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INTRODUCTION

Hepatitis-B (HBV) is a viral hepatitis responsible for the severe liver diseases such as, irritation, swelling, inflammation and liver cirrhosis. It has high morbidity and mortality rates especially in the developing countries including Pakistan. It is the debatable public health problem which infects almost 350 million people of the world.¹ The hepatitis-B surface antigen is a first surface marker in serum which can be identified within 2 to 8 weeks. The infected person has the evidence of liver malfunctioning that later on converts to jaundice.² Hepatitis-B is the blood-borne disease that may be transmitted from mother to their child, contaminated needles or surgical instruments. The infected body fluid can also be the source of HBV transmission from infected to healthy person.³⁻⁴ The healthcare personals are also at high risk of HBV transmission through the contaminated

PRE-OPERATIVE SURGICAL PATIENTS; SEROLOGICAL SCREENING OF HEPATITIS B, ITS KNOWLEDGE AND ATTITUDES IN QUETTA, PAKISTAN.

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ABSTRACT... Objectives: This study was designed to evaluate sero-prevalence of HBs Ag (Hepatitis-B surface antigen), its knowledge and attitudes in preoperative general surgery patients in Quetta, Pakistan. **Study Design:** A cross-sectional study. **Place and Duration of Study:** This study was carried out (April - September, 2016) and was screened in Balochistan Lab Brewery Road, Quetta. **Methodology:** 1000 preoperative patients from four ethnic groups predominantly present in surgical department of multiple hospitals of Quetta. Serum was subjected to chromatography immunoassay (ICT) and samples were reconfirmed through Enzyme linked immunosorbent assay. Demographical data, knowledge and attitudes were also collected using valid predesigned questionnaire. **Results :** Overall, prevalence 7.0% were observed with highest in Baloch population, 10.3 %, followed by Hazara community, 8.0 %, While Pashtoon were with 5.7 % and Panjabi population with 2.3 %. Statistically non-significant difference was observed in males and female population ($P < 0.05$). 03 of the patients were false negative with ICT when reconfirmed by ELISA. Overall, population show poor knowledge and attitudes in preoperative surgery patients toward Hepatitis B infection. **Conclusion:** The HBs Ag determination using rapid Immunochromatography device test should only be used for mass scale screening and results may be verified with ELISA to eliminate the chances of false negative cases.

Key words: Hepatitis B, prevalence, ICT, ELISA, Quetta, preoperative patients.

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needle pricks and injury during the sample handling.⁴⁻⁵ The precautions against hepatitis-B virus infection are taken when it is detected for the purposes of patient's surgery and treatment. The operation theater staff including surgeons, anesthetists, nurses and other attendants are also at higher risk of getting infection in preoperative patients. The World Health Organization (WHO) has estimated the chronicity of HBV infection as it is a spreading disease all over the globe.⁶

Immunochromatographic immunoassay is cheaper and less time consuming than ELISA used mostly in developing countries including Pakistan. Both of the techniques have enough accurate detection but, ELISA is found to have more sensitivity than the ICT in the HBV infection control. Pakistan, India and Bangladesh are with the highest prevalence of 2-8 % in the region.

Till now, 8 genotypes and 9 serological sub types of HBV have been identified that differs in different countries.⁷⁻⁸ Hepatitis B virus genotypes have specific distribution pattern in different geographical areas and present in different ethnic groups of the globe. Genotype A is mostly prevalent in South Africa, North America and West-North Europe, Genotype B in South-East Asia and genotype C in Korea, China, Vietnam and Japan.⁹⁻¹⁰ Genotype D is Pandemic and is more prevalent in Middle East and Mediterranean regions. In Pakistan Genotypes C and D is prevalent in general population.¹¹⁻¹² Very limited data is available in the province about HBV infection. This study was aimed to investigate the sero-prevalence of hepatitis-B viral infection in different ethnic groups, Pashtun, Baloch, Hazara community and Punjabi in preoperative patients of Quetta Pakistan.

PATIENTS AND METHODS

Study design and area

This cross sectional study was carried out in preoperative surgery patients in Quetta Balochistan, Pakistan. A total of 1000 (Male, 627; Female, 373) blood samples were collected from both genders and four different ethnic groups (Baloch, Pashtoon, Hazara and Panjabi) and were screened in Balochistan Lab Brewery Road, Quetta.

Sample collection

Five ml of whole blood was collected from each patient and allowed to clot. Blood was then centrifuged at 4000 rpm for 5 minutes and serum was separated and collected in a separate tube and tested for HBsAg on both ICT and ELISA method.

Principles and Procedure of Tests

All reagents were brought to room temperature before testing. The ICT based HBsAg test is an in-vitro immunochromatographic one step assay prepared for qualitative determination of HBsAg in human serum or plasma. The test cassette contain a membrane strip precoated with mouse monoclonal anti HBs-capture antibody on test

band region. The mouse monoclonal anti HBs-colloid gold conjugate and serum sample moves along the membrane chromatographically to the test region (T) and forms a visible line as the antibody-antigen gold particle complex forms. The HBs Ag test cassette has a letter of T and C as "Test Line" and "control Line" on the surface of the cassette. Both the test line and control line in result window are not visible before applying any sample. The control line is used for procedural control.

