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A STUDY ON PREVALENCE OF HEPATITIS-B: DETECTING HEPATITIS-B SURFACE ANTIGEN USING HEPA CARD AND CONFIRMATION BY ELISA.

Microbiology

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ABSTRACT

INTRODUCTION: Hepatitis B virus (HBV) is responsible for the most frequent chronic liver disease of infectious origin in human beings with chronically infected individuals worldwide. Chronic hepatitis B results in more than 6,00,000 deaths annually from the complications of end-stage liver disease and hepatocellular carcinoma (HCC). Detection of HBsAg is the most commonly used test for diagnosing acute HBV infections as well as for detecting carriers Immunochromatography assays (ICA) are economical and do not require special instrumentation for analysis and have been recommended for routine use in clinical microbiology laboratories for detection of HBsAg. It is simple to perform, is designed to be read by eye and does not require any expensive apparatus. When compared with a commercially available HEPA card kit for the detection of the same markers, ELISA was shown to be more sensitive for the detection of HBsAg.

MATERIALS AND METHODS: A cross sectional study was conducted at Great Eastern Medical School, Srikakulam, Andhra Pradesh, INDIA by collecting secondary data from laboratory registers of patients tested for HBsAg from March 2017 to March 2018. Data is tabulated and analysis is done using Microsoft Office Excel 2016 software.

RESULTS: Among 22000 patients of total study group, 157 are found positive for Hepatitis B Surface Antigen accounting for 0.71%. Out of 11000 male cases 102 are positive cases [0.925] and 4 results are in determined due to weak bands accounting for 0.036%, out of 11000 female cases 55 are positive cases [0.5%] and 2 are in determined cases accounting for 0.018% showing that sero-positivity is more in males. When 157 seropositive cases kept for ELISA all showed positive cutoff value accounting for 100%.

DISCUSSION: The present study targeted towards patients who were posted for surgeries during routine screening tests, patients with jaundice and gastrointestinal symptoms and HIV patients with an objective of screening for the infection with Hepatitis B virus by detecting HBsAg using HEPA card and confirming it by ELISA. Keeping all these facts in mind there is a need for intense IEC activities regarding Hepatitis virus, its mode of transmission and about the measures which prevents transmission from person to person. As the saying 'prevention is better than cure' it is better to prevent Hepatitis B infection than to treat with anti-viral drugs which are associated with many side effects and have low curative value. Our research is helpful for development of national control strategies to fight against Hepatitis B infection and provides basis for managing hepatitis B national prevalence surveys and control measures and there by reduces hepatitis B endemicity in Srikakulam in near future.

KEYWORDS

1. INTRODUCTION

Hepatitis B virus (HBV) is responsible for the most frequent chronic liver disease of infectious origin in human beings with approximately 257 million chronically infected individuals worldwide. Chronic hepatitis B results in more than 6,00,000 deaths annually from the complications of end-stage liver disease and hepatocellular carcinoma (HCC)[2]. The vast majority of new HBV infections occurs in highly endemic regions, such as China, Southeast Asia, and sub-Saharan Africa. HBV transmission can be mother-to-infant, person-to-person in young children (through open cuts and scratches) and adults, sexual, nosocomial, or blood-borne (for instance through sharing of infected needles or drug preparation materials), depending on the prevalence and risk groups in the area[3,4].

Diagnosis of HBV infection using serological markers varies depending on whether the infection is acute or chronic. Hepatitis B surface antigen (HBsAg) appears 1-7 weeks before biochemical markers of liver disease or jaundice become evident and remains in almost half of them even after 3 weeks after the onset of disease[5].

After initial infection, a proportion of patients fail to clear infectious material from the blood stream and become chronic carriers and in them, the HBsAg persists for longer periods, sometimes for life. Further, a large proportion of patients suffering from Hepatitis B may remain asymptomatic and can transmit the infection to healthy population[6].

Detection of HBsAg is the most commonly used test for diagnosing acute HBV infections as well as for detecting carriers Immunochromatography assays (ICA) are economical and do not require special instrumentation for analysis and have been recommended for routine use in clinical microbiology laboratories for detection of HBsAg. It is simple to perform, is designed to be read by eye and does not require any expensive apparatus. When compared with a commercially available HEPA card kit for the detection of the same markers, ELISA was shown to be more sensitive for the detection of HBsAg[7-9].

But detection of hepatitis B surface antigen by immunochromatographic essay is only a screening test there may be false positives or false negatives and the estimation of seroprevalence of hepatitis B infection will be misguided, so in our study we included ELISA test which is a confirmatory test.

The speed, sensitivity and simplicity of the ICA method makes it more attractive, particularly for large-scale surveillance studies with this background, the present study was undertaken to estimate seroprevalence of Hepatitis B surface antigen among patients at the study setting.

