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Seroepidemiology of Incidentally Detected Asymptomatic HBsAg Positive Subjects from Southern State of India – A One Year Study

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Authors' contributions

This work was carried out in collaboration between both authors. Author SF designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript and managed literature searches. Author AA managed the analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Introduction: Hepatitis B virus infection is assuming a silent epidemic phase in India. Majority of the chronically infected are asymptomatic and unaware of their status. This pool of individuals called; incidentally detected hepatitis B positive subjects [IDAHS], unknowingly transmits infection to their contacts for decades. In order to curb the spread of infection they need to be identified tested, followed up and treated if required.
Aim: To assess the prevalence of asymptomatic Hepatitis B virus infection and risk factors for acquisition of same. Serological and biochemical profile of these individuals and influence of demographic factors on these markers.
Study Design: A cross sectional and observational study.

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Place and Duration of Study: January 2014- January 2015 at Princes Esra Hospital of Deccan College of Medical Sciences.
Methodology: A total of 3260 blood samples were screened for hepatitis B surface antigen. Serum from IDAHS was later subjected to various serological and biochemical tests.
Results: Hepatitis B surface antigen was detected in 3.8% of the screened individuals. All were asymptomatic for the hepatitis B virus infection. Of the various risk factors enquired, intake of frequent intramuscular injections was the most common noted. Male predominance was seen for HBsAg and HBeAg as 56% and 100%. HBeAg and antiHBe were positive in 8% and 80% of individuals tested. Majority of the HBsAg and HBeAg positive subjects as 65% and 56% were young less than 30 years of age indicating the early age of acquisition of HBV infection and development of carrier state by 3rd decade of life. AntiHBc IgM and antiHBc IgG were positive in 20% and 98% of the individuals respectively. AntiHBs was above 10 IU/L in 4% of the tested. Serum alanine transaminase was raised in 12% of the individuals and all were HBeAg negative and antiHBe positive.
Conclusion: In light of above findings we suggest that a strategy is to be developed and implemented by public health care authorities to identify, evaluate, follow-up, link and treat these cases to contain the spread of infection.

Keywords: Hepatitis B virus; incidentally detected hepatitis B positive subjects; HBe antigen; anti HBe; anti HBc IgM; anti HBc IgG; anti HBs; serum ALT.

ABBREVIATIONS

Anti HBe : Antibody to hepatitis B virus E antigen
AntiHBc IgM : Immunoglobulin M against Hepatitis B virus core antigen
AntiHBc IgG : Immunoglobulin G against Hepatitis B virus core antigen
Anti HBs : Antibody to Hepatitis B virus surface antigen
CDC : Centres for Disease Control and Prevention
CHB : Chronic Hepatitis B virus infection
EPI : Expanded immunization program
HBV : Hepatitis B virus
HBeAg : Hepatitis B virus E antigen
HBsAg : Hepatitis B virus surface antigen
HBV DNA : Deoxyribose nucleic acid of Hepatitis B virus
IDAHS : Incidentally detected hepatitis B positive subjects
IEC : Information education communication
Serum ALT : Serum alanine aminotransferase
WHO : World Health Organization

1. INTRODUCTION

The epidemiology of HBV infection as per the World Health Organization [WHO] 2014 report shows that more than 2 billion people i.e. 1/4th of the world population is exposed to hepatitis B virus. Around 50 million new cases are diagnosed annually [1]. Fifteen to forty percent of these develop cirrhosis, decompensated liver disease and hepatocellular carcinoma [HCC] [2,3]. Over 360 million of the 2 billion exposed develop chronic hepatitis B infection [1]. Seventy percent of these chronic infections are inactive carriers and may not develop complications, and it is documented that 70% of them reside in Asia [4]. It is the 10th leading cause of death worldwide and 1.2 million die of HBV associated complications every year.

The epidemiological scenario of the same in the homeland is that India being an intermediate endemic region for hepatitis B virus infection is now facing the 'silent epidemic'. Every year around one million Indians are exposed to the risk of infection by HBV and 100,000 people die of it [5]. The point prevalence of HBV infection is 3.7% with 40 million HBV carriers in India. Majority of the infected people are unaware of their status and continue to spread the infection to others for decades increasing the cost of treatment of such cases and decreasing the productive manpower [5].

