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Comparative Study on Liver Enzymes (ALT & AST) In the Supervised and Non – Supervised Therapy in Patients of Pulmonary Tuberculosis

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I. Introduction

Tuberculosis is a specific infectious disease caused by Mycobacterium tuberculosis. The disease primarily affect intestine, meanings, bones, joints, lymph – nodes, spleen and other tissue of the body (K Park's Textbook of PSM, 2012, p. 164).

WHO in 1993 declared that "Tuberculosis is a global Emergency?" Because of pitfalls in control, despite the fact that causative organism was discovered hundred years ago, highly effective drugs available BCG vaccine ad free sputum confirmatory examinations, presently one third of world's populations is affected with TB, great majority developing countries.

Based on surveillance and survey data of WHO, there were 9 million new TB cases in 2004 and approximately 2 million died worldwide in ten year, majority (80%) from sub – Saharan Africa & Asia. 80% of all those struck by TB are in economically productive age group (15-49yrs) (WHO, 2004 Weekly epidemiological Record 23rd Jan 2004, 40.4.).

India is the highest TB Country in the world and accounts for nearly 1/5th (20%) of global burden of TB, 2/3rd of cases in SEAR. Every year approximately 1.8 million persons develop TB, of which about 0/8 million are new smear positive highly infectious cases. Tuberculosis kill about 0.32 million people every year. Two of every five Indian are infected with TB bacillus. Everyday about 5000 people develop the disease. Patients with infectious pulmonary tuberculosis disease can infect 10-15% in a year. (Govt. of INDIA, 2010 Annual Report, 2009-10 Ministry of Health & Family Welfare, New Delhi).

WHO has launched global plan for stop TB strategy (2006-2015), with objective to reduce incidence to minimum. DOTS (Directly observed Therapy, Short Term Programme), recognized as fastest expanding programme, launched in 1997, has covered whole country by March 2006.

DOTS refer to process where the patients are watched during swallowing of anti TB drugs, over period of 6-8 months by health personnel. DOTS is incorporated in RNTCP (Revised National TB Control Programme) in India, is comprehensive strategy for TB control. India has adopted and tested DOTS in various parts of the country since 1993 with excellent results. RNTCP now covers 120 million populations (TB India 2006 RNTCP Status Report. DOTS for All, All for DOTS, Central TB Division, Ministry of Health & Family Welfare, New Delhi)

TB is one of the most common diseases among HIV –infected persons worldwide and a major cause of death. In some African countries the rate of HIV infection among TB patients reaches 70-80% in certain urban setting (Harrison's Principles of Internal Medicine, 2012, p. 1349).

The first time drugs used by DOTS and non – supervised therapy are same, only in dosing & schedules are different.

Thus in DOTS (Supervised) Therapy – All Thrice a week.

Drugs	Adult	Children
Rifampicin	450 mg	10 mg /Kg
Isoniazid	600 mg	10-15 mg /kg
Pyrazinamide	1500 mg	35 mg /kg
Streptomycin	750 mg	15 mg /kg
Ethambutol	1200 mg	30 mg/kg

In Non – Supervised Therapy – Daily single dose.

Drugs	Adult	Children
Rifampicin	600 mg	10 mg /Kg
Isoniazid	300 mg	5 - 10 mg /kg
Pyrazinamide	15000 mg	25 mg /kg
Streptomycin	1000 mg	15 mg /kg
Ethambutol	1000 mg	15 mg/kg

First three drugs – Isoniazid, Rifampicin and Pyrazinamide are highly effective against Myco. TB but slightly hepatotoxic. They are extensively metabolized in liver and especially Pyrazinamide shows the hepatotoxic effect in many studies.

LIVER ENZYMES (ALT & AST)

Degree of hepatocellular damage is reflected by rise in Serum Transaminases (ALT & AST). Older patient with concomitant disease with history of Hepatitis and those using Alcohol should be checked first for Liver functions before starting therapy.

Amino transfer as are normally Intra cellular Enzymes with low limit found in plasma representing release of cellular contents during normal cell turnover. Thus elevated plasma level indicates damage to cells rich in these enzymes.

ALT is more specific for liver disease than AST & AST is more sensitive because liver contains more AST.

ALT catalyzes following reaction:

Alanine + α – ketoglutarate $\rightleftharpoons$ **Pyruvate + glutamate**

Enzyme catalyyes transfer of amino group from Alanine to

Pyruvate forming Pyruvate and glutamate. The reaction is readily reversible.

However, during amino acid catabolism, this anzyme functions in direction of glutamate synthesis.

AST – During catabolism, AST transfers amino group from glutamate to oxaloacetate forming aspartate, which is the source of nitrogen in urea cycle.

AST

Oxaloacetate + Glutamate $\xrightarrow[\text{AST}]{}$ **Aspartate + α – ketoglutarate**

Up to 20% of patients have small increase in Aspartate transaminases (up to 3 times the upper limit of normal) with no symptoms and are of no symptoms and are of no significance.

Rise in five to six fold serum transaminases and those with symptomatic hepatitis, treatment should be stopped and reintroduced one at a time after Liver function returns to normal.

Patient should be carefully educated about sign & symptoms of dark urine, loss of appetite, fever etc.

The British Thoracic Society has confirmed that 6 months short course chemotherapy, with an initial phase of 2 months of Isoniazid, Rifampicin, Pyrazinamide and Ethambutol followed by 4 months of Rifampicin and isoniasid is almost 100% successful in the treatment of pulmonary tuberculosis. The society has confirmed that Ethambutol can be replaced by streptomycin in the initial 2 months phase without significant difference in therapeutic outcome. With this regimen, 77% of the patients had sputum conversion to culture negative at 2 months compared with only 64% on the standard 9 months regimen.

Medications should be withheld in the following:

1. Transaminases levels > 3 times upper limit, with symptoms of hepatitis
2. Transaminases levels > 5 times upper limit of normal in asymptomatic persons. (Adopted by Minnesota, Department of Health; from materials developed by Charles P.; Fellow, National TB centre at Harlem Hospital, New York, Dec. 2003).

ALT and AST are present in the cytosol of the hepatocytes. The AST is also found in the mitochondria. Normal level of ALT is 0-45 unit/L and AST is about 0-49 unit/L. in the case of acute necrosis and ischaemia of various organs, e. g. myocardium, high ALT & AST suggests hepatocellular damage while slight rise occurs in cholestasis without cellular damage.

Drug induced hepatotoxicity is a potentially serious adverse effect of antituberculosis (ATT) regimen. A higher risk of hepatotoxicity has been reported in Indian patients (up to 11.5%) than in their western counterpart (up to 4.3%). The only measure available for managing hepatotoxicity is stopping the offending agents, once there is an evidence of liver damage and reintroducing the same after normalization of liver enzymes.

The pathogenesis of hepatotoxicity is not entirely clear but Isoniazid and Rifampicin induced damage may involve oxidative stress, lipid peroxidation, choline deficiency leading to lowering of phospholipids protein synthesis with alteration in cell wall configuration, reduced glutathione level and activation of CYP2E1. {Ref. Adhvaryu et al ; World Journal of Gastroenterology, 2008;14(30)}.

Observational versus Non-Observational Therapy

1. In observational therapy, patient takes medicines in front of health personal where as in daily therapy patient takes drugs at home.
2. There is proved relationship of drug dosing with hepatotoxicity. Overall dosage of drug is more in daily therapy.
3. In Non-Observational therapy, as patient become asymptomatic after 2 months, they tend to stop the drug, where as in Observational group, it's the duty of health personnel to compel the patient to complete the course and prevent relapse & resistance.
4. Thus Observational therapy is more compliant and adherent to the patients.

Work done on the incidence of hepatic toxicity and the clinical
efficacy in patients on DOTS Regimen, in comparison to daily regimen in this part of country is less. This fact attracted our mind to work on the present study. So it has been planned to observe and analyze the hepatic toxicity (especially on serum ALT and AST level) and clinical efficacy in patients of pulmonary TB (category 1) in the 6 months of therapy on DOTS regimen versus non – DOTS (daily regimen).

As RIMS Ranchi is a pioneer institute of Jharkhand state, and the population residing in this state are tribal people (27.6%) who are not only economically, socially and educationally backward but they mostly believe in the orthodox treatment of even most severe and complicated disease. The incidence and prevalence of pulmonary tuberculosis in these tribal belts are alarming. The good compliance and the ensured institution of drugs for pulmonary tuberculosis will certainly increase the cure rate of disease. The supervised short – course therapy would certainly have a good impact on the fact. Most of the patients on non- supervised therapy discontinue their treatment and turn to be treatment failure case.

