COMPARATIVE ANALYSIS OF IMMUNOCHROMATOGRAPHIC ASSAY WITH IMMUNOSORBENT RELATED ASSAY OF HEPATITIS B SURFACE ANTIGENTS

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ABSTRACT

Hepatitis is a type of systematic disease through primarily swelling in the liver. It can cause mutually type of disease acute and unending. The HBV is transmitted during contact with blood or other fluids which contaminated with HBV. According to WHO about 260 million people are living with infection, known as HBsAg in 2015, about 887 000 deaths accurse due to hepatitis B infection mostly from complications including hepatocellular carcinoma and cirrhosis. This study aimed to compare an immunochromatographic technique with ELISA. For revealing of HBsAg in NMCH, Jamuhar (Bihar), the selection of participants for this study was done on sampling the IPD and OPD patients of NMCH, Jamuhar, BIHAR, from 1st January 2018 to 29th April 2018.

Keywords: HBsAg, Hepatitis, ELISA, ICA, Hepatocellular, Cirrhosis, Systematic disease.

1.1 INTRODUCTION

The hepatitis is efficient disease with primarily swelling in liver. It can cause both type of disease sharp and chronic. This virus is prevalence high in East Asia and sub-Saharan Africa, where about 5–10% of the adult population is constantly infected with HBV. According to World Health Organization about 260 million people are living with HBV infection, known as hepatitis B surface antigen and in 2015, about 887 000 deaths accurse due to hepatitis B infection mostly from complications including hepatocellular carcinoma and cirrhosis. [1, 2]

The term ‘viral hepatitis’ it belongs to prime inflammation in the liver by any one of a heterogeneous cluster of hepatitis virus. By taxonomically hepatitis virus are unrelated. Except of hepatitis B virus which is a type of DNA virus, all the others hepatitis virus are RNA viruses. As all type of hepatitis virus cause a clinically similar kinds of acute illness which are indistinguishable. Therefore the differentiation is done on the bases of serological and molecular markers. In incidentally hepatitis may occur in much other viral infection, like in Lassa fever, Marburg, cytomegalo, herpes simplex, varicella zoster, measles, rubella and yellow fever. All of
this is not included in the category of viral hepatitis. On the basis of epidemiological and clinical criterion, two type of viral hepatitis recognized as more severe, first one occurred as epidemics, children and youthful adult. [3-5].

1.2 TYPE B HEPATITIS

Its is a sort of DNA disease size measure about 42 nm in diameter, with an on the outside envelope and contain an internal core size measuring about 27 nm in breadth, and it also contain viral genome and a DNA polymerase known as DNA dependent DNA polymerase. Because of its unique features HBV is belong to a separate people of Hepadnavridae (hepatotropic DNA viruses) and the Genus of Orthohepadnavirus [6,7]

1.3 DISCOVERY OF HEPATITIS B VIRUS

The innovation of hepatitis B virus was an accidental detection. The HBV is discovered by a scientist known as Blumberg in 1965. When he was studying on serum sample of two hemophiliac’s patients, who had received multiple blood transfusions. [8].

1.4 SYMPTOMS OF HEPATITIS B INFECTION

The hepatitis B cause both type of infection acute and chronic, in acute phase of infection only 30% to 50% of patients developed major symptom.

- Fever, a flu-like illness, Fatigue, Nausea, Vomiting, Loss of appetite, Joint pain
- Jaundice (yellowing of the skin and eyes), Dark urine, Clay-colored bowel movements, Pain in the upper right abdomen (due to the inflamed liver) [9]

1.5 TRANSMISSION

There are three important modes of Hepatitis B Virus transmission.

1. Parenteral transmission; Infection can occur due to use of objects that are contaminated with HBV infected blood. During medical, surgical and dental procedures, tattooing, or through the use of razors use of unsterile needles which are contaminated with HBV and similar object. Hepatitis B virus can be transmitted by transfusion of infected blood and blood products, and by organs transplantation like, liver, skin, kidney, and etc.

