

Molecular and Serological Assessment of Chronic HBV Carriers and Additional Burden of Applying Updated Guidelines in Pakistan

Agha Babar Hussain¹, Syed Azhar Ahmed⁴, Farooq Ahmad Khan², Eijaz Ghani¹,
Muhammad Ali Rathore¹ and Nadir Ali³

ABSTRACT

Objective: To assess the additional burden of the patients eligible for treatment, based on recommendations on viral load, in the light of 2009 version of AASLD guidelines, as compared to 2004 guidelines and to determine the frequency of HBeAg in chronic HBV carriers.

Study Design: Descriptive cross-sectional study.

Place and Duration of Study: Virology Department, Armed Forces Institute of Pathology, Rawalpindi, from November 2010 to January 2012.

Methodology: Persons with chronic HBV infection, reporting for HBV DNA PCR test, were included in the study and blood samples were collected. HBV DNA load was determined by Real Time PCR. HBsAg and HBeAg were tested by ELISA.

Results: Out of the 801 subjects positive for HBsAg, 74 (9.24%) were positive for HBeAg. Out of them, 113 (14.1%) had HBV DNA load > 100,000 copies/ml and were eligible for treatment according to AASLD 2004 guidelines. Forty one (5.1%) had HBV load between 10,000 and 100,000 copies/ml, and were additionally eligible for treatment as per AASLD 2009 guidelines. The 5.1% of 4.5 million estimated HBV carries in Pakistan comes to 229500.

Conclusion: There was a low HBeAg positivity and HBV DNA positivity in our chronic HBV infected persons. Moreover, there is an increase of 229500 potential candidates for HBV treatment in Pakistan based on viral load testing, according to the AASLD 2009 guidelines when compared with 2004 guidelines. The increase in the number of candidates for treatment may require an additional expenditure of tens of billions of rupees.

Key Words: Chronic hepatitis B virus (HBV) infection. American association for study of liver. Diseases (AASLD). HBeAg. HBV DNA load. HBeAg.

INTRODUCTION

About two billion people worldwide are estimated to have evidence of HBV infection, with 350 million chronic carriers.¹ The people with chronic HBV infection live in all parts of the world including USA, but the problem is endemic in certain regions.^{2,3} In Pakistan, the estimated number of HBV carriers varies from 6 to 9 million in different studies⁴ but according to Pakistan Medical Research Council (PMRC) survey, the prevalence is about 2.5%.⁵

The majority of HBV infected individuals clears the virus spontaneously, however, 15% to 40% of HBV infected individuals are likely to develop serious long-term sequela like cirrhosis, hepatic de-compensation and hepatocellular carcinoma (HCC) during their lifetime.⁶ So there are criteria for eligibility of patients for treatment based on molecular, serological and biochemical

markers like HBV DNA, HBeAg and ALT.⁷ In 2004 version of AASLD guideline, the response to antiviral therapy was defined as undetectable HBV DNA 20,000 IU/mL (10^5 copies/mL) in serum.⁸ In spite of this, the progressive liver disease was seen with HBV DNA levels as low as (3-5 log₁₀ IU/mL). In the 2009 guidelines by AASLD, it was recommended that the lower HBV DNA levels (3-5 log₁₀ IU/mL) may warrant treatment.⁷ PSSLD in Pakistan issued similar recommendations for Pakistan.⁹ As the patients, with low HBV DNA load, are now recommended for treatment, the number of patients requiring treatment has been increased.¹⁰ Thus, there was a requirement to assess the number of patients. The HBeAg is produced as a result of HBV replication and is recommended as to be a surrogate marker for HBV DNA, particularly when ALT is raised.¹¹ HBeAg thus complements the results of HBV DNA test. The research was thus planned at Virology Department of Armed Forces Institute of Pathology (AFIP), Rawalpindi, with the objective of determining the additional load of the patients as well as to study the latest trend of HBeAg positivity in these patients with chronic HBV infection.

