ijcrr
Vol 04 issue 12
Category: Research
Received on:20/05/12
Revised on:28/05/12
Accepted on:07/06/12

DRUG-INDUCED HEPATOTOXICITY AND GENOTOXICITY IN PULMONARY TUBERCULOSIS PATIENTS RECEIVING DIRECTLY OBSERVED THERAPY, SHORT-COURSE (DOTS)

Manish Pratap Singh[1], Ashish Kumar Saxena[2], Sarika Saxena[1]

[1]Department of Biotechnology, Madhav Institute of Technology and Science, Gwalior
[2]Medical officer. Balaghat

E-mail of Corresponding Author: saxena.sarika5@gmail.com

ABSTRACT

The aim of the study was to evaluate hepatotoxicity and genotoxicity in patients suffering from pulmonary tuberculosis. It was carried out on the pulmonary tuberculosis male patients from directly observed therapy, short-course (DOTS) Centre Fatehpur district (U.P) India. Patients were divided into three groups- 1) Before treatment 2) After treatment and 3) Control. Toxicity levels in the blood samples were determined by standard liver function tests (ALT & AST) by spectrophotometry. The study indicates significant increase in the level of liver transeaminases (ALT & AST, $P < 0.05$) in the after treatment group. Antioxidant analysis shows that the patients who suffer from oxidative stress have reduced Glutathione (GSH) and high Melonaldehyde (MDA) levels. After treatment with anti-TB drugs the level of GSH increases ($P < 0.05$) while level of MDA decreases ($P < 0.05$). Further genotoxic effect of anti-TB drugs was evaluated on lymphocytes by COMET assay which showed observable DNA damage in after treatment study group patients ($P < 0.05$). LDH expression profile of different isoforms of lactate dehydrogenase(LDH) on native polyacrylamide gel electrophoresis(PAGE) indicates that in pulmonary tuberculosis patients the LDH 3 isoforms was expressed more as compares to the other isoforms which may be due to damage in the lung cell lining by the mycobacterium. In some after treatment patients LDH 5 isoform level increased, which is an indication of liver injury due to hepatotoxic and genotoxic effect of anti-TB drugs on the patients.

Key Words: Antioxidants, Transeaminases, Comet assay, lactate Dehydrogenase, Lipid peroxidation

INTRODUCTION

Tuberculosis (TB), a ubiquitous, highly contagious chronic granulomatous bacterial infection, is still a leading killer of young adults worldwide. Pulmonary tuberculosis is India's biggest public health problem. Every year, approximately 1.8 million people develop tuberculosis, of which 0.8 million are new smear positive highly infectious cases. Directly observed therapy, short-course (DOTS) is the key to success for anti-tuberculosis chemotherapy nowadays. It involves the use of a multi-drug regimen with isoniazid (H), rifampicin (R) and pyrazinamide (Z) as essential drugs and a fourth drug, streptomycin (S) or ethambutol (E), is usually added in countries with high prevalence of drug resistance. These four drugs are administered together for 2 months (the intensive phase), followed by a continuation phase of 2 drugs, HR for 4 months (2HRZS or 2HRZE/ 4HR), in a fully supervised fashion.

The most frequent adverse effects of anti tuberculosis treatment are hepatotoxicity, skin

reactions, gastrointestinal and neurological disorders. Hepatotoxicity is the most serious one and is the focus of the present study [1]. Most studies on ATDH have been performed in Europe, Asia and the USA and the incidence varies between different world regions. Orientals are reported to have the highest rates, especially Indian patients [2, 3].

Significant transaminase elevations are reported in about 0.5% of all patients treated with isoniazid monotherapy [4, 5]. In general, rifampicin is a well-tolerated drug and hepatotoxicity occurs in about 1–2% of patients treated with prophylactic rifampicin monotherapy [6]. Hepatotoxicity is a major toxic effect of pyrazinamide. When the drug was introduced in the 1950s, a high incidence of hepatotoxicity was reported and the drug was nearly abandoned.

Being directly in the path of airborne materials, the lung tissue is particularly at risk from oxidative stressors such as cigarette smoke, atmospheric pollutants, and other in- haled environmental toxins [7]. GSH and GSH- associated enzymes present in the epithelial lining fluid (ELF) of the lower respiratory tract may be the first line of defense against such challenges [8, 9]. Sustained oxidative challenge to the lung results in depletion of GSH and other antioxidants from the lungs.