2. Perinatal transmission; The HBV can be transmitted from an infected mother to her newborn during the delivery process.

3. Sexual transmission; HBV is transmitted during blood and other unhygienic body fluids as sound as by body fluids. [10, 11].
• Hepatitis B virus is not spread through by casual contact or with food and water

1.6 DIAGNOSIS OF HEPATITIS

The laboratory diagnosis of hepatitis B is done by many different type of technique like

(1) Immunochromatographic Assay (ICA): For routine screening of HBV infection in Indian laboratory frequently done by immunochromatographic assay or by rapid card technique for detection of HBsAg, the rapid card or test kit is available readymade in market, it is a very simple technique for detection of HBV infection within 15-20 minutes. This kit is contain a nitrocellulose membrane, on this nitrocellulose membrane the hepatitis B antibodies are coated against the HBsAg in linear band label as (T) or test region and a second linear band is label as (C) or control band. For this technique we use serum sample about 50ul serum are add in rapid card kit and wait for 15-20 minutes. After the addition of sample the ample are rune to the test band (T) and control band (C) by capillary action. If in the test sample contain HBsAg it bind with coated antibody on test region and make a reaction which give the purple color band within 15-20 minutes. This band is also formed on control region for confirmation of the test. But if the test samples is does not contain HBsAg then the above complex is not formed and the purple color bond is not formed on test region only single bond is formed on control region, after 15-20 minute if not any band is formed then test is invalid and then repeat the test again. The reporting is done within 15-20 minutes after 20 minutes does not report the result. [12-14]

(2) Enzyme-Linked immunosorbsent assay (ELISA): The second most frequently used technique, this is a very use full technique for screening test of hepatitis B infection HBsAg, mostly it use in screening test of blood donor, because the hepatitis B virus highly risk of transmit through blood transfusion. Now day it mandatory to only transfused ELISA tested blood. This technique is complicated then rapid card technique because this is a multiple stapes method and highly sensitive technique for this required an ELISA radar and ELISA washer for operating of this instrument required electricity and a special staff who having knowledge about instrument and test procedure. This is a very sensitive and specific technique it can detect both antigen and antibody and it both type qualitative and quantitative technique. [15]

(3) Chemiluminescent immunoassay or (CLIA): This is frequently used assay it is also an immunoassays based on enzyme reactivity this technique is also qualitative detection of HBsAg in serum sample. [16, 17]

(4) Polymerase chain reaction or (PCR): The genetic material of Hepatitis B virus (DNA) can be quantified by using a specific technique known as polymerase chain reaction or (PCR) assays in serum or plasma. For this technique we use a real time PCR, for the quantitative detection of HBV by amplification of target DNA, for this we required a 20-25 basepair long primer which are complimentary to the gene sequence of hepatitis B genetic material or DNA and also required dtnp, polymerase, buffer solution and a PCR setup. [18]
1.7 PREVENTION OF HEPATITIS B

For avoidance of hepatitis B infection hepatitis B vaccination is mandatory. The WHO is recommended to give the hepatitis B vaccine to all the infants as rapidly as possible. Within 24 hours of after birth. Some environmental procedure can decreased the risk of infection to health care professional like doctor, laboratory staff, and others. [10, 11, 19]

1.8 VACCINE OF HEPATITIS

- The vaccine used for hepatitis B virus.

1) **Plasma derived vaccines**: this is an initial vaccine which is prepared by purification of HBsAg associated with 22nm Dane particles from the HBsAg optimistic carriers and it treated by virus inactivating agent like formalin, urea, and heat etc.

2) **Recombinant DNA derived vaccines**: this is a more specific vaccine this vaccine is made of HBsAg. The vaccine of HBsAg can be combined with other vaccines such as and pertussis diphtheria, tetanus combined with polio (DTP-polio), measles, mumps, and rubella (MMR), Calmette-Guérin bacillus (BCG), Homophiles influenza. [10, 11, 20, 21]

Hepatitis B virus.

- Different type of technique use for Hepatitis B virus detection.
  