METHODOLOGY

The minimum sample size for study was calculated with sample size calculator using one sample situation for estimating a proportion with specified absolute precision.

¹ Department of Virology / Chemical Pathology² / Haematology³, Armed Forces Institute of Pathology (AFIP), Rawalpindi.

⁴ Vice Chancellor, Baqai Medical University, Karachi.

Correspondence: Brig. Dr. Agha Babar Hussain, House No. 67-B, Khadim Hussain Road, Near Additional IG Police Office, Rawalpindi Cantt.

E-mail: drbabarhussain@hotmail.com

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Eight hundred and one persons reported with chronic HBV infection (with known HBsAg positive status for minimum of the last six months), consecutively at Armed Forces Institute of Pathology, Rawalpindi. HBV DNA tests were also included in the study from November 2010 to January 2012, after taking an informed consent, irrespective of their gender and age. The proposal was approved by ethical committee of AFIP for research. A proforma was filled for each patient, in which demographic details and relevant history of the disease was noted. The medical documents and previous test reports available with them at that time, were scrutinized along with interviewing the patients to find out the evidence for any co-infection. The patients with evidence of HCV and HIV co-infection were excluded; however, those with HDV co-infection were included in the study, because of the fact that HDV is a satellite virus which only causes co-infection or super-infection with HBV and cannot cause infection without HBV. Five ml of venous blood was taken from each patient, out of which 2 ml was dispensed in plain test tube and 3 ml in DNA/RNA free PCR tube. The sera were separated and stored at -70°C in plastic aliquots and PCR tubes, in a retrievable way until tested. All samples were tested for HBsAg (for confirmation of HBV carrier status) HBeAg and HBV DNA quantitative load.

HBV DNA viral load was determined by using the commercial kit of Bioneer Korea for Real-Time HBV DNA PCR (Quantitative). AccuPower[®] HBV Quantitative PCR Kit (96 reactions) is composed of a ready to use format for real time quantitative detection of HBV DNA in clinical samples using Exicycler[™] 96 Real time Quantitative Thermal Block (Bioneer Co., Korea). Of HBV premix containing all components, including HBV-specific primers, dual-labeled fluorogenic (TaqMan[®]) probe, DNA polymerase, dNTPs, and stabilizer to amplify specific 91 bp region of HBV genome. Some ingredients like Non-Template Control (NTC), Internal Positive Control (IPC) and PCR Grade water are supplied in different packs for use in amplification in addition to a set of 5 serially diluted standards.

HBV DNA was extracted from sera, using extraction Kit of Bioneer, (ExiPrep[™] Viral DNA/RNA Kit Ver 1.2) with the help of automated extraction system (Exiprep[™] 16) of Bioneer, as per manufacturer's instructions. Amplification was performed in 5 ul of the extracted DNA, using Bioneer's light cycler (Exicycler[™] 96). A set of 5 serially diluted standards (ranging from 1000 to 10,000,000) was used for quantification in each batch. Internal Positive Control (IPC) was added to every specimen for validation of each test. Non-Template Control (NTC) was used as negative control for each batch. The unit of HBV Standard DNA included in the kit was defined as copies/ul. Each IU in our methodology was equivalent to 10 copies. The sensitivity and the dynamic range of AccuPower[®] HBV Quantitative PCR

Kit were 10 copies /reaction and 8 log dynamic range, respectively, therefore, all the cases with more than 10 copies were reported as PCR positive.

Each sample was tested for HBsAg, by reagent Kit of Linear Spain ELISA, to confirm HBV carrier status. HBeAg was carried out by reagent kit of Amgenix USA, Microwell ELISA Diagnostic System. ELISA reader EL x 800, of Diasorin was used for taking OD readings. Positive and negative controls were run in each batch for quality control purposes. The cut-off value was calculated as per formula given in the kit insert for each reagent kit.

