# SEROPREVALANCE OF HBsAg AND HCV IN PATIENTS ATTENDING A TERTIARY CARE HOSPITAL IN NORTHERN INDIA


**Internship Training Report**

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**in partial fulfillment of the requirements**

**For the degree of**

**Master of Science in Clinical Microbiology**

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**May, 2016**

# DECLARATION

I hereby declare that the work embodied in this internship report was carried by me under the supervision of Dr. Anania Arjuna (Internal supervisor), Lovely Professional University and Dr. Shashi Sudhan Sharma (External supervisor), Government Medical College and Hospital, Jammu. This work has not been submitted in part or in full in any other university for any degree or diploma.

Name: Neeraj Sharma

Date: …….

Place: …

# ***CERTIFICATE***

This is to certify that ***Ms. Neeraj Sharma*** bearing **Registration Number**11405544 ***has*** completed her Master of Science in Clinical Microbiology internship under our guidance and supervision. This report is record of the candidate own work carried out by her under my supervision. I certify that the matter embodied in this report is original and has been not submitted anywhere for the reward of any other degree.

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# ABSTRACT

Viral Hepatitis is an inflammation of the liver due to the viral infections and there are groups of viruses that affect the liver of which the Hepatitis B and C are the causative agents of severe form of liver disease with high rate of mortality and morbidity. Viral Hepatitis is a major health problem worldwide and cause acute and chronic Hepatitis which can lead to the development of extensive liver scarring (cirrhosis), liver failure liver cancer and death. Viral Hepatitis is the tenth leading cause of cancer worldwide and the leading cause of death. The present study was done to evaluate the seroprevelance of HBV and HCV in individuals attending the Government Medical College and Hospital Jammu. The present study was conducted from October, 2015-March, 2016 in Viral Research &Diagnostic Laboratory, Department of Microbiology, Government Medical College and Hospital, Jammu. All the samples were screened for Hepatitis B surface antigen and anti-HCV antibodies using immuno-chromatographic method (SD Bio line) and the reactive samples were confirmed by using Enzyme Linked Immunosorbant Assay (ERBALISA). A total of 4390 cases were screened in past six month, from which 155 (3.53%) were positive. Out of these positive patients 115 (2.61%) were positive for HBsAg and 40 (0.91%) were found positive for HCV. Positive male patients accounts for HBsAg were 75 (1.70%) and female positive patients accounts for HBsAg were 40 (0.91%), whereas Positive Male for HCV were 27 (0.61%) and 13 (0.29%) female were positive for HCV. The present study reveals that the seroprevelance of Hepatitis B is 2.61% which is higher than the Hepatitis C that is 0.91%. Both Hepatitis B and Hepatitis C virus is found to be more prevalent in males than in females.