  - **Serological method**
    
    a) ELISA
    
    b) ICA
    
    c) CLEIA
  
  - **Molecular method**
    
    (a) Hybridization technique
    
    (b) Nucleic acid amplification technique
    
    -target amplification
    
    -signal amplification
    
    -probe amplification
• Survey on comparative study on ELISA and ICA technique for detection of HBsAg

Figure 2: Flow Chart for selection of related articles for this study.

2.3 HEPATITIS B VIRUS

2.3.1 History of hepatitis B

The discovery of virus an accidental discovery. The HBV is discovered by a scientist known as Blumberg in 1965. When he was studying on serum sample of two hemophilia’s patients, who had received multiple blood transfusions. He was getting a clearly defined line of precipitation this was named as Australia antigen[8,22].
3.3.2 Definition

HBV is a smaller virus it contain DNA as genetic material so that’s way is a DNA virus [23, 24]. On the basis of sequence of genetic material the Hepatitis B virus is classified into eight genotype, type A to H every genotype having particular geographical distribution. The HBsAg is composed of spheres and filament [25]. The genetic material of hepatitis B virus is a circular and partially double-stranded DNA of about 3.2 kilo base (kb) pairs[26]. It is a highly infectious viral infection. It can be damage liver by infecting the liver cells called hepatocytes. The virus is easily transmitted by hepatitis B–positive blood or by blood product and by semen or other body fluid. It can be transmitted by pregnant women to their babies mostly during birth if the mother having HBV infection. For prevention of infection person who have not been infected with HBV can be vaccinated against the hepatitis B virus. The hepatitis B cause both type of infection acute phase infection and chronic phase infection. The chronic hepatitis B infection can cause fibrosis in the liver[27, 28]

2.3.3 Epidemiology

The incidence of chronic hepatitis B infection worldwide is categorize in three sections, the first one is high endemicity, second is intermediate endimicity and the third one is low endimicity.

(1)High Endemicity; The incidence of hepatitis B virus infection in high endemicity differs considerably across the world [30]. Mainly infections are asymptomatic, in this area having little confirmation of acute disease related to hepatitis B virus, but in this area having high evidence of disease related to infection for example constant hepatic disease like cirrhosis and liver swelling such as hepatocellular carcinoma [31].

(2)Intermediate Endemicity; In this area the acute disease which is related to hepatitis B is common because the most of infections occur in children and adults, the chronic infections occurring in infants and children are maintained in high rates [32]. Mixed patterns of transmission exist, including adult, in the early hours childhood and infant.

(3)Low Endemicity; In these particular area the hepatitis B virus infects is about 5–7% of the population, and only 0.5–2% of the population are chronic carriers of hepatitis B infection [33]. In these areas, most hepatitis B virus infection crop up in adolescents and adults, including health care workers.

2.3.4 Classification

. This virus replicate by the reverse transcription of a viral RNA intermediate. It is blood borne infectious virus. It is more infectious than HIV virus[34]

2.3.5 Genetic material

The HBV is consist of two strand of DNA held in circular configuration. In which one strand are incomplete and one is complete. The positive strand of HBV is incomplete and negative strand are complete. This gives
appearance of partially double strand and partially single strand DNA genome. After electron microscopic examination of HBV infected serum sample three type of viral particles are visualized. [35-45].

2.3.6 Antigenic structure

2.3.6.1 HBsAg The HBsAg contains group specific antigen’a’ and two type specific antigens, d or y and w or r. the total four antigenic types of HBsAg first-adw, second-adr, third-ayw and fourth one is ayr, this all are useful markers for epidemiology.

2.3.6.2 HBcAg contains group specific protein which are does not detect in patient’s blood.

2.3.6.3 HBeAg is appear in serum along with HBsAg but this HBeAg are disappears within a few weeks. The HBeAg is a hidden and antigenic component of core. The HBeAg and HBcAg are immunologically coded with same gene.

