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COMPARATIVE STUDY OF HBV DNA LEVELS IN HBeAg POSITIVE AND NEGATIVE FEMALE PATIENTS SUFFERING FROM CHRONIC HEPATITIS B IN CHILD BEARING AGE.

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ABSTRACT... To compare HBV DNA values in HBeAg positive and negative female patients suffering from chronic hepatitis B of child bearing age. **Study Design:** Cross sectional study. **Setting:** Hospitals attached to Peshawar Medical College, Peshawar after taking ethical approval from the institution's ethical board. **Period:** October 2014 to March 2015. **Materials and Methods:** Blood samples were collected from 100 chronic hepatitis B females of child bearing age. Quantitative HBeAg was done by ELISA and HBV DNA values were estimated by PCR. The data obtained was statistically analyzed by using the Statistical Package for the Social Sciences (SPSS) version 17 and statistical significance between the variables was calculated using Fisher's exact test. **Results:** The mean age of the sample was 28.69 ± 6.83 years. A positive correlation ($p=0.000$) was found in HBeAg positive status and younger age group females (17-30). 84 cases were HBeAg negative but 42/84 had high levels of circulating Hepatitis B virus (2000 IU/ml). **Conclusion:** The present study established a positive association between HBeAg positive status and high HBV DNA values. However a statistically positive association was observed between HBeAg negative patients and high HBV DNA values (HBV DNA ≥ 2000 IU/ml).

Key words: Child Bearing age, ELISA, HBV DNA, HBeAg, Hepatitis B Virus

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INTRODUCTION

Hepatitis B caused by Hepatitis B virus (HBV) is becoming a global health issue with current estimates of 2 billion infected individuals and 387 million chronic carriers. Around one million HBV related deaths occur annually due to chronic hepatitis B, liver cirrhosis, liver decompensation and Hepatocellular carcinoma (HCC).¹ On the basis of prevalence of chronic hepatitis B around the globe, the world has been divided into three zones: high, intermediate and low prevalence zones. Pakistan falls in the area of intermediate prevalence zone with 2.5% of HBsAg positive population.²

HBV infection is one of the major health issues in Pakistan. The main routes of transmission of HBV is parenteral, vertical (mother to child transmission) and horizontal (sexual).³ The major mode of infection in endemic areas is vertical transmission that accounts for 50-60% of chronic

carrier. Vertical transmission either in utero or peripartum remains a constant source of increase in the reservoir of chronically infected individuals.⁴

The presence or absence of serological markers like HBsAg, HBeAg, Anti HBc, Anti HBs and Anti HBe are important in diagnosis, treatment planning and predicting the outcome of the disease.^{5,6} HBeAg is an important serological marker of HBV and is indicative of active viral replication. HBeAg positive carriers are regarded as a super carriers being highly infectious and are capable of transmitting the disease to others. They are kept under observation and are known candidates for antiviral treatment.⁷

HBV genome is highly susceptible to constant mutations. In chronic HBV carrier in addition to HBsAg, HBeAg may persist for years or even decades. Mutations occurring naturally mostly target precore and core promoter regions. Point

mutation in precore region at nucleotide 1896 (Adenine is substituted for Guanine) leads to the production of a stop codon and terminates the production of HBeAg molecule. As a result no HBeAg is found in the serum of the patients and the condition is termed as HBeAg negative Chronic Hepatitis B (CHB)(7). Loss of HBe antigen was first considered to be a good prognostic sign showing remission of hepatitis and suppression of HBV DNA. However with the advent of sensitive detection methods like PCR⁸, it showed that in a group of patients HBV DNA was detectable continuously or intermittently even in the absence of HBeAg.⁹

