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Comparison of ICT and ELISA Techniques for the Detection of HBsAg in Apparently Healthy Individuals in North West General Hospital Peshawar Pakistan

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Abstract

Background: The majority of cases of hepatitis B are clinically asymptomatic, making it the deadliest of the five kinds of hepatitis. Hepatitis B virus infection damages the liver over a long period without being detected. With an average prevalence rate of 2.70 percent, it is one frequent risk of liver problems in Khyber Pakhtunkhwa (KP), Pakistan.
Aim: We sought to examine the diagnostic effectiveness of the enzyme-linked immunosorbent assay (ELIA) and rapid immunochromatography test (ICT) for hepatitis B surface antigen (HBsAg) (ELISA).
Methodology: A total of 2434 Out Patients Department (OPD) patients within the age range 20-65 years (1451 (59.61%) males and 983 (40.38%) females) were screened for the hepatitis B virus infection using commercially available diagnostic kits at North West General Hospital, Peshawar, Khyber Pakhtunkhwa, Pakistan. Blood samples were collected in EDTA tubes from each subject after taking informed consent. The samples were centrifuged at 5000 rpm for 5 minutes to separate the serum. Samples were first examined for HBsAg using the quick ICT equipment (Accurate, UK) following the manufacturer's recommended technique, and then by ELISA.
Results: The result indicated that 42 patients (1.72%) were positive for HBsAg by ICT including 31 (73.80%) males and 11 (26.19%) females. Using a third-generation ELISA technology, follow-up was conducted. Blood samples of the patients declared positive by ICT were then tested by ELISA and it was found that 39 patients (1.60%) reacted positively

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including 29 (74.35%) males and 10 (25.64%) females.
Conclusion: It is vital to implement preventive and control measures as well as suitable evaluation and monitoring procedures. The ICT screening method gives false positive results and it should be confirmed by ELISA and PCR techniques.

Keywords: Hepatitis B, ICT, ELISA

1 Introduction

Hepatitis is the term for liver inflammation, and there are many different types, including hepatitis A, B, C, D, and E. The most dangerous and prevalent infectious liver illnesses are hepatitis B and C. 1 The hepatitis B virus (HBV) uses its reverse transcriptase to replicate through an intermediate of RNA that is partially double-stranded and circular (RT). 2. HBV infection is more prevalent in developing countries including Pakistan. Globally 2 billion people are suffering from HBV infection with 400 million people chronically infected.3 According to a 2015 WHO research, hepatitis B and C infections cause 96% of all hepatitis-related deaths and are responsible for 1.45 million annual deaths.4 According to studies, the majority of HBV-positive patients in poor nations are unaware of their serostatus. 5 Additionally, studies conducted in the community suggest that inadequate screening and treatment cessation may be to blame for the high prevalence of HBV. 4 The Global Health Sector Strategy (GHSS) on viral hepatitis places emphasis on the need for ambitious goals for the eradication of viral hepatitis (Asaduzzaman et al., n.d.) by 2030 and suggests a 90% decline in incidence and a 65% decrease in mortality rate owing to chronic hepatitis B and C infections. 4

Pakistan has a high prevalence of HBV infection due to its status as a developing nation. Nine million people have HBV infections as a result of recent cases (kyaw et al, 2020). 3 Islamabad has the highest prevalence of HBV (15.58% + 1.96), followed by Punjab (8.02% + 1.96), KP (2.12% + 1.96), and Sindh (1.82% + 1.96), in that order.3 The Pro-operative screening of HBV infection in developed countries is the standard practice and it has contributed to controlling the transmission of this insidious disease.6 Handling medical waste, abusing intravenous drugs, using contaminated medical equipment, and having sexual intercourse are risk factors for developing the HBV virus. Recombinant vaccines are available to protect against HBV infections (Mehmood et al, 2019). It is highly advisable to receive the HBV vaccine. Safe blood transfusion and safe sex methods are additional measures for preventing HBV infection.1 Different diagnostic methods including ICT, ELIZA, EIA, and PCR are used in the diagnosis of HBV infections(khan et al, 2019). ELIZA, In pricey labs and sizable tertiary care facilities, EIA and PCR techniques are employed. ICT rapid diagnostic methods are rapid, cheap methods and are a good choice for the diagnosis of HBV.7 (Allard et al, 2015).

Accurate and timely detection of the viral markers of highly infectious viruses like HBV is very important for controlling the transmission of viruses (khan et al, 2017). Therefore it is mandatory to validate different detection methods before use for diagnostic purposes.8 In developing countries both screening and diagnosis of HBsAg antibodies in acute and chronic HBV infection are done through ICT-based detection tests. However, ideally, more accurate and advanced methods such as ELIZA, EIA, or PCR are recommended for screening purposes (Hyder et al, 2012). Samples tested negative by rapid diagnostic tests are seldom retested, considering the cost of the retesting in poor resource settings(Mohammad et al, n.d). Consequently, for routine use, tests with high sensitivity and low positive predictive values (NPV) should be chosen above those with high specificity and high PPV.8 (Kalim et al, 2017).