**Keywords:** HBV, HCV

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**Neeraj Sharma**

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# CHAPTER- 1

# INTRODUCTION

## 1.1 HEPATITIS B VIRUS

Hepatitis B virus (HBV) is a well-known agent of acute and chronic hepatitis, with an estimated 350 million chronic carriers around the world (1). It is a major public health problem. About 30% of the world's population have been infected at some times in their lives with the Hepatitis B virus (2). Every year there are over 4 million acute clinical cases of HBV, and about 25% of the carriers, 1 million people a year die from chronic active Hepatitis, cirrhosis or primary liver cancer (3). More than 2000 million people alive today have been infected with HBV at some times in their lives. Of these, about 350 million remain infected chronically and become carriers of the virus (4-6). More than 780,000 people die every year due to the complications of hepatitis B, including cirrhosis and liver cancer. Hepatitis B is an important occupational hazard for the health workers (7). The spectrum of clinical manifestations of HBV infection varies in both acute and chronic disease. During the acute phase, manifestation range from subclinical hepatitis to anicteric hepatitis, icteric hepatitis and fulminant hepatitis. During the chronic phase, manifestation range from an asymptomatic carrier state to chronic hepatitis, cirrhosis and hepatocellular carcinoma. The outcome of HBV infection depends upon the age at infection, the level of HBV replication and the immune status of the host (8). Most adults infected with the virus recovers, but 5%-10% are unable to clear the virus and become chronically infected. The challenges in the area of HBV- associated disease are the lack of knowledge in predicting outcome and progression of HBV infection and an unmet need to understand the molecular, cellular, immunological, and genetic basis of various disease manifestations associated with HBV infection (7). The vaccine against hepatitis B has been available since 1982. The vaccine is 95% effective in preventing infection and the development of chronic disease and liver cancer due to hepatitis B (9). Current treatment strategies target the HBV polymerase enzyme. However, reports indicate that response depends on genotype and subtype. Also the variation in genotype is known to affect viral response and replication to therapeutic agents. It was shown very early that HBV would not grow in cell culture and was highly species specific, only infecting humans and some other primates. Hepatitis B virus (HBV) first detected in 1970 following the identification of the Australian antigen (10).HBV was the first member to be discovered of a family of a virus, later designated Hepadnaviridae. This family has since been divided into two groups, the Orthohepadnaviruses and the Avian Hepadnaviruses. These are hepatotropic, partially double-stranded DNA viruses. Their replication strategy is unique for animal DNA viruses and only shared by cauliflower mosaic virus (also a DNA virus), in that they use a RNA intermediate and a reverse transcription step (10). Based on sequence comparison, HBV is classified into eight genotypes, A to H (11).Type A is prevalent in northern Europe, sub-Sahara and western Africa. Type B and C are predominant in Asia, type D is common in Mediterranean area, the Middle East, and India, type E is localized in Sub Saharan Africa, type F (or H) is restricted to central and South America, type G has been found in France and Germany. Genotypes A, D, and F are predominantly in

Brazil and all genotype occurs in United States with frequencies dependent on ethnicity. The E and F strains appear to have originated in aboriginal population of Africa and New world respectively.

The evolutionary development of HBV into eight genotypes carry not only distinct geographical distribution but further their sub genotypes hold distinctive genomic markers at various parts of the HBV genome signifying different virological mechanism of hepato-carcinogenesis (12-14).

## 1.1.1 STRUCTURAL DETAILS OF HBV

The hepatitis genome, discovered by William S. Robinson of the Stanford University School of Medicine, is the smallest genome at 3.2 kb long (15). Ultra structural examination of sera from hepatitis B patient shows three distinct antigenic particles: a spherical 22nm particle, 42nm spherical particle (containing DNA and DNA polymerase) called Dane particle which is less frequently observed, and tubular or filamentous particles that vary in length. The small spherical and tubular particles are the unassembled components of the Dane particle: the infected form of virus (5). The most numerous are spherical particles, which are made exclusively of HBsAg as are tubular or filamentous forms, which have the same diameter but may be over 200nm long, they result from over production of HBsAg. The nucleocapsid composed of hepatitis B core antigen (HBcAg) complexes with virally encoded polymerase and the viral DNA genome (11). The 22nm diameter particle lacks nucleic acid hence is non-infectious, but these particles are highly immunogenic that induce the neutralizing anti-HBs antibody response. HBV is a partially double stranded circular DNA (dsDNA) virus, composed of a 27nm nucleocapsid core HBcAg, surrounded by the outer lipoprotein coat (also called envelope) containing the surface antigen (HBsAg) (16). The 27nm inner nucleocapsid core contains HBcAg, a 3.2kb circular partially double-stranded DNA, DNA polymerase, and protein kinase activity [5]. The major structural protein of a core is a 21 kD basic phosphoprotein called HBcAg. The nucleocapsid is round and exhibits icosahedral symmetry with the diameter of 30-35nm. The capsid consists of 180 capsomers, it can be penetrated by stains and some appears dark in the centre (17).