Lipid peroxidation products (LPPs) diffuse from the site of inflammation and can be measured in blood. The granulomatous destruction of the lung tissue itself may cause the liberation of toxic radicals, or indeed, it may be that the activated macrophages release highly reactive radicals which may then cause the local disruption of the essential structure including membrane lipids, deoxyribonucleic acid and proteins and hence, cause tissue destruction [10].

M. tuberculosis is expected to sustain a variety of potentially DNA-damaging assaults in vivo, primarily from host generated antimicrobial Reactive oxygen intermediates (ROI) and reactive nitrogen intermediates (RNI). DNA is a biological target for RNI and ROI, and interaction with toxic radicals is mutagenic [11]. Furthermore, damage to cellular components required for the protection or propagation of DNA can indirectly affect chromosomal integrity; while detoxification reactions might themselves yield endogenous damaging adducts.

The study was designed to evaluate the hepatotoxicity and genotoxicity of anti-tuberculosis drugs in tuberculosis patients.

MATERIAL AND METHODOLOGY

When tuberculosis is confirmed by the laboratory tests the patients are advised to receive DOTS therapy. In the present study, for preliminary examination detailed clinical history of all test subjects was recorded and filed. Thirty healthy individuals aged between 20 to 45 years who were symptomless, free from any clinical abnormality and those who were not taking any drug, constituted the control groups. Before start of treatment 5 ml blood was intravenously withdrawn from each test subject and collected in a vial. Similarly blood sample of control subjects was also collected and preserved. Serum was separated by centrifugation of whole blood at 1000 rpm for 15 min. Vials containing serum and whole blood were kept in an ice bucket and cold chain was maintained during transportation. Shaking was avoided to protect from haemolysis. After 3 months of therapy the same procedure was repeated again.

Study

The whole blood and serum obtained from patients before treatment and after treatment was analyzed in laboratory following standard protocols to study hepatotoxicity and genotoxicity induced by antitubercular drugs.

Tests for Hepatotoxicity:

Transaminases (AST- and ALT) [12]

The substrate (0.5 ml) was incubated at 37°C for 5 minutes followed by adding 0.1 ml of serum and incubation for 60 minute and 30 minute for AST

and ALT, respectively. For control tubes, 0.5 ml of substrate was taken and 0.1 ml of serum was added to it. Standard was prepared by mixing 0.1 ml of working standard with 0.4 ml of substrate, along with 0.1 ml of water. Blank was prepared after taking 0.5 ml of substrate, 0.1 ml water. DNPH was allowed to react at room temperature in all the tubes for 20 minute. 5 ml of 0.4 N NaOH was added and mixed well. The contents were incubated for 10 minutes at room temperature and optical density (OD) was read at λ 510 nm.

Lipid Peroxidation [13]

1.0 ml of blood in 1ml KCl solution was incubated at 37°C for 30 minutes. Proteins were precipitated by adding 1 ml of 10% TCA and then centrifuged at 2,000 rpm for 15 minute. 1 ml supernatant was taken as an aliquot in a separate tube to which 1 ml of TBA solution was added. The tubes were kept in boiling water bath for 10 minutes. After cooling the tubes, the optical density was read at λ 535 nm.

Reduced Glutathione [14]

0.1 ml of blood was taken in a tube to which 0.9 ml of distilled water and 1.0 ml sulphosalicylic acid was added. The contents were mixed thoroughly and then centrifuged at 5,000 rpm for 10 minutes. Now 0.5 ml of supernatant was taken in a tube and similarly blank and standards were prepared by taking 0.5 ml of distilled water and 0.5 ml of GSH standard respectively. To all the tubes, 4.5 ml of tris buffer and 0.5 ml of DTNB solution were added. After 6 minutes OD was read at λ 412 nm.

Native-page for studying lactate dehydrogenase enzyme expression profile

Glass plates of vertical gel electrophoresis apparatus were washed and sealed with 1% agar. Separating gel (8%) was poured in between the plates then immediately a comb was inserted. After polymerization the comb was removed and samples (50 microgram) were loaded in wells. Proteins were allowed to run in running buffer at constant voltage of 100 V. After electrophoretic separation, the gel was processed for LDH specific staining. The gel was soaked in a glass petri plate containing LDH staining dye [10 mg/ml NAD, 1 mg NBT/ml, 1 mg/ml PMS, 12 ml of Sodium lactate as substrate and 60 ml tris-HCl buffer (0.2M, pH-8.0)] and incubated at 37 ^{0}C for 10-15 min till LDH bands started appearing. Once all the bands became clearly visible, the reaction was stopped by adding tap water and the gel was fixed and stored in the 7% acetic acid, which then photographed with the help of gel documentation system.

