Chapter - SIX

Discussion
The present study was carried out on patients receiving combination therapy for the management of tuberculosis registered for DOTS program at various DOTS centres of defined area of RNTCP under Lala Ram Sarup (LRS) Institute of tuberculosis and respiratory disease. A total of 16 DOTS centre came under LRS, out of which 10 were situated in the Government dispensaries and 6 were under NGOs. Six centre, out of 10 under Government dispensaries were selected for patient enrollment in this study.

A total of 1011 patients were screened from all 6 centers of defined area of RNTCP under LRS Institute for the detection of any untoward reactions after drug administration. Out of 1011 patients, 351 patients reported adverse events. This accounts nearly one third of the screened patients reported adverse events which may or may not be related to medicines. Few patients experienced at least one adverse event while majority of patients reported more than one event during the period of study. On an average one patient reported at least 2.03 adverse events.

Trend analysis of TB incidence may help to identify its risk factors and target interventions to prevent it. But it is also important to identify possible seasonal pattern in the disease incidence, the knowledge of which may be used, to predict the future magnitude of the health problem, to develop an effective public health program and to set objectives and utilize available resources more effectively (Rios et al., 2000).

A total of 1011 patients were enrolled in this study, during the months of April, 2008 to December, 2008, which were divided in to three Quarters (2nd, 3rd and 4th quarter, 2008). The overall number of patients were enrolled in the 3rd quarter were higher as compared to number of patients enrolled in the 2nd quarter and 4th quarter. A significant difference in the number of patients enrollment among 3rd and 4th quarter was observed, whereas the number of patients enrollment among 2nd and 3rd quarter was not significant. TB is not widely known to have any seasonal patterns, but few studies have shown variable periods of peak seasonality in TB incidence/case notification rates in late winter to early spring in South Africa (Schaaf et al., 1996), during summer in UK (Douglas et al., 1996) and Hong Kong (Leung et al., 2005), during summer and autumn in Spain (Rios et al., 2000) and Japan (Nagayama and Ohmori, 2006). Relatively recently, it was demonstrated that TB
diagnosis peaked between April and June and reached a nadir between October and December in northern India and magnitude of seasonal variation had significant positive correlation with TB case rates (Thorpe et al., 2004). Similar finding were also reported by Aichtar and Mohammad (2008).

The incidence of drug side-effects may vary depending on a number of factors. These are the characteristics of the population of patients under treatment, the kind of regimens, drug combinations used, the frequency of drug administration and dosage. In the present study it was demonstrated that the overall incidence of side-effects was low with regimens containing isoniazid, rifampicin, pyrazinamide, streptomycin and ethambutol. A total of 1011 patients were enrolled from all six centre of RNTCP under LRS defined area and were divided in to three treatment category (Category I, Category II and Category III) on the basis of RNTCP guidelines. A significantly higher number of patients were enrolled among the category I as compared to category II and category III. But the percentage of adverse events was slightly higher among the category II patients as compared to category I and category III patients. This difference in frequency of adverse effect is due to different number of drugs in different category. Category II contains all five first line drugs (HRZES) and contributed slightly higher percentage of adverse events. Among all categories, lowest percentage of adverse events was reported in category III patients. Similar results were reported in literature that the difference in frequency of adverse effects between the two regimens differs and reported lower (i.e. 13%) in those containing isoniazid, rifampicin and ethambutol while higher (i.e. 16 %) in those which contains isoniazid, rifampicin, ethambutol, pyrazinamide and streptomycin. These different levels of toxicity are explained by the effect of streptomycin and rifampicin and not of pyrazinamide. Similar results were noted in other studies in which side-effects were observed in 10-12% with isoniazid, rifampicin and ethambutol but the incidence increased to about 25 % when pyrazinamide and streptomycin were added (Girling, 1978).

The demographic detail of patients includes age, sex, weight (kg), height (cm) and BMI were presented as mean ± SD. Among the demographic details the mean age of men
varies from 28.45 (±14.873) to 34.00 (±16.405) whereas of women varies from 23.00 (±11.205) to 39.14 (±25.354) years. Janmeja et al., (2005) reported that the majority of the subjects were young adults, mean age being 31.79 ± 11.13 years. In another study conducted in the Maulana Azad Medical College, New Delhi, it was reported that the mean age of study subject was 35.30 ± 12.00 years (Sharma et al., 2007). In the present study it was found that the maximum number of patient experienced adverse event belongs to the age group of 15 – 25 years followed by 26 – 35 years, i.e. they were young adults.

World wide, more men than women are known to be suffering from tuberculosis. In the present study male predominantly had tuberculosis than females and also higher incidences of adverse events were reported. Other studies reported in India (Arora and Bedi, 1989; Gaur et al., 2004) and abroad (Alvarez et al., 1987; Umeki, 1989; Dutt and Stead, 1993; Korzeniewska-Kosela et al., 1994; Perez-Guzman et al., 1999) have also reported similar male predominance. In relation to the gender of the patients, male predominance was found both in young adults and elderly patients. One possible explanation for this male predominance may be that in most countries young men usually have more social and labour activities than women, thus favoring the transmission of the disease. It matches with earlier reports which suggest that responses to TB differ between men and women. Barriers to early detection and treatment of TB may be greater for women than for men (Holmes et al., 1998). Another study conducted by Sharma et al., (2007) reported the prevalence of TB was higher among male as compared to female, because male have higher risk factors like smoking, alcoholism and drug addiction to get tuberculosis than female (Leung et al., 2004; Lonroth et al., 2008). Smoking accounted for 32.8% higher risk of tuberculosis among male, but female have only 8.6% higher risk of pulmonary tuberculosis (Leung et al., 2004).

