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MAGNITUDE AND PATTERN OF HEPATITIS B CARRIERS IN THE GENERAL POPULATION AT A TERTIARY CARE HOSPITAL.

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INTRODUCTION:

Hepatitis B virus (HBV) is a parenterally transmitted virus that has assumed great clinical significance of late. The clinical presentation of HBV ranges from asymptomatic or unapparent infection to acute liver failure. Subsequent to acute manifestations, there is generally elimination of the virus by the body's immune processes within 6 months, but the crux of the issue lies with the individuals who remain chronically infected, who could progress on to more severe forms of hepatic disease like cirrhosis and carcinoma, or may remain asymptomatic in the form of a carrier state [1].Around the world, it has been estimated that nearly 350-400 million people have been infected by HBV. HBV related chronic infections result in nearly 60% cases of cirrhosis and 80% cases of hepatocellular carcinoma, with end stage liver disease accounting for one in 40 deaths [2]. The incubation period for hepatitis B is 45-160 days, in which period transfer of the virus may occur to countless individuals, thus perpetuating the cycle of infection. Moreover, HBV is about a hundred times more infectious than the human immunodeficiency virus (HIV), and is easier to transmit by needle-stick injuries [3]. The Hepatitis B virus is the only DNA hepatitis virus, 3200 base pairs long, and one of the two Hepatitis viruses that can progress onto chronic liver disease and hepatocellular carcinoma. It is placed in the family Hepadneviridae type 1. It undergoes mutations at a rate of 10,000 bases/infection year [4]. The ultrastructure of the HBV particle shows the presence of an outer envelope and an inner core containing the viral genome and DNA polymerase. The particles causing antigenicity of the hepatitis B virus are the Hepatitis B surface Antigen (HBsAg) which is a surface component and the antigen present on the nucleocapsid Hepatitis B core Antigen (HBcAg). The Hepatitis B e-Antigen (HBeAg) is relatively the newest and arguably the most significant of the antigens. It is coded by the same gene as the HBcAg but is immunologically distinct. It was found to be a way to distinguish between carriers of high infectivity and carriers of low infectivity [5]. Most scientists agree that apart from the presence of HBsAg, the presence of HBeAg indicates high infectivity, known as 'Super carriers' or 'active carrier', and the absence of HBeAg along with presence of anti-Hepatitis B e-antigen (Anti-HBe) indicates the low infectivity 'Simple carrier' state or 'inactive/ healthy carriers'[5, 6]. The total HBV carrier pool in India is estimated to be around 43 million. Out of the estimated 400 million infected around the world, this gives India the dubious distinction of having onetenth of all carriers worldwide. Around 1 million new HBV carriers are added to this pool annually [7]. The average estimated carrier rate of HBV in India is 4.7% [8]. However, there have been vast inconsistencies and differences among different studies in India with different studies exhibiting a varying seroprevalence of 0.8% to 42.5% [9, 10]. The estimation of the number of carriers in most studies is mostly done by the detection of the HBsAg in individuals, but the detection of the presence of the HBeAg and anti-HBe has not yet gained mainstream attention. This study aims to estimate the carrier rate and pattern at a tertiary care hospital in Bangalore by detecting the presence HBsAg by the emiluminescence method, and the presence of HBeAg and the anti-HBe (IgG) among the carriers by the rapid immunochromatographic ssay method. Hepatitis B is still not infamous enough or publicized enough to warrant caution, compared to other viral disease of epidemic value like HIV. However, curbing HBV infection is possible and very much in the realm of reality as it is a vaccine preventable disease with a very high efficacy [11]. The WHO estimates that around 55 percent of children around the world receive the 3 doses of vaccine [12]. Coverage around the world, and in India, has remained low and targets ony very small subpopulations [13, 14]. We are progressing towards greater coverage for children by the inclusion of the Hepatitis B vaccine in the Universal Immunization Program instituted by the Indian Academy of Pediatrics [15], but its impact remains yet to be seen in subsequent cohorts of vaccinated children. Moreover, accination for high-risk adults has not yet entered the consciousness of most of the Indian medical field, so the greater population is still at a grave risk of getting infected, even though protection is one simple step away. Thus assessing the pattern of infectivity and carrier status would be helpful for identifying highrisk individuals and hence, preventing the spread of this disease.