Single Cell Gel Electrophoresis (COMET ASSAY) [15]

100 µl normal melting agarose was spread on frosted microscopic slide and allowed to solidify at 4˚C for 10 minutes. Whole blood and low melting agarose were taken in the ratio of 1:4, 100 µl was cased on precoated slides then kept at 4˚C for 20 minutes. After solidification the slides were immersed into the chilled lysis buffer for 1 h in dark at 4˚C. After completion of lysis step, the slides were placed for 20 minutes in an ice cold electrophoresis chamber containing alkaline electrophoresis buffer for unwinding. The electrophoresis was subsequently conducted for 20 minutes at 25 volt/300 mA. At the end of electrophoresis, the slides were washed thrice with neutralizing buffer for 5 minutes each and just before visualization, slides were stained with coating 40 µl ethidium bromide, rinsed with dH_2O twice and nucleus was observed under florescence microscope (Leitz Autoplan, excitation filter λ 595 nm, green filter).

All the preparations were done under dark/red light at 4˚C. Observations were recorded by software Lieica Qwin V_3.

RESULTS

The study was carried out in 30 known tuberculosis patients and 30 healthy control subjects from DOTS centre Fatehpur district U.P, India. All study groups was male and aged

between 20 to 45 years. The affected patients showed symptoms of prolonged fever, cough (with or without haemoptysis), anorexia, weight loss, but without other systemic symptoms tuberculosis or evidence of bronchiectasis. As tuberculosis was confirmed in the patients they were started with anti-TB drugs. The Directly Observed Treatment, Short-course (DOTS) constitutes the cornerstone of the current strategy for control of tuberculosis (TB). However as noted earlier, the three key drugs, isoniazid, pyrazinamide and rifampicin, used in the regimen are potentially hepatotoxic and may lead to drug-associated hepatitis.

Most of the hepatotoxic reactions are dose-related; some are, however, caused by drug hypersensitivity. The immunogenetics of anti-TB drug induced hepatotoxicity, especially inclusive of acetylaor phenotype polymorphism, has been increasingly unraveled. Drug-induced hepatic dysfunction usually occurs within the initial few weeks of the intensive phase of anti-TB chemotherapy.

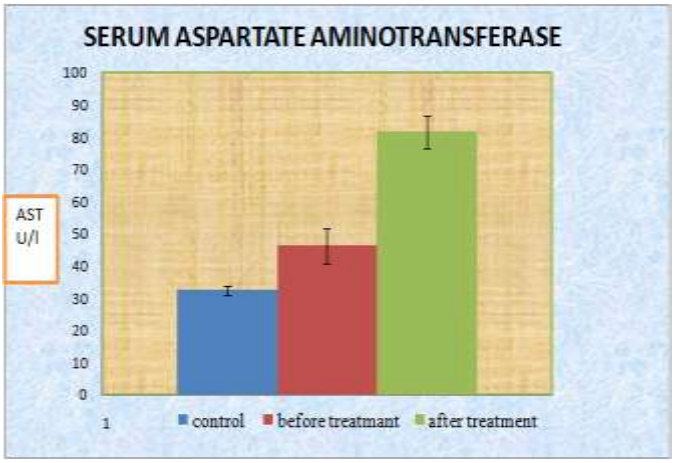
Results of the present work confirm previous findings about the subject and also support those using new parameters such as COMET assay.

Effect of Anti-TB Medicines on Hepatocellular Markers

.

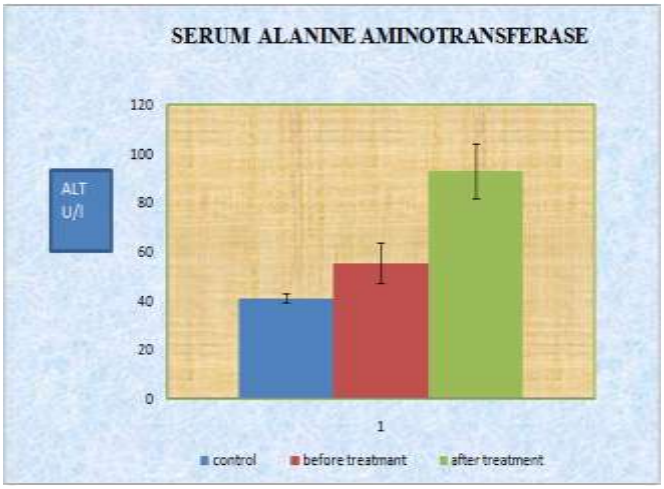
Increases in the levels of the liver enzymes alanine amino transferase (ALT) and aspartate amino transferase (AST) in serum, in combination with increased bilirubin levels, are generally considered to be the most relevant signal of liver toxicity. ALT is considered a more specific and sensitive indicator of hepatocellular injury than AST. An increase of ALT activity in the range of 2-4 fold and higher compared to concurrent control average or individual pre-treatment values in non-rodents, should raise concern as an indication of potential hepatic injury unless a clear alternative explanation is present.