II. Aims And Objecttives

1. Observation of alterations in liver function including changes in serum bilirubin, serum alkaline phosphates and serum aminotransferases in patient of pulmonary tuberculosis taking antitubercular drug according to supervised and non-supervised therapy.
2. Observation of changes in liver function in patient different age and sex of pulmonary tuberculosis taking anitubercular drugs under supervised and non-supervised therapy.
3. To established patient's drug dose efficacy and to adjust for safer regimen.

III. Materials & Methods

The present study " Comparative study on liver enzymes (ALT & AST) in the supervised and non – supervised therapy in patients of pulmonary tuberculosis" was carried out in 100 cases receiving Category 1 therapy. Patients were selected from outdoor patients and indoor patients from the Department of Medicine, Rajendra Institute of Medical Sciences, Ranchi

Only those patients were included in the study who were not associated with any other systemic disease.Before starting therapy, following pretreatment clinical evaluation counseling were done:

1. Voluntary HIV counseling and testing
2. History or any indication of hepatitis or cirrhosis
3. Any history of regular alcohol intake
4. Pregnancy of < 3 months postpartum
5. Abnormal baseline LFT

Now the study was made of 2 groups 'I' & 'II' excluding above cases.

A. Group-I(Supervised) – It comprised of patients (n=50) "TB & Chest Unit", RIMS, receiving DOTS, Category 1 Therapy.

B. Group –II)Non-supervised) – It comprised of patients attending regularly the OPD of Medicine and indoor of the Medicine of pulmonary tuberculosis receiving daily regimen of Anti TB drugs.

In Group I Category I was given thrice weakly, whereas Group-II taking Anti TB drugs with DAILY THERAPY. Patients under supervision were asked to report thrice weakly for 2 months and then monthly for 4 months, whereas patients of non-supervised therapy were asked to report monthly and were reassured for minor problems with drug intake.

Patients non reporting timely were excluded from study and sputum smear positive patients were included in the study.

Methodology for measurement of enzymes (ALT & AST)

The assay system for measuring transaminases activity contains two amino acids and two oxo-acids (keto acids). As there is no convenient method for assaying amino acids, formation or consumption of the oxo-acids is measured. Previously, various photometric substrates (2, 4 dinitrophenyl hydrazine and various dyes) were coupled with transaminase reactions, but nowadays considered obsolete.

Today, continuous monitoring methods are used to measure transaminase activity, by coupling the transaminase reaction to spedific dehydrogenease reactions. The oxo-acids formed in transaminase reaction are measured indirectly by enzymatic reduction to reduction to corresponding hydroxyl acids and the accompanying change in NADH concentration is monitored spectrophotometrically

Collection of sample

3ml of venous blood sample were collected by a dry sterilized syringe into a dry plain vial. The sample was allowed to clot for separation of serum. Then serum was centrifuged for 5 minutes at 3000 rpm. The clear supernatant serum was pipette into a clean dry test tube for ALT & AST measurement, which was done on RA50 system (semi – Auto Analyzer).

Storage and stability

Serum ALT and AST were carried out on the same day as activity of enzymes decreases progressively with time. Hemolysed or grossly contaminated samples were discarded.

MEASUREMENT OF ALT (ALAMINE TRANSAMINASE)

The present study, estimation of ALT and AST were done by UV kinetic method according to recommendation of expert panel of IFCC (International Federation of Clinical Chemistry)

Reagents

Reagent – 1 (Enzymes):-		
LDH	-	$\geq$1200 u/L
NADH	-	0.20 mmol/L
α – Ketoglutarate	-	16 mmol/L
Reagent – 1A (Buffer):-		
Tris Buffer (pH 7.5)	-	110 mmol/L
L- Alanine	-	550 mmol/L

Reagent Reconstitution

Reagents were allowed to attain room temperature. One bottle of Reagent -1 was dissolved with one bottle of Reagent 1A and mixed by gentle swirling. Reconstituted reagent is stable for 4 weaks when stored at 2^0-8^0 C.

System Parameter set as :

Reaction type	-	Kinetic
Reaction slope	-	Decreasing
Wavelength	-	340nm
Flow cell temp	-	37^0 C
Delay time	-	60 sec
No. of reading	-	4
Interval	-	60 sec
Sample volume	-	100 ul
Reagent volume	-	1ml
Path length	-	1cm
Factor	-	1746
Zero setting with	-	Distilled water

Principle

$$\text{L – Alanine} + \alpha\text{ – Ketoglutarate} \xrightleftharpoons{\textbf{ALT}} \text{L – glutamate} + \text{Pyruvate}$$

$$\text{Pyruvate} + NADH^+ + H^+ \xrightleftharpoons{\textbf{LDH}} \text{L – glutamate} + \text{Pyruvate}$$

As the reaction proceed, $NADH^+$ is oxidized to NAD^+. The disappearance of NADH is followed by measuring the decrease in absorbance at 340nm for several minutes. The change in absorbance per minute (ΔA/min) is proportional to the micromole of NADH oxidized and in turn to micromoles of substrate transformed per minute.

Procedure

Pipetting of working reagent and sample was done carefully into clean dry test tube as follows.:-

Reconstituted working reagent - 1ml
Serum sample - 100µl

Preparation was mixed and reading taken by semi auto analyzer ((RA-50 system)
Reference value – SGPT (ALT)
Serum / plasma – Upto 49/U/L (37^0 C)

MEASURMENT OF AST (ASPARTATE TRANSMINASE)

This is done by UV kinetic (IFCC) method in RA 50, semi- auto analyzer.

Reagents

Reagent - 1 (Enzymes):-
MDH - $\geq$ 600u/L
LDH - $\geq$ 900 u/L
NADH - 0.20 mmol/L
α – Ketoglutarate - 12 mmol/L
Reagent –A (Buffer): -
Tris Buffer (pH 7.80) - 88 mmol/L
L- Aspartate - 260 mmol/L

Reagent Reconstitution

Reagents were allowed to attain room temperature. On e bottle of Reagent -1 was dissolved with one bottle of Reagent 1A and mixed by gentle swirling. This reagent is stable for 4 weeks and stored at 2^0-8^0 C.

System Parameter set as :

Reaction type - Kinetic
Reaction slope - Decreasing
Wavelength - 340nm
Flow cell temp - 37^0 C
No. of reading - 4
Interval - 60 sec
Sample volume - 100 ul
Reagent volume - 1ml
Path length - 1cm
Factor - 1746
Zero setting with - Distilled water

Principle

$$\text{L – Aspartate} + \alpha\text{ – Ketoglutarate} \xrightleftharpoons{\text{ALT}} \text{L – glutamate} + \text{Oxaloacetate}$$

$$\text{Oxaloacetate} + NADH^+ + H^+ \xrightleftharpoons{\text{MDH}} \text{L – Malate} + NAD^+$$

AST = Aspartate amino transferase
MDH = Malate dehydrogenase

There is decrease in absorption at 340nm as NADH is converted to NAD. The change in absorbance per minute (A/min) is proportional to the micromoles of NADH oxidized and in turn to micromoles of substrate transformed per minute. Δ

Procedure

Pipetting of working reagent and sample was done carefully into clean dry test tube as follow:-

Reconstituted working reagent - 1ml
Serum sample - 100µl

Preparation was mixed and reading taken by RA-50 system (semi- auto analyzer).
Reference value – SGOT (AST)
Serum /plasma – Upto 46 U/L (37^0C)

IV. Results

TABLE – 1: BASELINE DEMOGRAPHIC CHARACTERISTIC OF TB PATIENTS

Characteristics	**Total patient (n=100)**
Age (Years)	39.79 ± 11.64
Sex (M/F)	68/32
WEIGHT (KG)	43.5±7.5

Above table shows the general demographic characteristics of the patient involved in the study.

TABLE – 2: CATEGORY OF PATIENTS

	No. of patients	**%**
Tribe	66	66
Non – Tribe	34	34

Above table shows the tuberculosis is commoner in tribal population in Jharkhand.