MATERIALS AND METHODS: This prospective study was conducted in the Department of Microbiology at a rtiary care hospital in Bangalore.

Study Design: Cross Sectional

Type of Study: Hospital based prospective study.

Study Period: 2 months, June - August 2015

Sample Size: 2061 samples

Sample taken randomly during in-patient routine screening, out-patient screening, or blood bank donor screening.

Exclusion Criteria: Subjects suffering from present liver disease, or HIV positive at point of blood collection.

All the blood samples were taken from samples that came in for routine screening at the Department of Microbiology and Hospital Blood Bank at the Tertiary Care Hospital. The samples were collected over a period of 45 days in the months of June-July 2015. The blood samples were collected by experienced technicians at the hospital and transported immediately to the Microbiology Department.

A 5 ml tube of whole blood was collected following standard procedures, using a sterile container, with no anticoagulant added, from each patient. Samples were allowed to clot for one hour at room temperature. It was then centrifuged for 10 minutes at approximately 1000g. An aliquot of 500µl of serum was made into labeled cryo-vials using clean pipette technique. Samples being tested immediately were stored at 4 degrees. Long term storage was done by freezing vials of serum in a deep freezer. Any time a serum sample was taken out of deep freeze, it was re-centrifuged before storage.

Samples were tested for HBsAg using automated chemiluminescence method [HBsAg Assay II, cobas® e 601, Roche Diagnostics, Germany]. The test works on the sandwich principle and takes a total duration of 18 minutes. In the 1st incubation period, 50 microliter of sample, two monoclonal Anti-HBsAg antibodies and a mix of monoclonal and polyclonal antibodies labelled with a ruthenium complex form a sandwich complex. During the second incubation, the complex gets bound to the solid phase after the addition and interaction of streptavidincoated Microparticles. This

reaction mix is aspirated into a measuring cell where the Microparticles are captured onto an electrode surface through magnetism. The unbound substances get removed and an electric current is passed through the electrode. This causes a chemiluminescent reaction which is detected and measured by a photomultiplier. Samples testing positive for HBsAg and meeting the inclusion and exclusion criteria were considered as HBsAg positives and were stored in deep freeze, in labelled cryovials. At the end of the experimental collection period, the samples positive for HBsAg which were stored were then tested for HBeAg and Anti-HBe antibody IgG using rapid immunochromatographic assay kit [Insight Device, Tulip Diagnostics, India]. The Anti-HBe IgG was tested as IgG is an indicator of long standing old HBV infection, whereas the IgM signified present infection. (Rapid immunochromatographic assay was used instead of enzyme linked immunosorbent assay due to laboratory availability and cost factors.) The Insight HBeAg test is a rapid, self-performing, immunochromatographic assay for the detection of in human serum. Sensitivity of HbeAg is 1ncu/ml, and Insight Anti-Hbe IgG test is a rapid, competitive, self-performing, immunochromatographic assay for the detection of HbeAg IgG in human serum. Sensitivity of Anti-Hbe IgG is 4ncu/ml. The two tests are based on the principle of serum agglutination on a membrane. For the HBeAg test, the conjugate pad contains Anti-HBe-colloid gold and mouse IgG-colloid gold. As the test specimen flows through the device, the Anti-HBe-colloid gold conjugate complexes with the HBeAg as well as the mouse IgG-colloid gold and travels further along the cassette to get immobilized by Anti-HBe and form a pink band at the Test region. The mouse IgG-colloid gold and unbound Anti-HBe move further to the Control region where it forms a band and serves as control. The test for the Anti-HBe is similar, but uses specific antibodies to the IgG instead. Samples positive for HBsAg and HBeAg were designated Super carriers. Those positive for HBsAg and Anti-HBe IgG, with absence of HBeAg were designated Simple carriers. All patients were advised to get their families vaccinated against Hepatitis B. Data was collated using the immunological tests and hospital data. All obtained data was used to determine rate of positivity for HBsAg and pattern (susceptible groups) of occurrence of HBsAg with regards to age and sex, as well as the presence of HBeAg and Anti-HBe IgG to determine the percentage of Super carriers and Simple carriers. Data was entered into Excel and analyzed with descriptive and inferential statistics.
Quality control was as per standard procedure recommended by MCI. All testing was conducted with informed consent and strict confidentiality has been maintained. Ethical clearance was obtained from the Institutional Ethics Committee after review of the study proposal.

