|| ISSN(online): 2589-8698 || ISSN(print): 2589-868X ||
International Journal of Medical and Biomedical Studies
Available Online at www.ijmbs.info
Volume 2, Issue 5; September-October: 2018; Page No. 51-57
Research Article

CO-RRELATATION OF ALT & AST LEVELS WITH HEPATITIS B VIRUS DNA VIRAL LOAD

Khan Salman[1], Madan Molly[2], Virmani Sunil Kumar[3]

[1] M.Sc.-Medical Microbiology, Department of Microbiology, Neta ji Subhash Chandra Bose Subharti Medical College, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India.

[2] M.B.B.S., M.D., Professor & Head, Department of Microbiology, Neta ji Subhash Chandra Bose Subharti Medical College, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India.

[3] M.B.B.S., M.D., Professor & Head, Department of Medicine, Neta ji Subhash Chandra Bose Subharti Medical College, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India.

Article Info: Received 16 September 2018; Accepted 12 October. 2018
Cite this article as: Khan, S., Madan, M., & Virmani, S. K. (2018). CO-RRELATATION OF ALT & AST LEVELS WITH HEPATITIS B VIRUS DNA VIRAL LOAD. *International Journal of Medical and Biomedical Studies*, *2*(5).
DOI: https://doi.org/10.32553/ijmbs.v2i5.41
Address for Correspondence: Salman Khan, M.Sc., PhD-Medical Microbiology, Department of Microbiology, Neta ji Subhash Chandra Bose Subharti Medical College, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India.
Conflict of interest: No conflict of interest.

ABSTRACT:

Objective:-The present study aimed to correlate the levels of ALT & AST with Hepatitis B Virus DNA Viral load by Real Time PCR in HBsAg Positive Patients

Methods: Study conducted on 4927 patients in Meerut, India, which was performed in central research station labortory of Microbiology at netaji subhash Chandra Bose Medical College and Hospital Between November 2016 to April 2018 The sera were separated and screened for HBsAg,HBeAg by ELISA kit and DNA Viral load.PCR Positive Sample were analysed for ALT and AST

Results: 245 positive for HBsAg. 55 (1.12%) were HBeAg positive. Out of 55 HBeAg cases, 35 were female and 92 female were negative for HBeAg, this was stastically significant (P< 0.039).In PCR, The results show that in 200 of patients were not detectable serum HBV DNA and 16(16%) were PCR positive, 12.18% were below 2000 IU/mL DNA levels, 1.7% were between > 2000 IU/mL to 20000 IU/mL HBV DNA levels and 2.1 % were >20000 IU/mL HBV DNA levels. ALT was significant (P<0.004) when correlate with DNA viral load. The correlation between AST & ALT was also significant (P< 0.025).

Conclusions: The study showed a strong correlation between ALT and HBV DNA viral load.In low-income countries, the management strategy, using HBsAg, HBeAg and ALT, seems adequate for the confirmation and diffrentiation of Hepatitis B virus inactive, active & chronic hepatitis B patients. Further studies are needed to standardize and improve the management of this group of patients

Keywords: Hepatitis B virus, HBsAg, RT-PCR, ALT,AST

1. INTRODUCTION:

The human hepatitis B virus (HBV) is a small-enveloped DNA virus causing acute and chronic hepatitis. Despite the availability of a safe and effective vaccine, HBV infection still represents a major global health burden, with about 350-400 million people chronically infected worldwide

and approximately 600,000 deaths per year due to HBV-associated liver pathologies **[1].** Between 15 and 40% of chronically infected individuals may develop severe liver disease and hepatocellular carcinoma (HCC), while the remaining become inactive carriers. **[2].**

HBV is present in blood, saliva, semen, vaginal secretions and menstrual blood of infected individuals and easily transmitted through contact with infected body fluids **[3].** Perinatal vertical transmission is the most common mode of transmission worldwide **[4]**. In households of a chronically infected individual, HBV infection can occur via person-to-person, nonsexual contact **[5]**.