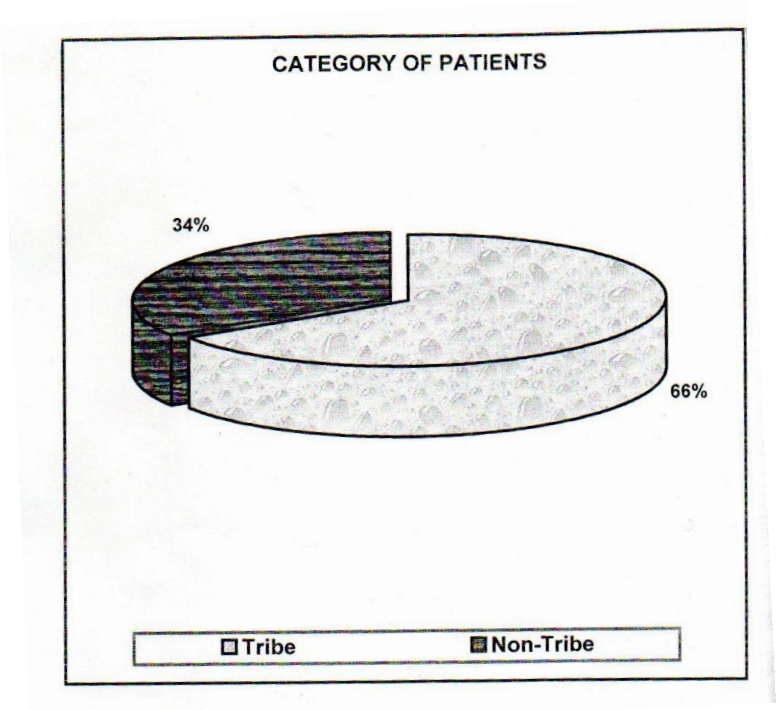


TABLE – 3: AGE & SEX DISTRIBUTION OF CASES (IN BOTH GROUP – I & GROUP – II)

Group	**Age group (years)**					
	15-30		**31-45**		**46-60**	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
Group – I	10	8	14	5	8	5
Group – II	10	6	14	4	12	4

Group – I = Supervised therapy (DOT therapy)
Group – II = Non supervised Therapy (Daily Therapy)
Total M/F in Group – I = 32/18
Group – II = 36/14

TABLE – 4: ENZYME LEVEL (ALT & AST) IN GROUP I & II CASES BEFORE STARTING THERAPY

Group	No of cases	ALT				AST			
		Range	Mean	S.D	Result	Range	Mean	S.D	Result
Group - I	50	12-26	18.2	5.3	t=1.07	15-28	20.5	3.94	t=0.31
Group - II	50	12-25	19.28	4.7	p=0.28	12-30	20.2	5.59	p=0.75

The above table shows that in Group – I cases (n=50) the rangeof serum ALT varied from 12-26 u/L mean value 18.2, S.D. = 5.3, S.E.M. = 0.75. The range of serum AST varied from 15-28 u/L with mean value 20.5, S.D. = 3.94 and S.E.M = 0.55

In Group – II cases (n=50), the range of serum ALT varied from 12-25 u/L, mean 19.28, S.D. = 4.7 and S.E.M. = 0.67. The range of serum AST level is 12-30 u/L, mean = 20.2, S.D. = 5.59 and S.E.M. = 0.79.

For ALT in group I & II 't' VALUE = 1.07 and 'p' value = 0.28, which is clinically not significant. Result of AST (P=0.75, . 0.05) is not significant.

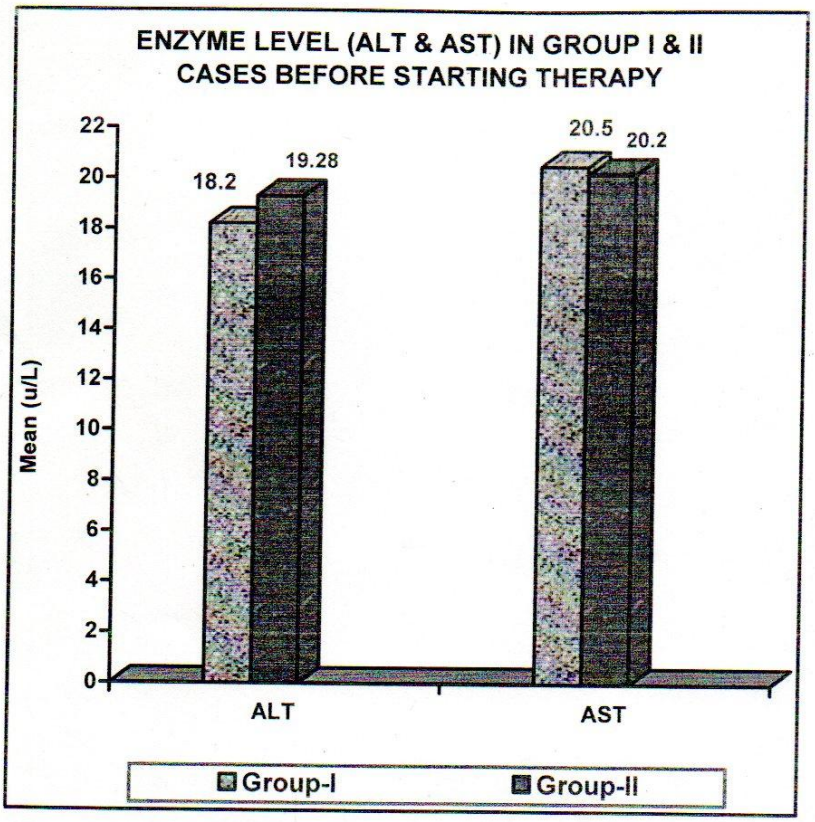
TABLE – 5: ENZYME LEVEL (ALT & AST) IN GROUP I & II CASES AFTER 1 MONTH OF THERAPY

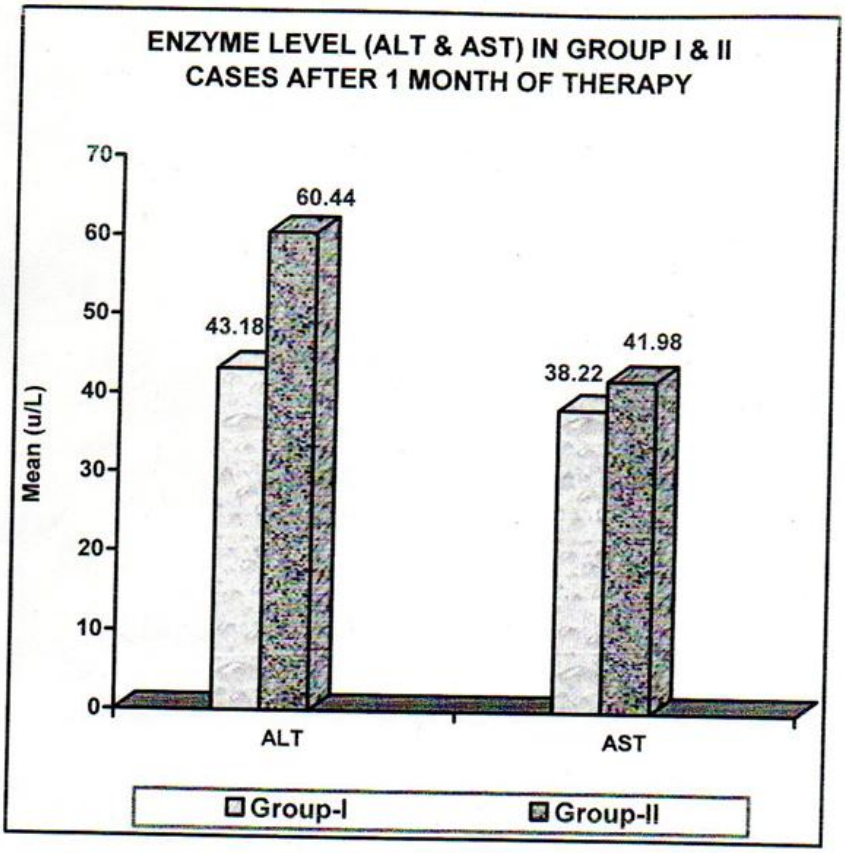
Group	No of cases	ALT				AST			
		Range	Mean	S.D	Result	Range	Mean	S.D	Result
Group - I	50	20-82	43.18	19.6	t=3.09	30-60	38.22	8.85	t=2.006
Group - II	50	30-240	60.44	34.21	p= 0.0026	24-60	41.98	9.87	p=0.04

The above table shows wide variation of ALT in 1 month of Anti – TB therapy. Group –I (n=50) shows range 20-82 u/L with mean 43.18, S.D. = 19.6. and S.E.M. = 2.8. The range of serum AST is 30-60 with mean 38.22 u/L, S.D. = 8.85 and S.E.M. = 1.25.