Women of childbearing age with HBeAg positive are highly viremic and can transmit the disease to their newborns. However women who are HBeAg negative status might have active viral replication going on despite of their HBeAg negative serology with increased HBV DNA levels due to precore mutations in viral genome.¹⁰ Around 90% of children born to these mothers with HBV DNA levels of more than 2000 IU/ml will develop chronic hepatitis B. 25% to 50% of children become infected before the age of 5 and another 5% in young adult hood as a result of post natal infection. 15-40% of these chronic carriers develop complications like HCC and liver cirrhosis.¹¹ This situation can only be prevented if active vaccination and immunoglobulins are administered in the first 12 hours of birth.¹²

Taking HBeAg negative hepatitis as non infectious is false sense of security which is the result of omitting an important parameter in HBV serology that is quantitative estimation of HBV DNA. Only HBV DNA estimation can decide whether virus is replicating or not. That is why this study was planned to compare HBV DNA values in HBeAg positive and negative female patients suffering from chronic hepatitis B in child bearing age by using PCR.

METHODOLOGY

Study Design

Cross sectional study.

Study Duration

Conducted from October 2014 to March 2015.

Sample Size

Samples were collected from 100 chronic hepatitis B females of child bearing.

Study Setting

Sampling was done at Prime Teaching hospital, Mercy Teaching hospital and Kuwait Teaching hospital. Processing of the samples was done at Prime Teaching Hospital and Department of Microbiology Peshawar Medical College, Peshawar.

Inclusion Criteria

The inclusion criteria for the study were females of child bearing age and who had Hepatitis B surface antigen positive for more than 6 months

Exclusion Criteria

Those females who did not give consent or had co-infection with HCV were excluded from the study.

Data Collection Tool

Questionnaire.

Data Analysis

SPSS version 17 was used to analyze data.

HBeAg (Cobas e 411 HBe Ag quantitative assay) was done by ELISA while Quantitative HBV DNA was extracted using INSTANT Virus DNA kit (AJ Roboscreen GmbH).

The data obtained was statistically analyzed by using the Statistical Package for the Social Sciences (SPSS) version 17.

RESULTS

Age

The mean age was 28.69 ± 6.83 years (Minimum age 17, Maximum 44 years). Age was divided into two groups, Group 1 (17-30 years) and Group 2 (31 and above)

HBeAg Status

Out of these 100 patients, 16 patients were HBeAg positive and 84 were HBeAg negative. HBeAg positivity and HBV DNA levels were significantly higher in patients with age of 17-30 years ($p=0.000$).

HBV DNA Levels

HBV DNA values were divided into four categories:
 Category 1: HBV DNA levels of 1999 IU/ml or less
 Category 2: HBV DNA values 2000- 9999 IU/ml
 Category 3: HBV DNA values 10,000-19,999 IU/ml
 Category 4: HBV DNA values of 20,000 and above
 There was statistically significant difference ($p=0.004$) between HBV DNA levels amongst the four groups.

In HBeAg negative patients, a comparison was made between two groups of HBV DNA levels (i.e. HBV DNA ≤ 2000 IU/ml and ≥ 2000 IU/ml). It did not show any significant statistical difference ($p=0.01$), However 50% ($n=42$) of these patients had viral load more than 2000 IU/ml.

DISCUSSION

Hepatitis B virus (HBV) is commonly associated with chronic carrier state especially in young age and children. This cross sectional study demonstrated that there is a very strong relationship between patients age, HBe positive

status and HBV DNA Levels ($p=0.02$). Our study is consistent with other studies which show a higher percentage of HBeAg positivity in women of child bearing age thus making them more prone to transfer the disease to their off springs.¹³ Transfer of HBV from mother to off spring is directly correlated with high levels of circulating viral DNA.^{14,15}

