing countries. No other disease has so much sociological, economic and health significance as Tuberculosis.

1.1 HISTORICAL BACKGROUND

The existence of Tuberculosis dates back to ancient times. Tuberculosis was found to be evident in neolithic man and its presence was recorded as early as 5000 BC by various countries. Egyptians had recorded Tuberculosis in a mummy of 21st dynasty. Chinese recorded observation of 'Laoping', which has close resemblance with the present day description of Tuberculosis. What is widely known today as Pulmonary Tuberculosis was described in India during 2000 BC.

Tuberculosis was named to indicate the formation of nodules or Tubercles. For many years the chronic form 'Pthisis or Consumption' was considered a
degenerative or hereditary disease, quite unrelated to the tuberculosis of childhood, which was obviously infectious. The infectious nature of this disease was first described by Girolamo Fracastoro of Verona (1443-1553). Franciscus Sylvius of Leyden (1614-1672) employed the term Tubercle and stated tubercles are often seen in lungs of consumptives. The signs and symptoms of tuberculosis were recorded by Richard Morton (1837-1898). Morgagni (1682-1771) described the pathological conditions of the lungs after dissecting the bodies of the consumptives. Pierre Desalt (1675-1740) was the first to discover that sputum spreads the disease.

The term Tuberculosis was later coined by Gaspard Laurent Bayle (1774-1816) and he traced the relation between Pulmonary tuberculosis and tuberculosis of other organs. Rene Theodore Laennec (1819), a consumptive himself, was the first to recognise the chronic form of tuberculosis as merely a further development of the same infection. Villermin (1827-1892) demonstrated that tuberculosis was due to a specific agent. Robert Koch (1882) disclosed to the world his epoch making discovery of the causative organism Mycobacterium tuberculosis. It is again Robert Koch, (1890) who produced tuberculin and described 'Koch phenomenon'. X-rays, discovered by
Professor Roentgen (1895) were put to clinical use by 1904 and the findings of Radiology and Bacteriology together helped in developing further knowledge of the disease, its control and prevention.

1.2 PREVALENCE

Tuberculosis is a necrotizing bacterial infection with protean manifestations and wide distribution. The lungs are the most commonly affected, but lesions may also occur in the kidneys, bones, lymphnodes, meninges or be disseminated throughout the body. Clinical disease may be caused shortly after infection called 'Primary Tuberculosis' or after a period of months and decades of dormancy.

The highest prevalence rate of tuberculosis is found in some countries of Asia where 60-80% of the children appear to be infected by the age of 14 as compared to 2-3% in the Western countries. The annual incidence of tuberculosis cases was as high as 250-500 or more per 100,000 population in Africa, Asia and Oceania as compared to rates as low as 15-20 per 100,000 population in USA, Denmark, Canada and The Netherlands (WHO report, 1980).
Mortality due to tuberculosis has fallen steadily in nearly all developed countries. The death rate has declined from 194.1 to 1.4 per 100,000 patients in USA from 1900 to 1977. In Australia it was 0.9 per 100,000 population in 1975 (N. Eng. J. Med. Report, 1980). On the other hand, in developing countries the death rates are declining slowly and in Asia it represents 3 - 10% of all causes of deaths as compared to 0.1% in U.K. and USA (Hawthorne 1979). In the South East region of Asia, tuberculosis mortality occupied the fourth place in 1971-82 while in Europe it was relegated to the 13th place (WHO, 1980).

But, in India due to the defective national reporting system no exact figures are available. But when all the surveys conducted are taken together some kind of light can be thrown on to the tuberculosis problem in India. The prevalence of infection is about 40% in all age groups, the minimum being at the younger age and the maximum being at 35 years. The risk of infection is of the order of about 2 - 4% per annum.

X-ray examinations of the lungs indicate the prevalence rate of the disease to be of the order of 2% of the people aged 10 and above, and of these about
20% (4 per 1,000 population) are bacillary i.e., sputum positive. The incidence of new cases is about one third of the prevalence - 0.13% of the total persons aged 10 and above. Both prevalence and incidence are higher in higher age groups and they are more in males than in females, the male to female ratio being from 3 : 1 to 5 : 1. Tuberculosis infection is more or less uniformly distributed in urban and rural areas and is more prevalent in lower socio-economic groups.