2.3.6.4 HBxAg is an antigen of HBV which are present in patients with severe chronic hepatitis and cancer. [46, 47, 48,49]

2.4 Different type of technique use for Hepatitis B virus detection.

2.4.1 Serological method

The researchers are have modified this technique even. In 2010 a novel monoclonal antibodies are used as capture layer and as a detector phase used a polyclonal biotinylated antibody to develop one new ELISA system.[50- 53]

2.4.2 Immunochromatographic assay.

Immunochromatography assay is a uncomplicated tool which is helpful to detect the virus absence or presence. The concept is a combination of chromatography and immunochemical reactions. It is rapid card test.

2.4.2.1 Components use in ICA

(1) Sample application pad.

(2) Conjugate Pad

(3) Substrate Membrane (Nitrocellulose)

(4) Adsorbent Pad
The adsorbent pad is works in end of the strip and maintains the flow of rate of liquid over the nitrocellulose membrane and stop back flow of test sample. All the components are fixed in a backing card. This card serves as a support and makes easy to handle the test strip. [54,55]

2.4.3 Chemiluminescent enzyme immunoassay

The CLEIA is this rapid immunoassay method in which uses of luminescent molecules labeled antibodies or antigen. The CLEIA is more sensitive than ELISA.[56-59]

2.4.4 Molecular methods

The Molecular methods used for diagnosis of HBsAg categorized as nucleic acid hybridization, nucleic acid amplification, sequencing and enzymatic digestion of nucleic acids.

2.4.4.1 Hybridization technique: The hybridization technique is highly specific technique, lack compassion. Composed of a peptide nucleic acid probe and a target DNA sequences in which the the peptide nucleic acid which combined with target DNA sequences which are more efficiently than DNA probes[60]

2.4.4.2 Nucleic acid amplification technique

For monitoring of HBV infection the quantitative detection is very important. The mostly molecular methods have been used for quantitation by different researchers [61].

2.5 SURVEY ON COMPARATIVE STUDY ON ELISA AND ICA TECHNIQUE FOR DETECTION OF HBsAg

After study of article related to comparative study of ELISA and ICA for detection of HBsAg the review of both serological techniques is following;

2.5.1 Material and method

The sample for detection of HBsAg ELISA and ICA are collected from the person came for blood donation in blood bank. The 3 to 5 ml of blood is collected in plane tube and after clot the sample are centrifuged and the serum are separated in another vile. The hemolised samples are not used.

2.5.2 Method for ICA

The different type of commercial kit of rapid card used in different laboratory for detection of HBsAg like J. Mitra Co. Pvt Ltd, Hepacard etc

The test result of ICA technique according to literature review on test done on 5416 serum sample in which 263 samples are gives positive result were 5153 sample gives negative results.
2.5.3 Method for ELISA

The different type of commercial kit for ELISA used in different laboratory for detection of HBsAg like J. Mitra Co. Pvt Ltd,

The test result of ELISA technique according to literature review on test done on 5416 serum sample in which 291 samples are gives positive result and 5125 samples are gives negative result.

The compared test results of two different type of serological technique in test 5416 samples. Immunochromatographic assay (ICA) and Enzyme Linked Sorbent Assay (ELISA) were used to test the HBsAg for qualitative results. The ICA technique is less sensitive and specific then ELISA technique. The false negative rate of ICA is 2.3% and of ELISA is 1.3%.

<table>
<thead>
<tr>
<th></th>
<th>ELISA</th>
<th>ICA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>291</td>
<td>263</td>
</tr>
<tr>
<td>Negative</td>
<td>5125</td>
<td>5143</td>
</tr>
<tr>
<td>TOTAL</td>
<td>5416</td>
<td>5416</td>
</tr>
</tbody>
</table>

Table 1: Showing positive and negative samples.