During HBV disease progression, after seroconversion (HBeAg (+) to HBeAg (-), HBeAg consists of two clinical forms; one known as chronic inactive with low persistant aminotransferase levels and HBV DNA levels (≤ 100,000 copies/ml) and second with no HBeAg, high ALT and HBV DNA levels (≥ 100,000 copies/ml). In low-income countries like Pakistan, Nepal, India etc many patients refused to do PCR and liver biopsy procedure due to poverty and cost of these tests. Beside these challenges, the growing concern is the early detection of viral hepatic disease and liver damage. For this purpose, in routine laboratory tests, elevated alanine aminotransferase (ALT) levels are used as indicators of liver cell injury and as non-invasive diagnostic tests **[6].** Elevated AST levels are usually predominant in liver cirrhosis with increased ALT levels **[7,8].** During assessment of liver disease due to hepatitis, serum AST and ALT levels are most commonly used serum markers to detect acute and chronic hepatocytes cytotoxicity **[9-11].** Now a days, the main emphasis of workers is early detection of liver damage due to chronic HBV, however, there is always questions about the effectiveness of these test because of their low sensitivity **[12,13].** Several studies in Italy, China, Korea and Hong Kong showed that ALT levels higher than the normal limits are strongly associated with an increased risk of liver cirrhosis in HBV infected patients **[14-17].** Recent studies revealed that in patients with HBeAg (-), high ALT levels greater than 0.5x to the upper limit of normal (ULN) relate to advance fibrosis and ALT > 30 IU/L and 19 IU/L in male and female respectively, **[9,10].** After HBeAg seroclearance, two disease states, not necessarily static, are possible: Some patients remain in an inactive carrier (IC) state, defined by the European Association for the Study of the Liver (EASL) as fulfilling the following criteria: (1) HBeAg negativity; (2) anti-Hbe positivity; (3) persistently normal ALT (PNALT) levels (<40 IU/mL, with measurements at least every 3-4 months during 1 year); (4) serum HBV DNA levels <2000 IU/mL. At the Management of Hepatitis B workshop in 2000, an arbitrary level of 20,000 IU/mL was adopted as the serum HBV DNA cut-off level distinguishing active and inactive chronic hepatitis.**[18]** EASL acknowledges that there can be inactive HBV carriers with DNA levels between 2000 and 20,000 IU/mL. HBV DNA levels in HBeAg-negative chronic hepatitis B can fluctuate from undetectable to >2,000,000 IU/mL**[19]** some inactive carriers occasionally have HBV DNA levels between 2000 and 20,000 IU/mL, a single HBV DNA level between 2000 and 20,000 IU/mL appears to be a ‘‘gray area’’ which can correspond to both active CHB or inactive carriers. These studies indicates ALT level as a reliable serum marker leading to fact that HBV natural history can vary from one population to another **[20]**. Thus, the aim of this study was to Correlatation of ALT & AST levels with Hepatitis B Virus DNA Viral load by Real Time PCR as the HBV is increasing in India.

2. Materials and methods

2.1. Study background and subjects

This was conducted on 4927 patients. Blood sample were collected in clean, sterile, small test tube from suspected HBV infections and its sequelae patients from Meerut and prosses in central research center labortory of Microbiology at netaji subhash Chandra Bose Subharti Medical

College and Hospital Between November 2016 to April 2018.

INCLUSION CRITERIA: Age between 21 – 65 years with suspected HBV infections and its sequelae patients. Ability to provide written informed consent indicating awareness of the investigational nature of this study.

EXCLUSION CRITERIA: If they were received any Immunization for HBV

Co-infection:- HBV – HCV, HBV- HIV, HBV – HDV , Liver disease due to other viruses, Alcohols, Diabetics, Autoimmune disease, Immuno-modulatory drugs (including systemic steroids, interferons, interleukins, or other cytokines)

2.2. Sample collection and processing

Five milliliter blood samples received in the Serology Section of Department of Microbiology from patients suspected of acute infectious hepatitis were analyzed. The sera were separated and screened for HBsAg by Hepa Card (J. Mitra & Co. Pvt. Ltd. New Delhi, India) and positive serum was stored in frozen (-20 °C) until tested for the viral markers. The positive serum samples for HBsAg by Hepa Card were tested again for HBsAg using commercially available ELISA kit (ERBA Transasia Bio-medicals Ltd. Daman, India). **[21]** Serum samples tested positive for HBsAg were tested for HBeAg (ELISA; Beijing Kewei Clinical Diagnostic Reagent Inc. Beijing, China). **[22]**

DNA isolation from the serum samples was performed using the "QIAamp DNA Mini Kit" (Qiagen, Germany) following the manufacturer's recommendations.**[23]**

The isolated DNA was amplified by Real Time PCR by using artus® HBV RG PCR Kit (Qiagen, Germany) following the manufacturer's recommendations **[24]**

All PCR Positive Sample were analysed for ALT and AST using UV(IFCC) kinetic method by Randox Laboratories Ltd Kit (140 London Wall,London,UK) following the manufacturer's recommendations **[25]**

2.3. Statistical analysis

Obtained data were analyzed by using the SPSS software for windows version 16. The comparison of data in respect of age groups and gender were performed by Z- test and Karl pearson corelation test was used for correlatation of DNA viral load with ALT & AST. $P < 0.05$ was consider to be statistically significant.