In group – II cases (n=50) the range of serum ALT varied from 30-240 with mean 60.44, S.D. = 34.21 and S.E.M. = 4.83. The range of AST varied from 24-60, with mean = 41.98 S.D. = 9.87 and S.E.M. = 1.39.

There is highly significant 'p' value for ALT and AST.




ENZYME LEVEL (ALT & AST) IN GROUP I & II
CASES AFTER 1 MONTH OF THERAPY
Mean (u/L)
70
60
50
40
30
20
10
0
60.44
43.18
41.98
38.22
ALT
AST
Group-I
Group-II

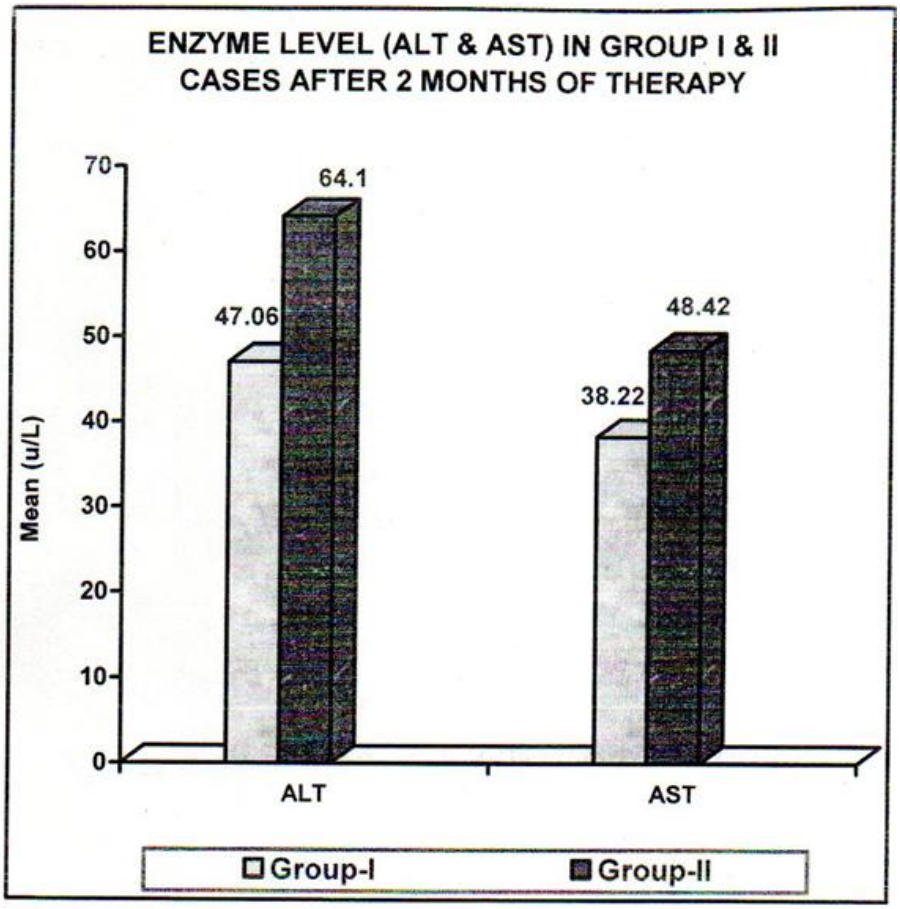
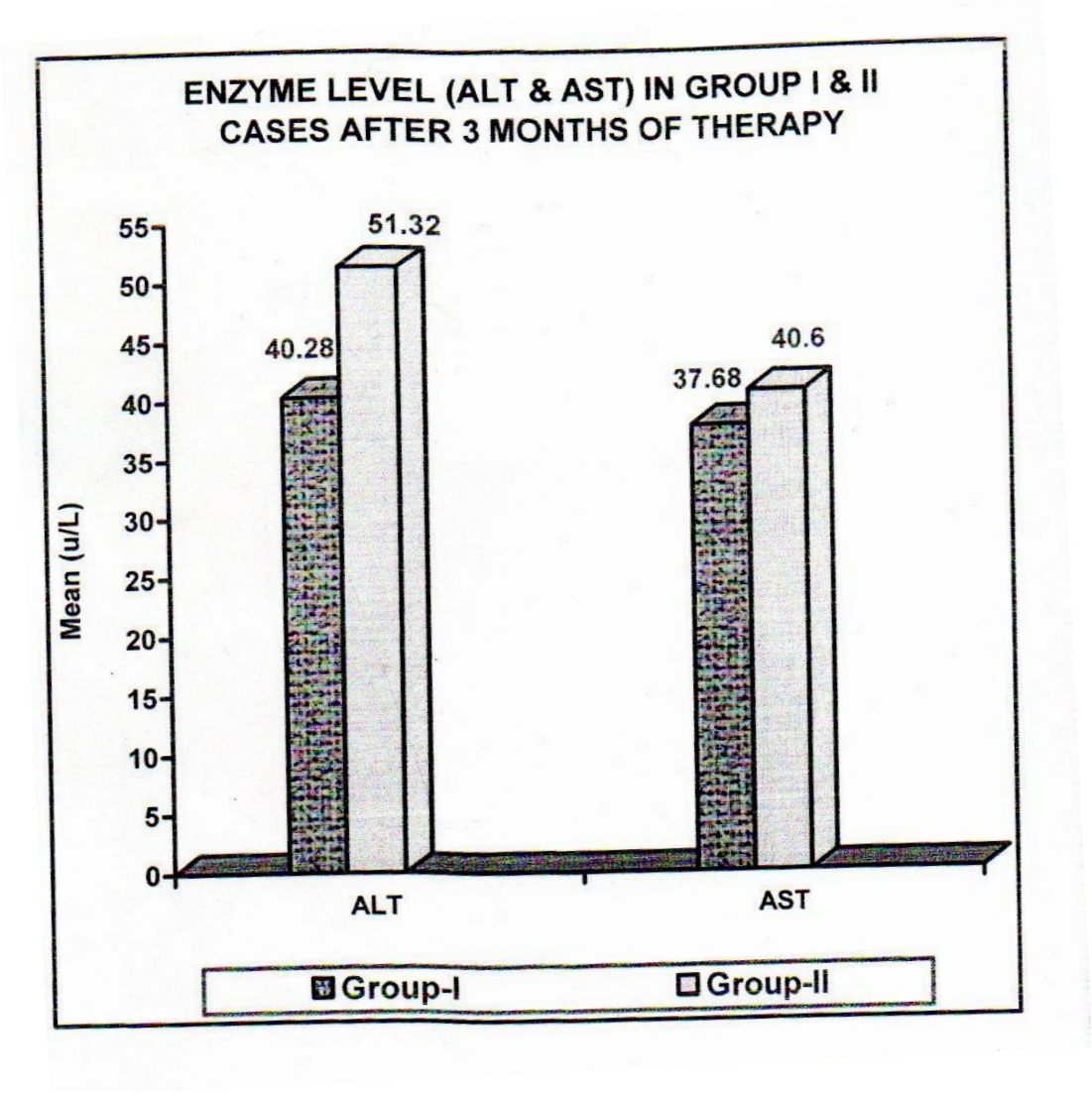

ENZYME LEVEL (ALT & AST) IN GROUP I & II
CASES AFTER 2 MONTHS OF THERAPY
Mean (u/L)
70
60
50
40
30
20
10
0
64.1
47.06
48.42
38.22
ALT
AST
Group-I
Group-II

TABLE – 6: ENZYME LEVEL (ALT & AST) IN GROUP I & II CASES AFTER 2 MONTHS OF THERAPY

Group	No of cases	ALT				AST			
		Range	Mean	S.D	Result	Range	Mean	S.D	Result
Group - I	50	25-160	47.06	25.62	t=2.95	30-60	38.22	8.85	t=4.82
Group - II	50	35-175	64.1	31.81	p= 0.004	24-72	48.42	12.05	p= <0.001

The above table shows in Group – I cases (n=50) the range of serum ALT varied from 25-160 with mean 47.06, S.D.=25.62, S.E.M. = 3.62. AST level varied from 30-60 with mean 38.22,S.D.= 8.85 and S.E.M. = 1.25.

In Group – II cases (n=50) range of serum ALT varied from 35-175 with mean 64.1,S.D. = 31.81 and S.E.M. = 4.49. The range of AST varied from 24.72, mean = 48.42, S.D.=12.05 and S.E.M. = 1.7.
'p' value is highly significant in both Group I & II for ALT and AST.



