**Discussion**

TB remains one of the major causes of disability and death worldwide (WHO report 2012). The essential services needed to control TB, based on diagnosis and treatment of infectious cases and incorporating the essential management tools, were developed and packaged as the DOTS strategy, which produces cure rates of up to 95% even in the poorest countries and prevents new infections by curing infectious patients. The number of countries adopting DOTS has increased dramatically over the decade and India now has the second largest DOTS programme in the world. Due to wide spread application of DOTS strategy in TB treatment, it is imperative to evaluate their risk on human health. Anti-TB chemotherapy under DOTS produces many adverse effects. Hepatotoxicity is one of the potential serious adverse effects related to anti-TB drugs, which is considered a worldwide serious medical problem among TB patients. Multidrug resistance/treatment failure, which is caused by hepatotoxicity during anti-TB therapy, is growing problem of serious concern in many countries around the world (Espinal *et al.*, 2001).

Several risk factors for the development of hepatotoxicity induced by anti-TB drugs have been suggested (Singla *et al.*, 2010). A higher risk of anti-TB-DIH has been reported in Indian population as compared to developed countries. The reasons for the higher rate of hepatotoxicity are still unclear. Therefore, this present study reports prevalence and risk factors for anti-TB-DIH in North Indian population.

In our study, the prevalence of anti-TB-DIH was observed in 14.3% (35/244) patients, which was higher as compared to other developed countries ranging from 3%-4% (Combs *et al.*, 1990). One previous study (Sharma *et al.*, 2002) reported that the risk of anti-TB-DIH in North Indian population was 16.2%. Similar to our study, Mahmood *et al.* (2007) also reported similar incidence cases (15%) of anti-TB-DIH, in Pakistan’s population. In Nepalese population, the prevalence of anti-TB-DIH was 8% (Shakya *et al.*, 2006). In Iranian population, the rate of anti-TB-DIH was 28% (Sistanizad *et al.*, 2011).
In our study, the time interval from the initiation and the onset of hepatotoxicity was 15-50 days in which, patients manifested as anorexia, nausea, vomiting, abdominal pain and jaundice. Makhlouf et al. (2008) also observed the similar clinical manifestations of anti-TB-DIH in patients and reported time interval of 15-60 days. In agreement to our study, Shakya et al. (2006) reported similar clinical symptoms of anti-TB-DIH within 12-60 days from the initiation of treatment. Mahmood et al. (2007) reported that the onset of anti-TB-DIH in almost two thirds of their patients was within 14 days from the start of therapy. In our study, we observed that age was not a significant risk factor for anti-TB-DIH. Similarly, other studies (Vuilleumier et al., 2006; Lee et al., 2005) in agreement with ours, showed no increased risk for the development of anti-TB-DIH regarding age. Also, in one recent study (Mohammad et al., 2011), no significant difference was to be observed between Iranian patients with and without DIH, with respect to age. However, another studies (Schaberg et al., 1996; Pande et al., 1996) reported old age as a risk factor for the development of hepatotoxicity, during treatment of TB. Our study clearly showed that hepatotoxicity induced by anti-TB drugs was more frequent in females as compared to males. Similarly, other studies reported that the prevalence of anti-TB-DIH was higher in female gender as compared to males (Attri et al., 2001; Sodhi et al., 1996). It has been suggested that slow acetylator are more prone to hepatotoxicity compared to rapid acetylator and females being a slow acetylator, are at higher risk for anti-TB-DIH (Weber et al., 1983). However, many previous studies showed no increased risk of anti-TB-DIH in women (Peters et al., 1994; Sharma et al., 2002; LoBue et al., 2005; Fountain et al., 2005). We observed that malnutrition (detected by body mass index <18.5 kg/m²) was a significant risk factor for anti-TB-DIH. Sharma et al., (2002) reported that low body mass index patients were significantly associated with the risk of anti-TB-DIH. Similarly, malnourished patients
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were found be highly susceptible for anti-TB-DIH in other previous studies (Scharer and Smith, 1969; Ferebee and Mount, 1969).

In our study, patients with extra-pulmonary cases were found to be more susceptible for anti-TB-DIH, as compared to pulmonary cases. This was also reported in another study (Marzuki et al., 2007), in which extra-pulmonary TB patients were more affected with anti-TB-DIH as compared with pulmonary TB patients. Whereas, Makhlouf et al. (2008) study indicates that patients with pulmonary TB were at an increased risk for anti-TB-DIH. In agreement to this study, Mahmood et al. (2007) also showed a significant association between pulmonary TB and the development of anti-TB-DIH.

Similar to the result of one previous study (Breen et al., 2006), we found that HIV positive patients were at increased risk for anti-TB-DIH. The decrement in the immune status of the HIV positive patients may be one of the reasons for higher risk of anti-TB-DIH (Yimer et al., 2008). HIV patients might also have a more severe and advanced TB infection, depending upon the stage of the HIV disease, which itself an independent risk factor for the development of anti-TB-DIH (Sharma et al., 2002).

