

ISSN: 0976-3031

*International Journal of Recent Scientific  
Research*

Impact factor: 5.114

**NARRATIVE AS A TEACHING TOOL AND  
COMMUNICATION FOR TEACHING CONTEMPORARY AND  
RECENT SOCIAL HISTORY**



**Mengo, Renée Isabel and Tenaglia, Paul Ruben**

**Volume: 6**

**Issue: 9**

**THE PUBLICATION OF  
INTERNATIONAL JOURNAL OF RECENT SCIENTIFIC RESEARCH**

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ISSN: 0976-3031

Available Online at <http://www.recentscientific.com>

*International Journal of Recent Scientific Research*  
Vol. 6, Issue, 9, pp.6079-6083, September, 2015

**International Journal  
of Recent Scientific  
Research**

## RESEARCH ARTICLE

# ASSOCIATION BETWEEN VIRAL INFECTIONS AND LIVER FUNCTION TESTS

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### ARTICLE INFO

#### Article History:

Received 15<sup>th</sup> June, 2015

Received in revised form 21<sup>st</sup> July, 2015

Accepted 06<sup>th</sup> August, 2015

Published online

21<sup>st</sup> September, 2015

#### Key words:

LFT, ALT, HbsAg, HIV and HCV

### ABSTRACT

In a developing and infectious disease prone country like India, increased prevalence of infection with HbsAg, HCV and HIV are on the increase. As of December 2014, World Health Organisation statistics shows globally 33% of population with HbsAg, 150 millions with HCV and 37 millions with HIV infections. Numerous studies done previously have indicated alterations in liver function due to infections caused by the above three types of deadly viruses. The aim of this study was to find out the association between viral infections and liver function test. This study was carried out with a reasonable number of patients involving both sexes in each type of infection. This study has proved that some analytes like ALT, GGTP, TP and Albumin showed alterations in viral infectious diseases as per the statistical significance obtained. Further Studies are required in this field with large number of viral infected patients to monitor alterations in Liver function tests in each type of infection and to make LFT as routine diagnostic investigation for such infected patients.

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### INTRODUCTION

Liver Function Tests (LFT) are often the first line of marker for diseases of the liver. Interpreting abnormal LFTs will be useful to diagnose any underlying liver disease in a common scenario in Primary Care. However, other tests of the liver such as liver biopsy should also be done to confirm the diagnosis of a particular disorder, and/or to monitor the activity of the disorder and response to treatment. People with Human Immunodeficiency Virus (HIV) who have a damaged immune system are also at risk of infection that may affect the liver, and therefore doing regular liver function tests in all viral infectious diseases will help detect these. Some anti-HIV drugs can cause side-effects that affect the liver. For people who are positive for Hepatitis C Virus (HCV), Alanine Transaminase (ALT) is the most commonly monitored enzyme among the liver function tests. A positive result on an antibody test along with elevated ALT levels may provide a fairly good indication that an individual is infected with HCV.

#### HIV & HBV

Abnormalities of LFT have been shown to be common in HIV/AIDS patients in developed countries. Studies have shown

that these abnormalities may be due to direct inflammation induced by the HIV virus on the liver cell. It may also be due to gall bladder disease and infection with bacterial, viral or other opportunistic agents and abnormalities of liver enzymes are common in HIV patients in this environment. It is therefore important to characterise the nature of this abnormality and to institute appropriate management. However, more studies are required in this field of HIV related liver disease in Niger (Ejilemele AA *et al.*, 2007). Mild to moderate increase in liver enzymes are common in HIV patients without HCV/Hepatitis B Virus (HBV) and absence of primary immunodeficiency is independently associated with elevations in both Aspartate Transaminase (AST) and ALT, while features typical of hepatic steatosis in Diabetes Mellitus (DM) and Body Mass Index (BMI) are only associated with increased ALP (Sterling RK *et al.*, 2008). Liver disease in HIV infected individuals encompasses the spectrum from abnormal LFTs, liver decompensation, with and without evidence of cirrhosis on biopsy, to Non-Alcoholic Liver Disease (NALD) and in its more severe form, Non-Alcoholic Steatohepatitis (NASH) and hepatocellular cancer (HCC). HIV can infect multiple cells in the liver, leading to enhanced intrahepatic apoptosis, activation and fibrosis. HIV can also alter gastro-intestinal tract permeability, leading to increased levels of circulating

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lipopolysaccharide that may have an impact on liver function (Megan Crane et al., 2012).