Risk of developing chronic HBV [CHB] after acute infection depends on the age at which the infection is acquired and the immune status of the host [3,6]. Clinically CHB is said to occur when HBsAg persist in the serum for more than six months with few exceptions [7]. Based on host and virus interactions CHB evolves into four different phases and two major forms of disease [8,9]. In majority of the 'e' antigen positive subjects the disease remains silent; therefore in the past it was designated as simply CHB, asymptomatic infection, inactive carrier and at times even just a carrier of hepatitis B irrespective of the disease activity [10]. Today with growing and substantial evidence it is clear that a good chunk of this people are virologically and histologically active and are at risk of developing decompensated liver disease and HCC [11-17]. To avoid ambiguity associated with the previous designations used for describing such people a new term was coined in early 1990's and 2000 as [IDAHS] incidentally detected asymptomatic hepatitis B positive which looks more precise. The definition includes a heterogeneous group of patients who are asymptomatic for liver disease and have been found to be positive for HBsAg in their serum during workup for unrelated symptoms or during routine check-up. Heterogeneous because it includes patients who have active as well as in active HBV infection, early as well as advanced liver disease, and patients with low as well as high viral load. The global picture of IDAHS shows that around 20-70% are 'e' antigen positive and 60-70% of them have elevated liver enzymes, whereas in India it is as 5-37% and 10-15% [3,18].

To assess the disease activity in IDAHS a wide array of laboratory tests are available right from serological, biochemical markers to molecular tests. The molecular assays like *in-situ* hybridization, immunohistochemistry [IHC] detect HBV cDNA whereas polymerase chain reaction [PCR] detect and quantitate HBV DNA in the clinical specimens. These assays provide the user valuable information on HBV pathology and helps in deciding and monitoring therapy [3,8]. But it becomes quite essential for the user to choose the most appropriate test without affecting the pocket of the patient and avoid advising irrelevant tests and tests with similar significance. Further the diagnostic tests selected must reflect the biological properties of the marker being detected [8]. Therefore many gastroenterologists over the world have come to a common conclusion that the minimum workup for CHB infection should include serum ALT, HBeAg, AntiHBe, and HBV DNA quantitative [3]. These tests help one to evaluate disease progress, determine the need for liver biopsy and indication for management [3]. Further several studies have linked disease progression rate in HBV infection with various serological and histological markers [3,18]. Recognized risk factor for progression are presence of HBeAg, advanced age , raised ALT, co-infections with other hepatitis viruses and diabetes mellitus [3,18]. Liver biopsy studies which grade inflammation and fibrosis as histological activity index [HAI] score provide much more relevant information about the ongoing liver damage than the serological markers and enzyme profile. As both 'e' antigen positive and negative subjects and with raised or normal serum ALT. have varying degrees of liver damage when studied by biopsy [11-17]. Nevertheless, in settings where estimation of HBV DNA levels is not feasible; HAI score with serum ALT levels is used for diagnosing, initiating and monitoring antiviral therapy [3,19-20].

With this background epidemiological and laboratory information on IDAHS, we can understand how crucial it is to identify evaluate, follow, report and treat this group of individuals. Aim – the present study was designed to assess the prevalence of incidentally detected hepatitis B positive subjects [IDAHS] and possible risk factors associated. Further we tried to check the serological and biochemical status of these individuals and variations with respect to age and gender.

2. MATERIALS AND METHODS

2.1 Aim

A cross sectional observational study was designed to assess:

1. The prevalence of hepatitis B positive asymptomatic individuals [IDAHS].
2. The most probable risk factors leading to infection.
3. Their serological and biochemical status.
4. Age and gender related variations in serological and biochemical markers.