The ELISA is qualitative detection of Hepatitis-B virus surface antigen (HBsAg) in human serum or plasma using pre-coated microplate with anti-hepatitis-B Virus surface antigen (anti-HBs). The test technique were followed as per recommendation of manufacturers (SD HBs Ag 3.0 ELISA). Briefly, 100 μ l of positive control, Negative control and sample were added into respective well followed by 25 μ l of enzyme conjugate. All the contents were mixed well and incubated at 37 °C for 60 minutes. Afterward all the wells were washed 5 times with 350 μ l of diluted washing solution giving at least 10 seconds soak time for each wash. A 100 μ l of TMB substrate mixture was be added into each well and the plate was incubated at room temperature for 10 minutes followed by 100 μ l stop solution. Then the wells were read for absorbance with 450 nm using spectrophotometer (Shimadzu).

Statistical analysis

The data collected were analyzed using statistical software SPSS. Version 21. Kruskal- Wallis test were applied for the evaluation of statistical significance in the data. While the p value less than 0.05 were taken as significant value.

RESULTS

A total of 1000 patients were recruited to evaluate the HBs Ag in the preoperative general surgery patients in Quetta, Pakistan. Overall, 70/1000 (7.0 %) seroprevalnce was recorded in 1000 (Male, 627: Female, 373) subjects (Table-I). On gender basis 44/627 (7.0 %) prevalence were logged in male while, 26/373 (6.90 %) in female patients. Similarly on age basis, 4/129 (3.1%) patients of

0-15 years age group were found positive. While, 33/363 (9.1 %) in 16-30 years followed by, 15/ 179 (8.3 %) in 31-40 age group seroprevalnce was reported. Whereas, 12/143 (8.3 %), in 41-50 years and, 6/18 (33.3%) in 51-60 years age group were found positive for HBs Ag (Table-I). Similarly, on the ethnical group bases the highest prevalence (10.3%) in Baloch population followed by Hazara 8.0 % population were noticed. While (5.7 %) in Pashtoon and (2.3 %) in Panjabi population were found. The two analytical techniques applied in this study, enzyme linked immunosorbent assay (ELISA) and Immunochromatography were also compared (Table-II). Out of 1000 samples, 67 and 70 samples were positive with ICT and ELISA, respectively. Whereas, 3 samples were false negative on ICT which were detected positive with ELISA indicating that ELISA technique is more sensitive and accurate.

More than fifty percent of patients were unaware of hepatitis, 70% of patients replied that they don't know that jaundice is the indication of hepatitis. Similarly 23 % of patients said that nausea, loss of appetite and vomiting could be the symptoms of viral hepatitis B infection. However 55 % of patients did not now that contaminated syringes and surgical instruments can transfer the infection. 41 % of patients knew that non-screen blood and its products can transfer the infection. Only 10 % of patients has given the positive answer to the question have you asked your dentist to use sterilize instruments before any procedure. Most of the patients didn't know that the Hepatitis-B viral infection disseminate through unsafe sex. Although 45 % of patients said that Hepatitis-B viral infection be transmitted by contaminated water/food. Similarly, all the patients were

asked different questions about their attitude with respect to hepatitis B infection. About 65 % of patients replied that they will reconfirm from another well reputed laboratory, if positive infection case is received and 78% said "NO" to the question that, do you think can hepatitis B virus infect you. Although, 50% patients were of the view to consult physician when asked, to whom you will talk about your illness .Wherever 65% patients were with the view to consult to the herb healer (HAKEEM).

Age group (Y)	No of patients With %	HBs Ag Positive %
0-15	129 (12.9)	04 (3.1)
16-30	363 (36.3)	33 (9.1)
31-40	179 (17.9)	15 (8.3)
41-50	143 (14.3)	12 (8.3)
51-60	18 (18.6)	06 (33.3)
Ethnic Groups		
Pashtoon	559 (55.9)	32 (5.7)
Baloch	330 (33.0)	34 (10.3)
Punjabi	86 (8.6)	2 (2.3)
Hazara	25 (2.5)	2 (8.0)
Gender		
Male	627 (62.7)	44 (7.00)
Female	373 (37.3)	26 (6.90)
Total Subjects	1000	70 (7.0)

Table-I. Demographic characteristics of HBV suspected preoperative patients in Quetta, Pakistan

Test	Total Samples	Positive	Negative	False Negative	Sensitivity
HBs on ICT	1000	67	933	3	95.70%
HBsAg on ELISA	1000	70	930	0	100%

Table-II. Comparison of ICT and ELISA analyzing hepatitis B antibodies in preoperative surgery patients in Quetta, Pakistan

Question asked to patients	Y		No	
	no. of patients	%age	no. of patients	%age
Have you know the disease termed as Hepatitis in past?	400	40	600	60
Do you think that due to hepatitis-B viral infection jaundice is one of the common indication?	300	30	700	70
Are the most common symptoms of hepatitis B viral infection are nausea, loss of appetite and vomiting?	230	23	770	77
Can contaminated syringes and surgical instruments can transfer the infection?	450	45	550	55
Can non screen blood and its products can transfer the infection?	410	41	590	59
Can you asked to your dentist to use sterilize instruments before any procedure?	100	10	900	90
Can Hepatitis-B viral infection disseminate through unsafe sex?	300	30	700	70
Can Hepatitis-B viral infection be transmitted by contaminated water/food?	450	45	550	55
Can Hepatitis B virus infection be self-cured by the body?	480	48	520	52
Is Hepatitis-B vaccination available?	800	80	200	20

Table-III. Knowledge of Hepatitis B in Preoperative surgery patients in Quetta, Pakistan.