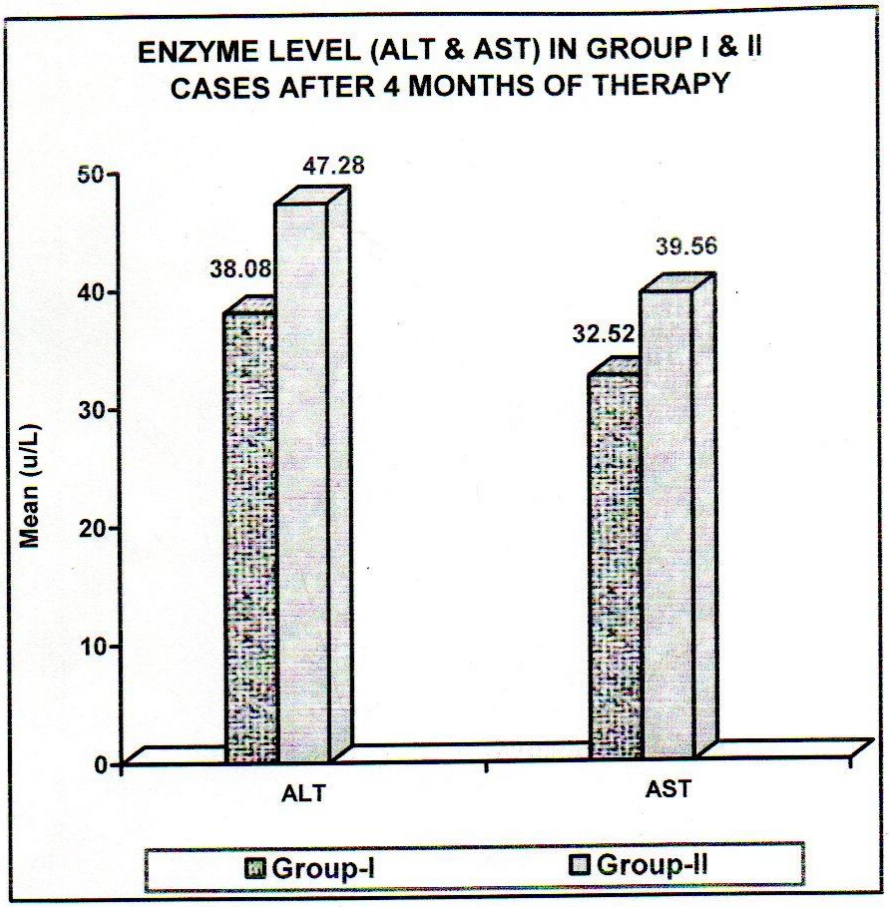


TABLE – 7: ENZYME LEVEL (ALT & AST) IN GROUP I & II CASES AFTER 3 MONTHS OF THERAPY

Group	No of cases	ALT				AST			
		Range	Mean	S.D	Result	Range	Mean	S.D	Result
Group - I	50	30-62	40.28	7.96	t=4.60	27-74	37.68	7.56	t=1.77
Group - II	50	28-98	51.32	14.97	p= 0.0001	24-65	40.6	8.83	p= 0.07

The above table shows in Group – I cases (n=50) the range of serum ALT varied from 30-62 with mean 40.28, S.D. = 7.96, S.E.M. = 1.12. AST level varied from 27-74 with mean 37.68, S.D. = 7.56 and S.E.M. = 1.06.

In Group – II cases (n=50) range of serum ALT varied from 28 -98 mean 51.32, S.D. = 14.97 and S.E.M. = 2.13. The range of AST varied from 24-65, mean = 40.6, S.D. = 8.83 and S.E.M. = 1.24.
Alt level in Group – I and II is highly significant.

TABLE – 8: ENZYME LEVEL (ALT & AST) IN GROUP I & II CASES AFTER 4 MONTHS OF THERAPY

Group	No of cases	ALT				AST			
		Range	Mean	S.D	Result	Range	Mean	S.D	Result
Group - I	50	24-55	38.08	9.21	t=4.51	23-42	32.52	4.71	t=6.07
Group - II	50	32-82	47.28	11.09	p= <0.0001	20-52	39.56	6.7	p= <0.0001

The above table shows in Group – I cases (n=50) the range of serum ALT 24-55u/L with mean 38.08, S.D. = 9.21, S.E.M.=1.3. The range of serum AST varied from 23-42u/L, mean 32.52, S.D.= 4.71 and S.E.M. = 0.66.

In Group –II cases (n=50) range of serum ALT varied from 32-82 with mean 47.28, S.D. = 11.09 and S.E.M. = 1.56. the range of serum AST is 20-52, mean = 39.56, S.D. = 6.7 and S.E.M. = 0.94.
The difference level of ALT and AST is highly significant.

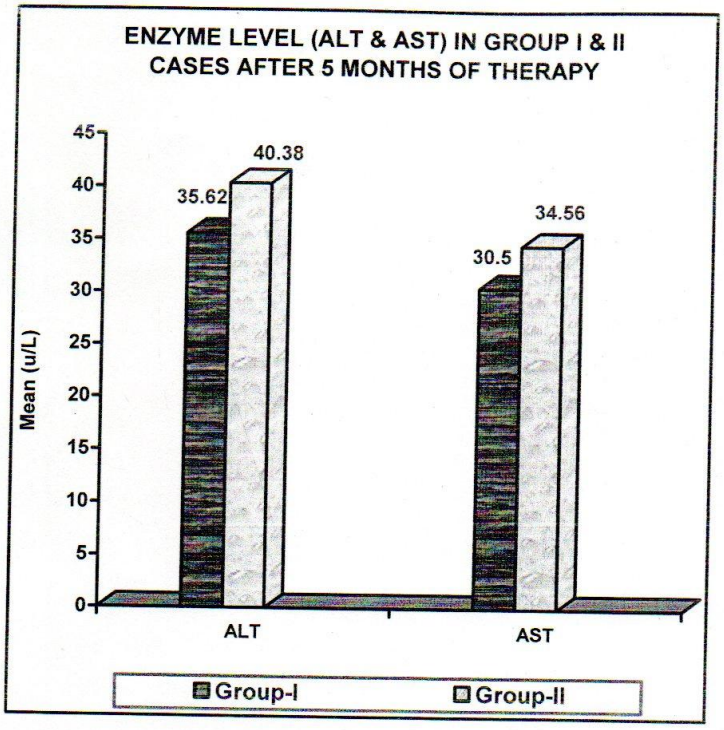


TABLE – 9: ENZYME LEVEL (ALT & AST) IN GROUP I & II CASES AFTER 5 MONTHS OF THERAPY

Group	No of cases	ALT				AST			
		Range	Mean	S.D	Result	Range	Mean	S.D	Result
Group - I	50	28-46	35.62	4.6	t= 3.79 p= <0.0003	24-42	30.5	4.40	t=4.66 p= <0.0001
Group - II	50	30-58	40.38	7.58		26-45	34.56	4.27	

The above table shows in Group – I cases (n=50) the range of serum ALT 28-46 u/L with mean 35.62, S.D. = 4.6, S.E.M. = 0.64. The range of serum AST varied from 24-42 u/L, mean 30.5, S.D. = 4.40 and S.E.M. = 0.62.

In Group – II cases (n=50) range of serum ALT varied from 30-58 with mean 40.38, S.D.=7.58 and S.E.M.= 1.07. The range of serum AST is 26-45, mean = 34.56, S.D. = 4.27 and S.E.M. = 0.60.
This shows highly significant difference of both ALT and AST in Group I & II.