3. Results

Of the 4927 serum sample, 2218 (45.01%) male and 2709 (54.98%) female were tested for HBsAg with the age range 21 – 65. In this study, it was observed that 245 were positive and 4682 were negative. In 245 positive cases 118 were male and 127 were female. In 4682 negative cases 2100 were male and 2582 were female. Highest positive case were found in the age group of 21-30. **[Table 1]** All 245 HBsAg positive cases was tested for HBeAg and DNA Viral load. HBeAg positive were 55(1.12%), 20 male were positive for HBeAg and 98 male were negative for HBeAg and Highest HBeAg positive male were found in the age group of 21- 30. Out of 55 HBeAg casees, 35 were female and 92 female were negative for HBeAg, this was stastically significant ($P< 0.039$) by using Z test. **[Table 2]** In RT-PCR, The results show that in 84% of patients were not detectable serum HBV DNA and 16% were PCR positive. Out of this 16% PCR positive patients,12.18% were below 2000 IU/mL HBV DNA levels, 1.7% were between > 2000 IU/mL to 20000 IU/mL HBV DNA levels and 2.1 % were >20000 IU/mL HBV DNA levels. **[Table 3]** All the 16 PCR positive patiants were analysed for AST & ALT to correlate with DNA viral load. ALT was significant (P value <0.004) when correlate with DNA viral load but the corelation of AST with HBV DNA viral Load was Non-significant (P value <0.330). The correlation between AST & ALT was also significant (P value < 0.025) by using Karl pearson corelation test. **[Table 4]**

Tables for, Correlatation of ALT & AST levels with Hepatitis B Virus DNA Viral load by Real Time PCR in HBsAg Positive Patients

Table 1: Distribution of Male and Female among HBsAg positive and HBsAg negative patients at different age group

Age group (Years)	HBsAg -Ve		HBsAg +ve		Total			Prevalance		
	Male	Female	Male	Female	Male	Female	Total	Male	Female	Total
21 – 30	934	840	69	74	1003	914	1917	6.88	8.1	7.46
31 – 40	604	744	33	29	637	773	1410	5.18	3.75	4.40
41 - 50	363	650	11	15	374	665	1039	2.94	2.26	2.50
51 - 65	199	348	5	09	204	357	561	2.45	2.52	2.50
Total	2100	2582	118	127	2218	2709	4927	5.32	4.69	4.97

Table 2: Distribution of HBeAg in Male and Female among HBsAg +Ve at different age group

Age group (Years)	Male	Female	Total
21 - 30	8	19	27 (49.09%)
31 – 40	7	13	20 (36.36%)
41 - 50	4	3	07 (12.73%)
51 - 65	1	-	1 (1.81%)
Total	20 (36.36 %)	35 (63.64%)	55 (1.12%)

Table 2: Distribution of HBV DNA viral load at different age group

S.N.	Age group (Years)			≥ cutoff (10 IU/ml)	Total
1.	21 - 30	**117**	**11**	**9**	**137**
2.	31 – 40	**49**	**5**	**7**	**61**
3.	41 - 50	**20**	**6**	**-**	**26**
4.	51 - 65	**14**	**-**	**-**	**14**
Total		**200**	**22**	**16**	**238**

Note: 7 samples was cancel due to low volume/ spoilage of sample

Table 3: Corelation of AST & ALT with HBV DNA Viral Load

S.N.	Age Age group	Liver Enzyme (IU/ml)		Viral Load (IU/ml)
		AST	ALT	
1	21 - 30	12	90	1.00413×10^6
2	21 - 30	35	64	2.75525×10^3
3	21 - 30	646	107	3.78439×10^5
4	21 - 30	55	45	2.6×10^1
5	21 - 30	49	47	5.725×10^1
6	21 - 30	108	253	9.0689×10^5
7	21 - 30	47	66	3.54425×10^3
8	21 - 30	>750	214	7.36287×10^5
9	21 - 30	310	116	8.39305×10^5
10	31 – 40	32	27	3.25×10^2
11	31 – 40	78	49	5.375×10^1
12	31 – 40	>750	149	7.7855×10^3
13	31 – 40	82	120	4.2315×10^3
14	31 – 40	12	56	4.35×10^1
15	31 – 40	169	41	1×10^1
16	31 – 40	13	36	1.125×10^1

4. Discussion

It is interesting to know that HBV evaluation depend on geographical association of the host and viral factors. Prati et al. proposed new cutoff value of ALT ≥ 30 IU/L in male and ≥19 IU/L in female **[10],** while Assy et al. 2009 reported ALT ≥ 30 IU/L in male and ≥ 19 IU/L in female along with HBV DNA levels ≥ 100000 copies/mL can classify a patient into the active carrier state **[26].** Although, serum AST levels are not thought to be incredibly useful predictor of HBV disease, we also evaluate their performance either they are useful or not for discriminating HBeAg (-) chronic active from inactive patients.