Oxidative stress plays an influential role in the pathogenesis of anti-TB-DIH, which is characterized by increased level of MDA with altered level of antioxidants (Chowdhury et al., 2001). Of the various anti-TB regimens, metabolic intermediates of INH metabolism are mainly responsible for the occurrence of anti-TB-DIH. These metabolic intermediates cause hepatocyte damage or necrosis through lipid peroxidation. In response to detoxify the toxic effects of these hepatotoxic metabolites, a large amount of glutathione is consumed, resulting in compromised anti-oxidative capacity (Wang et al., 2010).

In this study, before the initiation of drug treatment, there was no significant difference in the level of MDA, between patients with and without DIH. However, during the treatment period, we observed that MDA level was significantly higher in patients with DIH as
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compared to patients without DIH. Higher level of MDA (Lipid peroxidation product) indicates increased rate of oxidative stress in patients developing DIH. Mandai and Das (2005) also suggested that oxidative stress with increased lipid peroxidation products are involved in toxic and drug-induced liver injury.

Similarly, before the initiation of drug therapy, no significant difference was to be observed in the level of GSH, between patients with and without DIH. Whereas, significant lower level of GSH was to be observed after the initiation of anti-TB drug therapy in patients with DIH as compared to patients without DIH. Others studies, in agreement to ours, suggested that the level of GSH level was to be decreased in rats after the administration of INH or hydrazine (Jenner and Timbrell, 1994; Timbrell et al., 1982). Similarly, Chowdhury et al. (2006) observed that INH and RIF induced hepatotoxicity was associated with decreased level of GSH and increased rate of MDA production. Thus, the decreased level of GSH can be as a consequence of anti-TB-DIH.

SOD is an antioxidant enzyme, extensively used as a biochemical indicator of oxidative stress. In this present study, before the treatment period, when we compared between patients with and without DIH, with special reference to SOD level, no significant difference was observed. However, during the treatment period, the activity of SOD was significantly higher in patients with DIH as compared to patients without DIH. In other previous studies (Jenner and Timbrell, 1994; Timbrell et al., 1982), reduced concentration of SOD was observed after the administration of INH or hydrazine in rats. SOD catalyzes the dismutation of highly toxic superoxide radicals to H$_2$O$_2$. Increased generation of superoxide radicals lead to increased rate of lipid peroxidation. Thus, SOD has a protective role against free radical induced damage, and their induction can be understood as an adaptive response to oxidative stress.

In our study, we also examined the possible association of *GSTM1* and *GSTTI* null polymorphisms with the risk of anti-TB-DIH, in North Indian population. Many previous
studies suggest that these two genes (GSTM1 and GSTT1) have been genetically deleted in large number of human population (Bolt et al., 2006; Strange et al., 2000). Homozygous gene deletions of GSTM1 and GSTT1 genes are considered as predictors of xenobiotic or drug-related toxicities and disease states like aerodigestive cancers (Yue et al., 2004; Higuchi et al., 2007). According to Hussain et al. (2003), homozygous null mutations of these two genes, may modulate susceptibility to anti-TB-DIH. However, the risk assessment of GSTM1 and GSTT1 polymorphisms for anti-TB-DIH is specific for each population, because allelic and genotypic variations have been observed in different populations and ethnic groups in various parts of world (Leiro et al., 2008). Indian population is believed to be most diverse because of different socio-cultural traditions. Therefore, identification of these gene polymorphisms might have significant implications in Indian population, for predicting susceptibility to anti-TB-DIH.

In our study, GSTT1 genotype exhibited significantly higher frequency of gene deletion in cases than in controls. After adjustment for age, gender, body mass index and baseline values of liver function parameters, there was also a significant association between GSTT1 null genotypes and anti-TB-DIH, which indicates that subjects with GSTT1 null genotype had an increased risk of anti-TB-DIH. Whereas, we did not find any significant association between GSTM1 null polymorphism and anti-TB-DIH. Therefore, it could also be assumed that GSTT1 gene may play a more important role in detoxification of anti-TB drugs than GSTM1 gene.

The principal function of GST gene family is conjugations of hepatotoxic metabolites with reduced GSH. The intracellular binding reaction with GSH is catalyzed by the GST and leads to stable GSH-toxic products conjugates being transported out of the cell and excreted via feces and urine and thus reduces the toxic effects. Individuals with the GSTT1 null genotype resulting in either decreased or altered enzyme activity. The change in catalytic activity may
reduce in GSH-toxic products conjugates and excretion, which is further associated with enhanced rate of anti-TB-DIH (Sheweita, 2000).