ALT is an enzyme produced in hepatocytes, the major cell type in the liver. The level of ALT in the blood (actually enzyme activity is measured in the clinical laboratory) increases in conditions in which liver cells are damaged or die. As cells are damaged, ALT leaks out into the bloodstream. As summarized in fig 1 and 2, results indicate a significant increase in the activities of AST as well as ALT ($P \leq 0.05$) in tuberculosis medicine receiving group when compared with control and before treated group, which is confirmed by one way ANOVA.



Values are mean ± S.E., N = 30, $P < 0.05$ vs. normal control group.

Fig. 1 AST content in serum of tuberculosis patients before treatment and after treatment in correlation with control expressed in terms of U/l. Data was significant at ($P < 0.05$)

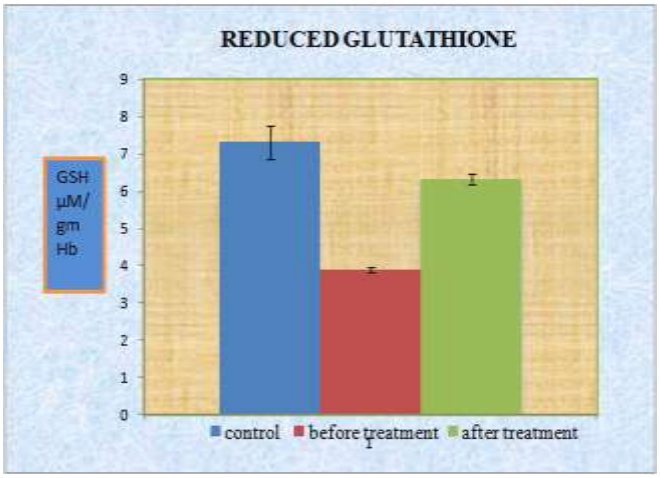


Values are mean ± S.E., N = 30. P < 0.05 vs. normal control group.

Fig. 2 ALT content in serum of tuberculosis patients before treatment and after treatment in correlation with control expressed in terms of U/l. Data was siginificant at (P < 0.05)

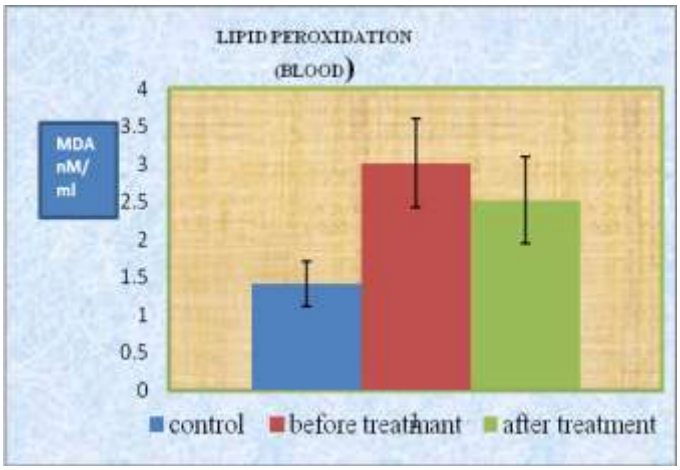
Effect of Tuberculosis Medicines on Lipid Per oxidation And Reduced Glutathione:

Whether oxidative stress is involved in antitubercular therapy is still a matter of debate, but it is considered to be an important parameter in measuring cell damage. Oxidative stress results from an imbalance between oxidants and antioxidants in favor of the oxidants. Non-enzymatic scavengers (antioxidants) as well as enzymatic systems (e.g. glutathione conjugation) are involved in the detoxification of reactive oxygen species. TB patients with Anti Tubercular Drugs Hepatotoxicity have been shown to have low plasma levels of reduced glutathione and high malondialdehyde, which is an oxidative stress parameter, which maybe as a result of oxidative stress from the anti-TB therapy. Fig. 3 and 4 depicts the effect of therapeutic agent of Tuberculosis medicine on LPO and GSH in liver. TB medicines induced intoxication significantly increased TBARS level and significantly decreased GSH content in blood as seen by statistical analysis ($P \leq 0.05$).