In the present study it was found that the highest number of male patients who have also experienced adverse events belong to the productive age group (i.e. 20 – 29 years). Lowest percentage of adverse events were reported in the youngest patients (i.e. <15 years). According to a study conducted by Sharma et al., (2010) tuberculosis is more prevalent
in the productive age group population and the impact of the disease is felt by the children and their families. In addition, progression from TB infection to disease may be faster in women of reproductive age than men of the same age (Murray, 1991). Nonetheless, there is an estimated 2:1 male to female ratio in the number of TB cases notified to public health authorities.

Most of the tuberculosis patients belong to poor family history and low income group population. In our study tuberculosis was reported to be high in patients educated up to High School followed by intermediate and primary school education. Tuberculosis among professional/PG and above education was found to be lowest. Highest percentage of adverse events were reported in patients belongs to education up to high school followed by inter or post high school diploma, primary school, Illiterate, graduate and lowest percentage of adverse events were reported in patients having education up to professional/PG and above. Similar findings was reported in a study conducted by Pandit and Choudhary (2006) where 50% patients were educated up to primary school and 23% were illiterate. Highest percentage of adverse events was also reported in patients who were education up to high school followed by illiterate, whereas lowest percentage of adverse events was reported in highly educated patients (i.e. professional/PG and above). The study revealed that the incidence of adverse events was significantly lower among those who have good knowledge about various aspects of disease and their treatment. In a report it was found that most of the tuberculosis patients having education up to secondary school or above but low cases of tuberculosis among patients having primary school education (Mweemba et al., 2008). In a survey it was reported that patients having higher education have more knowledge of tuberculosis and less likely to develop disease, whereas male have more likely to get tuberculosis. This may be due to more exposure of male at work place as compared to female. In contrast, lower figures for the same have been reported in earlier studies conducted in Delhi (Malhotra et al., 2002; Singh et al., 2002). In a study, it was found that the variation in prevalence of infection among high-school students in the United States varies according to the education level of parents and housing characteristics. This could be due to lower prevalence and incidence of
prevalence and incidence of tuberculosis in developed countries, thereby reducing concern about it in the general population of developing nations (Malhotra et al., 2002). Most of the tuberculosis patient belongs to low income group populations. In our study the incidence of adverse events were reported to be higher (59.54%) in lower income range (i.e. Rs/- 2041 - 6100) patients followed by income range of Rs/- 6101 - 10160 and Rs/- 10160 - 15820. In a study reported that forty-two percent of patients belong to low-income group and 55% were from the middle-income group (Janmeja et al., 2005).

Out of 209 patients, 113 patients were semi-skilled worker followed by 55 patients were unskilled worker belongs to income range of 2041 - 6100. Out of 93 (26.50%) patients, 44 patients, 20 patients and 11 patients reported adverse events were semi skilled worker, skilled worker and unemployed respectively, belong to income group of 6101 - 10160. In a study the patients are classified according to their profession in to two groups, unskilled and skilled profession and it was reported that tuberculosis was more prevalent in unskilled professionals than skilled professionals (Chadha and Bhagi, 2000).

In the present study incidence of adverse events were reported to be higher in semi skilled working group followed by unskilled working group and skilled working group.

Highest percentage of patients reported adverse events having education upto high school followed by primary school. It has been reported that tuberculosis was high in laborer. In a study conducted by Chadha and Bhagi (2000) the patients were classified according to their education into three groups illiterate, school educated and college educated and it was found that tuberculosis was more prevalent in school educated followed by illiterate and college educated patients. Out of 351 patients in unskilled working group, 71 (20.23%) patients reported adverse events; among them 25, 24 and 19 patients had education upto high school, illiterate and primary school or literate respectively.

Tuberculosis was reported to be high in lower middle class family. Out of 1011 patients 712 (70.43%) patients belongs to lower middle class family, followed by middle class family i.e. 231 patients. Chadha and Bhagi (2000) reported that 82% of tuberculosis patients belong to low socioeconomic status family. Among the socioeconomic class
highest percentage of adverse events were reported in the lower middle class family followed by middle class family.

Out of 1011 patients registered for this study, 78.04% patients were enrolled as new cases (patients receiving DOTS therapy first time) whereas 21.96% were retreated cases (patients receiving DOTS therapy second times or more) because of relapse, defaulted, failure or others. Among the new cases (i.e. 789 patients), 40.68% patients were new positive, 15.08% were new negative and 44.23% were new extra-pulmonary patients. Among the total retreated cases (i.e. 222 patients), 18.91% patients were relapse positive, 6.31% were failure positive, 37.84% patients were default positive and 36.94% were other category-II patients.