The purpose of this study was to determine the prevalence of HBV infection among OPD patients who visited NWGH(Kaur et al, 2000). The sensitivity of ICT and ELIZA techniques for the diagnosis of HBV was compared. It will give us an idea of the HBV infection burden in healthy-

looking populations in different areas of the KP(Qasmi et al, 2000). The information gathered from this study could be used to create plans and regulations for secure blood transfusions(Irwing et al, 2002).

2 Materials and Methods

Sample Size and Study Setting: A total of 2434 OPD patients visiting NWGH Peshawar, KP, Pakistan were included in the current study for four months spanning January 2021 and April 2021. 1451 (59.61%) of the 2434 patients were men, and 983 (40.38%) were women.

Sampling: Blood samples were collected from OPD patients visiting NWGH in dipotassium ethylenediaminetetraacetate After obtaining informed consent, using (K2-EDTA) evacuated vacutainer tubes from Becton Dickinson in Franklin Lakes, New Jersey.

Methods: Two diagnostic techniques, namely the ELISA technique and the one-step ICT quick test method, were used to test the OPD patients for HBsAg(Purday et al, 2007).

Immune-Chromatographic Test Method (ICT) for Detection of HBsAg; ICT tools were largely used to identify samples from HBV-infected patients during the screening of all the samples. Three drops of the serum were loaded into the sample well in an ICT device under sterile conditions. Samples were declared positive when two lines were produced on the ICT device i.e. control line and the test line.

Enzyme-Linked ImmunoSorbent Assay (ELISA) for Detection of HBsAg: The HBsAg kit (Equipar, Italy) based on one-step "Sandwich" ELISA was used to further analyze and confirm samples that tested positive for HBV using the ICT method. This gadget employs two distinct antibodies that are targeted at two distinct HBsAg epitopes. When one type of antibody is labeled with the enzyme horseradish peroxidase (HRP), the other is adsorbed onto the plate walls. The conjugate and samples were put simultaneously onto a microplate and incubated at 37°C. The specific immune complex on the surface of the solid phase was then found by adding chromogen to the microplates and re-incubating. The enzyme produced a color, which the ELISA reader then examined. The concentration of HBsAg in the samples directly relates to the color intensity.

3 Results

A total of 2434 OPD patients (20-65 years) were screened for HBsAg through ICT devices including 1451 (59.61%) males and 983 (40.38%) females. Out of 2434 patients, 42 (1.72%) tested positive for HBsAg by ICT device including 31 (73.80%) males and 11 (26.19%) females using a third-generation ELISA technology, follow-up was conducted. The Patients test positive by ICT device, were retested using ELISA and it was found that 39 patients (1.60%) including 29 (74.35%) males and 10 (25.64%) females reacted positively to the ELISA method (Table 01).

Table 01: Comparison of ICT and ELISA Method

HBsAg	**ICT Method (%)**		**Total**	**ELISA Method (%)**		**Total**
	Male	Female		Male	Female	
Positive/ Reactive	31 (73.8)	11 (26.2)	42 (1.72)	29 (74.4)	10 (25.6)	39 (1.60)
Negative/ Non-Reactive	1420 (59.4)	972 (40.6)	2392 (98.3)	1420(59.4)	972 (40.6)	2395 (98.4)

4 Discussion

In many nations, especially in developing nations, HBV has become endemic. It has been noted as a worrisome concern, particularly in Pakistan's rural areas. Many already have HBV, with a prevalence rate of 10%, but in rural regions, the percentage has been observed to be substantially

greater than the one mentioned.9

According to an Indian study, ICAS has a 100% specificity rate while only having a 93.4% sensitivity rate.10 Another study done on healthy Karachi residents revealed that the ELISA technique and ICT have similar sensitivity and specificity.11 Similarly sensitivity (97%) and specificity (100%) of ICT have been reported for the detection of HBsAg from Seoul. 12 Since the prevalence of HBV infection differs from region to region, the accuracy index of various ICA-based assays for the detection of HBsAg in serum may not be the same. The vast majority of these quick tests only employ recombinant proteins from the prototype virus. Several parts of the world are home to eight different HBV genotypes. These genotypes and subtypes exhibit a diverse geographic and epidemiological distribution. 1 In this situation, an ICA that does not support the specific subtype or subtypes will miss this type during testing. This could be the cause of one serum sample that tested positive on an ELISA but negative on one phase of the test. 13 Among the 42 HbsAg seropositive samples in the current investigation, 3 serum samples were identified via ELISA rather than ICT(Torane et al, 2008).

As a result of inadequate antigen coating, the fast test is unable to detect HBV reactive samples, genetic heterogeneity of HBV prevalent in a particular area, and different natures of the antigens. 14 Before being regularly used in clinical laboratories, fast tests must first be validated by testing them on a specific population to determine their efficacy for detecting subtypes and genotypes of HBV. To identify HBV, the ELISA is more reliable than fast testing. Only distant, underdeveloped areas and outlying medical facilities should offer fast tests. Infection with HBV is extremely deadly. The concern of silent disease transmission among people posed by the false negative result drives demand for more sensitive assays like ELISA and PCR.

5 Conclusion

Before being regularly utilized in clinical laboratories, fast tests must first be validated by evaluating them in a specific population to determine their efficacy for the detection of HBV subtypes and genotypes. Major elements that affect the epidemiology of any disease are awareness, prevention, and treatment. These elements are essential for effectively lowering the number of hepatitis infections in the future.

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