HBeAg negative chronic hepatitis B was once thought to be uncommon in our region but studies from UK, Hong Kong, India and Pakistan shows the prevalence of HBe negative CHB was around (30%-60%)¹⁶⁻¹⁸ A study that was conducted on 100 patients showed that 19% of patients with HBsAg positive status had HBeAg negative CHB. Another study conducted in Pakistan showed that 15% if HBeAg negative cases had high levels of HBV DNA due to the presence of pre core mutants.¹⁹ The frequency of HBeAg negative CHB patients was higher in our study than in other studies.^{20,21} In our study 84% of the females with HBsAg positive status were HBeAg negative. These patients were also tested for HBV DNA. 50% of HBeAg negative women had high HBV DNA levels (more than 2000 IU/ml). A reliable HBV DNA cutoff value to distinguish between active CHB and inactive CHB is still not clear but according to EASL guidelines people who have HBV DNA levels of more than 2000 IU/ml must be given treatment irrespective of their HBeAg negative status.^{22,23}

| | | Age Group 1 (17-30 years) | Age Group 2 (31-44 years) | P-Value |
|---------------|-------------|------------------------------|------------------------------|---------|
| HBe Ag | Positive | 11 | 5 | 0.16 |
| | Negative | 42 | 42 | |
| HBV DNA Level | ≤ 1999 | 21 | 23 | 0.27 |
| | 2000-9999 | 8 | 5 | |
| | 10000-19999 | 4 | 6 | |
| | >20000 | 22 | 11 | |

Table-I. Age Groups and their correlation with HBeAg and HBV DNA Levels

| HBeAg and HBV DNA Levels | Reactive Cases | | Non-Reactive Cases | | P-Value |
|--------------------------------|----------------|------------|--------------------|------------|---------|
| | Cases | Percentage | Cases | Percentage | |
| HBV DNA ≤ 1999 ($n=44$) | 2 | 2% | 42 | 42% | 0.0048 |
| HBV DNA 2000-9999 ($n=11$) | 1 | 1% | 10 | 10% | |
| HBV DNA 10000-19999 ($n=4$) | 0 | 0% | 4 | 4% | |
| HBV DNA >20000 ($n=41$) | 13 | 13% | 28 | 28% | |
| Total | 16 | 16% | 84 | 84% | 100 |

Table-II. HBeAg and HBV DNA viral load in different categories

Studies conducted have also concluded that such patients should be given treatment irrespective of their HBeAg status and ALT levels.^{24,25} This shows the importance of measurement of HBV DNA as it is critical for the diagnosis and management of HBeAg negative CHB. HBV DNA estimation through PCR is important and relevant in females of child bearing age for two major reasons:

1. Vertical transmission to baby during child birth may be prevented by appropriate preventive measures including safe antiviral treatment to the mother during last trimester, as decrease in maternal viremia is important as it lowers the risk of transmission to the new born.²⁶
2. The child born can be protected by giving active and passive vaccination during first 12 hours after birth. The high maternal HBV DNA level is the most important factor contributing to HBV perinatal transmission.²⁷⁻²⁹ If vaccination is not given on time these children have a 90% chance of developing complication in 3rd decade of life.
3. The attending doctors and medical staff should take all the required protective measures as these patients might be infectious.

It is also worth mentioning that in this study HBeAg positive cases were of lesser number i.e. only 16%. If only HBeAg is used as a sole marker of infectivity it will erroneously exclude a large number of HBeAg negative mothers with high circulating HBV DNA levels. Therefore it indicates that patients who are HBeAg negative should not be considered as less infectious as it may create a false feeling of "safety" in doctors and medical/paramedical staff dealing with these patients and thus makes them prone to acquiring the infection by adopting a causal or careless approach of lesser precautions.

CONCLUSION

The findings of the present study strongly suggest that HBV DNA quantification is of utmost importance in HBsAg positive patients irrespective of their HBeAg status. Detail population studies are also important to determine the true prevalence of HBeAg negative CHB. HBeAg negative patients should be evaluated for detection of HBV DNA by PCR so that proper treatment can be given on

time.





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|-------|--------------------|---|---|
| 1 | Mohsina Haq | Selection of topic, design and literature review wrote article. |  |
| 2 | Mahmud Ur Rehman | Approval of final version. |  |
| 3 | Najib-ul-Haq | Data collection. |  |
| 4 | Momina Haq | Abstract and recording. |  |