2. MATERIALS AND METHODS

A cross sectional study was conducted at Great Eastern Medical School, Srikakulam, Andhra Pradesh, INDIA by collecting secondary data from laboratory registers of patients tested for HBsAg from March 2017 to March 2018. Institutional Ethical Clearance for the study was obtained. Demographic characteristics like age, gender, clinical details and HBsAg test results of the patients were collected.

2.1 Detection Of Hbsag By Hepa Card

A venous blood sample of 5ml was collected from patients with standard precautions. The blood was allowed to clot for 45 minutes at room temperature and the serum was separated after centrifugation.

The serum was then subjected to one step rapid immunochromatographic assay (ICA) Alere Trueline TM (Alere Medical Pvt. Ltd) kit for detection of HBsAg following manufacturer instructions.

2.2 Detection of HBsAg by ELISA.

The HBsAg ELISA Test kit employs an antibody sandwich ELISA technique where monoclonal antibodies unique to HBsAg, are pre-coated on polystyrene microwell strips. The serum or plasma sample is added together with a second antibody, the HRP Conjugate, (horseradish peroxidase) and directed against a different epitope of HBsAg. Throughout the time of incubation, specific immunocomplex that may have formed (indicating presence of HBsAg) is captured on the solid phase. After washing, to eliminate serum proteins and unbound HRP-conjugate, chromogen solutions containing tetramethyl-benzidine (TMB) and urea peroxide are added to the wells. Next, the colorless chromogens are hydrolyzed by the bound HRP-conjugate to a blue-colored product while in the presence of the antibody-antigen-antibody (HRP) sandwich immunocomplex. Halting the reaction with sulfuric acid, the blue color then turns yellow. The color intensity can be gauged proportionally to the amount of antigen captured in the wells, and to the amount in the sample, respectively. The wells remain colorless if the HBsAg result is negative[10].

HBsAg ELISA Test Results Interpretations(S = the individual optical density (OD) of each specimen): Negative Results (S/C.O. <1): samples giving an optical density less than the Cut-off value are considered negative, which indicates that no hepatitis B virus surface antigen has been detected with this HBsAg ELISA, therefore the patient is probably not infected with hepatitis B virus.

Positive Results (S/C.O.>1): samples giving an optical density greater than or equal to the Cut-off value are considered initially reactive, which indicates that HBV surfaces antigen has probably been detected with this HBsAg ELISA.

HBsAg ELISA Assay Performance Characteristics: Analytical Specificity

1. No cross reactivity observed with samples from patients infected with HAV, HCV, HIV, CMV, and TP.
2. No interference from rheumatoid factors up to 2000U/ml observed.
3. No high dose hook effect up to HBsAg concentrations of 200000ng/ml observed during clinical testing.

Data analysis

Data is tabulated and analysis is done using Microsoft Office Excel 2016 software.

3. RESULTS

Among 22000 patients of total study group, 157 are found positive for Hepatitis B Surface Antigen accounting for 0.71%.

TABLE 1: Sex Wise Distribution Of Total Study Group.

Sex	Total Cases	Percentage
Male	11000	50%
Female	11000	50%
Total	22000	100%

TABLE 2: Sex Wise Distribution Of Seropositive Cases.

Sex	Total study group	Seropositive cases	Undetermined cases	Percentage of seropositive cases	Percentage of undetermined cases
Male	11000	102	4	0.92%	0.036%
Female	11000	55	2	0.5%	0.018%
Total	22000	157	6	0.71%	0.027%

Out of 11000 male cases 102 are positive cases [0.925] and 4 results are in determined due to weak bands accounting for 0.036%, out of 11000 female cases 55 are positive cases [0.5%] and 2 are in determined cases accounting for 0.018% showing that sero-positivity is more in males.

TABLE 3: Age Wise Distribution Of Seropositive Cases.

Age	Total study group	Seropositive cases	Undetermined cases	Percentage of seropositive cases	Percentage of undetermined cases
0-10	402	0	0	0%	0%
10-20	946	4	0	0.42%	0%
20-30	2010	10	0	0.49%	0%
30-40	3012	20	3	0.66%	0.099%
40-50	3924	37	1	0.94%	0.025%
50-60	4798	46	2	0.95%	0.041%
60-70	3118	26	0	0.83%	0%
70-80	2895	10	0	0.34%	0%
80-90	895	4	0	0.44%	0%
Total	22000	157	6	0.71%	0.027%

The seropositivity is more in age group 50-60 [0.95%] followed by 40-50 [0.94%]. In determined cases are more in age group 40-50 [0.099%] followed by 50-60[0.041%]

TABLE 4: Department Wise Distribution Of Seropositive Cases.

