

# Serological Markers of Hepatitis B Infection in Incidentally Detected HBsAg Positive Patients

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## ABSTRACT

**Objective:** To evaluate the serological profile of the incidentally detected HBsAg positive patients, to assess the severity of disease and to identify the risk factors for the transmission. **Design:** an observational study. **Place and duration of study:** the study was conducted at Bolan medical Complex hospital, Quetta, from January 2001 to December 2003. **Patients and methods:** All patients who presented to gastroenterology clinic of Bolan Medical Complex hospital Quetta and at few private clinics with positive HbsAg, detected incidentally, were tested for alanine transaminase (ALT), hepatitis Be antigen (HbeAg) anti HBe antibody and hepatitis-B virus DNA (HBV DNA) by polymerase chain reaction (PCR). Their risk factors for acquisition of infection were assessed with specific questions. **Results:** A total of one hundred and four (104) patients with HBsAg reactive were included, 89 were male and 15 were females. The mean age was 30 years, with minimum age of 07 years and maximum of 57 year. Out of 104 patients, 93 patients were tested for HbeAg, 14 (15%) were positive for HBeAg and 79 (85%) were negative. Anti HBe antibody was checked in 91 patients, and it was reactive in 72 (79%), while it was non-reactive in only 19(21%) patients. HBV DNA (qualitative) determination was done in 95 patients and it was detected in 11(11.6%) and 84 (88.4%) were negative for HBV DNA. No significant correlation is seen between seroconversion and ALT levels. Normal ALT levels were observed only in 36 patients having seroconversion i.e AntiHBeantibody positive, while it was also normal in 5 HBeAg positive patients. Common risk factors detected in these patients were intramuscular injections, surgery and dental treatment, however, in a large number, risk factors were unknown. **Conclusion:** Fifteen percent asymptomatic subjects with positive HBsAg were found to be HBeAg positive. Large number of patients 79% has seroconversion and only 11.6 % have HBV DNA detected in their serum. Intramuscular injections surgery and dental treatment were noted to be frequent risk factors in these subjects.

## INTRODUCTION

The first recognition of a form of hepatitis, which was transmissible through blood or blood products, was reported by Lurman in Germany in 1883.<sup>1</sup> He reported that jaundice developed in 15% of persons who had received a smallpox vaccine prepared from human sera. During World War II, a high incidence of jaundice was observed among soldiers who had received a yellow fever vaccine made from human serum.<sup>2</sup> In late 1960s, a unique antigen was identified in the serum of an Australian aborigine patient with acute leukemia. Subsequently this antigen, named 'Australia antigen', was recognized as hepatitis B

surface antigen (HBsAg) protein and found to occur most commonly in patients who had received multiple blood transfusions.<sup>3-5</sup>

Chronic hepatitis B is a common disease with an estimated global prevalence of approximately 5% of the world's population. There are wide ranges in the prevalence of HBV infection in different parts of the world. In Southeast Asia, China, Philippines, Indonesia, Middle East and Africa the prevalence is high, with HBsAg positivity rates ranging from 8% to 15%.<sup>6</sup> Regions of intermediate prevalence (2%–7%) include Japan, parts of South America, Eastern and Southern Europe. Prevalence is lowest (<2%) in the United States and Canada, Northern Europe and Australia. The true incidence of this disease in

Pakistan is not known, but according to different regional studies the prevalence of this disease is around 2.5-3%.<sup>9</sup> Meager data is available from province of Balochistan where HBsAg positivity is being observed in a significant number of asymptomatic persons screened either for blood donation, prior to vaccination or some other reasons.

The prevalence of HBV infection in a community is influenced by local factors including ethnic mix of the population, frequency of injection drug use and proportion of the population that engages in high-risk sexual activity. The disease is transmitted mainly parenterally or by intimate, often sexual contact.

The virus is not transmitted during intrauterine stage, as it does not cross the placental barrier. The infection is transmitted from the mother to the neonates at the time of birth through vertical transmission and due to close contact afterwards. It is well established that the risk of persistent infection is much greater in infants than in adults. The acquisition of disease during childhood is probably horizontal through kissing, shared utensils such as toothbrush & razors, and injections. Although only 1% to 3% of all reported cases of HBV infection in United States are thought to occur in children, 20% to 30% of all chronic HBV infections in United States occur in children under the age of 5 years.<sup>6</sup>

Unscreened blood transfusion is another major risk factor for the transmission of this disease. Inadequate sterilization techniques in operation theatres, dental surgery and endoscopy rooms may contribute in the spread of disease. Tattooing, acupuncture, ear piercing, manicures, I/V drug abuse, reuse of syringes etc., not uncommon in our society are also contributing towards spread of this disease.

