

## SEROPOSITIVE HBsAg FREQUENCY IN KARACHI AND INTERIOR SINDH, PAKISTAN

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### ABSTRACT

**Objective:** Viral hepatitis continues to be a major health problem in Pakistan which encourages appraisal of the current frequency of HBV surface antigenemia.

**Methodology:** A total of 35,257 individuals of both sexes presenting at a private diagnostic lab and its branches in key areas of Karachi City were screened for HBsAg during an 8-year period ending June 30, 2006. Also evaluated were 1,776 blood samples collected from residents of Hyderabad, Khairpur, Larkana, Nawabshah, and Sukkur in Sindh province. Detection of HBsAg in all serum samples as a marker of HBV was done using the Roche COBAS CORE HBsAg-II EIA system.

**Results:** A total of 1,735 HBsAg positive cases (4.92%) were detected in those assessed in Karachi, and 39 (2.19%) in the individuals tested in parts of interior Sindh. The youngest seropositive subject was a male 3-month old infant, the eldest a 70-year old man; the majority of carriers (78.9%) were aged 16-49 years, and 70.8% of those testing positive in Karachi were male, as were 69.2% in other Sindh cities.

**Conclusion:** HBsAg positivity in Karachi has steadily increased in frequency in our hands from 2.84% in 1998 to currently 4.92%. A significant majority of those positive in all age groups were male in gender warrants deliberation on the credible modes of infection, including homosexuality.

**KEY WORDS:** HBsAg, Incidence, Karachi, Sindh, Pakistan, Homosexuality.

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### INTRODUCTION

Hepatitis B continues to be a global public health problem despite efforts to eliminate this chronic viral disease through education, screening, and vaccination programs. It is currently estimated that 400 million people worldwide have chronic hepatitis B virus (HBV) infection,<sup>1</sup> the prevalence varies widely, with rates ranging from 0.1% to 20% in different parts of the world.<sup>2</sup> While "High" prevalence (hepatitis B surface antigen [HBsAg] positivity

rates >8%) regions where the viral infection is highly endemic include the Far East, parts of the Middle East and sub-Saharan Africa, and "Low" prevalence (<2% HBsAg positive) rates in the United States, Northern Europe and Australia, "Intermediate" frequency (2% to 7% HBsAg positive) is reported, among others, in Japan, the Indian subcontinent, and parts of central Asia.<sup>3</sup> Pakistan lies in the intermediate prevalence category; indeed, one version in 2003 apparently quoting a Meta analysis approximated an exposure rate of 35-38% with 4% being carriers,<sup>4</sup> subsequent to diverse reported frequency rates which were reviewed in 1998,<sup>5</sup> and 2002.<sup>6</sup> Also, several estimations published during 2002-2006 ranged among the subjects screened from a low 2% in 351,309 male volunteer blood donors in Karachi,<sup>7</sup> 2.4% in Karachi health care workers,<sup>8</sup> 2.68% in 5,207 individuals in Abbottabad,<sup>9</sup> to 3.53% among 5,371 young adults,<sup>10</sup> 6.5% in 387

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persons undergoing routine pre-op screening,<sup>11</sup> 8.3% among 903 Afghan refugees in Pakistan,<sup>12</sup> and a high 13.9% in 108 patients with chronic hepatitis.<sup>13</sup> Also, Zuberi (1996) estimated that 10% of healthy adults were HBV carriers<sup>14</sup> aside from former quotes of 2.8% in 383 health care personnel in 1977<sup>15</sup> and 3.42% among 1,111 professional blood donors in Karachi in 1974<sup>16</sup> using mainly the on hand counter-current immuno-electrophoresis procedure (CIEP) and prior to the advent of the Enzyme Immunosorbent Assay (EIA) widely in use today.

Using EIA, we had previously reported a frequency of 2.84% in 11,079 clients screened in Karachi during June 1988 and May 1998,<sup>17</sup> at a time when the prevalence rate in Pakistan was commonly quoted as high as 10%.<sup>14</sup> However, the frequency of HBsAg seropositivity in our sampling has subsequently steadily increased to approximately 4.92% in a total of 35,257 people tested since 1998. We have also noted that about 70.8% of those testing positive were males in all age groups, an observation that requires reflection on the likely modes of infection. Moreover, an estimated 69.2% of those testing HBsAg positive among a total of 1,776 residents of 5 cities in interior Sindh during June 2005 and June 2006 were also male in gender. That close physical contact and homosexuality may be of import amid the several means of spread of HBV infection in our environment is discussed.

## PATIENTS AND METHODS

*Subjects:* A total of 35,257 clients presenting at Dr. Essa's Laboratory & Diagnostic Center and its branches in key areas of Karachi City during June 01, 1998 and June 30, 2006 were screened for HBsAg; 19,568 of these were females (55.5%), 15,689 males (44.5%). The referrals representing the broad population of the metropolis included neonates and children, women undergoing prenatal screening, blood donors, students, doctors and professional health workers, hospitalized patients, and those with disturbed or normal liver function enzymes, among others. Also screened during

June 01, 2005 and June 30, 2006 were individuals and families fulfilling foreign-travel visa requirements residing in Hyderabad, Khairpur, Larkana, Nawabshah, and Sukkur in interior Sindh, totaling 1,776; these blood specimens were transported to Karachi for analyses on the day of collection.

*Blood specimens:* About 5ml blood samples from adults and 1-3ml from infants and children were collected in disposable sterile syringes, transferred into sterile test tubes, allowed to clot at room temperature, centrifuged at 1000rpm for 15mins, and the supernatant serum promptly removed from the packed cells and debris. Markedly hemolytic specimens which may offer false positive results were discarded and second blood samples obtained by contacting the relevant persons.