Consent form format

I, _____, age _____ years, gender_____, give consent for the use of my serum sample collected for further diagnostic testing for a research project. I understand that strict confidentiality will be maintained and that no extra cost will be charged to me.
Signature: _____
Date: _____
UHID/BB Number: _____ (For official use)

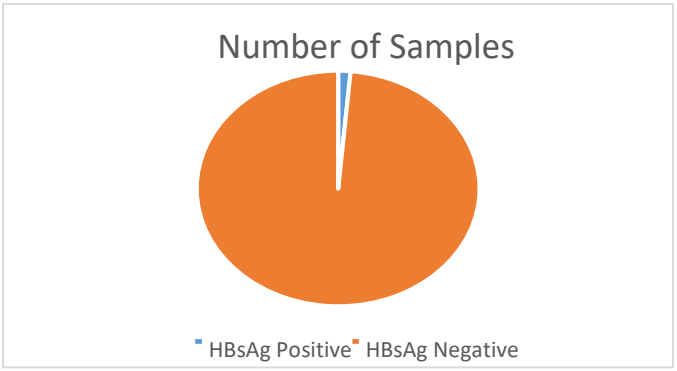
OBSERVATIONS AND RESULTS:

After immunological testing and statistical analysis of the data, the following observations were seen.

Gross Carrier Rate

The total number of samples tested for HBsAg by the chemiluminescence method was 2061, out of which 29 samples were found to be HBsAg carriers, satisfying the inclusion and exclusion criteria.This gives the gross rate of positivity as: [29/2061]*100=1.41%

Pie Chart 1:



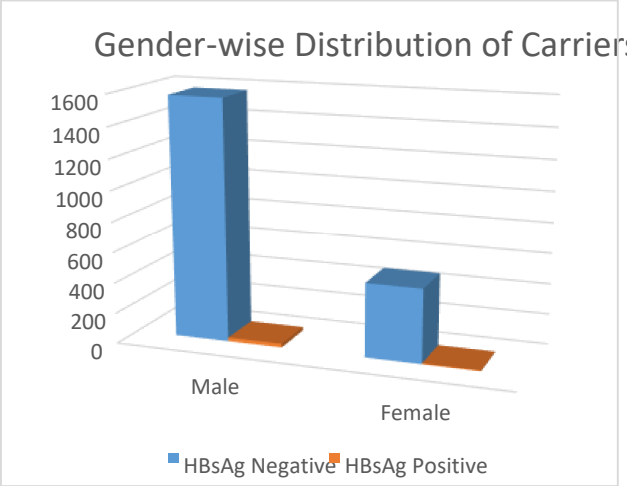
Gender-wise distribution of HBsAg carriers

The gender wise distribution of the positivity of HBsAg is as follows. There was a majority of male patients being tested, with a female to male ratio was 485:1596.

Table 1:

Gender	Total Number of Samples	Percentage of Total Samples	Positive	HBsAg Positive Percentage
Male	1596	77.44%	24	1.50%
Female	485	23.53%	5	1.03%

Bar Graph 1:
The findings of the gender-wise distribution are represented visually in the bar graphs.

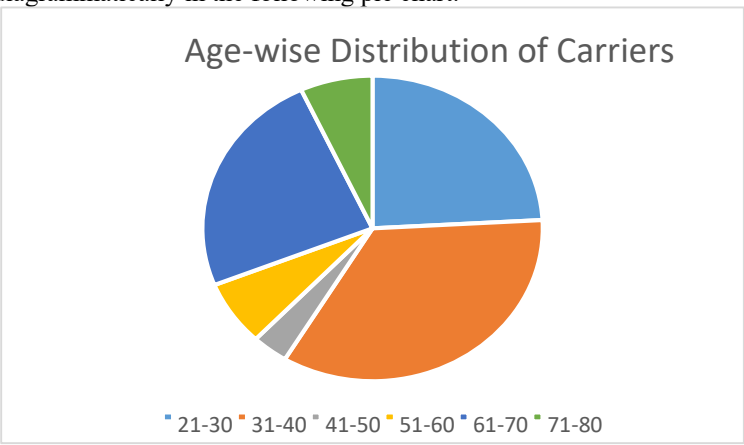


Age-wise distribution of HBsAg carriers
The 29 HBsAg positive samples were sorted according to age groups by decade and the following observations were seen. There were no positives in the under 20 year group. The age ranges of the positive samples was 24 years to 80 years of age.

