

ORIGINAL ARTICLE

Prevalence of Hepatitis 'B' in Apparently Suspected Individuals of Southern Punjab by Highly Sensitive Electro-chemiluminescence Immunoassay (ECLIA)

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ABSTRACT

Background: The prevalence of hepatitis B virus (HBV) infection is increasing worldwide. This study was aimed to assess the prevalence of HBsAg among suspected patients for hepatitis by community doctors attending collection centers of Aga Khan University Hospital's clinical laboratory Karachi in Southern Punjab.

Place and Duration: A retrospective study was conducted from January 2011 to June 2011 at Multan Stat Laboratory of Aga Khan University Hospital.

Methods: A total of 2819 suspected patients were received at collection centers and included in this study. The screening was performed in Aga Khan Multan Stat Lab by using the Elecsys HBsAg 11 assay kit on Cobas e 411 immunoassay analyzer. Data was entered and analyzed using SPSS version 16 statistical package.

Results: From the total of 2819 suspected hepatitis patients, 1809 (64.17%) were males and 1010 (35.83%) were females. HBsAg was found reactive in 623 (22.10%), borderline 10 (0.35%) and non-reactive in 2186 (77.55%). Out of the total individuals, 475 (16.84%) male and 148 (5.25%) female were reactive for HBsAg. Sex-wise distribution of HBsAg reactive cases was 76.24% male & 23.76% female with M: F ratio of 3.2:1. The overall seroprevalence was found to be 22.10%. BAHAWALNAGER(55.84%), RAJAN PUR(JP) (52.30%), MUZAFARGARH (38.63%) and DERA GHAZI KHAN (34.87%) were most affected districts HBV infection.

Conclusion: The overall prevalence of HBV is alarming high. Males were more affected. It is required to prevent the disease by giving education to the health personal as well as to general population.

INTRODUCTION

Hepatitis B is a major health problem worldwide especially in Asia, Africa, southern Europe and Latin America¹. About 2 billion people and 240 million chronic carriers of HBV have been estimated in the world by 2012². An estimated 600,000 new cases are diagnosed annually of which 82% are from developing countries³. Pakistan is highly endemic with HBV⁴ with nine million people infected with HBV⁵. Approximately 1 million people die each year from complications from HBV⁶.

HBV is a partially double-stranded circular DNA virus belongs to the hepadnaviridae family. Important factors contributing to HBV spread include unsafe use of therapeutic injections, blood transfusion, tattooing, mother to child transmission and unsafe sexual practices. In Pakistan, therapeutic injections administered in health care settings have been identified as major and consistently reported risk factors for HBV(7). The highest concentrations of infectious HBV are in

blood, serum and serum-derived body fluids, such as semen and saliva (8). Hepatitis B virus (HBV) is responsible for a substantial proportion of cases of post-transfusion hepatitis, liver cirrhosis and hepatocellular carcinoma(9). Detection of HbsAg indicates that the individual is infected / carrier of the virus¹⁰.

It was a retrospective study from 1st Jan 2011 to 30th Jun 2011 conducted at Agha Khan Multan stat lab. The objective of this study was to determine the frequency rate of HBsAg, and the gender that are predominantly infected reporting to eighteen collection points of Aga Khan laboratory Hospital belonging to Southern Punjab. This study will also assess trend of disease in various districts of Southern Punjab.

MATERIAL & METHODS

This retrospective study was carried out in the Aga Khan Stat laboratory Multan, with samples collected from all collection points in different areas in Southern Punjab from 1st Jan 2011 to 30th Jun

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2011. The study included blood specimens for routine testing for HBsAg received from individuals suspected for hepatitis by doctors at eighteen collection centers of Aga Khan Lab in different districts Dera Ghazi Khan(1), Rahim Yar Khan (3), Rajanpur (1), Layyah (1), Vehari (2), Muzaffargarh (1), Bahawalpur (1), Bahawalnagar (1), Multan (5), Khanewal (1), Lodhran (1) of Southern Punjab during study period. District Pakpattan is not included because there is no Aga Khan Collection center in Pakpattan .

A total of 2819 patients were received and included in this study. All ages and either sex were included. Samples were collected by using tubes containing separating gel and centrifuged prior to testing. The screening was performed in Aga Khan Multan Stat Lab by using the Elecsys

HBsAg 11 assay kit on Cobas e 411 immunoassay analyzer. The reactive and non-reactive controls of HBs Ag were run along with the samples.