## 1.2 Hepatitis C virus

Hepatitis C Virus (HCV), a blood-borne pathogen, is a major cause of hepatocellular carcinoma (HCC). The incident of this cancer is increasing worldwide (18). There are about 170 million chronic HCV carriers throughout the world (19).Hepatitis C can present as acute or chronic hepatitis. Most of the cases of acute hepatitis are asymptomatic (20). Symptomatic acute hepatitis with jaundice is seen in only 25% of patients and this virus usually does not cause fulminant hepatitis in immunocompetant individuals. Spontaneous viral clearance of HCV infection is usually with nearly 54-86% of the infected individuals progressing to chronic hepatitis (21).Hepatitis C virus (HCV) has been identified in 1989 (10). HCV is a single stranded positive sense RNA virus, 9.6kb in length and belong to family Flaviviridiae and genus Hepacivirus (22). There are six different genotype (strains) of the virus which have different genetic profiles (genotype1 to 6). Like human immunodeficiency virus (HIV) hepatitis multiplies very fast and attains very high level in the body. The genes that makes the surface protein of the virus also mutate quickly, and thousands of the genetic variations of the virus ('quasi-species') are produced daily. It is impossible for the body to keep up with the making effective antibodies against all the quasi-species circulating at one time. It is not possible yet to develop an effective vaccine since the vaccine must protect against all genotypes (23). The incubation period is long, 15-160 days, within a, mean of 50 days. About 75% of the infections are subclinical. Clinical infections with Hepatitis C is generally less severe, with milder symptoms, absent or less marked jaundice. About 50-80% of patients develop chronic Hepatitis. The affected individuals have persistence of the virus in their blood. They are at risk, of developing cirrhosis and hepatocellular carcinoma (16). The risk factors for HCV transmission includes; Injection drug use, Blood/blood products transfusion, Organ transplantation, Chronic haemodialysis, Occupational exposure among health care workers, Therapeutic injections. Major/minor surgeries, Shaving at barber, unprotected sexual contact, Vertical transmission (24, 25), HIV-infected person. People who receive body piercing or tattoos done by non -sterile instrument (26).

Approximately 70%-80% of the people with acute hepatitis C do not have any symptoms. Some people, however, can have mild to severe symptoms soon after being infected including: Fever, Fatigue, Loss of appetite, Nausea, Vomiting Abdominal pain, Dark urine, Joint pain, Jaundice (yellow color in the skin or eyes)Most of the people with chronic Hepatitis C do not have any symptoms. Chronic Hepatitis C is a serious disease that can result in long-term health problems, including liver damage, liver failure and liver cancer it is the leading cause of cirrhosis and liver cancer. Approximately 15,000 people die every year from hepatitis C related liver disease (27). The Hepatitis C virus particle consists of a core of genetic material (RNA), surrounded by an icosahedral protective shell of a protein, and further encased in lipid (fatty) envelope of cellular origin. Two viral envelope glycoprotein, E1 and E2 are embedded in lipid envelope.

Hepatitis C virus has a positive sense single-stranded RNA genome. The genome consists of single open reading frame that is 9600 nucleotide bases long (28).

# CHAPTER- 2

## REVIEW OF LITERATURE

Hepatitis B virus (HBV) is a well-known agent of acute and chronic hepatitis, with an estimated 350 million chronic carriers around the world (1). It is a major public health problem. About 30% of the world's population have infected at some times in their lives with the hepatitis B virus (2). Serological evidence of past or present Hepatitis B infection has been shown. HBV was the first member to be discovered of a family of a virus, later designated Hepadnaviridae. This family has since been divided into two groups, the Orthohepadnaviruses and the Avian Hepadnaviruses. These are hepatotropic, partially double-stranded DNA viruses (10).

Hepatitis C Virus (HCV), a blood-borne pathogen, is a major cause of hepatocellular carcinoma (HCC). The incident of this cancer is increasing worldwide (18). There are about 1 million chronic HCV carriers throughout the world (19).Hepatitis C can present as acute or chronic hepatitis. Approximately one -third of the world's population (20).

HCV is a single stranded positive sense RNA virus, 9.6kb in length and belong to family Flaviviridiae and genus Hepacivirus (21). There are six different genotype (strains) of the virus which have different genetic profiles (genotype1 to 6). Like human immunodeficiency virus (HIV) hepatitis multiplies very fast and attains very high level in the body.