Table 2:

Age Group (years)	Number of Carriers	Percentage of Total Carriers
0-10	0	0.0%
11-20	0	0.0%
21-30	7	24.14%
31-40	10	34.48%
41-50	1	3.45%
51-60	2	6.89%
61-70	7	24.14%
71-80	2	6.89%

This data is represented diagrammatically in the following pie chart.



Pie Chart: 2

Super carriers and simple carriers

Following the identification of the 29 carriers, they were tested for HBeAg and Anti-HBe IgG. They were designated as simple carrier if they were HBsAg positive, HBeAg negative and Anti-HBe positive. They were designated as super carrier if they were HBsAg positive, HBeAg positive and Anti-HBe negative.

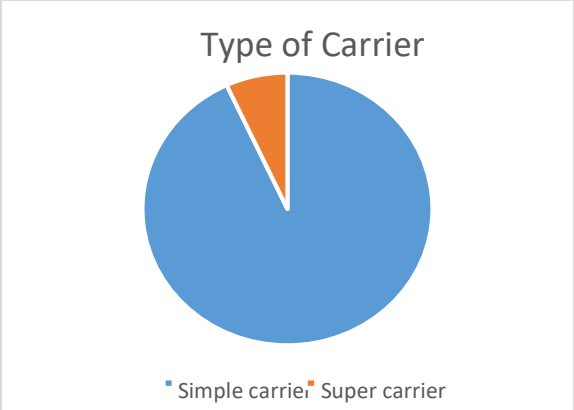
The following table shows the number of HBsAg positive samples sorted according to the type of carrier.

Table 3:

Type of carrier	Number of carriers	Percentage
Simple carrier	27	93.10%
Super carrier	2	6.89%

Out of the two carriers testing positive for HBeAg, one was male (age 52 years), and the other was female (age 63 years).

Pie Chart 3:



DISCUSSION:

Hepatitis B is a communicable disease with a heavy burden on any society, and carriers pose a great risk to the population. It is especially significant for public health as it is easily vaccine preventable, but sufficient measures are not being taken to combat this potentially deadly disease. As one of the leading causes of hepatocellular disease and liver carcinoma [2], it cannot be ignored and requires comprehensive strategies for its identification and control. In our study, we have found that out of a pool of 2061 individuals, 29 were found to be carriers of HBsAg, which gives a gross carrier percentage of 1.41%. This result is a lower figure than the quoted national average carrier rate of 4.7% [8]. However, this lower than average value may be due to the fact that this was study was conducted in an urban population in a major city, which does not have endemicity. A study by Batham et al showed a marked variation between tribal and non-tribal (urban) infectivity percentages (15.9% and 2.4% respectively), so the lower than average result tallies with that observation [8]. Other studies in populations in major cities have shown carrier percentages of as low as 0.61% and 0.87% in the India [9, 51]. As can be seen from Table 1, there was an approximate 1:3 ratio of females to males in the total samples tested, but the percentage of HBsAg positive in females and males was 1.03% and 1.50% respectively. This result showed that although the number of samples tested was different, the carrier percentage of males and females was relatively the same. The larger number of male samples was probably due to the fact that most voluntary donor at the blood bank were male. The age wise segregation of HBsAg positive individuals showed that a maximum percentage of individuals belonged to the 31-40 year age group (34.48%) as seen in Pie Chart 2. This was followed by 21-30 years and 61-70 years age group with 24.14% each. Following this was the 51-60 year and 71-80 year group with 6.89%, and lastly the 41-50 group formed 3.45% of the total carrier pool. The higher prevalence in the 31-40 age group may be due to unprotected sexual contact and unsafe needle sharing practices. However, the familial contact may also be a big contributor [5]. The relatively older age groups being tested positive may be due to the fact that a couple of decades are required for the development of chronicity of hepatitis B infection. An interesting find was that no HBsAg positive individuals below 20 years were found in the study period. This may be because of the HBV vaccines that have been rolled out since 2007 in India. Karnataka was one of the first ten states in which the vaccine policy was implemented [14], so it stands to reason that the policy is probably being implemented effectively. Contrastingly, other studies have shown a high rate of HBsAg positivity in children under 10 years as well [10], with indicates that this may be a region specific result. Moreover studies in India show a high rate of HBV infection in pregnant women [17], so perinatal transmission is still a major route of infection, but regular antenatal screening and birth dose of HBV vaccine seem to be effective in preventing its vertical transmission. Following HBsAg, 2 out of the 29 HBsAg positive individuals tested positive for HBeAg, as seen in Table 3, giving a percentage of 6.89%. The individuals were one male and one female. This indicates a state of active HBV DNA synthesis and high infectivity (Super carrier) [1]. Some other studies have shown HBeAg positivity rates ranging from 1% to 17.27% in various subpopulations. Most studies still use HBeAg assay only for prognostic value or

