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# Changing trends of Hepatitis B seromarkers amongst pakistani population: a laboratory-based review

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ORIGINAL ARTICLE

# Changing trends of Hepatitis B seromarkers amongst Pakistani population: a laboratory-based review

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## Abstract

**Objective:** To study the changing trends of hepatitis B markers tested at Aga Khan University Hospital clinical laboratory according to the internationally recognised classification of hepatitis B profile.

**Methods:** The retrospective study involved analysis of laboratory records of hepatitis B profiles of all patients collected from January 2001 to December 2008 at the Aga Khan University Hospital's clinical laboratory. Patients with complete profile tested were categorised according to the Centre for Diseases Control classification of hepatitis B profile. SPSS 16 was used for statistical analysis.

**Results:** A total of 185,825 patients had serological markers for hepatitis B tested. Mean-age of reactive hepatitis B surface antigen (HBsAg) patients was 30±12.5 years. HBsAg reactivity was significantly higher in males than females (34% vs 12%; p <0.0001). HBsAg showed a slight decline in the percentage reactivity during the 8-year study period, while a gradual increase in hepatitis B surface antibody (HBsAb) reactivity was observed. Of the total, 23% patients belonged to the 'susceptible to infection' category; 39% patients were classified as 'chronically-ill'; 12% patients were categorised as 'immune due to hepatitis B vaccination'. 3% patients were classed as 'acutely infected'. Overall, samples received from Peshawar, Quetta and Larkana showed very high reactivity rates.

**Conclusion:** The study substantiated the general perception that levels of HBsAg is showing a decreasing trend, while levels of HBsAb are increasing perhaps due to better vaccination of population.

**Keywords:** Hepatitis B profile, HBsAg, HBsAb, Serological markers. (JPMA 63: 826; 2013)

## Introduction

Hepatitis B virus (HBV) infection is a major health problem leading to significant morbidity and mortality. An estimated 400 million global population is chronically infected.[1] Most carriers of chronic HBV are from Asian, African and Mediterranean countries where perinatal transmission of the disease is high.[2] These chronic infections are responsible for around 40% of hepatocellular carcinomas in endemic regions.[3] Pakistan currently falls in the intermediate HBV prevalence group.[4]

In 2003, Pakistan Society of Gastroenterology issued a consensus statement on prevalence of HBV, according to which there are an estimated 4.5 million carriers in Pakistan with a carrier rate of 3-4%.[4] A more recently published meta analysis has revealed an overall HBsAg seroprevalence in healthy adults to be 2.4%.[5]

In 2002-03, HBV vaccination was introduced in the national Expanded Programme on Immunisation (EPI); attention was given to children under 1 year of age.[6] Impact of vaccination on our population has not been reported as yet. Thus, there is a dire need for studies that can show the disease in burden and to classify the affected population into clinically relevant groups such as susceptible, acute/chronic infection versus immunised group. Findings of such studies will help the policymakers in directing their limited resources effectively.

In the current study, we analysed a large data set of HBV serology performed over a period of 8 years. Main objectives were to analyse the trends of HBV serological markers and classify serological picture into clinically relevant groups, according to internationally recognised Centre for Disease Control (CDC) classification.[7] This is the first study from Pakistan that has attempted to assess trend of disease in various geographical regions and classify patients into different clinical groups.

## Materials and Methods

The retrospective analysis of HBV serology involved samples from 2001 to 2008 at the Aga Khan University Hospital (AKUH), a 591-bed tertiary care centre located in Karachi, Pakistan. During the study period 185,825 patients had serological markers for hepatitis B tested. Data was analysed during the year 2009. The clinical laboratory of AKUH has a network of sample collection centres located in 50 major cities and towns of Pakistan. This extensive networking makes the laboratory data representative of the country; hence disease burden was

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also analysed based on geographical locations within the country.

Classification was performed on patients that had all hepatitis B markers tested. They were categorized, according to CDC criteria, into 6 groups based on their serological profile:[8]

Group-1 (susceptible): HBsAg, hepatitis B core antibody (HBcAb; IgG) and HBsAb negative;

Group-2 (immune due to natural infection): HBsAg negative and HBcAb (IgG) and HBsAb were positive;

Group-3 (immune due to hepatitis B vaccination): HBsAg, HBcAb (IgG) were found negative with positive HBsAb;

Group-4 (acute infection): HBsAg, HBcAb (IgM) and HBcAb (IgG) positive along with negative HBsAb;

Group-5 (chronic infection): HBsAg and HBcAb (IgG) positive along with negative HBcAb (IgM) and HBsAb;

Group-6 (possibility of multiple interpretations): HBsAg and HBsAb negative along with HBcAb (IgG) positive results — may be recovering from acute HBV infection or may be distantly immune and test not sensitive enough to detect very low level of HBsAb in serum or may be undetectable level of HBsAg present in the serum and the person is actually chronically infected or/and may be susceptible with a false positive HBcAb (IgG).