**2.1 Evans Chavhunduka, *et al*., (2000)** in their study they determined the seroprevelance of HBV and HCV in Primary hepatocellular carcinoma at Parirenyatwa Referral Hospital, Zimbabwe. They evaluated the serological markers of the two viruses in the patients with primary hepatocellular carcinoma using commercially available enzyme-linked Immunosorbant kit. Primary hepatocellular carcinoma is one of the most common cancer in Zimbabwe. Hepatitis B and Hepatitis C are suspected to play a major role in causing this cancer. Sixty patients were presented to Parirenyatwa Hospital between October 1999 and August 2000 and were diagnosed as having PHC. Sera for the patient were collected from the Radioimmunoassay (RIA) Laboratory of Public Health Laboratory. Out of the 60 patients with PHC, 48.3% were seropositive for HBV and 20.0% were seropositive for HCV. Co-infection by HCV and HBV was found in 8% of the patients. Only 13.3% of the health control (blood donor) were positive for HBV. All the controls were negative for HCV. The conclusion of the study was that the high seropositivity of HBV and HCV in PHC in Zimbabwe suggested that the two viruses were major cause of the cancer.

**2.2 Ghavanini, *et al*., (2000)** determined the prevalence rate of Hepatitis B surface antigen (HBsAg) and anti-Hepatitis C virus antibodies (anti-HCV) among 7897 healthy voluntary blood donors in Shiraz, Islamic Republic of Iran. Sera was examined for HBsAg and anti-HCV antibodies using second generation enzyme-linked Immunosorbant assay. Positive sera of HBsAg were found in85% (1.07%). Of the individuals and anti-HCV antibodies were found in

47 (0.59%). They compared their results with those of other studies and conclude that the prevalence rate of HBsAg in their area has decreased in last 2 decades; from being an area of high prevalence, it is now one with moderate-to-low prevalence. Since there were few reports on the seroepidemology of Hepatitis C in the Islamic Republic of Iran, they could not asses the changes in prevalence of Hepatitis C.

**2.3 BeenuThakral, *et al*., (2006)** studied the clinical significance of anti-HCV antibodies in healthy blood donors. The study was conducted on 16,250 blood units. An overall seropositivity of 0.44% (72/16,250) was observed in donors. In contrast to drug abuse (6.4%) found minor percutaneous routes like sharing of shaving kits or visit to a road side barber (32%) as the major risk factor for HCV transmission. There was no prior history of blood transfusion in any donors; however history of some surgical procedures was present in25.8%.

**2.4 S ARaza, *et al*., (2007)** conducted a study to check the seroprevelance of Hepatitis B surface antigen (HBsAg) and antibodies against Hepatitis C virus (anti-HCV) in 27881 hepatocellular carcinomas (HCCs) from 90 studies. A predominance of HBsAg was found in HCCs from most Asian, African, and Latin American countries, but anti-HCV predominated in Japan, Pakistan, Mongolia and Egypt. Anti- HCV was found more often than HBsAg in Europe and in United States.

**2.5 Hong Zhang, *et al*., (2007)** in their study found that the prevalence of the HBV is higher in adults than in children. They determined the seroepidemology of HBV infection in an adult population in Jilin. China to guide effective preventive measures. A cross-sectional serosurvey was conducted throughout et al Jilin, China. A total of 3833 people were selected and demographic and behavioural information gathered. Serum samples were tested for HBV markers and liver enzymes. The conclusion of their study is that strong associations between HBV infection and immunity were observed regarding the gender, occupation, personal history of vaccination, and age in adults. There has been a decrease in the prevalence of HBV infection since the National Expanded Program on immunization. Mother-to-foetal vertical transmission of HBV is well controlled. Transmission between adults has become the most common mode of HBV spread. While the survey focused on a region in northeast China, it is likely that similar results may be found everywhere in the country. Identifying groups at risk for susceptibility can assist in the development of national strategies to target groups for cost-effective salvage vaccination programs for adults in the future.

**2.6 Syed Asad Ali, *et al*., (2009)** reviewed the medical and public health literature over a 13-years period (January 1994- September 2007) to estimate the prevalence of active Hepatitis B and chronic Hepatitis C in Pakistan. A weighted average of Hepatitis B antigen prevalence in paediatric population was 2.4% (range 1.7-5.5%) and for Hepatitis C antibody was 2.1% (range 0.4-5.4%). A weighted average of Hepatitis B antigen prevalence among healthy adults (blood donors and non-donors) was 2.4% (range 1.4-11.0%) and for Hepatitis C antibody was 3.0% (range 0.3-31.9%). Rates in high-risk subgroups were far higher.