Values are mean ± S.E., N = 30. P < 0.05 vs. normal control group.

Fig. 3 GSH content in blood of tuberculosis patients before treatment and after treatment in correlation with control expressed in terms of µM/gmHb. Data presented in fig. was significant at (P < 0.05)



Values are mean ± S.E., N = 30. P < 0.05 vs. normal control group.

Fig. 4 MDA content in blood of tuberculosis patients before treatment and after treatment in correlation with control expressed in terms of nM/ml. Data presented in fig. was significant at (P < 0.05)

Lactate dehydrogenase expression profile in native PAGE

Serum lactate dehydrogenase (LDH) concentration is an indicator of tissue injury. It can increase in a variety of interstitial diseases such as pulmonary tuberculosis. LDH level was found increased in all patients with active PTB as compared to controls. Total serum LDH and its tissue specific isoforms were estimated in TB patients before and after receiving drugs.

There are five isoforms of lactate dehydrogenase present in the almost all the tissues of the body and different isoforms of LDH express upon different injuries. After anti-TB drugs treatment TB patients had elevated level of LDH based on visible staining intensity of LDH1, LDH2 and LDH3 isoforms as compared to control. Expression profile indicated that the tubercular drugs significantly increased the hepatotoxicity in liver as after receiving drugs the LDH 5 level increased as compared to the level in control patients and before treatment patients. LDH 3 is expressed more in lung injury, and expression of LDH 3 increased significantly in both before treatment and after treatment group with comparison to control. This isoenzyme was expressed due to damage in the cell lining of lungs. Profile also indicated that the density of LDH 3 was more in before treatment group as compared to after treatment group. (Fig. 5).

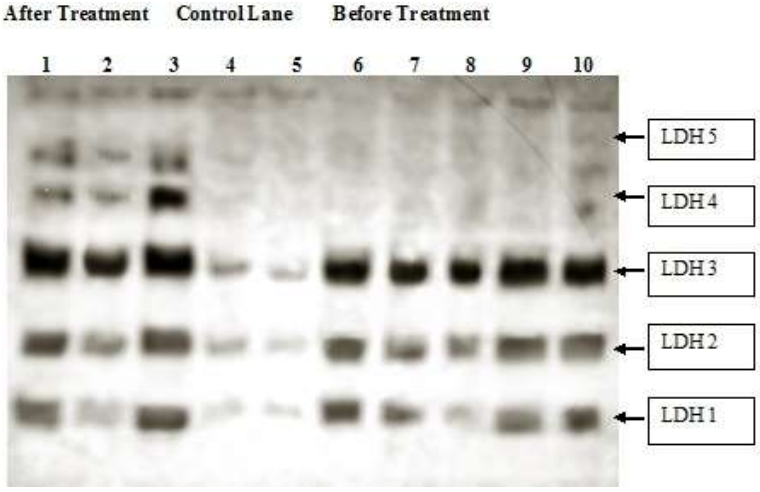


Fig. 5 Native PAGE expression profile of serum lactate dehydrogense of before treatment tuberculosis patients and after treatment tuberculosis patients

Assessment of DNA damage induced by Anti-TB drugs:

The single cell gel electrophoresis (comet assay) allows detection of DNA fragmentation in single cells, and was initially used for DNA damage estimation. Graph showed the effect of anti-TB drug on tail DNA damage, and tail length. The DNA damage was expressed as percent DNA migration in the tail and 20-25 nuclei were counted in each slide. In all anti-TB drug treated groups, the tail length and percent DNA was significantly increased (tail length 1.64). DNA damage was observed and the tail length as well as percent DNA damage was significantly high. All anti-TB drug groups showed almost an increase of 3 folds in the (tail length 1.64 and damage 4.05%). Before treatment tuberculosis patients were also noted (0.287 tail length and 1.48% damage) and control group (tail length 0.228 and damage 0.334%) respectively, controlled DNA damage which was also confirmed with the tail length (fig. 6).