Hospital staff including doctors, nurses, paramedics and others is at a higher risk of having the infection as compared to general population.

The asymptomatic subjects, who have been detected incidentally have now been labeled as "incidentally detected asymptomatic HbsAg positive subjects" (IDAHS)<sup>7</sup>

The worldwide prevalence of HBV infection is falling owing to vaccination, hygiene and campaign for awareness regarding risk factors.

Few studies are done in Pakistan to evaluate the serological profile of the asymptomatic subjects, who were incidentally detected to be positive for HbsAg and to evaluate the risk factors for the acquisition of disease.<sup>8,9</sup>

#### **AIMS AND OBJECTIVES:**

This study was carried out to primarily assess the severity of disease at the time of presentation i.e. chronic hepatitis B either active or carrier and cirrhosis on the basis of clinical findings, lab investigations and radiological evaluation, and also to identify the risk factors.

#### **Serological diagnosis**

HbsAg appears in the blood of the patient about 6 weeks after the acute infection and disappears by three months. Persistence of HbsAg in the blood for more than six months implies the carrier state. Most of the patients often go in to replicative phase.<sup>10</sup> These are usually labeled as "inactive HBsAg carriers".<sup>7,11</sup>

AntiHBs indicates recovery and immunity. It appears late, about three months after the onset but positive only in one third of HBsAg carriers.

HBe antigen's presence reflects high infectivity. It is transiently present in the acute phase but persistence for more than ten weeks strongly suggests development of chronicity.

Anti HBe's appearance is suggestive of relatively low infectivity and patient's likely recovery both from acute as well as chronic states.

HBV DNA is the most sensitive index of viral replication. It can be detected by polymerase chain reaction (PCR) both qualitatively and quantitatively. It is used for the diagnosis of chronic hepatitis B viral infection as well as during therapy to monitor the response.

#### **Inclusion criteria**

- All patients positive for HBsAg by ELISA
- Both sexes

#### **Exclusion criteria**

Patients having autoimmune disorders, alcohol abuse and chronic hepatitis C were excluded.

## PATIENTS AND METHODS

### Study population and sample selection

The population for this study was derived from patients, who presented to gastroenterology OPD of Bolan Medical Complex Hospital, Quetta and to private clinics with HBsAg positive reports from Jan 2001 till Dec 2003. The HbsAg status was reconfirmed by ELIZA method. In all the positive patients, after history and clinical examination, a series of blood tests and abdominal ultrasonography were advised. The blood tests included CBC, LFTs (especially ALT), HBe antigen, anti HBe antibody and HBV DNA by PCR (qualitative) (Table 1).

**Table 1: List of Investigations**

Sr. No.	Test
1.	HBsAg
2.	LFTs including Total Bilirubin, ALT, Alkaline Phosphatase, Albumin
3.	CBC including Hb, Platelet count, WBC etc.
4.	HBe antigen
5.	Anti HBe antibody
6.	HBV DNA by PCR (qualitative)
7.	Abdominal Ultrasonography especially for liver, portal vein and spleen

### Statistical analysis

Statistical analysis was performed on SPSS version 13. Chi-square test used to analyze the association between HBsAg and HBV DNA (Qualitative), HbeAg, Anti HBe anti body, serum ALT levels and abdominal ultrasound. The association between HBeAg and AntiHBe antibody, HBV DNA by PCR (qualitative), ALT Levels, and clinical features of chronic liver disease were also assessed. Alt Levels < 40 IU/L were set as normal. The relationship with the risk factors, like tattoo, blood transfusion, operation and dental treatment was also analyzed. A p-value of <0.05 was selected as significant.

## RESULTS

A total of one hundred and four (104) patients with HBsAg reactive were included. 89 were male and 15 were females. The mean age was 30 years,

with minimum age of 07 years and maximum of 57 year. History of dental treatment was given by 8 (7.7%) patients, history of blood transfusion in only 2 (1.9) patient. Tattoos were found in 2 (1.9%) patients. Past history of jaundice was given by 32 (30.8%) patients, while history of frequent IV injection and operation was present in 14 (13.5%) (Table 2).