**2.7 Sandhya Panjeta Gulia, *et al*., (2010)** conducted a study on blood donors for a period of 7 years to assess the prevalence of HBV infection. Hepacard, which uses the principle of one step

immunoassay was used for screening the donors. The objective of the study was to determine the seroprevelance of HBsAg in the local population in and around Viziangaram (Andhra Pradesh) from April 2003 – Oct 2010; to compare the prevalence of seropositivity of HBsAg with other regions in India; to compare the prevalence of HBsAg positivity in voluntary and replacement donor. A retrospective study was carried on 8601 blood donors of which 2560 (29.76%) were voluntary donors and 6401 (70.24%) were replacement donors. The prevalence of HBsAg seropositivity in replacement donors were found to be 2.45%; in voluntary donors 2.54% and overall prevalence was estimated to be 2.48%. The prevalence of HBsAg positivity were not to be found to be significant among voluntary and replacement donors ($p>0.05$). Whereas a significant result was observed in voluntary group over the study period ($p<0.001$). The conclusion of the study is that increasing prevalence of HBsAg can be reduced by more sensitive screening assays and proper donor selection.

**2.8 Abel Girma Ayele,*et al.*, (2011)**determined the prevalence and the risk factors of hepatitis B and C virus infections in patients with chronic liver diseases in three public hospitals in Addis Ababa City, Ethiopia. The study was conducted on 120 clinically diagnosed chronic liver disease patients. Possible associated factors with infections by the viruses were collected from the patient using questionnaire. Serum was screened for the presence of Hepatitis B surface antigen and anti-hepatitis C virus antibodies using qualitative immunochromatographic method. The conclusion of this study was that the HBsAg and anti- HCV antibody was high in patient below 50 years of age. Dental extraction at health facilities has 2.95 times association of acquisition of HCV infection than those who do not have history of dental extraction. Thus, all clinically diagnosed CLD patients should be tested for HBV and HCV serostatus. Proper sterilization of dental and surgical instruments must be carried out. To prevent the spread of HBV and HCV, people must be educated about these infections and modes of transmission.

**2.9 Muhammad Ikram Anwar, *et al.*, (2013)** studied the prevalence of active Hepatitis C virus infection among general public of Lahore. The study was conducted on 4246 blood samples 210 was confirmed positive for HCV. Gender wise active HCV prevalence revealed no significant difference. However, among the age groups the highest prevalence was observed in age groups 20-29(7.7%) and 30-39 years (6.4%) with odds of prevalence of 14.8% and 10.3% respectively. In age of 40 years decrease in levels of active HCV prevalence was observed.

**2.10 B.Vallab Ganesh Bhardwaj, *et al.*, (2014)** determined the seroprevelance of Hepatitis C virus infection among patient attending the rural teaching hospital in south India. The study population comprised of 10.136 individuals. The overall seroprevelance was found to be 0.68%. Among the positive cases, the seroprevelance in male sin females was 58% and 42% respectively and the frequency of HCV among age groups 0-20, 21-40, 41-60, >60 was 0% (0), 21.7% (15), 65.2% (45), 13% (9) respectively. Highest prevalence was observed among subjects of 41-60 years age group.

# CHAPTER- 3

# AIM AND OBJECTIVE

## 3.1 AIM

The aim of this study was to determine the seroprevelance of HBsAg and HCV over last six months.

## 3.2 OBJECTIVES

1. To detect the gender wise seroprevelance of HBsAg and HCV.
2. To determine the seroprevelance of HBsAg and HCV in ART referred patients.
3. To determine the seroprevelance of patients having HBsAg and HCV co-infection.

# CHAPTER- 4

# MATERIAL AND METHODS

## 4.1 MATERIAL

A list of material used during the study is mentioned in appendix- 1

## 4.2 METHOD

The present Study was conducted in the Viral Research & Diagnostic Laboratory under ICMR from October 2015 to March 2016 in Department of Microbiology, Government College Jammu, Jammu and Kashmir. The study involved 4390 patients from all ages and both sexes.