**Table 2: Comparison of ICA with ELISA.**

<table>
<thead>
<tr>
<th>Method use</th>
<th>Positive %</th>
<th>Negative %</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICA</td>
<td>4.855</td>
<td>95.145</td>
<td>100</td>
</tr>
<tr>
<td>ELISA</td>
<td>5.372</td>
<td>94.627</td>
<td>100</td>
</tr>
</tbody>
</table>

**MATERIAL AND METHODS**

**Collection of Sample**

The 3 ml of blood sample are collected in a plain or in containing clot activator tube form the patients coming for hepatitis B diagnosis in department of microbiology NMCH, Sasaram, BIHAR. Most of patients are coming from rural area near about 25km of hospital.
Preparation of sample

After collection of blood samples are allowed to clot at room temperature for 30 minutes and in water bath for 5 to 10 minutes. When the blood is clot the samples are centrifugation at 2600rpm for 5-10 minutes. After the centrifugation the serum or fluids part of blood are separated on the top of the tube. The separated serum is collect by piped in other tubes.

ICA TECHNIQUE

Immunochromatographic assay (ICA), namely lateral flow test, is a simple device intended to detect the presence or absence of the hepatitis B virus. The concept of immune-chromatography is a combination of chromatography and immunochemical reactions (separation of components of a sample based on differences in their movement through a sorbent).

Tabulation of result data

Table 3: Age category of patients showing positive and negative results

<table>
<thead>
<tr>
<th>Age category in year</th>
<th>Frequency</th>
<th>Percent (%)</th>
<th>Negative</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10</td>
<td>45</td>
<td>3.577</td>
<td>43</td>
<td>2</td>
</tr>
<tr>
<td>11-20</td>
<td>89</td>
<td>7.074</td>
<td>86</td>
<td>3</td>
</tr>
<tr>
<td>21-30</td>
<td>190</td>
<td>15.103</td>
<td>182</td>
<td>8</td>
</tr>
<tr>
<td>31-40</td>
<td>244</td>
<td>19.395</td>
<td>232</td>
<td>12</td>
</tr>
<tr>
<td>41-50</td>
<td>295</td>
<td>23.449</td>
<td>181</td>
<td>14</td>
</tr>
<tr>
<td>51-60</td>
<td>206</td>
<td>16.375</td>
<td>197</td>
<td>9</td>
</tr>
<tr>
<td>61-70</td>
<td>115</td>
<td>9.149</td>
<td>109</td>
<td>6</td>
</tr>
<tr>
<td>71-80</td>
<td>60</td>
<td>4.769</td>
<td>57</td>
<td>3</td>
</tr>
<tr>
<td>81-90</td>
<td>12</td>
<td>0.953</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>&gt;91</td>
<td>2</td>
<td>0.156</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>1258</td>
<td>100</td>
<td>1200</td>
<td>58</td>
</tr>
</tbody>
</table>
**Graph 2:** Graphic distribution of age group of patients.

![Graph showing age group distribution of patients]

**Table 4:** Showing male and female participants

<table>
<thead>
<tr>
<th>Sex</th>
<th>Frequency</th>
<th>Percent (%)</th>
<th>Negative</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>736</td>
<td>58.505</td>
<td>705</td>
<td>31</td>
</tr>
<tr>
<td>Female</td>
<td>522</td>
<td>41.495</td>
<td>495</td>
<td>27</td>
</tr>
<tr>
<td>Total</td>
<td>1258</td>
<td>100</td>
<td>1200</td>
<td>58</td>
</tr>
</tbody>
</table>
Graph 3: Graphic distribution of male and female patients.

Table 5: Area of patients living

<table>
<thead>
<tr>
<th>Area of living</th>
<th>Frequency</th>
<th>Percent (%)</th>
<th>Negative</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban</td>
<td>410</td>
<td>32.591</td>
<td>395</td>
<td>15</td>
</tr>
<tr>
<td>Rural</td>
<td>848</td>
<td>67.409</td>
<td>805</td>
<td>43</td>
</tr>
<tr>
<td>Total</td>
<td>1258</td>
<td>100</td>
<td>1200</td>
<td>58</td>
</tr>
</tbody>
</table>

Graph 4: Graphic distribution of urban and rural patients.

Table 6: Showing the patients from hospital department.