LFT abnormalities and fibrosis scores were only significantly higher in co-infected patients in the immune clearance and Hepatitis B surface antigen (HbsAg) negative chronic hepatitis phases. LFT abnormalities in Nigerians with Hepatitis B Virus (HBV) infection and co-infection with HIV was found to have a negative impacts on hepatic function (Iroezindu MO et al., 2013). The liver is a major part of reticuloendothelial system and is a site of HIV replication and is an organ for many opportunistic infections and so in HIV infected individuals, abnormal LFT can develop as a result of hepatic parenchymal disease. LFT are deranged in HIV positive patients as compared to control. Increased AST and ALT levels may identify patients requiring further investigations as a diagnostic and prognostic tool (Ivan Netto et al., 2009). A statistically significant difference was absent in the serum total protein levels between cases and controls. No significant differences were observed when the values for serum total protein, albumin and globulin and the albumin: globulin ratios among the two case were compared (Subir Kumar Dey et al., 2009).

Primary HIV-1 infection is often under-diagnosed because of its nonspecific presentations. Elevated AST and ALT levels are one of the clinical manifestations, but is infrequently reported in the literature and elevated levels may be an initial manifestation of primary HIV infection and is more common than expected. Primary HIV-1 infection will serve as one of the differential diagnosis to be considered in young men presenting with unexplained, new-onset liver function impairment (Chen YJ et al., 2010). There is an association between HIV viral load and aminotransferases as markers of hepatic damage leading to improved recognition, diagnosis and potential therapy of hepatic damage in HIV infected patients (José Antonio Mata-Marín et al., 2009). Recent reports of transmission by intravenous gamma-globulin preparations of A, B, C and non-A non-B hepatitis (NANBH), including several cases that progressed to severe liver damage and death, have raised concerns about the safe use of intravenous gamma-globulins. Transient minor elevations were observed for ALT, AST,  $\gamma$ -GT and ALP. None of the elevations were considered indicative of NANBH or of any chronic hepatic disease. Transient presence of hepatitis A, B and C antibodies were observed in some patients. All patients remained negative for HbsAg throughout the study. HIV antibodies resulted always negative in all patients (Antonelli A et al., 1992).

A more robust immune restoration was observed among HBV/HCV coinfecting subjects who developed liver enzyme elevation after antiretroviral (ARV) initiation compared with other groups. This finding suggests that ARV-related liver enzyme elevation may be related in part to immune reconstitution, as measured by changes in CD4 T-cell counts (Ofotokun I et al., 1992). Increased levels of ALT and AST were significantly associated with HBV/HIV coinfection status. Gender and liver function tests are important predictors for HBV/HIV coinfection and screening for HBV coinfection in HIV-positive patients is recommended (Eltony Mugomeri et al., 2015). The most common presentation was fever (90%), weakness (79%), weight loss (62%) and diarrhoea (62%). The

CD4 cell count was between 200-500/ $\mu$ L (33%). LFT showed hyperglobulinemia in patients having CD4 cell count <500/ $\mu$ L. Increased ALP was observed in 63% with CD4 cell count <200/ $\mu$ L. 66.6% had HbsAg reactivity, 33.3% had positive anti-HCV antibody and 50% had abnormal liver histology. One third of these had systemic opportunistic infections like tuberculosis. No correlation could be made between hepatic histology and LFT (Bhattachary N et al., 2006).

Co-infection of HIV and HBV is an emerging problem that should be addressed immediately. Hepatic damage in case of co-infected patients should not be assessed only on the basis of serum liver enzymes as their rise is not significant enough in these cases. Liver biopsy accompanied by liver function tests may provide a clearer picture of macroinflammation. Such co-infected individuals also face increased risk of hepatotoxicity from ARV. Individuals with HIV-HBV co-infection should have both the infections completely assessed in order to decide on the best therapeutic option for both viruses (Tamal Mukherjee et al., 2013).

### HbsAg

The prevalence of chronic hepatitis C (CHC) is 0.09%. The LFT abnormality in total subjects was 11.4%. The LFT abnormality of chronic hepatitis B (CHB) and CHC subjects was 21.72% and 63.2% respectively. The prevalence of CHB and C was lower than that of previous studies. The prevalence of CHB in the 2nd decade was still high (Kim SB et al., 2009). HBV DNA level may not indicate the severity of liver inflammation or fibrosis in chronic HBV infection. Patients with HbsAg negative often are complicated with more severity of liver fibrosis. In routine LFT both Bilirubin and ALT correlates with liver inflammation grading or fibrosis staging; but not with fibrosis staging alone (Xu QH et al., 2008). A significant dose-response relationship existed between liver function abnormalities and N, N-Dimethylformamide (DMF) exposure among workers in Taiwan, HBV carrier status or increased BMI had synergistic effects with DMF in causing abnormal LFTs and clinical chronic liver diseases (Luo JC et al., 2001).