All data was entered into SPSS 16.0. Frequencies were generated for categorical variables, and mean with standard deviation for continuous variables. Cross-tabulation was done to determine relationship between different groups.

## Results

A total of 396,348 patients were tested for HBsAg during the study period. Of these, 85215 (21.5%) tested positive by microparticle enzyme immunosorbent assay (MEIA) method. Mean age of population reactive to HBsAg was 29.8±12.5 years (Figure-1). Overall, 66261 (30%) males were reactive for HBsAg, while 18954 (11%) females were found reactive for this marker. Mean ages for males and females positive for HBsAg were comparable (30.0±12.4 vs 29.8±13.0 years). In the study population, 19236 (5%) were patients under 10 years of age. Majority 31955 (37.5%) of positive samples belonged to the 20-30 age group. Analysis of overall data showed progressive decline in percent positivity for HBsAg. It declined from 24% in 2001 to 18% in 2008 (Figure-2).

When analysed according to geographical location of sample collection site, the frequency of HBsAg positivity was lower for samples received from larger cities of Pakistan (15-21%), while it was relatively high for samples received from smaller cities (43-55%).

Besides, 69,331 patients were tested for hepatitis B 'E' antigen (HBeAg). Of them, 13381 (19.3%) were found reactive, with the majority (31%; n=4148) belonging to the 20-30 age group. Mean age and male-to-female ratio for positive serum samples was 25.0±14.0 years, 1.1 respectively. Overall, percentage of population reactive for this marker marginally decreased from 19% in 2001 to 18% in 2008.

As for HBsAb 58,549 patients were tested. Of these, 29626 (50.6%) were found reactive; 40% (n=11850) of the positive samples were received from the 20-30 age group. A gradual increase in frequency of patients reactive for HBsAb was noted; it increased from 47% in 2001 to 52% in 2008.

In terms of HBcIgG, 21098 patients were tested and 12448 (59%) were positive. The number of reactive patients remained steady during the study period; and the 20-30 age group provided the bulk of the positive samples (30%; n=3734).

With respect to HBeAb, 31,395 patients were tested, of whom 19779 (63%) were reactive, and 34% (n=6724) being from the 20-30 age group. There was an increase in the level of this marker from 57% in 2001 to 63% in 2008.

Table: Classification of hepatitis B profiles.

| CDC Category n (%) | 2001 n (%) | 2002 n (%) | 2003 n (%) | 2004 n (%) | 2005 n (%) | 2006 n (%) | 2007 n (%) | 2008 n (%) | Total |
|---|---|---|---|---|---|---|---|---|---|
| Susceptible | 377 (27) | 230 (26) | 142 (22) | 241 (21) | 233 (24) | 328 (25) | 189 (22) | 288 (23) | 2028 (24) |
| Immune due to natural infection | 156 (11) | 67 (8) | 54 (8) | 95 (8) | 81 (8) | 97 (7) | 71 (8) | 123 (10) | 744 (8.5) |
| Immune due to hepatitis B vaccination | 143 (10) | 98 (11) | 76 (12) | 114 (10) | 123 (12) | 180 (14) | 68 (8) | 220 (18) | 1022 (12) |
| Acutely infected | 42 (3) | 28 (3) | 14 (2) | 33 (3) | 24 (2) | 24 (2) | 25 (3) | 20 (2) | 210 (2.5) |
| Chronic infected | 582 (42) | 394 (45) | 311 (48) | 583 (50) | 456 (46) | 607 (46) | 455 (54) | 549 (44) | 3937 (46.5) |
| Multiple Interpretations possible | 78 (6) | 36 (4) | 38 (6) | 64 (6) | 57 (6) | 63 (5) | 37 (4) | 34 (3) | 407 (5) |
| Unclassifiable | 22 (2) | 24 (3) | 10 (2) | 29 (3) | 17 (2) | 12 (1) | 5 (1) | 4 (0) | 123 (1.5) |
| Total | 1400 | 877 | 645 | 1159 | 991 | 1311 | 850 | 1238 | 4390 |

