

Correlation between Hepatitis B e Antigen and Hepatitis B Virus DNA in HBV Infected Carriers.

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ABSTRACT

Background: Serum Alanine amino transferase (ALT) and estimation of the quantity of hepatitis B e Antigen (HBeAg) are the simple and cost-effective diagnostic methods used for the assessment of state of HBV infection in hepatitis patients. However, in most cases quantitative PCR is recommended for the confirmation of infection, prognosis and to precisely assess the real status of infection.

Objective: To study the frequencies of HBV and to assess the correlation among ALT, HBe Ag and HBV DNA in the diagnosis of hepatitis B carrier patients.

Materials & Methods: This retrospective study was conducted in Amreek Clinical laboratories, Saidu Sharif Swat. The duration of study was three years from Jan 2015 to Dec 2017. A total of 41 cases were collected and evaluated for various parameters such as types, age, gender and tissue involved. The study was done after the permission granted by the ethical committee. Informed written consent was taken from each patient.

Results: The results obtained were statistically analysed. We noticed HBeAg level increased with the raise in HBV DNA. The occurrence of HBV was higher in males (63.4%) in comparison to females (36.6%), young persons in the age group 20 - 50 years were the most affected (80.5%). Similarly, the occurrence of HBV was higher in married persons (63.41%) when compared to unmarried persons (36.65%), however majority of the patients were asymptomatic (92.7%).

Conclusion: It has been concluded from the current study that HBV carrier patients with the raised HBe Ag, are more likely to be positive for HBV DNA. This suggests that the combination of biochemical tests (ALT) and serological markers i.e. HBe Ag may be used as cost effective, easiest and an alternative option to HBV DNA quantification by PCR. However, more information's about the status of infection could be achieved if serological/ biochemical assays are coupled with HBV DNA estimation by quantitative PCR.

Key words: hepatitis B carrier, HBe Ag, Serum Alanine amino transferase, Polymerase Chain Reaction

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INTRODUCTION

Hepatitis is one of the deadly causes of deaths, affecting 3.5 billion individuals in the world and approximately 4.5 million in Pakistan each year.¹ About 350 million people have been infected, and more than 600,000 deaths occur annually from HBV infection globally.²

The prevalence of HBV is high among developing countries of Asia and Africa while its prevalence is low in developed countries such as America, Europe and Australia. Pakistan is also in the list of leading countries where large population is infected with HBV. The prevalence of HBV varies in different parts of Pakistan but the overall prevalence of HBV infection in Pakistan is 3-4%. The prevalence of HBV infection is high (5%) in Punjab and Sindh provinces of Pakistan followed by Baluchistan (4.3%) and Khyber Pukhtunkhwa (KP, 1%) provinces. Approximately, 12 million people have been infected with HBV and HCV in Pakistan. This disease may not be fatal if

diagnosed at its initial stages; however, late detection leads to cirrhosis and hepatocellular Carcinoma (HCC). Lack of proper health facilities, poor economic status, and less public awareness are responsible for high prevalence of HBV.³

HBV infection is diagnosed by several different methods. HBV surface antigen can be detected on immune chromatography (ICT) kit.⁴ ELISA method can be used for the determination of serological markers of HBV present on infected blood cells that include Hepatitis B surface antigen, anti-HBs Ag, Hepatitis B e antigen and anti-HBeAg and polymerase chain reaction that detect, quantify, and/or characterize HBV DNA genomes within an infected patient.⁵ Both of these methods are highly sensitive but expensive to use. But PCR still remains the most accurate, and standard diagnostic tool which not only detect HBV but also quantify and characterize it within the infected patient. But the facility of ELISA and PCR is not available in routine diagnostic laboratories because these assays are comparatively expensive and need to be performed by highly skilled, qualified, and well-trained person.⁶

MATERIALS AND METHODS

A total of 41 chronic HBV positive patients of different age groups (15-60 years) who belonged to Malakand Division, Khyber Pukhtunkhwa (KP)

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were enrolled in this study. These patients have been referred to Amreek clinical laboratory, Saidu Sharif, Swat, from January 2015 to December 2017. An informed written consent was signed from each participant of the study and the study was approved from ethical committee. Approximately 5 ml of blood sample was collected from each patient and centrifuged for serum at 4000 rpm for 10. Assays for serum ALT levels and HBeAg (performed on VITROS ECiQ (Enhanced Chemiluminescence by Jhonson&Jhonson, USA) were performed on the day of collection. Approximately 1 ml of serum from each studied sample was separated in a clean, dry, and sterilized Eppendorf tube and preserved at -20°C for HBV DNA quantification. HBV DNA was extracted using DNA extraction kit (Sacace biotechnology, Italy).