Microsoft excel and Statistical Package for Social Sciences (SPSS) version 16 for windows, was used for analysis of the data collected. Student t-test was applied to evaluate differences in proportions. P-value < 0.05 was considered significant. Results were presented as mean \pm SD. The eligibility for treatment was determined based on HBV viral load, in the light of the AASLD guidelines of 2004 and 2009 and additional load of the eligible patients, in the light of 2009 guidelines, was calculated. The frequency of HBeAg positivity in all 801 cases was determined and the mean viral load in HBeAg and HBeAg negative individuals was compared. Moreover, estimate of the likely additional expenses involved on management of these cases were made, based upon the figures quoted in different international studies.

The institute catered for the necessary reagents, testing kits and other expenses for this research work.

RESULTS

In this study, 624 (77.9%) out of 801 cases were males and 177 (22.1%) were females. The age of HBV carriers ranged between 1 - 80 years, with a mean of 35.42 ± 12.4 . All 801 cases were positive for HBsAg and out of them, 74 (9.2%) had detectable HBeAg, whereas 727 (90.8%) were negative. The HBV DNA load testing of 801 samples revealed that 450 (56.2%) were having no detectable HBV DNA, and 351 (43.8%) cases had varying degree of HBV DNA, detectable, with an overall mean of $1.24 \times 10^7 \pm 1.10$ copies/ml. The viral load

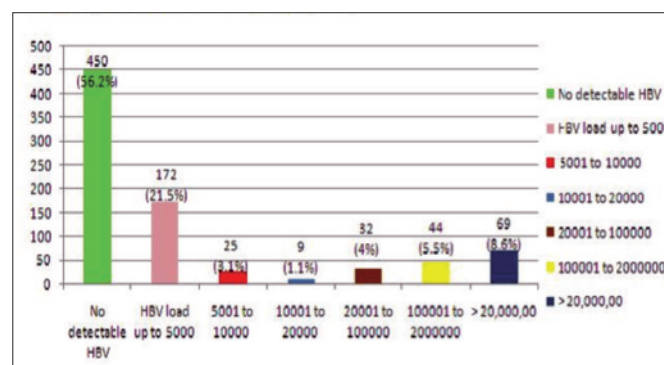


Figure 1: Frequency of HBV DNA levels in copies/ml (n = 801).

ranged between 0 (for cases with undetectable HBV DNA) and 2.4×10^9 . The frequency and percentages of different ranges of HBV DNA levels have been given in Figure 1. The mean viral load in HBeAg positive cases was 74617601.55 ± 1.66 , as compared to 6107734.05 ± 1.01 in HBeAg negative individuals ($p < 0.001$). There were 41 (5.1%) cases with viral load between 10,000 and 100,000 copies/ml, out of which 5 were positive for HBeAg and 36 were negative.

DISCUSSION

The Virology Department of AFIP, Rawalpindi is one of the few laboratory setups in Pakistan which are ISO 9001/2008 certified as well as 15189 accredited. In this study, at virology department, 43.8% cases had varying degree of HBV DNA load which was similar to a recent study conducted in India,¹² in which 40% of HBV carriers were positive for HBV DNA by PCR. However, this percentage was less than 64.3%, reported by Khan *et al.*¹³ The percentage of HBeAg positivity (9.2%) in this study was also lower than the earlier reported percentages.¹⁴⁻¹⁶ This difference might be due to the difference in nature and number of study subjects because the study population of Khokhar *et al.* was newly diagnosed cases of HBV, whereas in Bangladesh study, the sample size was only 72 cases versus 801 in this study. Although, HBV DNA positivity and HBeAg positivity in our patients were less than above quoted studies but the results of all tested parameters, including HBV DNA and HBeAg in this study, complement each other.