In the present study the test subjects were:

Inclusion criteria: Subjects registered for serological profile before surgery, antenatal women and haemodialysis patients for regular viral screening. Few of them were

referred from other departments or were ones who wanted to clear the doubts about a positive HBsAg report from another diagnostic centre. A total of 3240 subjects were screened for HBsAg during the year January 2014 to January 2015. And those who gave a positive HBsAg 124 [3.8%] result were included as IDAHS after confirming their asymptomatic status. Serum from these individuals was analysed for various hepatitis B markers and measured for serum alanine transaminase levels.

Exclusion criteria: Patients with symptomatic HBV disease, chronic liver disease due to autoimmune hepatitis or alcoholic liver disease were excluded from the study.

After an oral informed consent 5ml blood was collected in BD vacutainers under strict aseptic precautions from all the subjects. Blood samples were allowed to clot and serum separated into aliquots for further testing in microbiology and biochemistry laboratory of Princess Esra Hospital of Deccan College of medical sciences. HBV markers in the form of HBeAg, AntiHBe, HBsAg, and AntiHBc IgM and IgG were checked and the marker of liver damage, serum ALT levels was determined.

Risk factors for acquisition of hepatitis B infection were enquired from all the study subjects by administering a short questionnaire on most likely mode of transmission. This included questions on:

1. Mother to child transmission
2. Frequent intramuscular injections
3. Past history of surgical procedures
4. Past history of blood transfusion
5. Unsafe sex
6. Tattooing
7. Ear piercing
8. Drug abuse
9. Dental procedure

2.2 Serological Test

Were performed in microbiology laboratory. Presence of Hepatitis B surface antigen was detected using the commercial kit provided by J.Mitra and Co. Pvt. Ltd. India. The test is based on the principle of antigen capture or sandwich Elisa using lateral flow immune chromatography in which positive result is detected by the appearance of red coloured band along with the control band. The sensitivity and specificity of the test is 100% and 99.4%. HBeAg was detected using competitive capture or sandwich Elisa provided by Beijing Wantai Biologicals Ltd. from China. Antibody response to HBeAg, HBsAg HBcAg was detected using kits from Dia Pro - Diagnostics Ltd. from Italy based on the principle of competitive Elisa. Quantitative Elisa was used to demonstrate the titer of antiHBs and AntiHBc IgM.

Serum ALT was determined using Beckman system in biochemistry laboratory. Serum alanine aminotransferase levels above 40 IU/L in males and above 30 IU/L in females were taken as significant.

2.3 Quality Assurance

Test was run in presence of internal and external quality control samples to ensure validity.

2.4 Statistical Analysis

Data was analysed using EPI-INFO: 7 version from CDC.

3. RESULTS

Of the 3240 subject screened, 124 individuals 3.8% were found to be positive for hepatitis B surface antigen and all were asymptomatic for the disease.

Of the various risk factors enquired, intake of frequent intramuscular injections from registered medical practitioners [RMP] accounted for 34% as the most common risk factor followed by dental procedure in 29%, past history of surgery in 14%, history of twice a week haemodialysis in 4.8%, very few admitted history of blood transfusion 1.6% and in the rest 21.4%, the cause could not be established.

3.1 Demographic Features

The study subjects were in the age group 9-90 years. The mean age of the subjects noticed was 42.4±17.6. The male to female ratio was 1.29. The mean age for men noticed was greater as 44.6±18.0 when compared to females as 39.87±16.8.

It is observed that most of the IDAHS were in the age group 21-30 followed by 41-50 years. Least number of individuals were positive in the two extremes of age i.e. 0-10 and 80-90 years as seen in Graph 1.