<table>
<thead>
<tr>
<th>Department</th>
<th>Frequency</th>
<th>Percent (%)</th>
<th>Negative</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPD</td>
<td>486</td>
<td>38.633</td>
<td>463</td>
<td>23</td>
</tr>
<tr>
<td>OPD</td>
<td>772</td>
<td>61.367</td>
<td>737</td>
<td>35</td>
</tr>
<tr>
<td>Total</td>
<td>1258</td>
<td>100</td>
<td>1200</td>
<td>58</td>
</tr>
</tbody>
</table>
**Graph 5:** Graphic distribution of IPD and OPD patients.

![Graph 5](image)

**Table 7:** Comparison of ICA technique with ELISA technique

<table>
<thead>
<tr>
<th>Test technique</th>
<th>Positive (%)</th>
<th>Negative (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td>5.166</td>
<td>94.834</td>
<td>100</td>
</tr>
<tr>
<td>ICA</td>
<td>4.61</td>
<td>95.39</td>
<td>100</td>
</tr>
</tbody>
</table>

**Graph 6:** Graph showing the assay technique ans % distribution.
In this study according to sex the 4.21% male are showing REACTIVE test result and 5.17% female are showing REACTIVE test results, according to living place of patients 3.66% urban patients showing REACTIVE test and 5.07% rural patients are showing REACTIVE test results. And according to hospital department the 4.73% IPD patients are showing REACTIVE test results and 4.53% OPD patients are showing REACTIVE test results. The female patients are having more reactivity than male patients for HBsAg and also rural patients having more reactivity than urban patients.

**CONCLUSION**

The hepatitis B is a DNA virus it is highly contagious virus because it transform from one person to another person by contact with blood and another body fluids. The high risk to it can transfer in infants from his mother. So for that we need techniques which can give 100% specific and sensitive screening results. So for that we need to compare study of techniques which mostly used in Laboratory for diagnosis of hepatitis B virus. So then we can know about the Techniques which give high specificity and sensitivity for detection of hepatitis B virus.

In this study we did a comparative study between ELISA and ICA technique for detection of hepatitis ANTIGEN in Microbiology Department of Narayan Medical College and Hospital, Sasaram, Bihar.

We done study on 11 literature on topic related to detection of hepatitis by ELISA and ICA, in which we get that the study is done on total 5416 serum sample in which 291 sample are gives Positive results for HBsAg by ELISA techniques and 263 sample only gives Positive results by ICA techniques. After the review of previous study we gate an idea about the technique which gives more specific and sensitive results. So according to my review study the ELISA techniques are more sensitive and specific than ICA techniques, because the ELISA have given more Positive results then ICA.

In my study the started from 1st January 2018 and Completed on 29th April 2018. In which we have collected total 1258 blood samples from the patients come for Hepatitis B virus diagnosis in Microbiology department of NMCH Bihar. the total 1258 samples are tested by both techniques ELISA and ICA in which we get 65 samples are Positive given by ELISA techniques and only 58 sample are Positive given by ICA techniques. So, the ELISA technique are gives more sensitive results then the ICA technique because 7 specimen in 1258 serum samples are gives negative result by ICA technique that gives positive results by ELISA Technique. But both the techniques are having same specificity, because the specimen which gives positive results by ICA technique also positive by ELISA technique.
So according to my study the ELISA technique is more sensitive than ICA technique but having same specificity.

So, according to comparison between literature review and our study the both shows the ELISA technique is more sensitive then ICA technique but the percentage of different of sensitivity is more in our study then study of review literature and the specificity is same in ELISA and ICA technique but according to review literature the ELISA technique is more specific then ICA technique.

So, according to our project work or study the ELISA is more sensitive then ICA Technique with equal specificity. So, for screenings diagnosis of Hepatitis B surface antigen in Laboratory the ELISA techniques are more useful technique then ICA. It can reduced the chances of false Negatives results, and it help in control the transmission of the Hepatitis B virus infection from infected person to healthy person by direct blood contact and by mother to his infant.

So, according to our study the best technique is Enzyme Linked Immunosorbent Assay (ELISA) then Immunochromatographic assay (ICA).

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