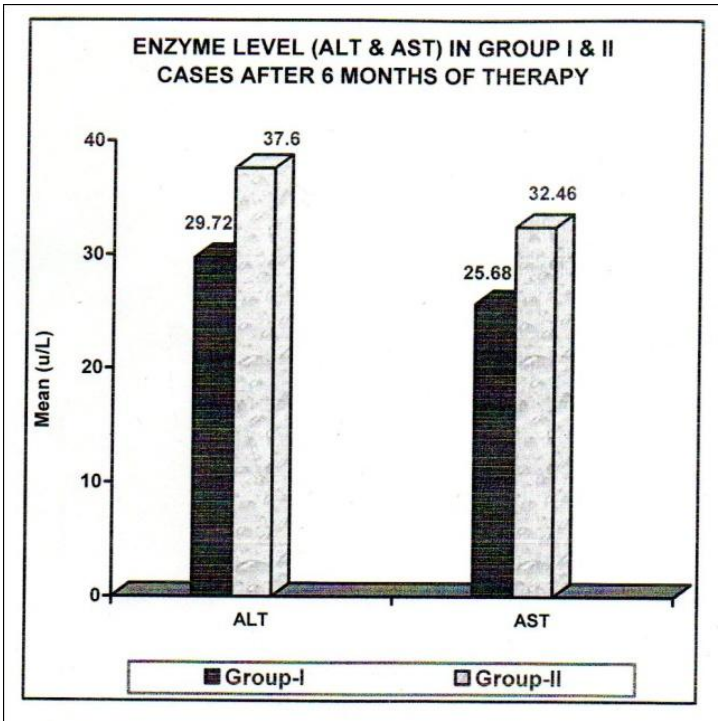


TABLE – 10: ENZYME LEVEL (ALT & AST) IN GROUP I & II CASES AFTER 6 MONTHS OF THERAPY

Group	No of cases	ALT				AST			
		Range	Mean	S.D	Result	Range	Mean	S.D	Result
Group - I	50	22-42	29.72	7.07	t= 5.27 p= <0.0001	20-36	25.68	3.75	t=6.85 p= <0.0001
Group - II	50	24-54	37.6	7.85		20-46	32.46	5.9	

The above table shows in Group – I cases (n=50) the range of serum ALT level varied from 22-42, mean 29.72, S.D. = 7.07,S.E.M.=1.00. The range of AST is 20-36, mean25.68, S.D. = 3.75 and S.E.M. = 0.53. The Group – II cases (n=50), the range of serum ALT varied from 24-54 with mean 37.6, S.D. = 7.85 and S.E.M. = 1.11. The range of serum AST is 20-46, mean = 32.46, S.D. =5.9 and S.E.M. = 0.83.
Both ALT and AST difference is highly significant.

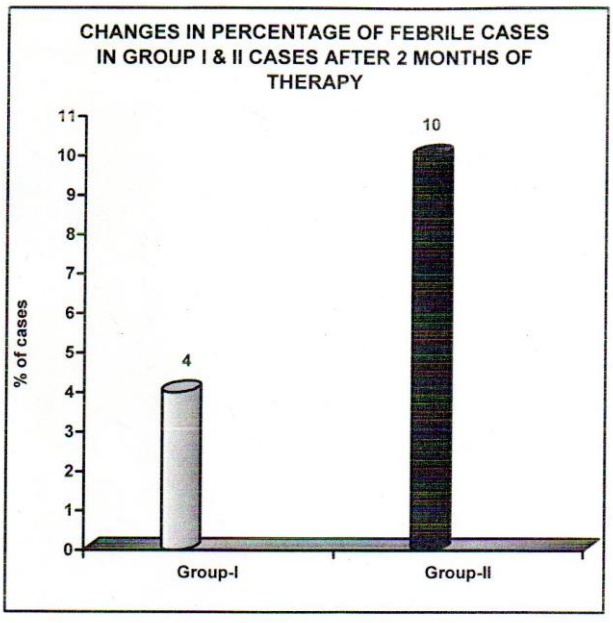


TABLE – 11: MALE /FEMALE DISTRIBUTION OF HEPATOTOXICITY

Group	Sex	No. of cases	Cases of Hepatotoxicity
Group – I	Male /Female	32/18	1:1
Group –II	Male /Female	36/14	2:2

Overall M/F hepatotoxicity rate = 4.4% /9.37%

TREATMENT OUTCOME IN GROUP I GROUP I AND GROUP II

TABLE – 12: CHANGES IN PERCENTAGE OF FEBRILE CASES IN GROUP I & II CASES AFTER 2 MONTHS OF THERAPY

Group	Total No of Cases	No. of febrile cases after 2 months	%	Fisher exact test
Group – I	50	2	4	P value = 0.436
Group –II	50	5	10	

Above table shows 2 febrile cases in Group – I 2 Months of therapy and 5 cases in Group –II. 'p' value is not significant.

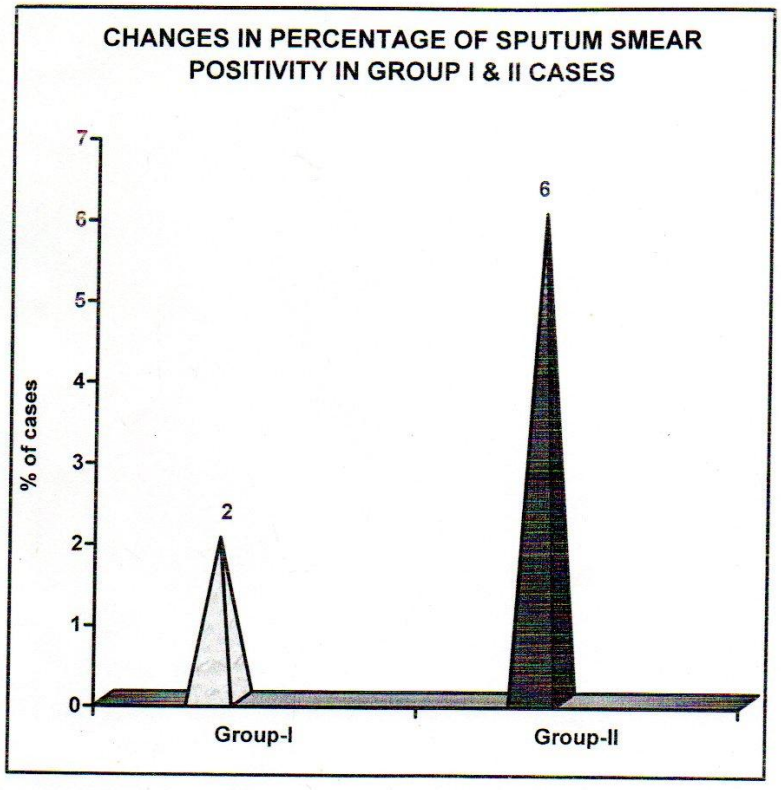


TABLE – 13: CHANGES IN PERCENTAGE OF SPUTUM SMEAR POSITIVITY IN GROUP I & II CASES

Group	Total No of Cases	Sputum smear + ve cases after 2 months of intensive treatment	%	Fisher exact test
Group – I	50	1	2	Odds ration = 3.12 p value = 0.33
Group –II	50	3	6	

Above table shows patients with 2 months of intensive phase, 1 patient remained sputum +ve in Group – I (supervised therapy) whereas 3 patients remained sputum +ve in Group – II (non- supervised therapy) which is clinically significant but statistically not significant.

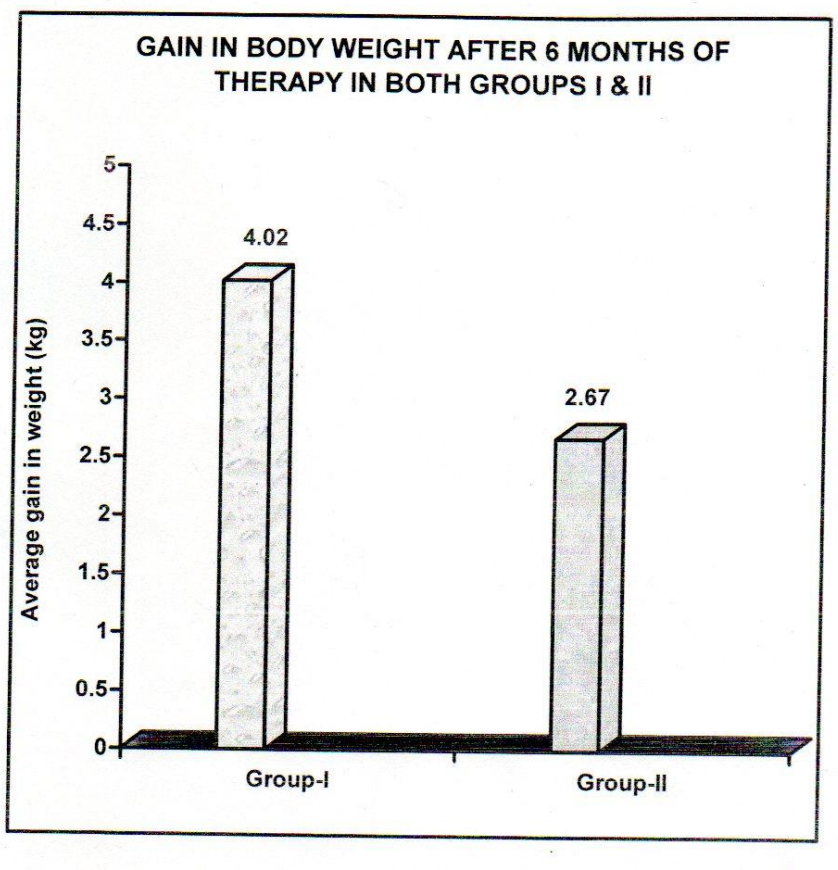


TABLE 14: GRAIN IN BODY WEIGHT AFTER 6 MONTHS OF THERAPY IN BOTH GROUPS I & II

Group	Total no. of cases	Mean weight before therapy (kg)	Mean weight after therapy (kg)	Average gain in weight (kg)	Result (paired 't' test)
Group –I	50	4.02±6.74	47.04±6.78	4.02	t= 2.97 p=<0.005
Group –II	50	43.54±8.22	46.21±8.28	2.67	t=1.61 p=0.1088

Above finding shows increased gain in weight in Group – I patient. There is average gain in weight in Group – I is 4.02 kg whereas in Group – II gain in weight is 2.67kg.
Result is highly significant in Group – I but not significant in Group – II.