The Graphed prisms software (Version 5.0) was used for calculating correlations among the groups. The Origin Pro software version (8.5.0) was used for making graphs.

RESULTS

Out of a total 41 HBV carrier patients screened, the occurrence of Hepatitis B was higher in male patients (63.41%) as compared to female patients (36.59 %) (Figure 1).

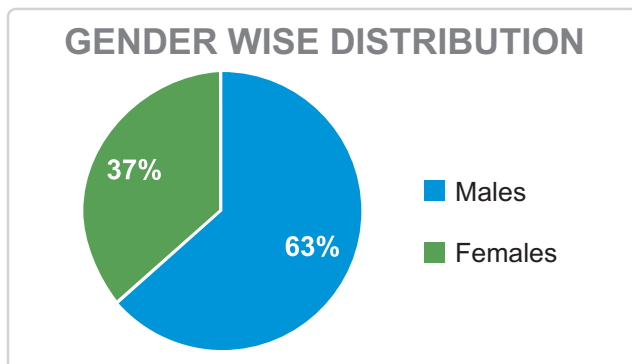


Fig 1. Gender wise distribution of Hepatitis B

The highest frequency of hepatitis B was observed in the age group 20-50 years (80.5%), followed by age group <20 years (17%). However, only single patient (2.5%) was in the age group >50 years (Fig 2).

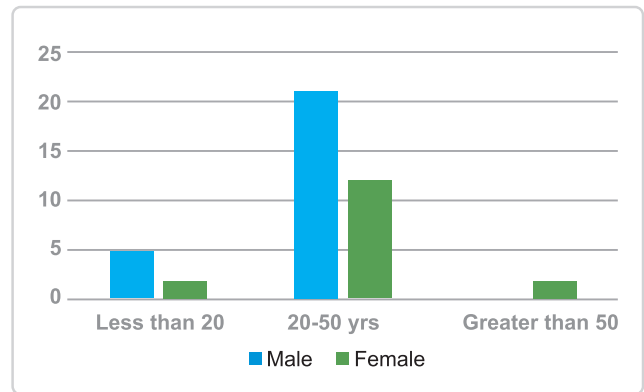


Fig 2 Age and gender wise distribution of Hepatitis B Patients

Majority of the patients were asymptomatic (92.7%) and merely few of the patients (7.3%) had typical symptoms of hepatitis B. The majority of the patients were male with a male to female ration of 1.6:1. The incidence of hepatitis B was high in married patients (63.4%) when compared to unmarried patients (36.6%) in the studied area.

Out of 41 (n= 41) carrier patients, 28 (68.29%) were reactive for HBeAg, 13 (31.71%) were non-reactive for HBeAg, and 26 (61.44%) were positive for both HBV DNA and HBeAg while 12 (29.26%) carrier patients were negative for both HBV DNA and HBeAg. Merely 2 (4.87%) of the carrier patients were reactive for HBeAg. However, HBV DNA was not detected in these two patients. Similarly, 1 (2.46%) of the carrier patients were non- reactive for HBeAg but were positive for HBV DNA (Table 1).