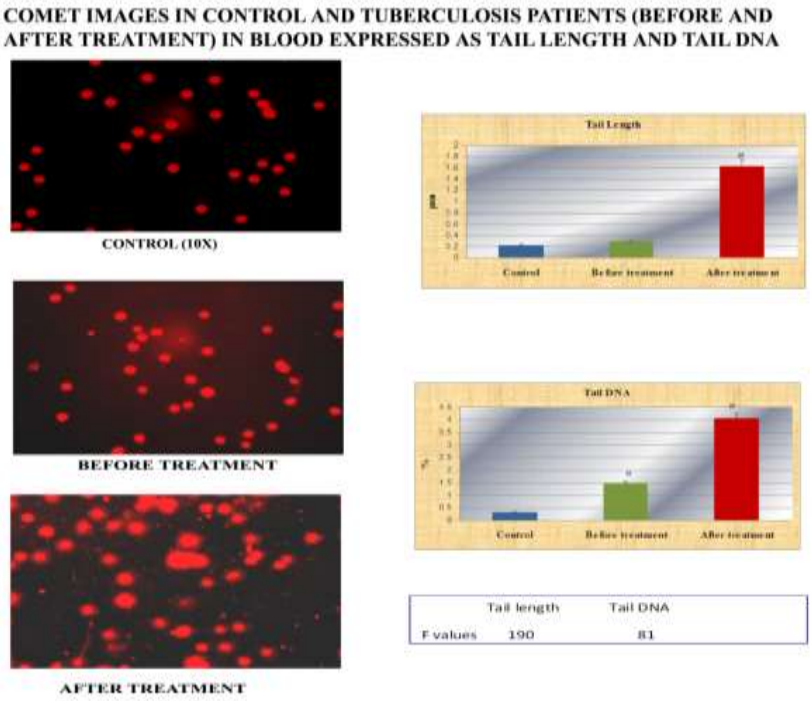


Fig.6 COMET assay analysis of different study groups. (N=30, P= 0.001 at 5 %)

DISSCUSSION

This study was performed to analyze hepatotoxicity and DNA Damage in pulmonary tuberculosis patients for evaluation of the genomic effects of anti-TB drugs. There is clear evidence of a genotoxic activity of these drugs in human lymphocytes, when evaluated by COMET assay. The results of our study also show significant differences between all study groups. Anti-TB drug-induced Hepatotoxicity is one of the most prevalent drug-induced liver injuries. Identification of patients at increased risk for drug induced hepatotoxicity is important because hepatotoxicity causes significant morbidity.

In malnutrition, glutathione stores are depleted which makes one vulnerable to oxidative injury as in a malnourished person liver metabolizes drug at a slower pace. In a study done in India, incidence of Hepatotoxicity was found to be three times higher in malnourished patient's mortality and modification of drug regimen may be required [16].

Patients enrolled in the study were given combination of anti-TB drugs which makes it difficult to conclude which drug was the main culprit for causing hepatotoxicity. Although, INH is the major drug incriminated to induce hepatic injury, role of other possible hepatotoxic drugs (RMP and PZA) can also be speculated. Previous studies conducted have proven that the risk is in the order of INH + RMP>INH>PZA>RMP>E [17].

INH and RMP in TB patients significantly raise the risk of anti-TB drug–induced hepatitis [18, 19]. The exact mechanism of hepatotoxicity caused by PZA is not known. RMP is considered to be less hepatotoxic but is a powerful enzyme inducer, which may enhance INH Hepatotoxicity [20, 21]. Formation of hydrazine, which is the key intermediate of INH metabolism and which is a potent acylating agent capable of causing liver necrosis is facilitated by RMP. In some meta-analysis study INH and RMP given together produce hepatotoxicity more than INH alone [22]. Since INH, RMP and PZA are always

given in combination; it is difficult to diagnose the drug causing hepatotoxicity.

The biochemical mechanism and pathogenesis of drug-induced hepatotoxicity are still not entirely clear for most offending agents. These uncertainties also apply to the hepatotoxicity induced by anti-TB agents. While a dose-related toxicity may exist, a direct correlation between serum drug levels and hepatotoxicity has not been well reported. Thus, the clinical relevance of therapeutic monitoring of serum Rifampicin and Isoniazid concentrations in managing anti-TB drug-associated toxicity is still being explored [23].

The present study is a comprehensive evaluation of concentrations of circulating antioxidants and markers of oxidative stress in tuberculosis patients. Our results show lower antioxidant potential such as reduced glutathione and enhanced lipid peroxidation products (MDA) in before treatment TB patients. After receiving the tubercular drugs the patients showed marked increase in glutathione concentration and decrease in lipid peroxidation products. Our findings further support a role for oxidative stress in the pathogenesis of tuberculosis and suggest lower anti oxidant capacity and higher oxidative stress in the TB patients than in healthy human control [24]. In the present study, it was observed that the free radicals activity increased and total antioxidant status (enzymatic and non-enzymatic) was low in all TB cases, irrespective of treatment status, indicating that there is an oxidative stress. The decrease was more pronounced in the untreated (TB) indicating that the anti-oxidants were nearly completely utilized to scavenge the superoxide free radicals.