According to RNTCP guidelines cure rate expected to be >85%. In the present study cure rate among the new pulmonary positive patients was found to be 89.41% whereas treatment completion rate among new pulmonary negative and extra-pulmonary cases were 92.44% and 96.56% respectively. In a study, it was reported that cure rate were 91% in category-I patients and 73.3% in category-II patients (Chadha and Bhagi, 2000). Similar findings were also reported by Arora et al., (2003); Gaur et al., (2004); Filho et al., (2007); Pardeshi and Deshmukh, (2007).

Expected death rate should be less than 5% according to RNTCP guideline. In the present study, death rate among the new positive, negative and extra-pulmonary cases were found to be 0.62%, 0.84% and 1.15% respectively. The overall death rate among new case was found to be 0.89% which was significantly lower than the expected death rate according to RNTCP guidelines (Central TB Division, 2009).

Treatment failure rate among the new pulmonary positive and extra-pulmonary cases were found to be 6.85% and 0.29% respectively. The overall failure rate was 2.92%. According to the RNTCP guideline expected failure rate should be less than 4%. In the present study failure rate in new pulmonary positive patients was found to be slightly higher than expected failure rate but the overall failure rates among the new pulmonary
tuberculosis as well as new extra-pulmonary tuberculosis patients was reported significantly lower than the expected failure rate (Central TB Division, 2009).

According to the RNTCP guidelines default rate should be less than 5%. Default rate among the new positive, negative and extra-pulmonary cases were found to be 2.49%, 6.72% and 2.01% respectively. The overall default rate was found to be 2.92%. In the present study default rate was reported to be higher among the new negative pulmonary tuberculosis patients but the over all default rate was found to be lower than the expected default rate according to the RNTCP guidelines (Central TB Division, 2009).

In the retreated patients, cure rate among relapse positive, failure positive and default positive patients were 73.81%, 42.86 and 63.10% respectively, whereas death rate among relapse positive, failure positive and default positive patients were 4.76%, 7.14% and 4.76% respectively. Failure rate among relapse positive, failure positive and default positive patients were 9.52%, 42.46% and 10.71% respectively. Default rate among relapse positive, failure positive and default positive patients were 11.91%, 7.14% and 20.24% respectively. In the retreated case, among other category-II patients; treatment completion rate, failure rate and default rate were 89.02%, 2.44% and 7.32% respectively.

A study from Delhi reported treatment success rate in Category-I and Category-II patients as 91.0% and 73.0%, respectively (Chadha and Bhagi, 2000). Similar treatment success was also reported in other studies from Mumbai (Yatin et al., 2000), Lucknow (Prasad et al., 2000), Bangladesh (Kumareson, et al., 1998), Delhi (Gaur et al., 2004) and Karnataka (Jagota et al., 1998). While a study from Bangalore (Sophia et al., 2004) reported a treatment success of 67.9% in Category-I patients, which was likely due to high default rate.

Compliance among the tuberculosis patients is very important. Non-compliance leads to drug resistance which is more difficult to treat because drugs are highly intolerable, duration of treatment is more and drugs are very costly. The over all compliance observed in the present study was good and our results are in accordance with WHO
guidelines. The World Health Organization recommends at least 85% cure rate of all diagnosed TB cases. In order to achieve this cure rate, compliance needs to be in the order of 85-90% (Murray et al., 1991). Similar findings were reported in earlier study conducted in Zambia (Mweemba et al., 2008). Another study revealed that the compliance of DOT was significantly high among those who have good knowledge about various aspects of disease (Pandit and Choudhary, 2006). Some of the studies have also portrayed the same fact that the factors like age, sex, work and education had no association with adherence of treatment (Gad et al., 1997). But Johansson et al., (1996) had reported that patient’s economic situation is an important determinant of compliance and noncompliance and compliance of DOT was significantly high among those who have good knowledge (health education) about various aspects of disease. Similar observations have been documented by other authors as well (Gad et al., 1997; Tekle et al., 2002; O’Boyle et al., 2002; Thomas, 2002). There are varieties of reasons why patients fail to take their medication. The symptoms of TB commonly resolve within a few weeks of starting TB treatment and many patients then lose motivation to continue taking their medication. Regular follow-up is important to check on compliance and to identify those patients, who developed any adverse event with drug therapy. Patients need to be advised about the importance of taking their tablets regularly, and the importance of completing treatment, because of the risk of relapse or development of drug-resistance.

According to socio-economic status, highest number of tuberculosis patients belong to lower middle class family and contributing high number of Adverse Drug Reactions followed by patients belongs to middle class family. In the lower middle class family out of 712 (70.43%) tuberculosis patients 80 (7.91%) patients reported ADRs whereas in the middle class family out of 231 (22.84%) tuberculosis patients only 15 (1.48%) patients reported ADRs. ADRs were not reported in patients of upper class family. The trend of occurrence of ADRs decreases as socio-economic status advances and especially seen in tuberculosis patients.

In our study tuberculosis was reported to be high in patients having education up to high school followed by intermediate and primary school education. Out of 1011 tuberculosis
Chapter 6

Discussion

patients, 404, 187, 178, 139, 75 and 28 patients having education up to high school, Inter or post high school diploma, primary school, Illiterate, Graduate and Professional/PG and above respectively. Out of 404 tuberculosis patients having education up to high school, only 41 patients experienced ADRs. Whereas out of 187 tuberculosis patients having education up to inter or post high school diploma, only 12 patients reported ADRs.