TABLE – 15: CASES OF HEPATOTOXICITY

Group	No. of TB patients	No. of patients with hepatotoxicity	%	Result
Group – I (Observational therapy)	50	2	4	Odds ratio = 2.08 P=0.40
GROUP – II (Non – Observational therapy)	50	4	8	

Above table shows the cases of hepatotoxicity in Group – I in 2 whereas 4 in Group – II. With odds ration 2.08, 'p' value is not significant.

Tuberculosis remains a world wide public health problem despite the fact that the causative organism was discovered more than 100 years ago and highly effective drugs and vaccines are available making tuberculosis a preventable and curable disease.

The main features responsible for the disease to reemerge as a major public health problem are poor compliance to chemotherapeutic regimen, illiteracy, lack of awareness of cause of illness, poverty and homelessness of attendants, the compliance is even more poor in intravenous drug users, alcoholics, HIV positive and hepatitis – B infected patients.

The present study (prospective study) is Comparative study on liver enzymes (ALT & AST) in the supervised and non supervised therapy in patients of pulmonary tuberculosis" was carried out on the 100 cases of category I of pulmonary tuberculosis patients.

Patients were selected for 2 groups of study. Group I comprised of 50 patients which were on supervised therapy taking drugs.

Rifampicin 450mg, Isoniazid 600mg, Pyrazinamide 1500mg and Ethambutol 1200mg all thrice weakly in intensive phase (2 months) then Rifampicin and Isoniazid thrice weakly for 4 months of continuation phase.

Group II (non-supervised therapy) were comprised of 50patients of pulmonary tuberculosis taking drugs-Rifampicin 600mg, Isoniazid 300 mg, Pyrazinamide 1500mg and Ethambutol 1000mg dialy for 2 months (intensive phase) and again continuation therapy with Rifampicin and Isoniazid for 4 months. Before starting drug therapy, liver function test was done in all patients (abnormal liver enzymes, alcoholic, hepatitis B positive, preexisting liver disease patients were excluded from the study).

General demographic characteristics of the patients were having mean age 39.79$\pm$11.64 years, 68 males, 32 females and mean weight 43.5$\pm$7.5kg (Table 1).

There were 66 tribal patients 34 non-tribal patients signifying prevalence of tuberculosis in low socioeconomic group in the state (Table 2).

A prospective cohort study evaluation of anti tuberculosis drug induced liver enzyme elevation was undertaken monthly in both group I & II for 6 months. Patients were instructed to report any sign and symptoms of hepatotoxicity like anorexia, nausea, vomiting, fever and jaundice.

In both group I and group II, enzyme level before therapy was within normal limit. In group I, mean ALT level was 18.2$\pm$5.3 IU/lit. with S.E.M. O.75 and in group II mean ALT level was 19.28$\pm$4.7 IU/lit. S.E.M. 0.67 with 't value 1.07 and 'p' value 0.28 which is not significant (because $p>0.05$). whereas mean AST value in group I was 20.5$\pm$3.94 IU/lit. with S.E.M. 0.55. In group II mean AST level was 20.5$\pm$5.59 IU/lit. S.E.M 0.79 't' value 0.31 and 'p' value 0.75 which is not significant (because $p>0.05$). Thus difference of values is not significant (Table 4).

Al value are within normal range of ALT & AST (normal range ALT – 0.49 IU/lit. AST – 0.45 IU/lit.).

After I month of therapy it was analyzed that most of the patients in both groups showed increase in liver enzymes ALT & AST but mostly were asymptomatic with < 3 fold elevation of serum transaminases. Mean value of ALT in group I was 43.18$\pm$19.6 IU/lit. and in group II it was 60.44$\pm$34.21 IU/lit. with S.E.M 2.77 and 4.83 respectively ('t value 3.09, p value 0.0026) i.e highly significant. The difference of mean is highly significant statistically but not significant clinically because 2-3 times of upper limit of normal (ULN)were of not much significant after anti tuberculosis drugs.

Patients who showed elevation in transaminases (>3x ULN but < 5x ULN) were continuing their anti tuberculosis regimen but under strict observation. Fortunately their enzyme level normalized within a month of continued treatment in both groups.

Mean AST level in group I were 38.22$\pm$8.85 IU/lit. and in group II 41.98$\pm$9.87 IU/lit. with t value 2.006 an p value 0.04 (significant). This shows also the difference in AST level in both groups but was within normal range (Table 5).

During the study period, 2 patients in group I and 4 patients in group II developed features of hepatotoxicity, detected by symptoms and confirmed by liver function test. The time interval from initiation of therapy to onset of hepatotoxicity was 12-60 days (median 28 days) (Table-15).

Symptoms shown by all 6 patients (2 in group I and 4 in group II) developing hepatotosicity was almost nausea, vomiting, abdominal discomfort, anorexia and jaundice. Due to this anti-tubercular therapy were stopped for a weak until their clinical and biochemical picture were found normal, reinstitution of anti tuberculosis therapy was done in sequential fashion, rifampicin and Isoniazid. Pyrazinamide was withdrawn completely because. PZA may exhibit both does dependant and idiosyncratic hepatotoxicity (Am. Thoracic Society 2006). These values are in accordance with the observation made by Hong Kong chest Service/British Medical Research Council 2006. The drugs, which can be safely given in liver disease, include Aminoglycosides, Ethambutol, Quinolones and Cycloserine.

The incidence of hepatotoxicity in both froups (group I and group II) is much higher than previous studies from USA and UK Timbrell JA, 1985). The incidence of hepatotosicity has been reported to be higher in developing countries and factors such as acute and chronic liver disease, poor nutrition, wide spread parasitism, chronic infections, indiscriminate use of various drugs, ethnic factors, severity of disease of genetic predisposition may play a role individually or collectively (Kumar A, 1991; Ugno J R, 1998).

At the 2 months of therpy, there was again variantion in the liver enzymes. In group I, Mean ALT level was 47.06 $\pm$ 25.62 IU/lit. with S.E.M 3.62 and in group II it was 64.1 $\pm$ 31.81 IU/lit. with S.E.M 4.49. the t value

was 2.95 and p value was 0.004, which is statistically significant. There was increase level of enzymes in both group I and II but more in group II.

Mean AST level in group I was 38.22 ± 8.85 IU/lit. with S.E.M 1.25 and in group II it was 48.42 ± 12.05 IU/lit. with S.E.M 1.7, t value 4.82 and p<0.001 which is highly significant statistically but not much significant clinically because there are less that three times ULN (with clinical symptoms) (Table 6).

However, when drug induced liver injury occurs following the use of 4 drug combination regimen, it is impossible to quantify the contribution of each drug in the development of drug induced liver injury (Harshad Devarbhavi, 2012).

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 Isoniazid + Rifampinic > Isoliazid > Pyrazinamid > Rifampicin > Ethambutol (Krishnaswami K 1997).

ALT is specific to hepatocellular injury and the level of ALT were more than AST throughout the study. The overall incidence of ALT and AST is higher throughout in the study in the dialy regimen group than thrice weakly group. The highest level of ALT was found in supervised therapy was 160 IU/lit. and in non-supervised therapy, 240 IU/lit. Like wise highest level of AST in first group was 60 IU/lit. whereas in second group was 72 IU/Lit.