1.3 BACTERIOLOGY

The tubercle bacillus belongs to the genus *Mycobacterium* of the family Mycobacteriaceae, order Actinomycetales and class Schizomycetes. Members of this genus have the distinct property of acid-fastness.

*Mycobacterium tuberculosis* causes tuberculosis in man and is occasionally found to infect other animals like pigs, cattle, and monkeys. The bacterium does not usually multiply outside the host tissue. *Mycobacterium tuberculosis* is a non-motile, non-sporing organism and has no capsule. It is usually 1 - 4 μ long and 0.3 - 0.6 μ wide. The cell wall contains lipids, proteins, and carbohydrates with specific biological characteristics. Apart from a cell wall, it contains a
thin cytoplasmic membrane and core cytoplasm. Electron microscopic studies have shown presence of ribosomes in cytoplasm.

The nucleus contains a coiled double stranded DNA molecule.

The bacilli are strict aerobes and thus most commonly affect the organs with relatively high oxygen tension.

1.4 PATHOLOGY

The spectrum of anatomical lesions seen in tuberculosis lung disease can broadly be separated into two classes. One occurring predominantly in children and the other in adults and elderly. Observations on tuberculosis lung disease in man and in the experimental animal, indicate that the reaction of the pulmonary tissue to invasion by tubercle bacilli and morphologic nature and course of the lesion, which eventually develops, depends on the status of host versus parasite balance. The latter in its term is largely an outcome of the interaction between variables like the specific immunity and allergy acquired by the host, the general resistance inherent in him and the bacterial virulence.
Host resistance to tuberculosis is both general and specific, the latter being constantly accompanied by varying degrees of allergy. Thus considering immunity to tuberculosis in its broad sense as a situation where the host parasite balance is in favour of the host and where allergy operates as an additional factor with variable intensity, it is obvious that the morphologic expression of the disease in an individual with little or no immunity would be distinctly different from that in a partially or completely immune person. The specific immunity is acquired only after birth, usually by the initial infection, which in India occurs mostly within the first ten or fifteen years of life, producing certain types of lesions in this non-immune set-up. Subsequent infections or subsequent lodgement of the same infection, at another site in the lung will on the other hand, usually produce different types of lesions because of the milieu of the immunity and allergy. The former type of lesions are called as primary or childhood disease while the latter as reinfection.

PRIMARY DISEASE AND PRIMARY COMPLEX: It is also known as 'Ghon lesion'. The parenchymal lesion, known as the primary focus, forms at the site of the lodgement of the microbe and within a short period an extension, through
the lymphatics, takes place into the draining lymphnodes, the combination of parenchymal and lymph-nodal lesions being termed the 'primary complex'. Primary foci occur in well aerated parts of the lungs, mostly at the terminal ends of the airways, thus involving the sub-pleural areas of the middle portions of both the lungs. It is therefore frequent to find these lesions in the middle lobe, upper part of the lower lobe and lower part of the upper lobe eventhough other areas are also involved. The apical and sub-apical regions are very rarely affected.