According to international guidelines, a correct approach to chronic HBV infection requires an accurate differential diagnosis between chronic hepatitis and the inactive carriers through HBV DNA for at least one year. Some data suggest that the use of HBV DNA and ALT alone to define an inactive carrier, without resort to liver biopsy, may not detect significant histological disease in about 10% of patients.**[27]**

Previous studies reported raised serum ALT levels from ULN (Upper limits of Normal) can predict liver dysfunction with 90% specificity and 56% sensitivity **[28],** but according to Kim et al. prior testing of ELISA along with ALT level can better predict liver function as compared to only ALT levels **[29].** This is particularly important because without performing PCR and liver biopsy, the decision as to predict HBeAg (-) chronic inactive is difficult. Serum ALT, AST and HBV DNA levels were found to be highly significant. Their performance was assessed by using different cut off values irrespective of patient's gender. We observed same results as described by Prati et al. **[10]** and Assy et al. **[26].**

The results show that in 84% of patients most were not detectable serum HBV DNA and 16% were PCR positive. Out of this 16% PCR positive patients,12.18% were below 2000 IU/mL HBV DNA levels, 1.7% were between > 2000 IU/mL to 20000 IU/mL HBV DNA levels and 2.1 % were >20000 IU/mL HBV DNA levels. In all the 16 PCR positive Patients, ALT was significant when

correlate with DNA viral load but the corelation of AST with HBV DNA viral Load was Non-significant, leading to the conclusion that inflammation increases in patients with elevated HBV DNA levels as HBeAg has immunomodulatory action **[30].**

In recent study by Kim et al. (2011) validate the performance of ALT and HBV DNA, and found that these markers may also used for discriminating patients with HBeAg (-) active carriers from inactive **[31].** Recent studies showed that for HBeAg (-) patients, low HBV DNA levels are associated with less liver damage although some studies were unable to observe such relationship **[32,33].** These findings suggest that HBV DNA load and ALT are most convenient techniques to predict active chronic HBV patients.

Ethical approval & Funding

Ethical approval for the study was taken from institutional research ethical committee.

No funding to declare.

Acknowledgements

The authors are grateful to Dr. Arvind Shukla, Assistant Professor of Community Medicine Neta ji Subhash Chandra Bose Subharti Medical College, Swami Vivekanand Subharti University, Meerut, (Uttar Pradesh), India for their support.

References

1. WHO. Epidemiology of hepatitis B virus. Division of Health and Development, WHO. 2013
2. Manno M, Cammá C, Schepis F, Bassi F, Gelmini R, Giannini F, et al. Natural history of chronic HBV carriers in Northern Italy: morbidity and mortality after 30 years. Gastroenterology. 2004;127:756-63.
3. Lavanchy D.,Hepatitis B virus epidemiology, disease burden,treatment, and current and emerging prevention and control measures. J Viral Hepat. 2004;11:97–107.
4. Stevens E, Neurath RA, Beasley RP, et al. HBeAg and anti- HBe detection by radioimmunoassay: Correlation with vertical transmission of hepatitis B virus in Taiwan. J MedVirol 1979;3:237–41.
5. Alter MJ.Epidemiology of hepatitis B in Europe and worldwide. J Hepatol 2003;39: 64–9.
6. Pratt DS, Kaplan MM: Evaluation of abnormal liver-enzyme results in asymptomatic patients. N Engl J Med 2000, 342:1266-1271.
7. Dufour DR, Lott JA, Nolte FS, Gretch DR, Koff RS, Seeff LB: Diagnosis and monitoring of hepatic injury. I. Performance characteristics of laboratory tests. Clin Chem 2000, 46:2027-2049.
8. Green RM, Flamm S: AGA technical review on the evaluation of liver chemistry tests. Gastroenterology 2002, 123:1367-1384.
9. Kim HJ, Oh SW, Kim DJ, Choi EY: Abundance of immunologically active alanine amino-transferase in sera of liver cirrhosis and hepatocellular carcinoma patients. Clin Chem 2009, 55:1022-1025.
10. Prati D, Taioli E, Zanella A, Della Torre E, Butelli S, Del Vecchio E, Vianello L,Zanuso F, Mozzi F, Milani S, Conte D, Colombo M: Updated definitions of healthy ranges for serum alanine aminotransferase levels. Ann Intern Med 2002, 137:1-10.
11. Sheth SG, Flamm SL, Gordon FD, Chopra S: AST/ALT ratio predicts cirrhosis in patients with chronic hepatitis C virus infection. Am J Gastroenterol 1998, 93:44-48.
12. Brodkin CA, Daniell W, Checkoway H, Echeverria D, Johnson J, Wang K, Sohaey R, Green D, Redlich C, Gretch D, et al: Hepatic ultrasonic changes in workers exposed to perchloroethylene. Occup Environ Med 1995, 52:679-685.
13. Kelleher TB, Afdhal N: Assessment of liver fibrosis in co-infected patients. J Hepato 2006, 44:S126-S131.
14. Kim HC, Nam CM, Jee SH, Han KH, Oh DK, Suh I: Normal serum aminotransferase concentration and risk of mortality from liver diseases: prospective cohort study. BMJ 2004, 328:983