With the continuation of therapy with Rifampicin and Isoniazid at third month, it was observed that liver enzymes were declining. The mean ALT level of group I was 40.28±7.96 IU/lit. with S.E.M 1.12 and in group II it was 51.32±14.97 IU/lit with S.E.M 2.13, t value 4.16 and p value = 0.0001 which is highly significant. The level is still different and significant in the above 2 groups. These date show more tendencies towards liver damage in the non-observational therapy than observational therapy. Like with mean AST leavel in group I was 37.68±8.83 IU/lit. with S.E.M 1.06 and 1.24 respectively, t value 1.77 and p value 0.07 which is non – significant (>0.05). these fall to normal range. At the monthly observation of therapy, it was found that all the patients in the group I and group II, having declining liver enzymes in serum.

In the patients of hepatotoxicity, after recovery, low does of INH and Ethambutol were reintroduced and after one weak Rifampicin was added. None of the 6 patients has reoccurrence of hepatotoxicity later on. So this cohort study revealed that it is possible to reinstitution of potentially hepatotoxic agenst after recovery.

Mechanism for this adaptation is not known. It was observed that gradually introducing the drugs by giving them in increasing number and dosage is the reason for the successful retreatment procedure (L.P.Omerod, C.Shinner; hepatotoxicity of ATT 1996).

Normally pyrazinamide is avoided for reintroduction because some studies have reported fatal hepatic necrosis caused by PZA (Durand F et al, Hepatology 21:926-932.

Thus in my study it was experienced that timely detection and temporary with drawl of the offending agent can completely cure anti – TB drug induced hepatotoxicity.

At the 4th month of therapy, in group I there was mean ALT 38.08±9.2 IU/lit with S.E.M 1.3 and in group II 47.28±11.09 with S.E.M. 1.56 paired 't' value was 4.51 with p < 0.0001 (highly significant). AST level in group I was 32.52±4.71 with S.E.M. 0.66 and in group II 39.56±6.7 with S.E.M 0.94, 't' value is 6.07 and p value < 0.0001 (highly significant). These values signify that even in 4 months there are significant differences in liver enzymes, although they came under normal level. Thus with continuation of therapy there was normal level of enzymes with higher normal in group II (Table-8).

Again with continuation of therapy there was gradual decrease in liver enzymes in both groups. In the 5th month of therapy, mean ALT in group I was 35.62±4.6 and in group II 40.38± 7.5 with 't' value 3.79 and p = 0.0003 which is highly significant. With still increased dose in group II, there is increased derangement of enzymes level. Mean AST level in group I and group II (in u/L) were 30.5±4.40 and 34.56±4.27 respectively with 't' value 4.66 and p<0.0001 (highly significant)

At the completion of therapy (6 months) the mean of ALT in group I & II (in unit/L) were 29.72±7.07 and 37.6±7.85 with paired 't' test 5.27 and p value < 0.001. AST level 25.68±3.75 and 32.46±5.9 in group I and II respectively, 't' value is 6.85 and p<0.001 (highly significant). Thus even on completion of therapy, there is difference in ALT and AST in group I and II, although both were within normal limit (Table-10). These values are in accordance with K.D.Chang, C.C.Lang & C.M Tan. European Resp J 2007; De Souza AF, 1996; Singh J 1996, Altman 1993.

Table 11 is showing the male femal ratio and percentage risk of hepatotoxicity in the study. There are total 68/32 male/female with 32/18 in group I and 36/14 in group II.

Overall percentage hepatotoxicity rate were 4.4% 9.37% in the male/female population. Previous studies by Snider DE, 1992; DE Souza AI, 1996; Singh J 1996 showed the independent predictor for drug induced hepatotoxicity for female gender.

Although women have tradiationally been considered more susceptible to develop TB drug induced liver injury, recent reports suggest that men outnumber women in the incidence of TB drug induced liver injury.

This likely reflects the demographic disparity where more men that women are under treatment for tuberculosis. However female gender is a positive predictor of more severe liver disease including death (Devarbhavi H, Dierkhising R, Kremers WK et al Hepatology 2010;52:798-9.

Difference in incidence of DIH (drug induced hepatotoxicity) in male and female is due to :

1. Pharmacokinetic variantion, probably slower biotransformation and subsequent clearance of exogenous molecules due to lower level of microsomal enzymes.
2. It is also believed that women are slow acetylators. Ratio of slow acetylator enzymatic pattern in male: female being 1:4. Due to being a slow acetylator, females are more predisposed to risk of hepatotoxicity (Gramhagen Riska, 1995; Marwin W, 1998).

Treatment outcome in both observational and non-observational gherapy were compared after the 2 months of intensive phase. Number of febrile cases after the intensive phase was 2 with 4% in group I and 5 in case of group II. With fisher exact test p value showing 0.436 (> 0.005) which shows non-significant data. This might be due to the irregularity of medicines, defaulter cases in group II (possiblility of defaulter in group I is nol, as they were continuously under supervision) or having superadded viral infection (Table-12)

Likewise percentage of sputum smear positivity in group I were 1 (2%), and in group 2 were 3 (6%) showing fisher exact test p = 0.33, odds ration is 3.12 means group II were 3.12 times more liable to sputum positivity after 2 months in my study, p value is 0.33 (>0.5) in non significant. The result could be attributed to the patient's extensiveness of disease, inability of taking drugs due to poor socioeconomic condition or defaulter as previous cases. Study show that adherence to treatment for 6 months or more is difficult in patients and can't be relied upon patients without supervision because many of patients after getting relief from symptoms, tend to stop the drugs (Table 13).

The table -14 showing mean weight before therapy (in Kg) 43.02$\pm$6.74 in group I and 43.04$\pm$6.78 after completion of therapy with paired 't' test = 2.98 and p = 0.0037 (< 0.05). Data shows highly significant, effectiveness of therapy. With the healing of lesion and curing the symptoms there is significant gain in weight

In group II, mean weight (in kg) before therapy were 43.54$\pm$8.22 and 46.21$\pm$8.28 after therapy with 't' value 1.61, p = 0.108 which is not significant. This signifies the more compliance of group I patients towards therapy.

In the last table (Table-15), it is showed the incidence of hepatotoxicity in both group I and II which is 4% in observation therapy whereas 8% in non-observational therapy with odds ratio 2.08 and p value 0.40 which is not significant.

The value is higher than the previous studies done in UK and USA (LP Omerod; S Skimer, Hepatotoxicity of ATT 1996, 5:111). But the value is lower than the studies done in Nepalese population (8% in supervised therapy) (Shakya R, Rao BS. Ann Pharmacother, 2004;38(6); 1074-9).

These differences in different countries might be due to difference in racial factors, nutritional status, body mass index genetic factors, ethnic factors, indiscriminate use of drugs and more advanced TB infection. A high incidence of viral hepatitis has been reported to coexixt in patients with TB in developing countries, resulting in misdiagnosis of ATT-induced hepatotoxicity.

ATT can cause varied degree of hepatotoxicity from a transitory asymptomatic rise in transaminases to a acute liver failure in both daily and thrice weakly therapy. The frequency of hepatotoxicity in different countries varies widely from 2-39% shown in previous studies (Anand AC, Seth AK et al. risk factor of hepatotoxicity during anti TB treatment. MAJAFI, 2000).

Whether dosing schedules affect the risk of drug induced hepatitis may bear clinical relevance. A nested case control trail study showed that standard thrice weakly anti tuberculous treatment increased the risk difference might be clinically significant for patients with cavitary TB (K C Chang, Yew WW, Tan CM. A nested case control study on treatment related risk factors for early relapse of TB. Am J Resp Crit Care Med 2004; 170:1124-1130).

Previous randomized controlled trails involving combination therapy with isoniazid, pyrazinamide and rifampicin in the initial phase showed that dialy treatment may be nore hepatotosic than thrice weakly treatment but this is not applicable for the standard 6 months. Moreover there is paralleled rise in transaminases with drugs.

V. Conclusion

On the basis of above observation made in the prospective cohort study, it can be concluded that liver enzyme alteration is most common finding in the patient receiving anti tubercular chemotherapy. Revised WHO Regimen, Directly Observed Therapy Short (DOTS) course is most cost-effective therapeutic programme, especially for developing countries like India. Hence, every patient of pulmonary tuberculosis should be given antitubercular drugs under direct supervision, as it increased the patient adherence to treatment regimens, less

increase of ALT and AST, thus less prone to develop hepatotoxicity, more compliant to patient, increased effectiveness with easy availability in all government hospitals and awareness of patient about the disease.

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