In the present study it was demonstrated that the incidence of ADRs was low with regimen containing isoniazid, rifampicin, pyrazinamide, streptomycin and ethambutol. Out of 1011 tuberculosis patients enrolled under DOT for treatment, only 102 (10.09%) patients reported adverse drug reactions during DOTS therapy.

Hepatotoxicity: It is well known that isoniazid, rifampicin and pyrazinamide are the most common potential drugs that cause hepatic injury. In the present study the most common drug which has to be terminated due to hepatotoxicity was rifampicin followed by isoniazid. Hepatotoxicity was reported in 22 (2.58%) patients, which needed discontinuation one of the anti-tuberculosis drugs. Isoniazid was discontinued in 10 (0.99%) patients, whereas rifampicin was discontinued in 12 (1.19%) patients. Severe hepatotoxicity leading to discontinuation of at least one of the three standard drug or sometimes all three drug for few weeks, so that the liver enzymes become normal or near to normal. Then anti-tuberculosis drugs were reintroduction one by one as per the treatment guidelines. Rechallenge order were decided by the treating physician. Pyrazinamide was the most common drug discontinued due to hepatotoxicity followed by rifampicin and isoniazid. Severe hepatotoxicity was more frequent among the younger patients as well as older patients. Although there was no significance difference in gender in those patients who developed hepatotoxicity compared with those who did not. Rifampicin causes cholestasis at both the sinusoids and canaliculi of the liver because of defect in uptake by hepatocytes and defect in excretion, respectively (Haddad and Winchester, 1983). Hepatitis occurs in 1% or less of patients, and usually in the patient with pre-existing liver disease. Hepatitis is due to a toxic metabolite of monoacetyl hydrazine, which binds covalently to liver proteins (Black et al., 1975). The rate of acetylation is genetically determined. Acetyl-isoniazid is further hydrolysed to
isonicotinic acid and acetylhydrazine, both of which are excreted in the urine. Isonicotinic acid is conjugated with glycine. Acetylhydrazine is further metabolised to diacetylhydrazine and may be converted by the hepatic microsomal enzymes to the reactive metabolite (i.e. hydrazine) which are thought responsible for INH-induced hepatotoxicity. Acid labile hydrazones of isoniazid are formed with α-ketoglutarate and pyruvate, but since these do not appear to any extent in the blood, they are thought to be produced in the bladder (Ellard et al., 1972; Russell, 1972; Boxenbaum and Riegelman, 1974). In some patients an allergic mechanism has also been proposed: acylation of hepatic macromolecules by acetyl hydrazine may lead to the release of antigenic macromolecules which induce the formation of antibodies directed against the liver leading to hepatotoxicity (Davies, 1981). With regard to the risk of hepatotoxicity, the combinations of isoniazid and rifampicin are of low toxicity, even when pyrazinamide is added, particularly in patients who are not alcoholics or have no previous liver damage (Girling, 1978; Zierski and Bek, 1980).

Hyperuricemia: It is well known that anti-TB therapy with pyrazinamide as well ethambutol affects the uric acid level and leads to polyarthralgia. In the present study 102 (10.09%) patients reported ADRs, out of which only 11 (1.08%) patients reported hyperuricemia, which needed discontinuation of one of the anti-tuberculosis drugs. Isoniazid and pyrazinamide were discontinued in 2 (0.18%) patients, whereas ethambutol was discontinued in 7 (0.69%) patients. It is well known that anti-TB therapy with pyrazinamide as well ethambutol affects the uric acid level (Dukes, 1986). Hyperuricemia has been found in up to 66% of patients receiving ethambutol (Postlethwaite et al., 1972) and there have been reports of acute gouty arthritis precipitated by ethambutol in some patients (Self et al., 1977). It was believed that renal clearance of urate may be reduced in about 50% of patients receiving ethambutol and pyrazinamide and also, acute gout has been precipitated in patients with gout or impaired renal function (Reynolds, 1989). Treatment with pyrazinamide led to a rise of serum uric acid concentrations in about 56% of patients within 2 months of drug administration. This was not associated with clinical symptoms, probably owing to the relatively short
duration of pyrazinamide administration. This drug can cause arthralgia when given for a longer period (Girling, 1978).

**Peripheral neuritis:** Peripheral neuropathy cause due to the damage to nerves, characterized by numbness and pricking pain in the hands or feet. Symptoms of peripheral neuropathy include pain, numbness, tingling, weakness, loss of muscle control, burning, loss of feeling etc, reported more in patients suffered from nutritional deficiency. In the present study 11 (1.08%) patients reported peripheral neuropathy. Peripheral neuropathy due to isoniazid was reported in 8 (0.79%) patients whereas with ethambutol in 3 (0.30%) patients. Peripheral neuritis may precede or accompany ocular damage. Changes are more severe in the sensory than in the motor nervous system. Peripheral neuropathy secondary to chronic exposure is due to the deficiency of pyridoxine and pyridoxal phosphate. INH produces pharmacological changes in pyridoxine metabolism (Biehl and Vilter, 1954). This may occur due to increased renal excretion of pyridoxine by formation of INH-pyridoxine hydrazones, which competitively inhibit pyridoxine kinase, the activating enzyme that converts pyridoxine to the physiologically active pyridoxal phosphate and inactivation of the pyridoxal containing enzymes (Wood and Peesker, 1972).