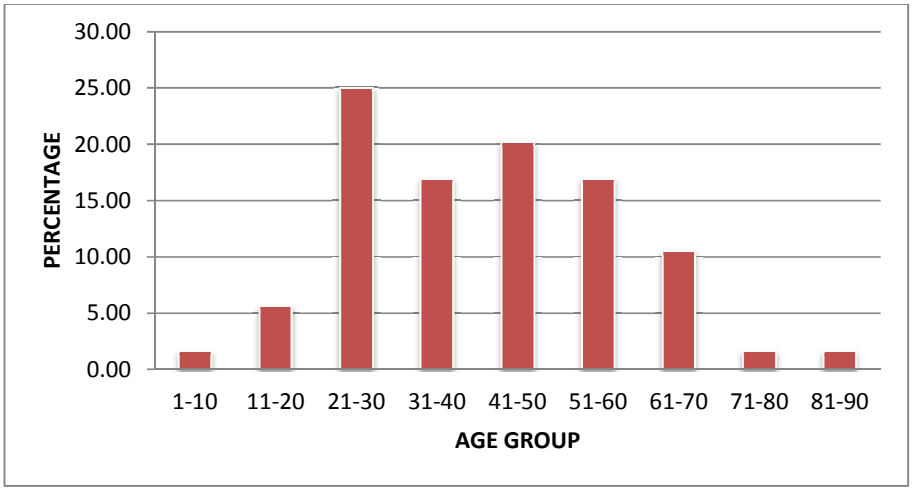
As far as gender is concerned more number of males 56% were found to be positive for HBsAg than females 44%. Anyhow females in the age group 21-30 years out numbered males of this age group for HBsAg seropositivity. Nevertheless, maximum number of subjects for HBsAg seropositivity for both the sexes were in the age group 21-30 years followed by 41-50 years as seen in Graph 2.

When screened for various HBV markers. Tests for antibodies to HBV revealed that majority of the subjects i.e. 98% were positive for AntiHBc IgG. AntiHBe was positive in 80%. AntiHBs was more than 10 IU/L in 5 subjects [4%] only. AntiHBc IgM was detected in 20% of the individuals out of the 114 tested. Of these 23 antiHBc IgM positive individuals, 15 were positive for both antiHBe and antiHBc IgM, five were positive for HBeAg and antiHBc IgM and IgG and rest three were positive for antiHBc IgM and IgG as seen in Graph 3.

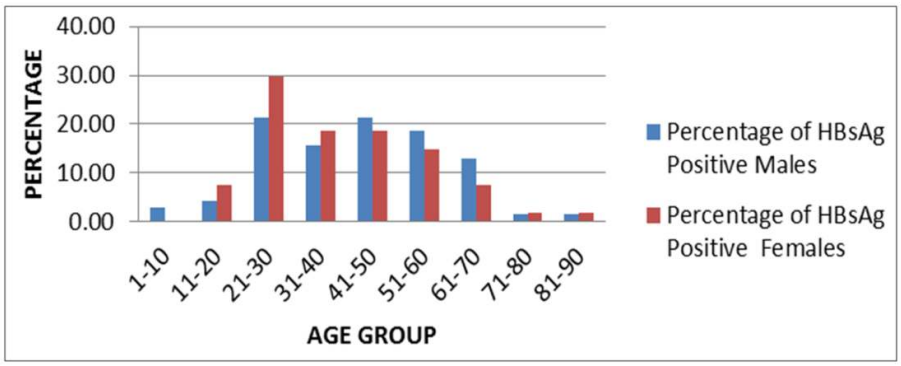
3.2 Test for HBe Antigen

Could be checked in only 115 subjects and 9 [8%] gave a positive result indicating replicative and infectious state of disease as seen in Graph 3. All these subjects were antiHBe negative but five of them were antiHBc IgM positive too. Only one subject in the study was analyzed for HBV DNA by quantitative PCR who was HBeAg and antiHBe negative but antiHBc IgM and IgG positive. Serum tested for HBV DNA revealed viral load as 1.4 x 10^{8} copies /ml.

Serum alanine transaminase level was estimated in 116 subjects only, and was found to be raised in 14 individuals accounting for 12% of the total tested. Further it is noted that none of the subjects with raised serum ALT levels were e antigen positive, on the contrary all were e antigen negative as seen in Graph 3.