Presence of HBV-DNA in maternal blood during the third trimester of pregnancy is significantly associated with maternal serum ALT levels in HbsAg-negative chronic HBV-infected pregnant women. Women with an ALT/sodium ratio greater than 0.092 have the higher probability of HBV-DNA presence in maternal blood whereas an ALT/sodium ratio greater than 0.11 could discriminate those women with HBV-DNA levels higher than 2000 IU/ml (Elefsiniotis et al., 2013). HBV was predominantly associated with underlying Chronic Liver Disease (CLD) among this group of patients in India and suggest that HBV coinfection in HCV-infected patients should not be excluded by negative HbsAg status alone (Saravanan S et al., 2009). Prevalence of HbsAg positive cases in Guilan province was higher than in other studies. Although frequency of HCV-Ab was similar to other studies, frequency of increased ALT was less, and that all hemophiliacs should be vaccinated against HBV and should have regular program for checking HCV (Mansour-Ghanaei et al., 2002).

All rheumatic patients who plan to take antiTumorNecrosis Factor-alpha treatment should undergo a test for HBV serology, including HbsAg, and have a close follow up with an LFT test during therapy. Further prospective studies for hepatitis B viral load using HBV-polymerase chain reaction in patients who are HbsAgpositive are needed to identify whether the abnormal LFT comes from the reactivation of occult HBV infection (Kim YJ *et al.*, 2010).A significantly higher number of genotype D-infected patients were anti-HBe positive and had elevated ALT levels (42% of genotype D-infected patients but 0% of patients infected with genotypes B and C). Genotype D strains with mutations in the core promoter and precore regions were significantly correlated with elevated ALT levels in the patients. The differences were not age related and hence genotype D appears to be associated with more active disease(Kidd-Ljunggren K *et al.*, 2004).

### HCV

In serial studies, patients with high-risk results for oral calcium chloride or Perfused Hepatic Mass (PHM) had nearly 15-fold increase in risk for clinical outcome. Less than 5% of patients with "low risk" Quantitative LFTs (QLFT) experienced a clinical outcome. QLFTs independently predict risk for future clinical outcomes. By improving risk assessment, QLFTs could enhance the noninvasive monitoring, counseling and management of patients with chronic HCV(Everson GT., *et al* 2012).HCV infection with elevated AST levels is a significant risk factor for severe veno-occlusive disease (VOD) after marrow transplant. However, the decision to proceed to transplantation in HCV-positive patients must balance the absolute risk of death from VOD against the risks of the underlying disease.

In long-term survivors, HCV infection is not associated with excess mortality over 10 years of follow-up(Strasser SI *et al.*, 1999).In a study, LFT results in fascioliasis and positive HCV showed AST/ALT increased in 42.9%, globulin increased in 21.4% and decreased in 42.8%. A/G decreased in 28.6% and increased in 57.2%. LFT of pure 27 fascioliasis patients showed that AST /ALT increased in 29.6%, globulin increased in 3.7% but decreased in 37.0% and A/G ratio increased in 48.1% (Wahib AA *et al.*, 2006).

The mean AST/ALT ratio in the cirrhotic patients was higher than in the noncirrhotic patients. A ratio > or =1 had 100% specificity and positive predictive value in distinguishing cirrhotic from noncirrhotic patients, with a 53.2% sensitivity and 80.7% negative predictive value. The ratio correlated positively with the stage of fibrosis but not with the grade of activity or other biochemical indices. Of the cirrhotic patients, 17% had no clinical or biochemical features suggestive of CLD.The AST/ALT ratio is a dependable marker of fibrosis stage and cirrhosis in patients with chronic HCV infection (Sheeth SG *et al.*, 1998).In 97% of patients with CHC, Sustained Virological Response (SVR) is durable without evidence of disease progression, although some degree of hepatic fibrosis may persist and patients with pre-treatment cirrhosis are at continuing low risk for hepatocellular carcinoma (Koh C *et al.*, 2013).

## MATERIAL METHODS

A total of 52 non hospitalised patients consisting of males and females in the age group of 21 to 74 years attending the infectious diseases clinic and who were investigated for liver function tests aswell as HbsAg, HCV and HIVwere enrolled for this study. As the sole aim of this study was to find out the association between LFT and HbsAg, HCV and HIV, inclusion or exclusion criteria were not followed.