Table 1. Association between HBeAg and HBV DNA

HBeAg vs. HBV DNA	Count	%age
Detectable HBV DNA with reactive HBeAg	26	63.4
No detectable HBV DNA with non-reactive HBeAg	12	29.3
HBeAg reactive with non-detectable HBV DNA	02	4.87
HBeAg non-reactive with detectable HBV DNA	01	2.43
Total	41	100

Table 2. Positive tested parameter (percentages wise)

Tested Parameter	Count	%age	Total Count
HBeAg Reactive	28	68.29%	41
HBeAg Non-reactive	13	31.71%	
HBV carrier with normal ALT level	16	39.02%	41
HBV carrier with abnormal ALT level	25	60.98%	
Patients with detectable HBV DNA	27	65.85%	41
Patients with non-detectable HBV DNA	14	34.15%	

All the parameters were tested quantitatively and a significant increase in HBeAg values was observed with increase in HBV DNA. The

statistical analysis represents a positive correlation between these two parameters. (Table 3)

Table 3. Statistical analysis

S.No	Correlation groups	P (two tailed) Value	Pearson (r) Value	Status of correlation
1	Association of HBeAg with HBV DNA (group of male patients)	0.0011	0.603	Positive
2	Association of HBeAg with ALT level (group of male patients)	.0002	0.675	Positive
3	Association of HBV DNA with ALT level (group of female patients)	0.0003	0.649	Positive
4	Association of HBeAg with HBV DNA (group of male patients)	0.002	0.73	Positive
5	Association of HBeAg with ALT level (group of male patients)	0.01	0.65	Positive
6	Association of HBV DNA with ALT level (group of male patients)	0.0055	0.678	Positive

DISCUSSION

Liver function tests and serological markers are the simple and cost effective diagnostic methods used for the assessment of state of HBV infection in hepatitis patients. Serological markers such as HBeAg have been used previously to test the status of HBV infection, HBeAg is associated with HBV DNA replication.⁷ However, in most cases quantitative (q) PCR is recommended for the confirmation of infection, prognosis and to precisely assess the real state of infection. The qPCR estimates the virus DNA (viral load) and is particularly important in chronic hepatitis B patients, but this method of analysis is much expensive and more laborious. The current study was designed to determine if the simple and low cost assays such as liver enzyme test (for ALT) and serological marker (HBeAg) could be used as an alternatives to HBV DNA detection/ estimation

by PCR.⁸ Moreover, it may be more informative for the physicians to couple serological markers/ liver function tests with HBV DNA detection methods. This will give a more comprehensive image of the status of HBV infection.⁹

In our study the male individuals of HBV infection are greater than females. This may due to the predominance and unique life style of male population in the society (males may be more likely exposed as they are more interactive than the females in the studied area).¹⁰ Similarly, the HBV infection was more common in the married individuals as compared to unmarried individuals. This may due to the fact that married individuals have more exposed routes for virus transmission.¹¹ The occurrence of HBV infection was higher in young people age ranging from 20 to 50 years as compared to teens and elders. The

higher incidence of HBV in young people may be due to their greater exposure to the outside environment as compared to old people and children.¹² It was also observed that majority of the HBV carrier patients remained asymptomatic. Thus, it could be suggested to have a regular screening for HBV in the healthy and asymptomatic individuals so that the diseases could be diagnosed at early stage and should be treated accordingly.¹³

In our study there was significant association of serological parameter (HBeAg) with HBV DNA titer. The level of HBeAg increased significantly with the raise in HBV DNA. Similarly, the ALT level was elevated in HBV carrier patients who have been reactive for HBeAg and had a detectable level of HBV DNA. Our current analysis is in agreement with the previous study conducted in Bangladesh where they also showed a positive correlation between the HBeAg (anti-HBe), liver enzymes (ALT & AST) and HBV DNA. This suggests that Hepatitis B carrier patients with positive HBeAg and raised ALT level are more likely to be positive for HBV DNA.¹⁴

CONCLUSION

It has been concluded from the current study that HBV carrier patients with the raised ALT level and HBeAg are more likely to be positive for HBV DNA. This suggests that the combination of biochemical tests and serological markers may be used as an alternative option to HBV DNA quantification by PCR, and could be considered for antiviral therapy of patients. However, more information's about infection status could be achieved if serological/ biochemical assays are coupled with HBV DNA quantification by PCR.

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DATA SHARING STATEMENT: The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under

Ahmed I, Ahmad S:	Concept and design of study, Collection of data, statistical analysis
Khan W, Lal A:	Writing of manuscript, critical review of manuscript
Akbar F:	Analysis and interpretation of data, statistical analysis
Ali M:	Data collection, bibliography

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.