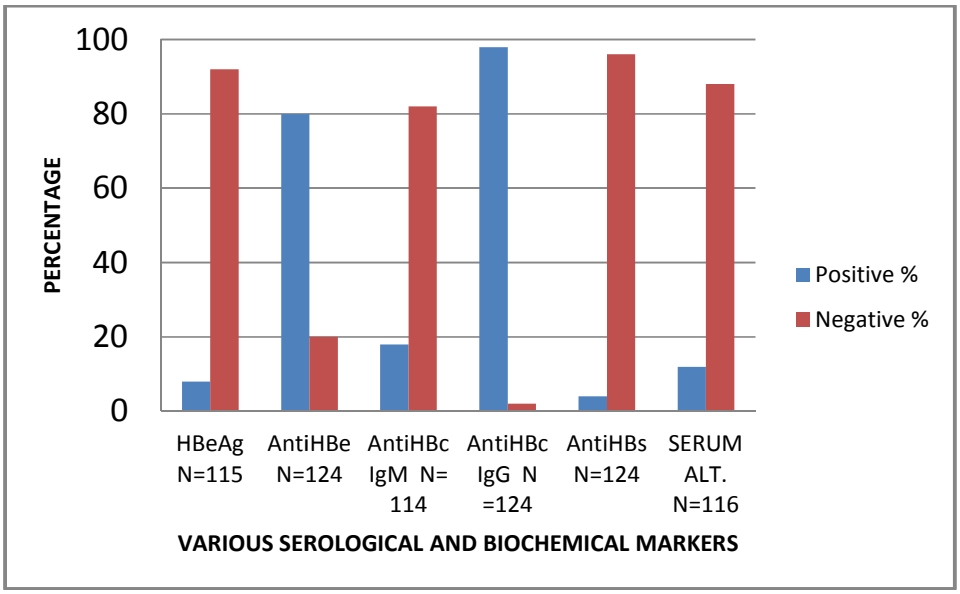
Graph 1. Influence of age on HBsAg seropositivity in IDAHS



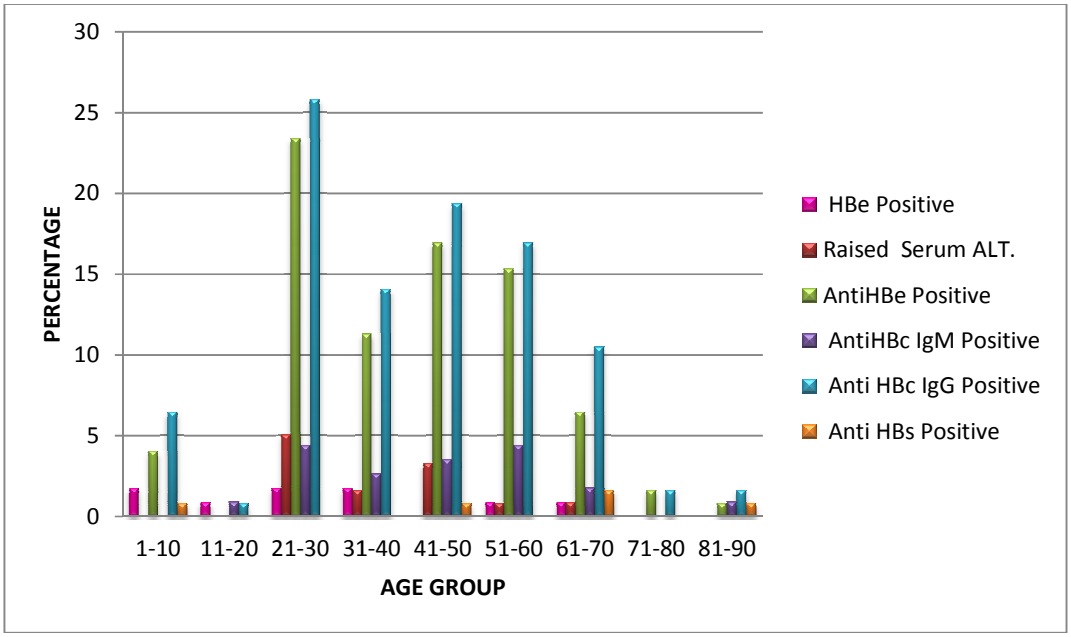
Graph 2. Influence of age and gender on HBsAg seropositivity in IDAHS

Influence of age on various serological markers when plotted on graph showed that majority i.e. 60% of the individuals tested gave a positive serological and biochemical test result in the age group 21-30 years which is 23.39% for antiHBe, 4.39% for anti HBcIgM, 25.81% for AntiHBc IgG,5.06% for serum ALT.and 1.74% for HbeAg as seen in Graph 4. Further it was noticed that of the total HBeAg positive subjects 56% of them were less than 30 years of age as seen in Graph 4.