Malnutrition may also influence anti-oxidant status and oxidative stress [25]. TB patients are unable to produce sufficient amount of anti-oxidants to cope up with the increased oxidative stress in them.

Serum LDH still remains as one of the important parameters to assess the cell/tissue specific stress or pathology, caused by *Mycobacterium tuberculosis*. Expression profile indicates that the anti-tubercular drugs significantly increase the hepatotoxicity in liver because after receiving drugs the LDH 5 level increases significantly as compared to the control patients and before treatment patients. Some before treatment group also show little intensity of LDH 4 and LDH 5 because the mycobacterium also induces some injury to patients who may be suffering from any other disease. LDH 3 is more expressed in lung injury, and expression of LDH 3 significantly increased in both before treatment and after treatment group with comparison to control. This isoenzyme is expressed due to damage in the cell lining of lungs. Profile also indicates that the intensity of LDH 3 was more in before treatment group in comparison to after treatment group.

Tuberculosis, as a chronic bacterial infection, is expected to induce chromosomal damage in the host by production of cytokines and active metabolites of host immune cells and also by production of microorganism exo/endotoxins [26]. The information available on whether tuberculosis infection per se is clastogenic or not is little and contradictory [27, 28]. Moreover, drugs commonly used in tuberculosis treatment may have a mutagenic effect on the human genome, as may any other foreign chemical agent. This is of greater concern in the newly employed short course regimens, which expose patients to a greater number of drugs, namely Isoniazid (INH), Rifampicin (RIF), Ethambutol (ETB) and Pyrazinamide (PZI).

Since the discovery and general use of anti-TB drugs, their possible mutagenic effects, either alone or in combination, have been studied by many investigators. Mutagenic effects of INH in combination with other drugs have been observed by other investigators [29]. Similar results were obtained in patients exposed to long term anti-TB regimens and short term therapy [30] who are in accord with our results.

CONCLUSION

In the present study it was found that anti tubercular drugs exert significant genotoxicity and hepatotoxicity on the pulmonary tuberculosis patients. This may poses a challenge for physicians treating tuberculosis. The hepatotoxicity may either be pre-existent or may develop during the course of disease or as an adverse reaction to anti-TB drugs. If, however in serious situations, it is considered necessary to continue anti-tubercular drugs, special precautions need to be taken. The long duration of TB treatment is one of the main problems to overcome. Improvement of the bactericidal effect of the anti-TB drugs will reduce treatment length and consequently increase treatment adherence and efficacy.

ACKNOWLEDGEMENT

Authors would like to acknowledge the kind help and advice received from Dr. Sunita Sharma during the research work and Mr. Harshal Mishra for valuable comments and suggestions. The authors are highly indebted to Department of biotechnology MITS Gwalior for providing us the research facilities to carry out this work.

Finally, authors acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. The authors are also grateful to authors / editors /publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

REFERENCES

1. Frieden T.R., Sterling T.R., Munsiff S. S., Watt C.J., Dye C., Tuberculosis, Lancet, 2003, 362, 887–99
2. Yee D., Valiquette C., Pelletier M., Parisien I., Rocher I., Menzies D., Incidence of serious side effects from first-line anti-TB drugs among patients treated for active tuberculosis, Am. J. Respir. Crit Care Med., 2003, 167, 1472–7
3. Breen R.A., Miller R.F., Gorsuch T., Adverse events and treatment interruption in tuberculosis patients with and without HIV co-infection, Thorax , 2006, 61, 791–4
4. Nolan C.M., Goldberg S.V., Buskin S.E., Hepatotoxicity associated with isoniazid preventive therapy: a 7-year survey from a public health tuberculosis clinic, JAMA, 1999, 281, 1014–18
5. Fountain F.F., Tolley E., Chrisman C.R., Self T.H., Isoniazid hepatotoxicity associated with treatment of latent tuberculosis a 7-year evaluation from a public health tuberculosis clinic, Chest, 2005, 128, 116–23
6. Villarino M.E., Ridzon R., Weismuller P.C., Rifampin preventive therapy for tuberculosis infection: experience with 157 adolescents, Am. J. Respir. Crit Care Med., 1997, 155, 1735–8
7. Kidd P.M., Phosphatidylcholine, a superior protectant against liver damage, Alternative Med Rev., 1996, 1,258-274
8. Deleve L.D., Kaplowitz N., Importance and regulation of hepatic glutathione, Seminars Liver Dis., 1990, 10, 251-266
9. Pacht E.R., Timerman A.P., Lykens M.G., Deficiency of alveolar fluid glutathione in patients with sepsis and the adult respiratory distress syndrome, Chest, 1991, 100, 1397-1403
10. Jack C.I.A., Jacken M., and Hind C.R.K., Circulating marker of free radical activity in patients with pulmonary tuberculosis, Tubercle and Lung Disease, 1994, 75, 132-137
11. Zhuang J.C., Wright T.L., DeRojas-Walker T., Tannenbaum S.R., and Wogan G.N., Nitric oxide-induced mutations in the HPRT gene of human lymphoblastoid TK6 cells and in Salmonella typhimurium, Environ.Mol. Mutagen, 2000, 35, 39–47
12. Reitman S., Frankel S., Amer. J. Clin. Path., 1957, 28: 56