for determining HBV DNA synthesis activity [29, 30, 35, 36], but it would be helpful to also classify carriers based on their infectivity when trying to determine cost effective measures of prevention and treatment of Hepatitis B. The assessment of HBeAg and infectivity status has practical uses in transfusion centers for counseling HBsAg positive blood donors, for determining priority targets of immunization and advising the medical professionals on the care and precautions during the care of high infectivity carriers. However, as with any study, this study is also limited by the fact that it only assesses one major tertiary care hospital in Bangalore, Karnataka. This limits the population studied to an urban one, which affects prevalence rates no doubt. However, this result is useful for further epidemiological studies and to study the effectiveness of any further measures to combat Hepatitis B. Thus, we can see that although many measures are in place to combat the spread of Hepatitis B, there are still many carriers hidden in the population. This indicates that preventive measures have to be stepped up and implemented in a more widespread manner.

CONCLUSION:

Hepatitis B is a major public health issue in India and around the world. Ever since its emergence into importance 30-40 years ago, many efforts have been made to combat it and curb its spread. With devastating consequences like hepatocellular carcinoma and liver cell death, especially seen among the children who get infected, it is a great burden on our society. The main point of contention is its easy spread with a very minute viral load, by means as simple as saliva, mucus or even sweat. Although the main modes of spread continue to be parenteral routes like through mother to child, tainted blood from blood banks, unprotected sexual contact and injectable drug use, measures need to be put in place to ensure that high infectivity individuals are isolated and made non-infectious as soon as possible. The studies on prevalence of HBsAg have given results which are consistent in their inconsistency, but that is probably due to the vast and diverse nature of populations across India. However, we can conclude that the rate is still very high compared to the rest of the world, especially first world countries. Thus, any preventive measures which are applied throughout the country must also be tailored to fit the needs of specific regions, if they are to be truly effective. As Hepatitis B is very easy to prevent but difficult to treat, the emphasis of any national policy should be on vaccination and prevention rather than treatment. Efforts have been made by our government, with the introduction of compulsory Hepatitis B vaccination under the Universal Immunization Program, but the implementation of any program in India remains sporadic and disproportionately skewed. More emphasis also needs to be made on adult vaccination, especially among healthcare workers, high-risk groups and those in endemic areas, as well as education on safe practices for food handlers, blood banks and promiscuous individuals. The only way we can lessen the burden of this disease is by constant vigilance for its prevention.

SUMMARY:

This study was conducted to find the Hepatitis B carrier rate at a tertiary care hospital in Bangalore. The samples collected were tested for HBsAg using chemiluminescence assay and positive samples were further tested for HBeAg using radioimmunoassay. Samples were then classified and analyzed statistically. It was seen that 1.41% of individuals are carriers of HBsAg and among them, 6.89% are positive for HBeAg. The 31 to 40 age group formed a majority of the positive samples with 34.48%. There was no significant difference among male and female prevalence rates. This result shows that the prevalence of Hepatitis B is still very high, and carriers with high infectivity must treated with extra caution and care to help prevent the spread of this disease.

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