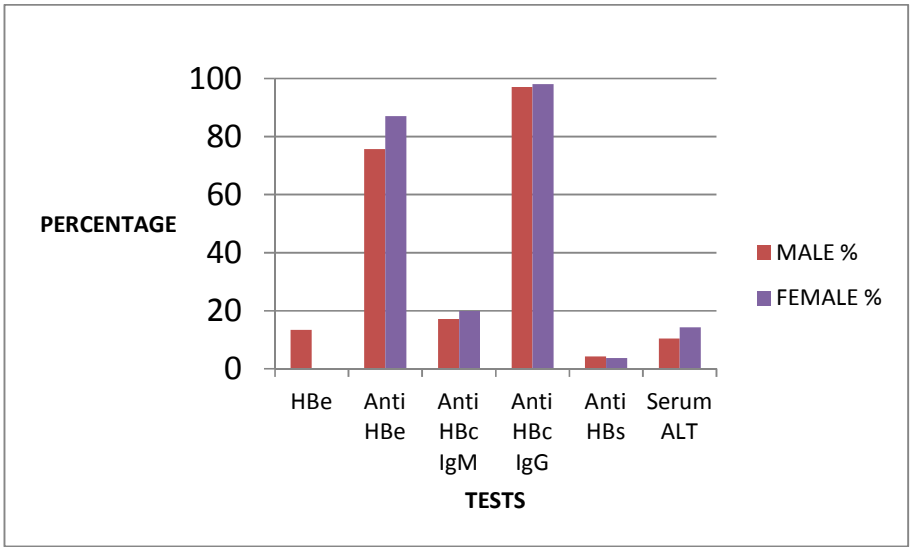
In Graph 5 the most noticeable feature seen is complete predominance of male gender for HBeAg positivity, no other parameter showed such a profound impact by gender.



Graph 3. Prevalence of HBV and enzyme markers in IDAHS



Graph 4. Impact of age on various HBV and enzyme markers



Grpah 5. Impact of gender on prevalence of various HBV markers in IDAHS

4. DISCUSSION

Viral hepatitis caused by hepatitis B virus is a major public health problem worldwide. In India it is the second most common cause of infective hepatitis after hepatitis E virus [5]. Prevalence of HBV in our study is 3.8% which is almost similar to the national point prevalence rate of 3.7% [5,9]. Most common risk factors for acquisition of hepatitis B, were identified as frequent intramuscular injections, dental procedure and previous surgery, which is similar to the reports by Kokhar N. as 32.1% for injections, surgery 25% and dental procedure 6.3%. Malik K. has reported surgery and injections together as the most common risk factor in in his study as13.5% [21-22]. Nearly 8% of the tested IDAHS were positive for HBeAg, which similar to the results reported by Puri AS. as 5-37%,Ijoma U as 8.6%, MM Rahman as 9.3% [18,23,24] but less when compared to the reports by Reza. M. 11.7%, K S Rashmi as 12.89%, Malik K. as 15%, Shiha G 18%, V.Dixit as 21%, Kokhar N. as 21%, Rahman M. A as 23% Chan HL as 31% and Chandra et al. as 45% [11-12,21-22,25-28]. So our findings are suggestive of decline in the prevalence of HBeAg positive IDAHS and a simultaneous increase in the 'e' antigen negative asymptomatic chronic hepatitis B patients. Decrease in 'e' antigen positive CHB cases reflects success of immunization program, mutations in the virus or improved sensitivity of detection methods. However absence of 'e' antigen clinically indicates an inactive state of the disease but possible development of pre core mutants at the molecular level which can be determined only by further testing for HBV DNA in these individuals Ping Chen et al. [20].