Principle of The Test

The HBsAg CLIA kit is based on a solid phase sandwich enzyme-linked immunosorbent assay. The assay system utilizes one anti-HBs monoclonal antibody (anti-HBS Mab) for solid phase (microtiter wells) immobilization and another anti-HBs polyclonal antibody (anti-HBS Pab) as antibody-enzyme (horseradish peroxidase) conjugate reagent. The HBsAg present in the reference standards and serum or plasma are "sandwiched" between the two antibodies. Following the formation of the coated antibody-antigen-antibody-enzyme complex, the unbound

antibody-enzyme labels are removed by washing. Upon the addition of the substrate, the horseradish peroxidase activity bound in the wells is then assayed by chemiluminescence reactions. The Related Light Unit (RLU) of the reaction is proportional to the concentration of HBsAg present in the specimen.

Statistical Analysis

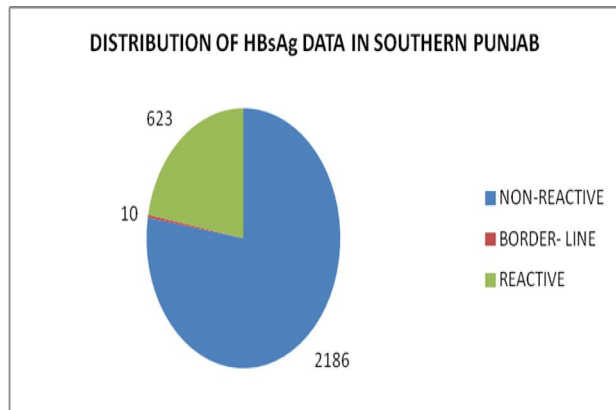
Data were collected from computerized record of Aga Khan Laboratory University Karachi . Permission was obtained from the Head of Stat Laboratories, Aga Khan University hospital before the data collection. Data was analyzed using SPSS version 16.0. Percentages were calculated directly for HBsAg in different gender and for district-wise individuals having HBsAg positivity .

RESULTS

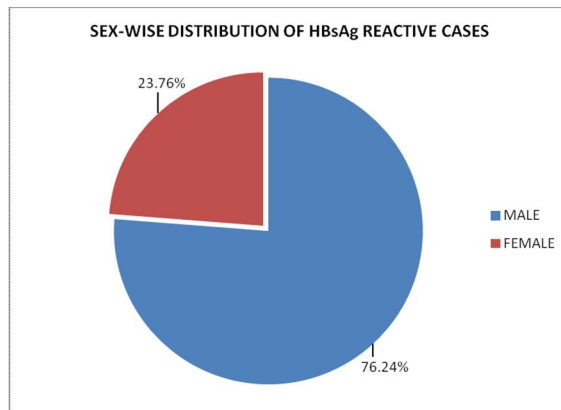
Table 1 shows that out of the total 2819 individuals, 1809 (64.17%) were males and 1010 (35.83%) were females: HBsAg was found reactive in 623 (22.10%), borderline 10 (0.35%) and non-reactive in 2186 (77.55%) . Sex-wise distribution of HBsAg reactive cases was 76.24% male & 23.76% female with M: F ratio of 3.2:1. Out of the total individuals, 475 (16.84%) male and 148 (5.25%) female were reactive for HBsAg. Out of total male subjects, 26.26% were reactive for HBsAg, and out of total female subjects 14.65% were HBsAg reactive. The overall seroprevalence was found to be 22.10%.

Table 1

Total no. Of patients screened for HBsAg	2819
Total no. Of male patients screened for HBsAg	1809 (64.17%)
Total no. Of female patients screened for HBsAg	1010(35.90%)
Total no. And percentage of HBsAg non reactive patients	2189 (77.65%)
Total no. And percentage of HBsAg borderline patients	10 (0.35%)
Total no. And percentage of HBsAg reactive patients	623 (22.10%)
Total no. Of HBsAg reactive male patients	475
Percentage of HBsAg reactive male patients from total patients	16.84%
Total no. Of HBsAg reactive female patients	148
Percentage of HBsAg reactive female patients from total patients	5.25%
Percentage of HBsAg reactive male patients from hbsag screened male patients	26.25%
Percentage of HBsAg reactive female patients from hbsag screened female patients	14.65%



Graph 1

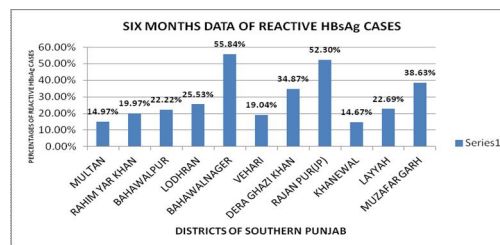


Graph 2: Graph 1 shows distribution of HbsAg data in patients from southern punjab, and graph 2 shows sex-wise distribution of HbsAg reactive cases.