**Hypersensitivity:** A total of 14 (1.38%) patients reported hypersensitivity during DOTS therapy under RNTCP. Hypersensitivity was reported with isoniazid, rifampicin and pyrazinamide in 5 (0.49%) patients, 6 (0.59%) patients and in 3 (0.30%) patients respectively. Hypersensitivity reactions may occur usually characterized by an allergic reaction like pruritis, urticaria, angioedema, shock, shortness of breath and usually occurs after intermittent therapy. Various exanthemas, Stevens-Johnson syndrome, "toxic" epidermal necrolysis, purpura-like vasculitis, acute thrombocytopenic purpura, joint pain, drug fever, and leukopenia have been attributed to hypersensitivity. Hypersensitivity may be due to antibody-mediated immune reactions of type-B. It is dose independent reaction and may occur at any time during therapy. It has also been suggested that some of the adverse effects associated with isoniazid and rifampicin may be attributed to its metabolite monoacetyl hydrazine and desacetyl rifampicin respectively. These reactions may arise during combined treatment with other tuberculostatics and it is therefore
difficult to determine which drug is responsible. Chronic systemic lupus erythematosus with polyarthralgias, fever, skin rash, lymphadenopathy, hepatosplenomegaly, pleural and pericardial effusions, haemolytic anaemia has been reported (Rothfield, 1982; Greenberg, 1972; Grunwald, 1982). LE cells may be found and antinuclear antibodies are found in 5-33% of treated patients (Rothfield, 1982).

**Visual toxicity:** The most serious potential adverse effect of ethambutol is ocular toxicity manifested by optic or retro-bulbar neuritis, which exists in two forms and may affect one or both eyes. In the present study 3 (0.30%) patients reported visual toxicity due to ethambutol and needed discontinuation of offending drug. Adverse effects to ethambutol appear to be uncommon with doses of 15 mg/kg body-weight (Reynolds, 1989). Ethambutol may produce decreased visual acuity which appears to be due to optic neuritis and to be related to dose and duration of treatment. Mental confusion, visual hallucination and optical neuropathy have been reported in cases of acute overdosage. In severe cases even blue-yellow defects occurred which may result in achromatopsia (Dukes, 1986). Visual-evoked potential testing is reported to be the most reliable method for early detection of ocular abnormalities. Decrease in visual acuity induced by ethambutol was reversible when administration of the drug was discontinued. In rare cases, recovery may be delayed for up to one year or more and the effects may possibly be irreversible in these cases. Patients should be advised to report promptly to their physician any change in visual acuity. Patients developing visual abnormality during ethambutol therapy may show subjective visual symptoms before, or simultaneously with, the demonstration of decreases in visual acuity, and all patients receiving ethambutol should be questioned periodically about blurred vision and other subjective eye symptoms. Recovery of visual acuity generally occurs over a period of weeks or months after the drug has been discontinued. Patients have then received ethambutol again without recurrence of loss of visual acuity (Ordell, 1989). The effects are generally reversible when administration of the drug is discontinued promptly. Optic neuropathy is virtually unknown when ethambutol is given in doses of up to 15 mg/kg body-weight and is rare at doses of up to 25 mg/kg. However, a patient developed rapid progressive deterioration of vision only 3 days after beginning therapy with ethambutol 800 mg daily.
(about 15 mg/kg body-weight) and this patient remained blind over one year after the initial reaction (Karnik et al., 1985). The underlying cause of visual alterations appears to be a disturbance of metabolism due to depletion of copper and zinc which serve as prosthetic groups for many enzymes. The eye normally contains a considerable store of zinc, amounting to 0.5% of the weight of the eyeball. Much of the zinc is in the pigmented cells of the outer zone of the retina, where it serves as a metal prosthetic group for retinol (alcohol) dehydrogenase. Sub-clinical impairment of colour discrimination was reported to be relatively common in 54 patients receiving about 15 mg/kg body-weight of ethambutol daily as part of anti-tuberculosis chemotherapy when compared with 50 patients receiving other anti-tuberculosis drugs (Reynolds, 1989). Ethambutol may produce constriction of visual field, central and peripheral scotoma, and green-red colour blindness which may be associated with retrobulbar neuritis (Dukes, 1986; Reynolds, 1989).

Cutaneous reaction: Cutaneous reactions were reported in 13 (1.29%) patients receiving DOTS therapy under RNTCP needed discontinuation of one of the offending drugs. In the present study cutaneous reactions were reported with isoniazid, rifampicin and pyrazinamide in 3 (0.30%) patients, 7 (0.69%) patients and 3 (0.30%) patients respectively. Through the inhibitory effect of rifampicin on cellular immunity, rifampicin may interfere with cutaneous reactivity to intradermal tuberculosis (Alford, 1990). Discolouration of skin to glowing red-orange; pruritus at doses five times the therapeutic dose - called "red man syndrome". It was reported in literature that chronic daily therapy led to self-limiting rash in up to 5% of cases. Eczematous patches, flaccid bullae and crusted plaques on the skin which are reversible when rifampicin was discontinued.