15. Kim WR, Flamm SL, Di Bisceglie AM, Bodenheimer HC: Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. Hepatology 2008, 47:1363-1370.
16. Sherman M: Risk of hepatocellular carcinoma in hepatitis B and prevention through treatment. Cleve Clin J Med 2009, 76:S6-9.
17. Sung JJ, Chan HL, Wong ML, Tse CH, Yuen SC, Tam JS, Leung NW: Relationship of clinical and virological factors with hepatitis activity in hepatitis B e antigen-negative chronic hepatitis B virus-infected patients. J Viral Hepat 2002, 9:229-234.
18. Lok AS, Heathcote EJ, Hoofnagle JH. Management of hepatitis B: 2000 --- summary of a workshop. Gastroenterology. 2001;120:1828-53.
19. Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. Hepatology. 2009;50:661-2.
20. Fattovich G, Bortolotti F, Donato F: Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. J Hepatol 2008, 48:335-352
21. Transasia Bio-medicals Ltd. Ringawada, Daman India
22. Beijing kewei clinical diagnostic reagent inc. Gucheng Xi Rd, Shi Jing Shan District, Beijing, China
23. QIAamp® DNA isolation Mini kit 2016, Germany www.qiagen.com.
24. *Artus*® HBV RG PCR Kit 2014, QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, GERMANYwww.qiagen.com.
25. AST & ALT by Kinetic UV method based on IFCC recommendations. Randox Laboratories Ltd Kit ,140 London Wall,London,UK.
26. Assy N, Beniashvili Z, Djibre A, Nasser G, Grosovski M, Nseir W: Lower baseline ALT cut-off values and HBV DNA levels better differentiate HBeAg- chronic hepatitis B patients from inactive chronic carriers. World J Gastroenterol 2009, 15: 3025-3031
27. Villa E, Fattovich G, Mauro A, Pasino M. Natural history of chronic HBV infection: special emphasis on the prognostic implications of the inactive carrier state versus chronic hepatitis. Dig Liver Dis. 2011;43:S8-14.
28. Ferraris R, Colombatti G, Fiorentini MT, Carosso R, Arossa W, de la PM: Diagnostic value of serum bile acids and routine liver function tests in hepatobiliary diseases. Sensitivity, specificity, and predictive value. Dig Dis Sci 1983,28:129-136.
29. Kim HC, Nam CM, Jee SH, Han KH, Oh DK, Suh I: Normal serum aminotransferase concentration and risk of mortality from liver diseases: prospective cohort study. BMJ 2004, 328:983.
30. Milich DR: Immune response to hepatitis B virus proteins: relevance of the murine model. Semin Liver Dis 1991, 11:93-112
31. Kim ES, Seo YS, Keum , Kim JH, An H, Yim HJ, Kim YS, Jeen YT, Chun HJ, Um SH, Kim CD, Ryu HS, Lee HS: A Threshold HBV DNA Level for Discriminating Patients with Hepatitis B e Antigen-Negative Chronic Hepatitis B from Inactive Carriers. Hep Monthly 2011
32. Chan HL, Wong VW, Wong GL, Chim AM, Lai LH, Sung JJ: Viral genotype and hepatitis B virus DNA levels are correlated with histological liver damage in HBeAg-negative chronic hepatitis B virus infection. Am J Gastroenterol 2002, 97:406-412
33. Sakugawa H, Nakasone H, Nakayoshi T, Kawakami Y, Yamashiro T, Maeshiro T, Kinjo F, Saito A: Correlation between serum transaminase activity and virus load among patients with chronic liver disease type B. Hepatol Res 2001, 21:159-168