Department	Total cases	Sero positive cases	Percentage of seropositive cases	Undetermined cases	Percentage of undetermined cases
Medicine	5302	35	0.66%	1	0.018%
Surgery	7796	48	0.61%	2	0.025%
OBG	1398	21	1.5%	2	0.14%
Ortho	904	6	0.66%	0	0
ENT	499	5	1%	0	0
Ophthalmology	4905	27	0.5%	1	0.02%
Causality	1196	15	1.25%	0	0
Total	22000	157	0.71%	6	0.027%

Out of 157 seropositive cases highest number are from surgery department [0.61%] followed by medicine department [0.66%].

Maximum undetermined results are from surgery [0.025%].

TABLE 5: Distribution Of Cases Based On Clinical Symptoms.

Clinical diagnosis	Total	Seropositive cases	Undetermined cases
HIV	1462	80[5.47%]	2[0.13]
Jaundice	1243	20[1.6%]	0[0]
Gastrointestinal symptoms	963	24[2.49%]	2[0.2]
Routine screening for patients posted for different surgeries	18332	31[0.16%]	2[0.16]
Total	22000	157[0.71%]	6[0.027%]

Table 6: Percentage Of Seropositive Cases Confirmed By ELISA.

Number of seropositive cases	Number of cases confirmed by ELISA	Percentage of confirmed cases
157	157	100%

When 157 seropositive cases kept for ELISA all showed positive cutoff value accounting for 100%

TABLE 7: Percentage Of Undetermined Cases Confirmed By ELISA.

Total no of undetermined cases	No of cases positive by ELISA	Percentage of cases positive by ELISA	No of cases negative by ELISA	Percentage of cases negative by ELISA
6	4	66.6%	2	33.3%

So the total number of seropositive cases are 161 accounting for 0.73%.

4. DISCUSSION

The present study targeted towards patients who were posted for surgeries [routine screening tests], patients with jaundice and gastrointestinal symptoms and HIV patients with an objective of screening for the infection with Hepatitis B virus by detecting HBsAg using HEPA card and confirming it by ELISA.

As this was the earliest Ag to appear in the infected person's serum/blood and last parameter to disappear, it is more sensitive test than antibody detection. To rule out the false positive results we were confirming it by ELISA which was highly sensitive and specific.

When we discuss about the sex wise incidence of present study, more positivity was seen in male population. This might be due high risk

sexual behavior, occupational exposure and increased chance of blood transfusions due to road traffic accidents.

Coming to age wise distribution of study group more incidences is seen between 50-60years followed by 40-50years. It could be due to injudicious use of unsterile needles, unsafe sexual practices and due to unscreened blood transfusions. When we observed the study group no one have the infection between 0-10years age group indicating that there was 0% vertical transmission which might be due proper antenatal screening.

When we studied various groups for the prevalence of this infection we found more cases in HIV patients indicating that increased co–association of HIV infection with Hepatitis B infection.

Keeping all these facts in mind there is a need for intense IEC activities regarding Hepatitis virus, its mode of transmission and about the measures which prevents transmission from person to person. As the saying 'prevention is better than cure' it is better to prevent Hepatitis B infection than to treat with anti-viral drugs which are associated with many side effects and have low curative value.

TABLE 8: Comparative Study Of Seroprevalance Of Hepatitis B Infection In Different Studies.

AUTHOR	Year of study	No of samples tested	No of positive cases	Prevalence rate
Trupthi et.al[11]	2016-17	6905	39	0.56%
Bulle et.al[7]	2015	4649	73	1.57%
Patil et.al[12]	2010	767	23	2.99%
Quadri et.al[6]	2010	4283	70	1.63%
Narayanaswamy et.al[13]	-	3182	105	3.3%
Sood et.al[14]	2007-08	3196	28	0.87%
Present study	2017-18	22000	161	0.73%

The prevalence rate of hepatitis B infection of present study [0.73%] was nearly equal to prevalence rates of sood et.al study [0.87%].

5. CONCLUSION

Over last two decades there was a significant improvement in control of HBV infection due to considerable progress of virology tools for screening and diagnosis, implementation of vaccination programs and due to recent advances in pharmaceutical field in development of effective antiviral therapies that inhibit viral replication for long duration.

Our study showed that HBV is endemic in Srikakulam. It emphasizes the need for universal vaccination to all children and establishment of strategies to prevent mother to child transmission. Our research is helpful for development of national control strategies to fight against Hepatitis B infection and provides basis for managing hepatitis B national prevalence surveys and control measures and there by reduces hepatitis B endemicity in Srikakulam in near future.

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