Flu-like syndrome: Flu-like syndrome was reported in 11 (1.08%) patients with rifampicin. Intermittent therapy with rifampicin is a common denominator for inducing renal failure as in acute interstitial nephritis and usually proceeded by fever and flu-like symptoms. Though fever has been described as an infrequent adverse reaction typical 'flu' like syndrome consisting of fever, chills, malaise, shivering, dizziness, headache and bone pains occurring within a few hours. The 'flu' syndrome has usually been associated
with intermittent use of rifampicin, though a report of 'flu' like symptoms due to isoniazid was also published (Motion et al., 1989). Rifampicin-induced "flu" syndrome typically begins 2-3 hours after drug ingestion, lasts for up to 8 hours and usually requires no treatment. When it persists, reduction of dose, a change to daily administration and rarely interruption or termination of rifampicin has been advocated. The underlying mechanism of ethambutol induced 'flu' syndrome cannot be postulated, though, like Rifampicin, it may have an immunological origin (O'Mohoney and Chew, 1973). In a review of nearly 2,000 patients on EMB reported 'flu' syndrome (Pitt, 1977).

**Ototoxicity:** Ototoxicity was the most important adverse reaction reported by streptomycin. Ototoxicity includes tinnitus, balance disturbance, pain in the ear, dizziness, vertigo etc. In the present study ototoxicity was reported in 5 (0.49%) patients, needed discontinuation of one of the offending drugs. Berg (1951) in a review on ototoxicity produced by streptomycin states that histological examination of the auditory system of numerous affected species by several authors gave either negative findings, or indications that the lesion was localized to the central nervous system, or indications that the peripheral sensory epithelium of the labyrinths was affected. Consequently, it is difficult to draw definitive conclusions on the site of streptomycin-induced lesions. Berg postulates that the primary lesion is in fact the vestibular sensory epithelium, rather than the Organ of Corti, and that changes in vestibular nerves and central vestibular nuclei are secondary to this effect, resulting from an ascending atrophy (Berg, 1951). Patients presenting with ototoxic adverse effects after treatment with streptomycin, dihydrostreptomycin or kanamycin, for various clinical conditions, were studied in a retrospective study. Vertigo was reported by the end of the first week of treatment in 25/26 patients treated with streptomycin alone at doses between 0.25 and 2 g/person/day (~3 to 36 mg/kg/day). At lower streptomycin doses (15 mg/kg/day) for about 7 days, only one case of vestibular damage was reported when over 1000 patients were treated (Erlanson and Lundgren, 1964).

**Fever:** Fever was the most common reported adverse drug event due to anti-tuberculosis therapy but discontinuation of one of the offending drugs were needed in only special
cases. In the present study isoniazid was discontinued in 9 (0.89%) patients due to fever whereas streptomycin was discontinued in only 1 (0.09%) patients.

Five mechanisms of drug fever have been identified. Fever may arise from a drug effect thermoregulation, drug administration-related reactions, the drug’s pharmacological action, idiosyncratic response and hypersensitivity reactions; this latter is the most common mechanism of drug fever (Lipsky and Hirschmann, 1981; Tabor, 1986; Hanson, 1991).

Psychiatric changes: Some of the patients observed with psychiatric behaviors during anti-tuberculosis therapy and needed discontinuation of the offending drug or some modification in the treatment regimen to prevent development of neurotism (psychological illness). In the present study only 2 (0.18%) patients reported psychological illness due to isoniazid. A rare but extremely uncommon case of psychoses with isoniazid or ethambutol were reported during anti-tuberculosis therapy. In a report psychotic symptoms developed on start of ethambutol initially and later with isoniazid (Prasad et al., 2008). The mechanism of production of isoniazid-related psychiatric disorders is not clearly known, but isoniazid is known to interfere with several metabolic processes essential for the normal functioning of the neuron (Holtz and Palm, 1964). INH produces pharmacologic changes in pyridoxine metabolism (Biehl and Vilter, 1954). This may occur due to increased renal excretion of pyridoxine by formation of INH-pyridoxine hydrazones, the hydrazones competitively inhibit pyridoxine kinase, the activating enzyme that converts pyridoxine to the physiologically active pyridoxal phosphate and inactivation of the pyridoxal containing enzymes. Isoniazid causes deficiency of vitamin B₆ by causing excessive excretion of the vitamin, which in turn leads to a disturbance of normal tryptophan metabolism. Isoniazid also inhibits the activity of brain pyridoxal-5-phosphate (produced in the body from pyridoxine), which leads to a decrease in brain gamma-aminobutyric acid and other synaptic transmitters, resulting in neurologic ill effect (Wood and Peesker, 1972; Girling, 1982).

Naranjo’s probability scales are used for measuring the probability of an adverse drug reaction, and then employed to subjectively assess the likelihood that the observed reaction were the result of prescribed regimen. A total of 351 patients reported adverse
events were classified as definite ADRs, probable ADRs, possible ADRs and doubtful ADRs according to Naranjo's probability scale. Out of 351 (34.72%) reported adverse events; 102 (10.09%) were definite, 59 (5.83%) probable, 123 (12.17%) possible and 67 (6.63%) were doubtful. We have reported a case of maculopapular rash in patients receiving standard DOT therapy and the adverse reaction was categorised as probable ADR (Khayyam et al., 2010).