HBeAg production is associated with virus replication, so there is low replication of virus leading to less production of HBeAg in these cases.^{7,11} The low percentage of HBV DNA positivity close to the one given by Aakanasha *et al.*, may be demonstrating a new and different pattern of chronic HBV infection in the region. This might partly be attributed to the population bias, as majority of our clients were government servants and their families, relatively more educated and undergoing regular follow-up of their disease since long. The long periods of infection might be associated with more clear-cut loss of HBeAg and HBV DNA. Moreover, the less virulent strain of HBV reported in Pakistan¹⁷ and improvement in testing techniques might be other factors but these aspects need further in depth research. The mean viral load in HBeAg positive cases was 74617601.55 ± 1.66 , as compared to 6107734.05 ± 1.01 in HBeAg negative individuals ($p < 0.001$). This was in line with the other similar studies.¹⁸

The 41 persons additionally eligible for treatment in the light of 2009 AASLD guidelines, are 5.1% of the total of 801 cases under study and constitute additional economic burden in terms of transportation, further investigations, treatment and hospitalization if AASLD guidelines of 2009 are complied with.¹⁹

The healthcare cost for different categories of chronic HBV was calculated in different US based studies in 1998 and 2001. In a 1995 US study, the lowest annual healthcare cost was \$4175 for a patient of compensated cirrhosis, whereas it was, \$22072 for a patient with de-compensated cirrhosis.²⁰ The expenses on liver transplantation were estimated to be \$89076.²¹ In a recent study, based on a survey of tertiary care set-ups in China, direct annual cost in US dollars for severe hepatitis B, chronic hepatitis B, compensated cirrhosis, de-compensated cirrhosis and primary liver cancer was \$10834, \$4552, \$7400.28, \$6936 and \$10635, respectively.²² The majority of patients in the Chinese study were insured.

There is no large scale health insurance program for masses in vogue at governmental or private level and in most of Pakistani tertiary-care hospitals adequate arrangements do not exist to maintain relevant data of the patients and expenses incurred on each of them. As the aim behind this study was to provide an idea about additional burden, so calculation has been made taking minimum figure out of these quoted studies, which is 4175 USD. The minimum total number of patients with chronic HBV infection in Pakistan according to PMRC survey is 2.5%.⁸ So there are at least 4.5 million HBV carriers in the country of 180 million people. Five point one percent of 4.5 million comes to 229500, which constitutes additional load of the patients eligible for the treatment if the 2009 AASLD guidelines are followed. The lowest figure in above quoted US and Chinese studies, 4175 USD is roughly equivalent to 417500 PKR at a rate of 100 rupees for one dollar. If the annual cost of each patient is multiplied with the total likely additional cases (229500) the answer in above quoted studies comes to more than 95 billion rupees. This expenditure, however, cannot be totally attributed to the adherence to the revised AASLD guidelines as there is possibility that some of these 5.1% HBV carriers might had also required some expenses on routine monitoring and management, particularly those who would undergo reactivation.⁶

As such, even if one attributes a half of the above mentioned expenses to change in guidelines, still the likely additional expenditures are huge. The cost of Interferon (IFN) preparations (particularly pegylated IFN) and different oral anti-virals like adefovir, entecavir and tenofovir, available in the market, is high and duration of therapy may be up to 5 years on case to case basis. The expenses on laboratory tests are also high. Requirements for liver biopsy, ultrasonographic examinations, various other tests/procedures, transportation and hospitalization are also substantial. In peculiar circumstances of Pakistan, which is facing multiple problems like poverty, un-employment, illiteracy, recession, security situation due to war on terror, population explosion, inadequate growth of economy because of energy crisis

and serious lack of resources, such a huge additional expenditure is very difficult to adjust.

CONCLUSION

There was an overall tendency towards lower HBeAg positivity and HBV DNA positivity in Pakistani subjects with chronic HBV infection. Moreover, there is a likely increase of 202500 potential candidates for HBV treatment based on viral load testing, according to the AASLD guidelines update of 2009 as compared to 2004 guidelines. This increase in number of candidates for treatment may require an approximate additional expenditure running in multiples of ten billion rupees, which is a huge expenditure in the wake of current socio-economic situation of the country. There is, thus a need for in-depth appreciation of the situation and allocate resources according to the magnitude of the problem. There is also a requirement to undertake research and modify the guidelines according to the local conditions, along with obtaining benefits from international recommendations.

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