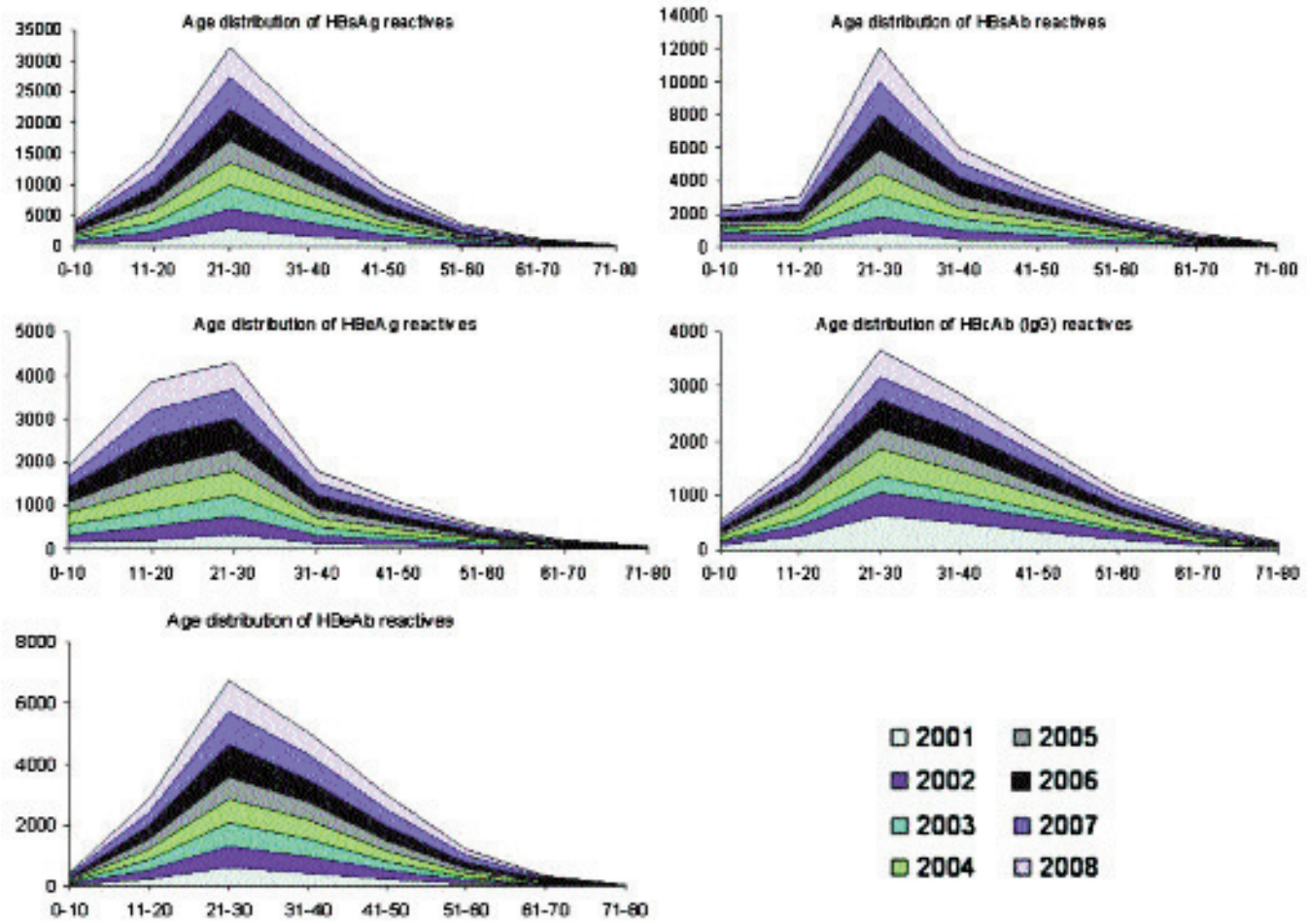


**Figure-1:** Cumulative graph showing age distribution of patients reactive for different hepatitis B seromarkers.

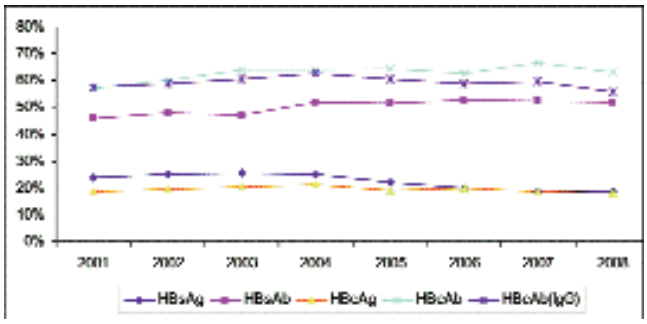


**Figure-2:** Year-wise trends of HBsAg, HBsAb, HBeAg, HBcAb (IgG) and HBeAb in patients tested from 2001 to 2008.

Complete hepatitis B profile was done on 4390 patients and were further analysed for clinical classification according to the CDC criteria.[7] Of the total, 1038 (24%) patients were classified as 'susceptible to infection'; 8% (372) patients were classified as 'immune due to natural infection' 591 (13%) were 'immune due to vaccination'; 93 (2%) and 2067 (47%) showed profile suggestive of 'acute' and 'chronic' infection respectively; 191 (4%) were classified as having multiple interpretations of their profiles; and 44 (1%) cases on an average could not be classed into any category and were labeled as unclassified (Table).