Hartwig preventability scales are generally used to measure the preventable and non-preventable nature of adverse events. In the present study a total of 37 (36.27%) adverse events were non-preventable in nature whereas 65 (63.73%) preventable in nature. We have reported a case of maculopapular rash in patients receiving standard DOT therapy and the adverse reaction was non-preventable (Khayyam et al., 2010).

Severity of drug related adverse events were measured according to Hartwig severity scales. A total of 351 adverse drug events were reported in the present study. Out of total, 225 (22.26%) were mild, 105 (10.38%) moderate and 21 (2.08%) were severe ADRs. We have reported a case of maculopapular rash in patients receiving standard DOT therapy and the adverse reaction was categorised as moderate of level 3 (Khayyam et al., 2010).

Biochemical changes during drug therapy are common because every drug is an active chemical entity which has their pharmacological as well as toxic effects on body leading to drug adverse effects. In the present study blood samples of patients who developed adverse events were taken to study the biochemical changes during DOT therapy. Blood sample of patients were taken before for the base line assessment and after drug administration when any adverse events occur for liver function test, kidney function test and CBC as per the protocol.

Monitoring of liver function tests is very important in patient receiving DOT therapy, because component of DOT have reported to cause hepatotoxicity. In the present study baseline ALT (SGOT) and AST (SGPT) were found to be 23.77± 13.198 and 29.40 ± 13.752 respectively. After standard DOT therapy initiation, sample of blood was taken for monitoring from patients who developed any untoward reaction. first blood sample of patients was taken when adverse events appeared and second blood sample were taken...
when adverse events resolved or disappeared and in some patients a third blood sample were also taken whose value not reached to normal even when symptoms was resolved.

The baseline LFT was either normal or slightly increased in majority of cases, who experienced adverse events. Out of 351 patients AST and ALT values (expressed as Mean ± SD) were found to be within the normal range when adverse events appeared and resolved in 210 patients (i.e. 26.58± 13.079 and 31.57± 11.742) and 234 patients (i.e. 31.90 ± 22.815 and 28.53 ± 18.195) respectively, whereas AST and ALT values were found to be raised, 1 – 2 times than upper normal limits when adverse events appeared and/or resolved in 47 patients (i.e. 46.62 ± 14.593 and 41.13 ± 10.13) and 56 patients (i.e. 47.98 ± 19.725 and 34.23 ± 12.311) respectively. AST and ALT values were found to be raised 2 – 3 times than upper normal limits when adverse events appeared but lowered when resolved and came to normal in the next follow up tests in 59 patients (i.e. 73.84 ± 27.432; 43.55 ± 11.990 and 23.43 ± 12.377) respectively. AST and ALT values were found to be raised 3 – 5 times than upper normal limits when adverse events appeared but lowered when resolved and came to normal in the next follow up tests in 19 patients (i.e. 177.16 ± 49.344; 98.54 ± 18.443 and 48.44 ± 12.788) respectively. In a clinical trial the median aspartate aminotransferase levels was 3 – 10 times the upper normal limits in 16 participants taking 9 months of isoniazid and 2 – 10 times among 3 participants taking 4 months of rifampin. Corresponding alanine aminotransferase levels was 6 – 16 times and 4 – 15 times upper normal limit respectively (Menzies et al., 2008).

In the present study baseline alkaline phosphate (ALP) were found to be 227.23 ± 91.864, whereas after DOT therapy initiation ALP values were found to be within the normal range when adverse events appeared and/or resolved in 228 patients (i.e. 229.14 ± 105.653 and 229.14 ± 105.653). But after DOT therapy initiation ALP value was found to be raised 1 – 2 times than upper normal limits when adverse events appeared and/or resolved in 69 patients (i.e. 303.96 ± 117.055 and 268.66 ± 123.864). ALP value was found to be raised 2 – 3 times than upper normal limits when adverse events appeared.
and/or resolved but lowered and came to normal in the next follow-up tests in 43 patients (i.e. 441.00 ± 192.470; 349.34 ± 178.406 and 281.45 ± 155.677). ALP value was found to be raised >3 times than upper normal limits when adverse events appeared and/or resolved but lowered and came to normal in the next follow-up tests in 11 patients (i.e. 577.22 ± 212.798; 391.11 ± 157.225 and 314.33 ± 167.443).

In the present study baseline uric acid value (Mean ± SD) was found to be 5.85 ± 1.391, whereas post-treated uric acid value was found to be with in the normal range in patients reported adverse events in 265 patients (i.e. 5.35 ± 0.625 and 4.56 ± 0.968). But post-treated uric acid value was found to be between 6 - 8 mg /dL when adverse events appeared and/or resolved and came to normal in 52 patients (i.e. 7.68 ± 0.821; 6.21 ± 0.533 and 5.42 ± 0.750). After DOTS therapy initiation uric acid value was found to be more than 8 mg /dL in 34 patients who reported adverse events when appeared, resolved and came to normal were 8.48 ± 0.532, 7.23 ± 0.651 and 6.79 ± 0.893 respectively. Discontinuation of one or all the anti-tuberculosis drug(s) were needed only in 11 patients in which blood uric acid level was reported more than 8 mg/dL.