In agreement to our study, a recently published study (Leiro et al., 2008) conducted on Caucasian subjects, with 35 cases and 60 controls, showed a significant association between the null GSTT1 homozygosity and anti-TB-DIH, whereas no significant association was to be found between the homozygous GSTM1 null polymorphism and anti-TB-DIH. In contrast to ours, Roy et al. (2001) suggested that subjects with GSTM1 null genotype had an increased risk for anti-TB-DIH. Similarly, Huang et al. (2007) also reported that homozygous null mutation at GSTM1 loci was a significant risk factor for anti-TB-DIH.

Also, in this present study we investigated the combined effect of GSTM1 and GSTT1 polymorphisms on the risk of anti-TB-DIH. We found that the distribution of the combined genotypes were not statistically significant between cases and controls. After adjustment potential confounders, there were also no significant association between the combinations of GSTM1 and GSTT1 polymorphisms and the risk of anti-TB-DIH. Chatterjee et al. (2010) analysed association between combined effect of GSTM1 and GSTT1 null polymorphisms with anti-TB-DIH and reported that homozygous deletion of both genes GSTM1 and GSTT1 were not significantly associated with anti-TB-DIH. Similarly, Leiro et al. (2008) also observed no relation between combined GSTM1 and GSTT1 null polymorphisms and the risk of anti-TB-DIH.

Therefore, GSTT1 homozygous null polymorphism may be a potential risk factor for anti-TB-DIH in our Indian population. Regular monitoring of liver biochemical tests may be considered in patients with GSTT1 null polymorphism genotype who are subjected to anti-TB drugs.

Drug metabolizing enzyme-CYP2E1, plays an imperative role in the progression of anti-TB-DIH. Because of genetic polymorphisms, CYP2E1 metabolic enzyme differs greatly between
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individuals. The three genotypes of \textit{CYP2E1} are classified as C1/C1, C1/C2 and C2/C2 by RFLP using \textit{RsaI} restriction enzyme. Of the three genotypes of \textit{CYP2E1}, particularly C1/C1 genotype is associated with high activity of \textit{CYP2E1}, which is further directly responsible to develop anti-TB-DIH (Cai \textit{et al}., 2012). Therefore, this present study reports the possible association between three genotypes of \textit{CYP2E1} and the susceptibility to anti-TB-DIH in North Indian population.

In the present study, the frequency of wild type genotype \textit{CYP2E1} C1/C1 compared with the mutant genotype (\textit{CYP2E1} C1/C2, \textit{CYP2E1} C2/C2), was found to be higher percentage in cases group as compared to controls. According to Huang \textit{et al}. 2003, individual with wild type C1/C1 genotype exhibit higher activity of \textit{CYP2E1} than those with mutant \textit{CYP2E1} C1/C2 or C2/C2 genotypes and therefore, may produce more hepatotoxins and raises the possibility for the development of anti-TB-DIH. However, in our study, no significant association was to be observed between \textit{CYP2E1} C1/C1 genotype and the susceptibility to anti-TB-DIH. Similar to the finding of our study, Cho \textit{et al}. (2007) found no relationship between \textit{CYP2E1} C1/C1 genotype and anti-TB-DIH in their Korean population. These results are inconsistent with those of Vuilleumier \textit{et al}. (2006), who showed positive correlation between \textit{CYP2E1} C1/C1 genotype and anti-TB-DIH. Similarly, Lee \textit{et al}. (2010) reported the association of \textit{CYP2E1} C1/C1 with the severity of anti-TB-DIH.

In our study, among the three genotypes of \textit{CYP2E1}, the mutant type \textit{CYP2E1} C2/C2 was found to be in minimum percentage in both groups. Similarly, one previous study (Teixeira \textit{et al}., 2011) that was conducted on the Brazilian population showed rare percentage of mutant \textit{CYP2E1} C2/C2 genotype and this genotype was not to be associated with the susceptibility of anti-TB-DIH. Rare cases of mutant \textit{CYP2E1} C2/C2 was also observed in Taiwanese population, which was studied by Huang \textit{et al}. (2003).
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Also, no significances difference was to be observed in this present study, when hepatotoxic patient’s age, sex, body mass index, socio-economic status, disease classification and level of liver function parameters were analysed between wild type (C1/C1) and mutant type (C1/C2 +C2/C2) genotypes. Similarly, Huang et al. (2003) analysed patient’s age, gender, body mass index, and pretreatment liver biochemical tests between CYP2E1 C1/C1 and mutant type (C1/C2+ C2/C2) genotypes and found no significance difference in patient’s baseline characteristics and pretreatment level of liver biochemical parameters between these genotypes. Lee et al. (2010) also determined the characteristics of hepatotoxic patients with respect to three genotype of CYP2E1 and observed that patients with CYP2E1 C1/C1 genotype had a higher mean serum AST level, but not ALT level, than patients with the C1/C2 and C2/C2 genotypes.