Diuri CS 1300 B and Dialab reagents were used to measure LFT and VitrosEQI analyser was used to measure HbsAg, HCV and HIV analytes. While the accuracy of all LFT results obtained in this study were validated by the use of Bio-Rad accuracy controls at two levels, theaccuracy for HbsAg, HCV and HIV tests were validated using Ortho Clinical Diagnostic commercial controls available. For statistical analysis of data, a software downloaded from the website <http://www.vassarstats.net> was used to calculate correlation coefficient (r) , students 't' distribution (t) and probability (p) between normal and viral infections groups.

## RESULTS

**Table I** Mean analyte values for Normal and Infectious groups

	Groups	TB	DB	TP	ALB	ALT	ALP	GGTP
n=35	NORMAL	0.67	0.14	6.91	4.02	20.31	82.69	26.54
	HbsAg	0.72	0.26	6.67	3.95	55.14	85.74	63
n=8	NORMAL	0.71	0.13	7.16	4.04	17.63	83.75	29
	HCV	1.94	1.54	7.25	3.88	81.75	86.75	191.90
n=9	NORMAL	0.69	0.13	7.13	4.08	17.33	82.89	30
	HIV	1.01	0.47	7.11	3.59	44.67	109	44.44

**Table II** Statistical Parameters ( r, t & p) ; Normal Vs Infected patients

Groups	Analytes	Comparison	r	t	p
HbsAg (n=35)	TP	Normal Vs	0.7016	5.6560	0.0000150
	ALB	Infected	0.4939	3.2630	0.0012835
	ALT		0.4694	3.0540	0.0022230
HCV (n=8)	GGTP	Normal Vs	1.3687	2.299	0.031
		Infected			
HIV ( n=9)	DB	Normal Vs	-0.5470	-1.729	0.06374
	TP	Infected	0.9026	5.547	0.00043

The Statistical results obtained for all viral infected patients – HbsAG (n=35), HCV (n=8) and HIV (n=9) and normal patients for both Male and Female are presented in Table- I. Table I givesthe mean values for each analyte studied (normal as well infected group). From the data presented in this Table I, visual observation indicate elevations in the mean values of TB, DB, ALT, GGTP in HCV and HIV and decrease in ALB in infected patients compared to normal group.

Table II shows the statistical comparisons between infected and normal groups. In HbsAg infected group, higher significant association was found in TP, ALB and ALT, and in HCV infection, a moderate significant was observed only for GGTP with P=0.03 and in HIV infected patients DB and TP showed significant associations with P= 0.06 and <0.0001 respectively. From this statistical data, it is clear that liver function is indeed affected and majority of LFT tests like TP, ALB, ALT, GGTP and Direct Bilirubin are altered in viral infected patients. Albumin shows a negative association in HIV infected patients

suggesting that liver synthetic capacity of ALB is affected in HIV infection.

## DISCUSSION

Many previous studies have confirmed that increase in liver enzymes occur in HIV infected patients and elevated Transaminases levels are common in such cases (Megan Crane et al., Ivan Netto et al., Chen YJ et al., Antonelli A et al.). Our study outcome also confirms such previous observations and majority of LFT tests are altered in all three type of infections. Previous studies have shown that in HbsAg and HCV infected patients, abnormalities in LFT are observed in TB and ALT (Kim SB et al., Xu QH et al., Kim YJ et al.). As per our study, in HbsAg infection LFT alterations were observed in TP, ALB and ALT and in HCV infection GGTP g et al tered. Our study has clearly shown that majority of LFT tests were altered in viral infectious diseases when compared to normal controls. The outcome of our study suggests that LFT should be made a routine test for infectious diseases involving HbsAg, HCV and HIV.

## CONCLUSION

This study was done using a reasonable number of patients infected with HbsAg, HCV and HIV to find out the alterations in LFT. Statistical analysis done clearly demonstrated that some principal analytes like ALT and GGTP were found to be elevated in such infections. Out of routine 7 LFT, 5 principal analytes viz TP, ALB, ALT, GGTP and DB were found to be altered in HbsAg infections, TP, ALB, ALT in HCV infections, notably GGTP and DB and TP in HIV infections. Further studies with large number of patients are required to confirm the inclusion of LFT as a routine test in all three types of infections.

## Acknowledgement

The authors would like to thank Dr. Mitra Ghosh, Chief of Laboratory Services at Apollo Speciality Hospitals, Vangaram for giving us permission to undertake this study.

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**How to cite this article:**

Rajeswari S *et al.* 2015, Association Between Viral Infections And Liver Function Tests. *International Journal of Recent Scientific Research*, 6 (9), pp.6079-6083.

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*International Journal of Recent Scientific  
Research*

ISSN 0976-3031



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