Table 2: District-Wise Male and Female Distribution of Total Patients for HBsAg Screening

DISTRICTS	TOTAL MALE	TOTAL FEMALE	TOTAL PATIENTS
1 MULTAN*	700(65.92%)	362(34.08%)	1062
2 RAHIM YAR KHAN**	421(62.74%)	250(37.26%)	671
3 BAHAWALPUR	103(60.23%)	68(37.77%)	171
4 LODHRAN	30(63.83%)	17(36.17%)	47
5 BAHAWALNAGER	44(57.14%)	33(42.86%)	77
6 VEHARI***	42(50%)	42(50%)	84
7 DERA GHAZI KHAN	167(70.17%)	71(29.83%)	238
8 RAJAN PUR	51(78.46%)	14(21.54%)	65
9 KHANEWAL	67(61.47%)	42(38.53%)	109
10 LAYYAH	91(55.83%)	72(44.17%)	163
11 MUZAFAR GARH	93(70.45%)	39(29.55%)	132
GRAND TOTAL	1809(64.17%)	1010(35.83%)	2819
MULTAN* (MN-MP-MT-MU-SJ-05 POINTS)			
VEHARI*** (VEHARI-BUREWALA-02 POINTS)			
RAHIM YAR KHAN ** (RAHIM YAR KHAN-SADIQABAD-KHANPUR-03 POINTS)			

Table 2: shows total number of suspected patients and % ages of male and female subjects in different districts of Southern Punjab. Maximum samples(1062) were collected during study period in Multan (5 collection points) followed by (671) in Rahim Yar Khan (3 collection points) and Dera Ghazi Khan(238).



Graph 3: shows BAHAWALNAGER(55.84%), RAJAN PUR(JP) (52.30%), MUZAFARGARH

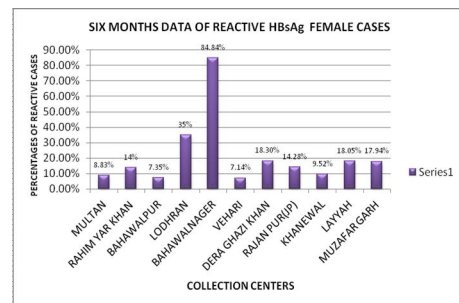
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(38.63%) and DERA GHAZI KHAN (34.87%) were most affected districts for HBV infection.

Table 3: District-Wise Data of Female Patients for HBsAg

DISTRICT	NEG FEMALE	POS FEMALE	% POS FEMALE	BORDERLINE FEMALE	TOTAL FEMALE
MULTAN	329	32	(8.83%)	1	362
RAHIM YAR KHAN	215	35	(14%)	0	250
BAHAWALPUR	63	5	(7.35%)	0	68
LODHRAN	11	6	(35%)	0	17
BAHAWALNAGER	5	28	(84.84%)	0	33
VEHARI	39	3	(7.14%)	0	42
DERA GHAZI KHAN	58	13	(18.3%)	0	71
RAJAN PUR(JP)	12	2	(14.28%)	0	14
KHANEWAL	38	4	(9.52%)	0	42
LAYYAH	58	13	(18.05%)	1	72
MUZAFAR GARH	32	7	(17.94%)	0	39
	877	148	(14.65%)	2	1010

Table 3 and graph 4 show that the maximum %age (84.84%) of HBsAg reactive female subjects was detected in district BAHAWALNAGER, followed by LODHRAN (35%), DERA GHAZI KHAN (18.3%), LAYYAH (18.05%), MUZAFARGARH (17.94%), RAJAN PUR (14.28%), RAHIM YAR KHAN (14%), KHANEWAL (9.52%), MULTAN (8.83%), BAHAWALPUR (7.35%) while the lowest frequency was observed in district VEHARI (7.14%).