*Screening:* Most sera were screened the same day of collection, or refrigerated overnight at 4-6C and tested the following day using the HBsAg II EIA (Roche) solid phase qualitative enzyme immunoassay in the COBAS CORE analyzer, which affords a diagnostic specificity of 99.9% for random samples and 95% confidence interval with specificity 98.1 - 99.9% for hospitalized patient sera. Also, portions of random samples were sent for cross-check verification to a reputable lab in Karachi, in order to fulfill quality assurance requirements of a private ISO-certified diagnostic lab.

*Subject data:* Clients were self-motivated or routine referrals, and beyond recording age and gender, no attempt was practicable to obtain any history of contact or likely mode of infection associated with those whose blood tested HBsAg positive.

## RESULTS

Approximately 1,735 of the 35,257 referrals of all ages tested in Karachi during the 8-year study period were HBsAg positive, suggesting a frequency rate of 4.92%. Of the 1,776 subjects screened in interior Sindh cities, only 39 were seropositive, offering a current frequency of 2.19%.

The youngest carrier was a male infant 3 months old, while the oldest was a 70-year old male; subjects tested ranged in age from the newborn (n=24) to two senior citizens aged 99 and 102 years, both female.

The approximate percentages of positive antigenemia and related male gender in five age groups, with most cases (78.9%) detected between the ages of 16-49 years, are as follows:

Age	HBsAg (%)	Male (%)
03 m-14yrs	5.4	79.4
16-29yrs	41.1	76.8
30-49 yrs	37.8	73.9
50-59 yrs	9.8	68.5
60-70 yrs	5.9	52.4

A majority (n=1,229) of the 1,735 HBsAg positive cases in Karachi (70.8%) were male in sex, as were 69.23% of those testing positive in interior Sindh.

The results recorded with approximately 180 randomly selected HBsAg reactive and non-reactive sera subjected to cross-check at a reputable lab were confirmed.

## DISCUSSION

The general impression during the 1970s and 1980s was that the incidence of HBsAg positivity as an indicator of HBV carriage was as high as 7-10% in the general population of Pakistan.<sup>14</sup> This was at a period when the routine method available for detection of HBsAg in blood was CIEP, and prior to the arrival of other systems of testing such as Latex particle agglutination, Immuno-chromatography, EIA, and PCR. Of these, the first alternative introduced was the rapid, easily applied and less expensive latex agglutination technique; pertinent kits were marketed by a number of countries, some of which are still in routine use in a majority of minor labs in the country and which offer variable percentage of false positive results.<sup>17</sup> This method of screening may likely have contributed to the previous apparently exaggerated rates quoted, even though some studies at the time, among others, gave more plausible lower frequency figures such as 2.8%<sup>15</sup> and 3.42%.<sup>16</sup>

Using the more sensitive EIA, we had earlier reported a frequency rate of 2.84% among referrals in Karachi during 1988 and 1998,<sup>17</sup> but have since noted a steady increase of seropositivity during the last eight years to approximately 4.92% currently. Whether the increase is due to the enhanced spread of HBV, or is in keeping with the increase in population and public awareness with more individuals opting for testing, is unclear; interestingly, the prevalence rate (2.19%) we have recorded among inhabitants of five cities in interior Sindh, although calculated in lesser study clusters, remains low.

A noteworthy observation in our lab data is that a majority of seropositive people detected in Karachi (70.8%) and also in interior Sindh (69.2%) were male, as were 54% of 550 children aged 6months-15years detected seropositive in Bahawalpur urban slums<sup>8</sup> and 73.4% of 893 patients in Hazara Division.<sup>18</sup> Indeed, HBV seems to have a collective predilection for males.<sup>2,3</sup> This gender bias, although acknowledged, necessitates deliberation of possible risk factors involved in HBV transmission as occurring in our environment. No doubt spread of the virus is known to occur in several ways: transfusion of infected blood and blood products; via semen and vaginal fluids; mother to child; frequent sharing of contaminated needles, syringes, razors, toothbrushes, and dental floss; use of unsterile needles for acupuncture or tattooing and ear/nose piercing; reuse of unsterile glass syringes; needle stick accidental inoculation; previous surgeries or dental procedures, etc.<sup>19</sup> And while there were no definite relationships between ABO blood groups and HBsAg carrier state, possibly poor personal hygiene as a contributory factor were observations in one study.<sup>16</sup> Where males are concerned, other relevant means of spread cannot be ignored that include undertaking circumcision or regular shaves by street or saloon barbers who reuse fixed blades or razors,<sup>7,20</sup> contamination of skin abrasions during self-flagellation in religious rites, and anal or oral homosexual contact. While semen is a known carrier of the virus, Shah et al.<sup>13</sup> have

mentioned “unhygienic sexual relation” as a possible means of spread in their study group; whether this refers to exposure of mucous membranes to saliva, which is known to harbor HBV, in wet-kissing or lubrication to facilitate the sex act, is unclear. And whether a significant percentage of seropositive males are indeed “carriers” of the virus is currently a subject of conjecture.

But across Asia, homosexual behavior has been widely reported in both Islamic,<sup>21,22</sup> and non-Islamic countries. Even in contexts where homosexuality has long been denied, there may be well developed homosexual networks and subcultures such as those documented between male sex workers and their clients in Pakistan,<sup>23,24</sup> a nation where sex between men is strictly forbidden by law and religion. Section 377 Penal Code indeed criminalizes homosexuality in Pakistan, and the law punishes such acts with a minimum of two years in prison. Yet gay relationships do take place, even in the most conservative or repressive countries; discreet liaisons between individuals of varying ages, or simple sharing a bed with a cousin or friend are commonplace, as are the promiscuous frailties of adolescence.

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