AntiHBc IgG an indicator of infection, exposure to HBV and which also signifies chronic infection was positive detected in 98% of the subjects which is similar to the reports by Ijoma U. as 97% and Shiha G. as 100% [11,23]. It was absent in only 2% of the tested subjects who were AntiHBc IgM negative too, the most probable reason for this could be due to decline in the antibody titter which has fallen below the detectable level.

AntiHBc IgM was detected and quantitatively raised in 20% of the subjects. Fifteen i.e. 65% of these individuals were positive for antiHBc IgG and antiHBe indicating acute flares or exacerbations of chronic HBV infection. Five i.e. 22% of them were HBeAg and antiHBc IgG positive but antiHBe negative indicating ongoing liver damage and flare up of chronic active infection. In rest 3 i.e. 13% who were negative for both HBe and antiHBe but positive for both antiHBc IgM and IgG needed further evaluation for HBVDNA and liver damage by biopsy and in fact one of them showed very high HBV DNA levels of 1.4 $x10^8$ copies/ml when determined, emphasizing the role of HBV DNA estimation in these IDAHS.

Several studies have documented seroprevalence of antiHBe to be as 53-90% [23,29-30]. In our study 80% of the tested individuals gave a positive result, which is similar

to the reports by Kokhar N as 78.6%, VK. Dixit as 79%, Malik K. as 79%, Shiha G. as 81.6%, Rashmi K. S. as 81% [11,12, 21,22,24,], but less when compared to reports by Rahman MM. as 86% and Reza M. as 88% [24,25]. Some authors have reported it to be positive in less percentage of individuals like Chandra et al as 53%, Chan HL as 69% and. Rahman M. A. as 59% Ijoma U as 75% [23,27,28,31]. Variations in the expression of 'e' antigen in chronic HBV are related to the genotype of the virus Saudy et al. [32]. 'E' antigen disappears early in patients with genotype D because of the early stop codon mutations which is common in Asia [32]. Presence of antiHBe indicates seroconversion from replicative to non-replicative state. Therefore it is obvious from the study findings that majority of the infected individuals are 'e' antigen negative and most of them have normal ALT levels. Despite the seroconversion some individuals continue to suffer from progressive liver damage as demonstrated by presence of viremia in about 97% of the individuals by PCR and histological evidence of varying degrees of chronic HBV infection in 61.4% by liver biopsy and 27.2% by IHC as reported by [11]. Therefore antiHBe is not a true indicator of non-replicative state of the virus. Presence of core antigen or HBV DNA in liver in absence of 'e' antigen in circulation indicates pre core or promotor region mutation in the virus. Therefore estimation of HBV DNA levels or liver biopsy holds good to characterize and manage this group of individuals [11,20].

AntiHBs is considered as an indicator of immunity following exposure or immunization, in our study we have found 4% of the individuals to be positive with a significant titer of antibodies > 10 IU/ml. Shiha G. has reported it to be positive in 7% of the subjects tested [11]. Co-existence of HBsAg and anti HBs occurs in chronic infection and has been reported in high percentage of individuals as 24% in Iran [11] Here again presence of antiHBs along with HBsAg and antiHBc IgG indicates either early seroconversion or could be due presence of less dominant types of surface antigens of the virus which is not neutralized by the existing antibody [11].

Serum alanine transaminase which is known as a marker of liver damage was seen to be raised in 12% of the subjects which is similar to the reports of Rahman MM. as 12.33% [24]. This is less when compared to reports by other authors Reza M. as 17.8%, Chan HL as 22%, Kokhar N. as 26%, VK Dixit as 27%, Rahman M.A.as 30-40%, Chandra et al. as 76% and Al Mehtab M et al. as 50% [12,13,21,25,27,28,31]. It has been established that high levels of ALT in 'e' antigen negative subjects correlates well with histopathological changes in the liver in the form of varying degrees of inflammatory changes and fibrosis therefore liver biopsy in these individuals is mandatory as it helps in determining liver damage, planning management and forecasting expected prognosis [11-17,20].