REINFECTION TUBERCULOSIS: This variety of disease results from reinfection of an individual, earlier exposed to Mycobacterium tuberculosis infection, by organisms from exogenous and endogenous source. Exogenous infection, as in primary disease, commonly results from inhalation of the organism, while endogenous infection occurs from pre-existing lesions within the lung which might otherwise appear inactive or dormant, but still harbour a few bacilli, that under certain circumstances, get stimulated to grow and disseminate. The small nodular forms of lesions, the apparently healed up lesions and the healed Simon's foci are examples of such quiescent, but potentially active
In the reinfection tuberculosis, the tubercle bacilli encounter a certain amount of host resistance, which the individual acquires subsequent to the primary infection. A variable degree of allergy also operates. Consequently, eventhough the initial lesion is again a small focus of lobular pneumonia, the process takes on a far more chronic course and results in lesions in many ways different from those observed in primary disease. Only on those extremely rare occasions where the immunity acquired during the first infection is negligible or was at the time of second infection tapered off to almost non-existence, does a lesion similar to primary complex develop. More vulnerable parts of the lungs namely the sub-apical portions and upper parts of the upper lobe are preferentially affected in reinfection tuberculosis. As in the primary complex, the early pneumonic change quickly passes on to necrosis and the active lesion at any given time may present in one of three morphologic forms. In approximately half of the cases the disease occurs as nodules, and in the other half as more florid fibrocaseous tuberculosis with or without cavity while miliary tuberculosis forms an insignificant minority (Nayak et. al., 1970).
It is generally recognised that not all persons infected with tubercle bacilli develop clinical disease. The pathological state is not static, but dynamic and is the end result of the interaction between the host and parasite. Several factors contribute to this 'host-parasite' relationship such as constitutional, hormonal and environmental factors. Among the three, constitutional factors play a major role. Till recently tuberculosis was thought to be hereditary. It was realised later that it is not the disease but the resistance to disease that is hereditary. This resistance is genetic in character and the individual has no control over it. Genetic resistance to tuberculosis among humans seems to vary in different parts of the world. Hence it is imperative to conduct genetic studies on general populations and affected individuals to identify the high risk groups. This would help in the control and prevention of this major social and health problem in India.

1.5 GENETIC STUDIES IN TUBERCULOSIS:

Socio-economic and environmental factors, though known to influence the occurrence of tuberculosis, the available literature suggests a possible major role of genetic factors in the control of host responses to
Mycobacterium tuberculosis (Lurie 1964 and Comstock 1978). Kallman and Reisner (1943) have shown an appreciably higher concordance of pulmonary tuberculosis in monozygotic twins than dizygotic twins. Comstock (1978) also observed similar findings among twins, thus giving the evidence in favour of genetic determination. The segregation of pulmonary tuberculosis in families emphasises the importance of heredity. Further more in studies comparing white and black population with tuberculosis, blacks have been reported to be more sensitive to tuberculin PPD and higher mortality from tuberculosis.

These ethnic differences among the prevalence and mortality rates of tuberculosis and the possible genetic determination of susceptibility generated interest among geneticists to probe this aspect. Many studies have been made on the relationship between the human major histocompatibility complex and tuberculosis. Though some studies reported a relationship between a particular HLA type and susceptibility to tuberculosis (Selby et. al., 1978, Lamya i(1979), Al-Arif et. al., 1979, Khomenko et. al., 1980, Mehr et. al., 1980, Jiang et. al., 1983) others could not find any such
relationship (Rosenthal et. al., 1973, Singh et. al., 1983) Although many studies have concluded that HLA has a significant biologic influence on tuberculosis, the mechanism has not yet been understood.

1.6 CYTOGENETIC STUDIES IN TUBERCULOSIS:

To date very few cytogenetic studies have been conducted with regard to the disease as such (Jaju and Ahuja 1983, Rohrborn et. al., 1978). Though both groups reported similar results, it was felt that the number of samples studied were small and since not many investigations were carried out, there exists a lacuna with regard to the chromosomal damage in relation to the bacterium Mycobacterium tuberculosis. Thus, the present study was undertaken to study this aspect in a larger sample of population.

1.7 CHEMOTHERAPY AND ITS EVOLUTION:

With the advent of chemotherapy, a new era has begun in the treatment of tuberculosis. Before the discovery of anti-bacterial drugs for tuberculosis, therapeutic measures were aimed at increasing the resistance of patient by means of Sanatorium regimens, high protein-calorie diet, good airy accommodation,
nursing care and collapse therapy. With the introduction of chemotherapy, elimination of bacilli has become far more important than the patient's immune response.