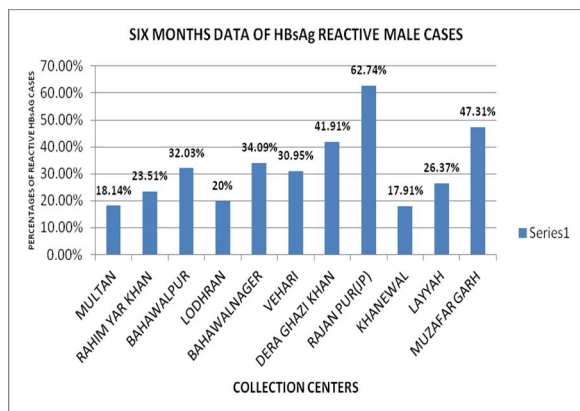


Graph 4

Table 4: District-Wise Data of Male Patients for HBsAg

DISTRICT	NEG MALE	POS MALE	%AGE POS	BORDERLINE MALE	TOTAL MALE
MULTAN	571	127	(18.14%)	2	700
RAHIM YAR KHAN	321	99	(23.51%)	1	421
BAHAWALPUR	69	33	(32.03%)	1	103
LODHRAN	24	6	(20%)	0	30
BAHAWALNAGER	29	15	(34.09%)	0	44
VEHARI	28	13	(30.95%)	1	42
DERA GHAZI KHAN	96	70	(41.91%)	1	167
RAJAN PUR(JP)	19	32	(62.74%)	0	51
KHANEWAL	55	12	(17.91%)	0	67
LAYYAH	66	24	(26.37%)	1	91
MUZAFAR GARH	48	44	(47.31%)	1	93
	1326	475	(26.25%)	8	1809

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Graph 5

Table 4 and graph 5 show that the maximum %age of HBsAg reactive male subjects was detected in district RAJAN PUR(JP) (62.74%) followed by MUZAFARGARH (47.31%), DERA GHAZI KHAN (41.91%), BAHAWALNAGER (34.09%), BAHAWALPUR (32.03%), VEHARI (30.95%), LAYYAH (26.37%), RAHIM YAR KHAN (23.51%), LODHRAN (20%), MULTAN (18.14%) while the lowest frequency was observed in district KHANEWAL (17.91%).

DISCUSSION

A lot of work has been published about the prevalence of hepatitis in different regions of Pakistan. Prevalence of chronic hepatitis B (HBV) virus infection shows variation among regions. Many studies are available about the prevalence of HBsAg in hospital patients or from the volunteer healthy blood donors but not in patients suspected as having hepatitis. In our study the suspected patients for hepatitis, referred by consultants for investigation were screened for HBsAg. The results were expected to have higher rates.

In the present study, we found that the overall HBsAg positivity in the individuals of Southern Punjab was 22.10%. The result is comparable with the studies in which the highest rates of infection 44.54% were found in 2010 followed by 30.13% 2009 and 25.32% in 2008 respectively⁷. It is possible that the disparity in reported frequency rates can be due to better diagnostic technique and to dissimilar screening methods applied.

Many studies have been done in different parts of the world to assess the magnitude and dynamics of disease transmission¹¹. Most of the studies have reported higher prevalence among males which is also true in our study.

This study reveals the gender-wise prevalence associated with HBV in the Southern Punjab. Regarding the sex distribution of HBV infection there were more male 76.24% patients than female 23.75%. This was compatible with work from Naz et al 2002 reported a high prevalence in males 68.3% than females 31.7%¹². Moosa et al. 2009; and Awan et al. 2010; reported a high (59.1%, 58.3%) prevalence in males than females (40.9%, 41.7%) respectively^{13,14}. Higher HBV Infection in males as compared to female may be due their being employed outside their homes, visiting barber shops and also their involvement in blood transfusion practices. While women are mostly involved in house hold activities based on the social, cultural and religious preferences and influence.

Wide variations were observed in district-wise distribution of results within Southern Punjab. The higher %ages of positive HBsAg were noted in districts BAHAWALNAGER, JAMPUR, MUZAFARGARH and DERA GHAZI KHAN. Comparing these findings with the study carried out during 2005 in southern Punjab, the maximum cases with positive HBsAg in young adults were noted in district Muzaffargarh (15)

Alam et al reported prevalence of HBV in adults belonging to central Punjab. It was 5.85% for Sargodha, 7% for Jhang and 7.3% for Faisalabad (16) which is low as compared to our study in southern Punjab.

CONCLUSION

Despite the presence of Hepatitis B vaccine, new Hepatitis B virus infection remains common worldwide. With the increasing rate of Hepatitis B infection in Pakistan, the use of screening has become a vital tool in preventive measure for the disease.

In order to prevent HBV infection in our country government should take aggressive steps towards awareness programs involving both the media and public sectors organizations. It is required to prevent the disease by giving education to the health personal as well as to general population. Awareness programs regarding Hepatitis B at School level should be started to save our younger generation. Hepatitis B vaccination to non infective individuals should be compulsory in our country. For its further effective control the federal government, the World Health Organisation and other funding agencies need to work in collaboration.

LIMITATIONS OF STUDY

This study took place at collection points and Agha Khan Multan stat lab in Southern Punjab, and the results may not apply to other regions of Pakistan.

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AUTHORS' CONTRIBUTIONS

JA designed the study and advised about the protocols. JA carried out sampling, experimental procedures and manuscript preparation. ZF and FF critically reviewed and approved the manuscript. All authors read and approved the final manuscript.

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