In the present study baseline hemoglobin value among the male and female were found to be 12.02 ± 1.364 and 10.70 ± 1.640 respectively. The post-treated hemoglobin value, in male patients, reported adverse events when appeared and disappeared were found to 11.23 ± 1.332 and 12.42 ± 2.186 respectively, whereas in female patients, reported adverse events when appeared and disappeared were found to 10.57 ± 2.759 and 11.21 ± 1.372 respectively. The baseline total leukocyte count was found to be 8579.41 ± 3204.443 whereas After DOTS therapy initiation total leukocyte count when adverse events appeared and symptoms of adverse events resolved was found to be 9732.35 ± 2542.412 cells/mm³ and 9075.00 ± 3786.129 cells/mm³ respectively. In a clinical trial it was reported that there is reduction in leukocyte count of at least 1.00 cells/mm³ in 41% participants taking rifampin (with a full set of measurements), and 10% had a reduction of 2.50 cells/mm³ or more (Menzies et al., 2008).

In the present study baseline neutrophil (N), lymphocyte (L) and Eosinphils (E) was found to be 64.59 ± 11.794, 18.40 ± 9.855 and 3.25 ± 1.581 respectively. The post-
treated neutrophil, lymphocyte and eosinophils when adverse events appeared were found to be $77.32 \pm 12.923$, $19.40 \pm 9.855$ and $6.34 \pm 1.438$ respectively. But the post-treated neutrophil, lymphocyte and eosinophils when symptom of adverse events disappeared were found to be $70.98 \pm 15.420$, $19.65 \pm 10.916$ and $4.55 \pm 1.820$ respectively. There were no significance difference was reported in patients developed adverse events in the baseline or after DOTS therapy initiation in DLC. In a clinical trial it was reported that the baseline neutrophil counts in the two treatment arms were $1.90 \times 10^9$ cells/L and $1.76 \times 10^9$ cells/L, which decreased to nadir levels of $0.66$ and $0.81 \times 10^9$ cells/L, respectively, after 4 to 5 weeks of therapy. Both participants remained asymptomatic, and neutrophil counts returned to near-baseline levels within 2 weeks after therapy was stopped (Menzies et al., 2008).

The analytical method developed for determination of plasma concentration of isoniazid in patients receiving DOT therapy with a modified method of Saudeg et al., (1996). The method was modified and partially validated according to ICH guidelines. This method was found suitable and stable for this analytical procedure throughout the experiment. There was much less and insignificant interference with our analytical method as plasma samples were screened for interferences by other biological components and did not show any peaks interfering at the retention time of isoniazid. System selectivity was conducted by injecting three lots of blank plasma and an interfering peak was observed at the retention time of the drug but the response of the interfering peak was very less and insignificant. After three injections the coefficient of variation (% CV) for area was found to be 0.8%. The method was accurate and precise for analyzing plasma samples as within-day variation ranges from 0.86% to 4.13% and day to day variation ranges from 1.38% to 6.04%. The accuracy of the method within batch ranges from 98.50% to 105.21% and between batch ranges from 100.37% to 108.28%. This was in concordance with ICH guidelines requirement for validation of analytical method validation. The method demonstrates linearity over a concentration range of 0.25-4\(\mu\)g/ml and the total recovery was found to be 96.21-102.89%. The analyte of interest (isoniazid) was stable for three-freeze thaw cycles and for forty hour on bench top cycles. Hence this method is accurate, precise, linear and able to show stability and passes the partial validation.
criteria set by ICH guidelines document for analytical method validation. Therefore it was used for analysis of isoniazid in plasma sample.

The patient's data as well as bimodal distribution of proposed parameter shows that the isoniazid method gave a clear-cut classification of the study population into slow and fast acetylators. Computation of antimode allowed to assign the slow acetylator status in 20 patients (55.56%) and fast acetylator status in 16 patients (44.44%). Our results are consistent with earlier study conducted on Gujarati and Marathi population in Mumbai and they found that 46.8% populations are slow acetylator while 54.2% populations are fast acetylator (Kshirsagar et al., 1987). In another study conducted in All India Institute of Medical Science New Delhi and they found that 66% populations are slow acetylator while 34% populations are the fast acetylator (Singh et al., 1996). Similar study conducted in the North Indian populations and reported that 14.55% population are slow acetylator, 46.36% population are intermediate acetylator and 39.09% population are fast acetylator (Gupta et al., 1984). Similar results were also reported from earlier studies and they showed that subjects with low plasma levels after administration of the drug have a rapid acetylation of the drug in the liver (Salako, et al., 1977; Evans, 1983).

The rate of inactivations of isoniazid has been shown to be determined genetically (Harris et al, 1958; Peters, 1960; Sunahara, 1962; Evans, 1968). Marked racial differences in the incidence of rapid and slow inactivators of isoniazid have been reported by various investigators, ranging from 95% of rapid inactivators amongst Eskimos (Armstrong and Peart, 1960) to 32% among Swedish (Hanngren et al., 1970). Frequency of rapid inactivators of isoniazid was 89% among Koreans, and 36% among Finns (Mattila et al., 1967). These variations in the incidence have been due to ethnic differences. In the present study there was very slight difference between distribution of slow and fast acetylator among the patients, so dose of INH should be modify on individual basis according to the patients acetylator phenotype and/or plasma isoniazid concentration to increase the efficacy and optimize the adverse effects.