In the present study we noticed that all HBeAg positive subjects were males, other authors too have reported a male predominance of 'e' antigenemia like Rahman M.A. in 83%, Rashmi K.S. in 56.7% Ping Chen in 21.9% respectively [20,26-27]. The most probable explanation offered by authors for this is pathogenesis - related mechanisms involving sex hormones and other social activities [20]. Further it was observed that majority of the HBeAg positive subjects around 56% were young less than 30 years of age years indicating early mode of transmission that is perinatal or childhood exposure to infection which is similar to the reports by Kokhar N. as 57%, where he proved by liver biopsy that 59% of them had significant histological changes in the liver [21]. Rahman M.A. reported it to be positive in 60% of 21-40 years age [26]. Further other serological markers were also psoitive by majority in 21-30 years of age which is similar to the report by Rahman M. A. in 52.5% [26].

The bottom line of the discussion here is the basic work up of any IDAHS should include estimation of HBV DNA baseline levels besides other parameters as mentioned rightly by D Amrapurkar and VK Dixit et al. but how far it is practicable [3,12]. Affordability becomes an issue in the workup and follow-up of these cases. Several studies have demonstrated HBV DNA viral load to be $\geq 10^5$ copies /ml in 70- 90% of the 'e' antigen positive subjects with raised ALT levels for like Kokhar N. in 89%, Ping Chen et al. in 91% and Chandra et al. [20,21,28]. Moreover the viral load has been found to be raised even in 40-70% of the 'e' antigen negative individuals. Chandra et al reported it to be as 61%, Rodrigues et al. as 49%, Ping Chen as 45%, Chaudhuri S as 34.2%, Kokhar N. as 30%, Chan HL as 17% and Malik K.as11.6% [20-22, 29,31,33-34]. Therefore estimation of HBV DNA level looks to be more sensitive than serum HBeAg to diagnose and assess viral replication. It is possible that DNA level may remain high in

the 'e' antigen negative group because these individuals were in unrecognized disease phase or that the 'e' antigen titer had simply fallen below the threshold levels but not below the threshold functional impact [20]. In designing antiviral treatment it is essential not to rely on 'e' antigen levels alone though it indicates replication but is not sufficient enough to assess replication because pre core mutations suppress the expression of 'e' antigen while having no effect on DNA replication. Hence our study emphasizes the need to measure the viral load first at the time of diagnosis and then during follow up period for proper management of these individuals. Though some authors have insisted that in resource poor settings where estimation of HBV DNA levels is limited due to prohibitive cost, HBeAg detection and ALT levels can be used best to describe HBV activity and infectiousness [20].

5. DRAW BACKS

Estimation of base line HBVDNA levels an essential parameter in IDAHS workup could not be performed on all subjects in the study due to prohibitive cost as majority of the subjects belonged to below poverty line or low middle income group. Liver ultrasound and biopsy as further investigations to assess tissue damage needs patient consent and hospitalization, which again was not feasible. Therefore we need to rely on HBeAg and serum ALT levels even in 21st century, which are not always conclusive of viral activity and liver damage.

6. CONCLUSION AND RECOMMENDA-TIONS

HBV infection continues to be a major public health problem in endemic areas. With a decline in 'e' antigen positive hepatitis B virus chronic infection there is a simultaneous up rise of 'e' antigen negative subject's .Therefore it becomes essential to identify, evaluate, follow, report and treat them in order to contain the spread of the infection and reduce substantial morbidity and mortality associated with it. In first place the existing prevention and control program for hepatitis B needs to be intensified and strengthened similar to the one adopted by CDC as "KNOW HEPATITS B" [35] by vaccinating infants, children, adults and high risk group with complete dose coverage. Secondly by improving hepatitis B virus surveillance, identification, screening and linkage to treatment and care facilities of the subjects detected to be suffering from chronic progressive liver disease in terms of raised ALT and viremia. The public health care sector must try to bring down the cost of such relevant tests for the benefit of society. The surveillance program should evolve strategy to report and follow up IDAHs subjects for at least minimum one year. Finally HBV awareness in general population and high risk group needs to be enhanced through information education and communication i.e. IEC approach.

CONSENT

An oral informed consent was taken from all the patients before enrolling them in study.

ETHICAL APPROVAL

The study was approved by the ethical committee of the institute before commencement and references.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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