Hepatitis B attitude questions	no. of patients	%age
What would be your reaction if lab give to you a Hepatitis B viral infection positive report?		
Reconfirm by Superior technique.	300	30
Reconfirm from well reputed lab.	650	65
Do not giving any importance to positive report.	50	5
Do You think can Hepatitis B virus can infect you?		
Yes	220	22
No	780	78
What will be your reaction if you found hepatitis B virus positive?		
Fear	150	15
Surprise	200	20
Sad	650	65
To whom you will talk about your illness?		
Physician	500	50
Spouse	50	5
Friends	450	45
The Hepatitis B diagnosis and treatment is how much expensive, what do you think?		
Reasonable	200	20
Somewhat expensive	200	20
Expensive	600	60
If you are diagnosed with hepatitis B, what thing will get you worry?		
wonder of death	280	28
Fear of disease dissemination to family	550	55
Expanse of treatment	170	17
You know that your friend has symptoms of Hepatitis B viral infection, what will you do?		
Consult to Doctor	300	30
Consult to herb healer	650	65
Go to Homeopathic	50	5

Table-IV. Attitude towards Hepatitis B viral Infection in Preoperative surgery Patients, Quetta, Pakistan

DISCUSSION

The HBV is well known for serious morbidity and mortality. The prevalence of 5 to 10 % have been reported from Sub Saharan Africa as well as Asia. Similarly, It is estimated that 2 to 5% prevalence rate of HBV is present in India and Middle East, while North America and Western Europe has less than 1% chronic HBV infection.¹³

Overall, 7% prevalence of HBV infection have been reported in this study from general population in preoperative patients in Quetta, Pakistan. In Asia, Africa, Latin America and southern Europe about 2 billion people are infected with HBV.¹⁴ In Pakistan 9 million people have been infected with HBV.¹⁵ In present study, higher seropositivity (10 .3%) have been reported in Baloch ethnic group followed by Hazara community (8.0 %) and Pashtoon tribes (5.7 %) (Table-I). our findings corroborate with Nadeem et al, who also conducted a study in Baloch population refrain areas reported an average higher prevalence (9.8 %) in general population.¹⁶ High rates of hepatitis B in these tribes are ascribed to unscreened blood transfusion, reuse of contaminated syringes, and delayed or no vaccination. As the population is scattered in patches living in remote areas and most of the population is illiterate with low socio economical status. On gender bases statistically non-significant prevalence in male, 44 /627(7%) and female 26/373 (6.9%) patients was recorded .This show clearly that both the sexes are equally at risk of getting infection. In this study, three samples were found false negative with ICT but, positive through ELISA rendering it more sensitive and specific than ICT.¹⁷

In the current study, Poor knowledge of Hepatitis B virus was observed in the preoperative surgery patients as very low numbers of patients knew about the disease. The lack of knowledge of Hepatitis B in the study population can be due to lack of awareness and poor literacy rate in the community. Our study is similar to what is reported from other different areas of Pakistan.¹⁸ Similarly, Poor knowledge of Hepatitis B was also observed throughout the world.¹⁹ In addition the attitude of the patients was also show that

the Hepatitis B disease cannot infect them, very expensive to treat and most of the patients will use alternative medication like herb therapy if infected with Hepatitis B. In conclusion striking cause of Hepatitis B is the limited knowledge, awareness and poverty in in general surgery patients in Quetta, Pakistan. Mass scale screening program should have to be launched using ICT and reconfirmation through ELISA or other advanced molecular technique. Similarly, proper and regular screening of healthcare staff including surgeons, lab workers, theater anesthetics and all other assistants of the operation room to be regularly vaccinated. Before any surgical procedure patient should screen through ELISA for the determination of HBsAg in preoperative surgery patients. Proper control and eradication should have to be launched and public awareness program are advised using electronic and print media could be helpful to minimize the infection in the society.








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AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
1	Muhammad Umer	Conceived the study, take part in sample collection and study design.	
2	Ferhat Abbas	Helped in study design and critical review of manuscript.	
3	Muhammad Shafee	Analyzed the data and helped in manuscript writing.	
4	Abdul Rauf	Helped in statistical analysis and critical review of the data.	
5	Najeebullah	Helped in data collection and interpretation.	
6	Muhammad Naeem	Performed screening and helped in immunoassay technique.	
7	Ghazalla Siddique	Helped in data collection and interpretation.	
8	Amir Muhammad	Analyzed the data and helped in manuscript writing	