Out of these 104 patients, having HBsAg positive, the HBeAg was done in 93 patients, and it was reactive in 14 (15%) non reactive in 79 (85%) (Table 3). AntiHBe antibody was checked in 91 patients. The antibody was reactive in 72 (79%), while it was non reactive in 19 (21%) (Table 3). HBV DNA by Polymerase chain reaction was performed in 93 (89.4%) patients, and it was detected in 11 (10%) patients, while was undetected in 84 (90%) patients (Table 3).

Alanine aminotransferase (ALT) levels were done in 92 patients. The values of ALT were divided in to four groups (Table 4)

**Table 2: Risk factors for the transmission.**

Variable	Count	%	p-value
H/O Dental treatment	8	7.7	>0.05
H/O blood transfusion	2	1.9	>0.05
H/O operation/injection	14	13.5	>0.05
Tattoos	2	1.9	>0.05
H/O Jaundice	32	30.8	<0.05
H/O IV drug abuse	00	00	>0.05
H/O unsafe sexual exposure	00	00	>0.05

**Table 3: Results of serological marker.**

Serological Marker	Reactive	Non Reactive	Not Done
HbeAg	79	14	11
Anti HBe antibody	72	19	13
HBV DNA Qualitative	11	84	09

**Table 4: Group distribution of ALT levels.**

Groups	ALT Levels
Group 1	½ to 1 x ULN
Group 2	1-2 x ULN
Group 3	2-3 x ULN
Group 4	> 3 x ULN

In 74 HbeAg non reactive patients, 38 patients having normal ALT levels, while the values were 1-2x ULN in 23 patients and much higher in 13 patients.

ALT values were normal in 36 patients who have antiHBe antibodies reactive, while the values were 1-2x ULN in 22 patients. Significantly high ALT levels (>3 x ULN) were observed in 8 patients.

In 78 HBV DNA qualitative negative patients, ALT levels were normal in 41 patients, while were high in 37 patients (Table 5).

A cross tabulation analysis of anti HBe antibodies reactive samples to both HbeAg and HBV DNA results showed HbeAg non-reactive in 68, while HBV DNA negative in 64 persons, which is highly significant (p value < 0.01).

Only two HbeAg negative patients were having HBV DNA reactive, thus indicating a precore mutant strain. While in nine HBV DNA reactive patients, HbeAg was also reactive.

Table 5: Comparison of ALT to serological markers

Serological Marker	Normal ALT	High ALT	p Value
HbeAg (non reactive)	38	36	>0.05
Anti HBe antibody reactive	36	33	>0.05
HBV DNA Negative	41	37	>0.05

## DISCUSSION

Out of 91 subjects who were tested for antiHBe antibodies 72 (79 %) had these antibodies reactive in their serum, indicating sero-conversion in them.

Only 14 % of incidentally detected HBsAg positive patients were positive for HbeAg, thus reflecting active disease. These patients need treatment for chronic hepatitis B.<sup>12</sup> Previous studies in Pakistan showed 18-21% HbeAg positive.<sup>8,9</sup> Much higher rates of 45% HbeAg positive are shown in a study from India.<sup>13</sup>

HBV DNA qualitative were detected only in 11 (10%) patients, only two of these were negative for HbeAg, thus indicating less number of precore mutant. The reason may be that none of them had any treatment for hepatitis B in the past, because the emergence of these mutants is more related to

the use of oral lamavidine.

No significant correlation between ALT levels and seroconversion status was observed. Only 36 patients had ALT < 40 IU/L, while normal ALT levels were also noted in 5 HbeAg positive patients. Thus to rely on the ALT levels as an indicator for progression of disease is no more useful. The term "Healthy carrier" should not be used as these patients can develop chronic liver disease and even hepatocellular carcinoma. A more appropriate term now is "incidentally detected asymptomatic HbsAg positive subjects" (IDAHS)<sup>7</sup>

The major risk factors were past history of injections (14%) and dental treatment (7.8%). A significant number of patients had given history of jaundice (31%), but they did not have history of any injection therapy or surgery prior to that.

## CONCLUSION

Our study showed 14% HbeAg positive disease in incidentally detected HBsAg positive subjects, and 10% having HBV DNA detected in their serums. Serum ALT levels have no significant correlation with the severity of disease. Chronic hepatitis B is a progressive disease and can end up as liver cirrhosis or even hepatocellular carcinoma. Thus all the HBsAg positive patients should be checked for ALT, HbeAg, antiHBe antibodies and HBV DNA by PCR, in order to offer them proper and timely treatment.

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