## Discussion

The study showed that HBV infection is a disease of children and young adults in Pakistan with maximum disease acquisition in the first to third decades. Throughout the study period, maximum number of specimens submitted to the laboratory were from these age groups and were found positive for various seromarkers. Early exposure is of great concern as childhood acquisition of disease results in higher morbidity and mortality and is a major burden to a poorly-funded healthcare system of a developing country. Poverty and prevailing lower socioeconomic

conditions along with inadequate healthcare facilities are known factors in the dissemination of HBV infection in early childhood. As per published reports, Pakistan is amongst those countries where reuse of syringes for therapeutic purposes and intravenous (IV) medications is highly prevalent.[9,10] Use of inadequately disinfected and unsterilised equipment in majority of the healthcare facilities is another major issue playing role in disease dissemination.[11,12] These factors have also been implicated as a major cause of disease transmission in our neighbouring countries which bear similar burden of HBV infections.[2,13,14] In contrast to the findings of our study, reports from Taiwan and China have quoted vertical transmission in neonates as the main cause of childhood acquisition of disease.[15,16] Similarly, surveillance data published from the developed world show sexual (homosexual, heterosexual, multiple partners) and IV drug addiction as the main mode of disease transmission. This is also reflected by the fact that major disease burden in these countries is among older age groups, that is in the third and fourth decades of life.[8,17]

Our data demonstrates that seroprevalence is three-fold higher amongst the male population. Previously published community data from this region also shows higher seroprevalence of HBV among males, but not at this scale.[18] Circumcision in early childhood, regular barber shop visits and higher rates of IV drug addiction are major risk factors which places males more exposed to blood-borne infections. Additionally, greater excess to health care facilities and IV medications further exposes them to such diseases and diagnosis.[8-11,19,20]

In countries with high seroprevalence of HBV infection, such as Thailand and Taiwan, vaccination programme was launched in the 1980s. Multiple long-term followup reports from these countries are showing dramatic change in its acquisition and epidemiology.[21,22] In Pakistan, vaccination was included in the EPI in 2002-03.[6] In our study, it was noted that during the second half, that is from 2005-2008, a rise in HBsAb and fall in HBsAg and HBeAg occurred. Recently, similar findings were reported in the national prevalence survey of Hepatitis B and C conducted by the Pakistan Medical Research Council.[23] This may reflect results of vaccination among infants and children and may also be a direct result of the increased trend of immunisation in the general population due to their better understanding and awareness of the disease through electronic media and governmental campaigns, especially in urban regions of Pakistan. This finding is further endorsed by the application of CDC classification, which also shows that in the later part of the study period, there is a rising trend in the vaccinated group, fall in acutely infected population and susceptible population. This trend is encouraging and indicates that a comprehensive hepatitis control programme focused on improved awareness and easy availability of vaccines can possibly reduce disease transmission and burden in endemic regions of the country.

Another interesting finding of this study was a high number of positive serum samples received from the central region of Pakistan which comprises southern Punjab, northern Sind and parts of Balochistan. This region is also known for high prevalence of hepatitis D and human immunodeficiency virus (HIV) infections.[24,25] These findings indicate that blood and body fluid borne disease transmission is perhaps occurring consistently and causing micro-epidemics in various regions of Pakistan. These regions are in dire need of preventive strategies such as immunisations and improvements in healthcare units. Implementation of infection control in all health centres, mass education on the use of syringes and to avoid unnecessary injections might also prove useful.

The strength of our study was its good sample size and the findings report decreasing trend of disease acquisition in local population for the first time in Pakistan. However, there are certain limitations and inherited weaknesses of the study. Firstly, it is a retrospective evaluation of laboratory data; secondly, we were not able to randomise the included population; and the data includes healthy people tested for reasons other then being diseased, that is, jobs, travel etc.

## Conclusion

Acquisition of HBV infection seems to be occurring at an early age with wide variation in its prevalence in different geographical regions within Pakistan. There is a decreasing trend of disease transmission, but for its further effective control the federal government, the World Health Organisation and other funding agencies need to work in collaboration. EPI needs to be strengthened across the country and policy of mandatory vaccination to all should be adopted in areas with very high prevalence of disease. Further, countrywide community-based surveys are required to validate these trends and assess the true effects of immunisation in our population.

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