The anti-tubercular drugs are broadly classified as 'standard or first line drugs' since these are used in the initial phase of newly diagnosed patients, and 'reserve or second line drugs' used for those patients who had developed resistance to the standard drugs. Streptomycin (SM), Para amino salicylic acid (PAS), Isoniazid (INH) and Thiacetazone (TAZ) belong to the former group while Ethionamide, Purizinamide, Thambutol and Rifampin (RIF) belong to the latter group.

Though chemotherapy is largely successful in the cure and control of tuberculosis, its success depended on the prevention of emergence of drug resistant bacilli. Resistant strains of the bacilli have been found to emerge with each single drug used in chemotherapy. These strains became a problem in the elimination of bacilli because single drugs become non-effective on these strains. This prompted the use of double drug combinations in chemotherapy which was observed to be more effective than single drug therapy.
Double drug combination are useful not only in the elimination of bacteria, but also in decreasing the risk of drug resistance.

Though Isoniazid and Streptomycin have been found to completely prevent further growth of sensitive bacilli, neither Para amino salicylic acid nor Thiacetazole were so effective. This led to an occasional failure of double drug chemotherapy with INH + PAS or INH + TAZ. In order to overcome this problem a new concept of chemotherapy, triple drug combinations were introduced in the treatment of tuberculosis which were observed to be more effective than double drug therapy (British Medical Research Council 1962, East African / British Medical Research Council 1966 MacDonald 1968, , Pamra et. al., 1974).

The phenomenon of drug resistance is the consequence of the size of the bacterial population because larger the number of bacilli, greater the chances of emergence of mutant varieties. Under such circumstances the risk of emergence of resistant varieties of bacilli is very high and hence chemotherapy in the early phases of treatment should be intense and
after an initial phase, when the bacterial population is reduced to such an extent wherein it no longer contains resistant varieties, a less intensive chemotherapy should be used. This biological concept led to the emergence of two phase chemotherapy. The intensive phase consisted of three or four drugs and second continuation phase with two drugs. The optimum duration of the intensive phase depends on the severity of the disease and the rapidity of bacterial conversion.

This concept of two phase therapy was proved to be 100% effective than single phase therapy and patients with resistant bacilli responded favourably to this therapy (Crofton 1958, 1960, Ross et. al., 1958, Thomas et. al., 1960, International Union against Tuberculosis 1964, Thomas 1964).

Failure of the patients to self administer the drugs at home regularly brought about another new concept of intermittent chemotherapy, which is effective in solving this problem because these regimens are easy to supervise, less expensive and less toxic. Many studies have proved that these intermittent regimens are also equally effective in the control of tuberculosis (Corper and Cohn 1947, Grumbach et. al., 1952, Palmer et. al., 1956, Bloch 1961, Madras Study 1964).
Over the years continuous changes in the combinations and durations of different anti-tubercular drugs have been tried to control the tuberculosis. The latest among these efforts is the introduction of short term chemotherapy. These regimens confer an obvious advantage because the patients need not take drugs over a prolonged period of one and half to two years which is difficult and expensive. Besides continuous medication might result in toxic effects also. These difficulties are overcome in this short term chemotherapy, wherein the patient is treated only for six to nine months with three or four drugs in the first intensive phase and two drugs in the second continuous phase.

Though chemotherapy is an effective tool in control of tuberculosis, it has got its own disadvantages. Drugs are known to cause chromosome damage, when individuals are exposed to them for a long duration. Most of the chemicals are known mutagens or clastogens and on exposure produce chromosome aberrations and sister chromatid exchanges. Most of the anti-tubercular drugs have been analysed for their effects on the genetic material. Many reports are available on the individual effects of INH, SM, TAZ, RIF, and combination of these drugs. But to date, no
studies have been reported on the effect of short term chemotherapy regimens on genetic material. Hence the present study is undertaken to assess the clastogenic / mutagenic property of these drugs.

Thus the present study aims at

(1) assessing the chromosome damage in untreated tuberculosis patients with reference to Mycobacterium tuberculosis, and

(2) assessing the effect of anti-tubercular drugs on human chromosomes.

In order to achieve these aims, parameters such as chromosomal aberrations, sister chromatid exchanges, mitotic index and cell cycle kinetics were estimated in the present study.