13. Sharma S.K., Krishnamurthy C.R., Production of lipid peroxidation of brain, J. Neurochem, 1968, 15, 147–149
14. Brehe J.E., Burch H.B., Enzymatic assay for glutathione, Anal Biochem., 1976, 74,189–197
15. Singh N.P., McCoy M.T., Tice R.R., Schneider L.L., A simple technique for quantitation of low levels of DNA damage in individual cells, Experimental Cell Research, 1988, 175 (1), 184-191
16. Pandle J.N., Risk factors for hepatotoxicity from anti-TB drugs; A case control study, Thorax, 1996, 51, 1326
17. Krishnaswamy K., Prasad C.E., Murthy K.J., Hepatic dysfunction in undernourished patients receiving INH and RMP, Trop Geogr Med., 1991, 43, 156-160
18. Dorteh S., Askgaard D.S., TB chemotherapy: The need for new drugs. Hepatotoxicity caused by the combined action of INH and RMP, Thorax, 1995, 60, 213- 214
19. Altman C., Biour M., and Grange J., Hepatotoxicity of antitubercular agents: Role of different drugs, Presse Med., 1993, 22, 1212-1216
20. Garibaldi R.E., Drusin S.H., Ferebee., INH-associated hepatitis; report of outbreak, Am rev Respir Dis., 1972, 106
21. Singh J., Garg P.k., Thakur V.S., Anti-TB treatment induced hepatotoxicity: Does acetylator status matters, Indian J. Physiol. Pharmacol., 1995, 39, 43-46
22. Steele M.A., Burk R.F., DesPrez R.M., Hepatitis with INH and RMP: a meta analysis, Chest, 1991, 99, 465-471
23. Yew W.W., Therapeutic drug monitoring in anti-TB chemotherapy, 1998, 20, 469–72
24. Rock C.L., Jacob R.A., Bowen P.E., Update on the biological characteristics of the antioxidant micronutrients: vitamin C, vitamin E and the carotenoids, J Am Diet Assoc., 1996, 96, 693-704
25. Hemilia H., Kaprio J., Pietinen P., Albanes D., Heinonen OP., Vitamin C and other compounds in vitamin C rich food in relation to risk of tuberculosis in male smokers. Am J Epidemiol., 1999, 150(6), 632-641
26. Gopal Rao V.V.N., Venkatarama, Gupta E.V., and Thomas I.M., Chromosome damage in untreated tuberculosis patients, Tubercle, 1990, 71, 169–172
27. Jaju M., Jaju M., and Ahuja Y.R., Cytogenetic effects of chemotherapy with three combinations of anti-tubercular drugs involving isoniazid, thiacetazone, para-aminosalicylic acid and streptomycin of human lymphocytes: chromosome aberrations, sister chromatid exchanges and mitotic index, Hum. Genet., 1983, 64, 42–49
28. Ekmekci A., and Sayli A., Cytogenetic study of tuberculosis patients before and after tuberculostatic drug treatment, Mutat. Res., 1995, 334, 175–183
29. Jaju M., Jaju M., and Ahuja Y.R., Combined action of isoniazid and para-aminasalicylic acid in vivo on human chromosomes in lymphocyte culture, Hum Genet., 1981, 56, 375–377
30. Gopal Rao V.V.N., Venkatarama, Gupta E.V., and Thomas I.M., Chromosomal aberrations in tuberculosis patients before and after treatment with short term chemotherapy, Mutat. Res., 1991, 259, 13–19