1. Each patient was screened for HBsAg and anti-HCV antibodies by immunochromatographic method SD BIOLINE HBsAg One Step HBsAg test and SD BIOLINE HCV One Step anti-HCV test (Alere Medical PVT. Ltd).
2. Positive sera was stored in aliquots each at -20$^{O}$C for further use. The positive samples were further confirmed for HBsAg and HCV by the method of ELISA.
3. The positive sera were tested for HBsAg and anti-HCV antibodies by 3$^{rd}$ generation ErbaLisa SEN HBsAg and ErbaLisa Hepatitis C (Transasia Bio-Medicals Ltd).
4. All the tests were performed with the manufacturer's instructions.

## SPECIMEN COLLECTION, STORAGE AND PRECUTIONS FOR RAPID TEST.

1. Serum is extracted from the whole blood in the collection tube (NOT containing anticoagulants) by venepuncture, leave to settle for 30 minutes for blood coagulation and then centrifuged to get serum specimen of supernatant.

2. If serum specimen is not tested immediately they should be refrigerated at 2-8$^{0}$C. For storage period longer than 2 weeks, freezing is recommended. They should be brought at room temperature prior to use.

## SPECIMEN COLLECTION AND STORAGE FOR ELISA

ErbaLisa SEN HBsAg is recommended to be used only for testing of human serum. Collect the specimens aseptically. Extract serum as soon as possible to avoid haemolysis. Samples containing aggregates must be centrifuge prior to use.

Fresh serum samples are preferred. Undiluted serum can be stored at 2-8$^{0}$C for a week or frozen at -20$^{0}$C until use. Frozen specimen should be completely thawed and centrifuged. The test should be performed on a clear supernatant collected after centrifugation.

Avoid repeated freezing and thawing of the specimen.

Heat inactivated, haemolysed and icteric hyperglycaemic samples may yield erroneous results.

## 4.2.1 SD BIOLINE HBsAg ONE STEP HBsAg TEST FOR QUALITAIVE DETECTION OF HBsAg IN HUMAN SERUM

## PRINCIPLE

The test cassette contains a membrane strip, which is pre-coated with the 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 SD BIOLINE HBsAg 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 the Control line in the result window are not visible before applying any samples. The Control Line is used for the procedural control. The control line should always appear if the test procedure is performed properly and the reagents of the control line are working. The SD BIOLINE HBsAg can identify HBsAg in plasma and serum specimens with a high degree of sensitivity.

## PROCEDURE

1. Bring the required number of SD BIOLINE foil pouches and specimen to room temperature prior to testing and remove the test device from foil pouch, and place it on flat, dry surface.

2. Using micropipette add 100ul of specimen into the sample well (S) or using disposable dropper add 3-4 drops of serum or plasma in the sample well.

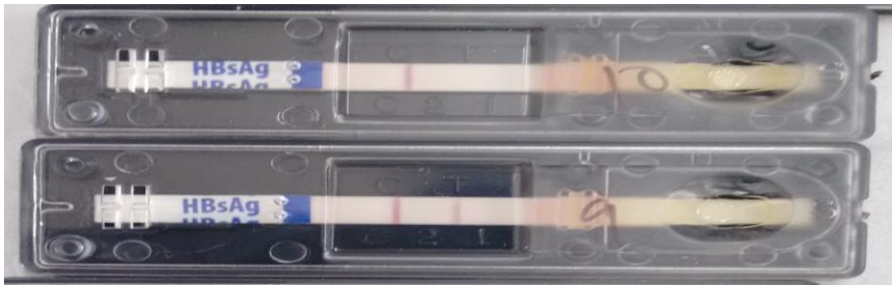
3. As the test began to work, you will see purple color moves across the result window in the centre of the test device. Interpret the test results at 20 minutes.

4. A positive result will not change once it has been established at 20 minutes. However, in order to prevent any incorrect results, the test results should be interpreted after 30 minutes.

### INTERPRETATION OF THE RESULT

1. A color band will appear at the left section of the result window to show that the test is working properly. This band is the "Control Band".

2. The right section of the result window indicates the test results. If another color band appears at the right section of the result window, this band is the "Test Band".




Rapid test for HBV

**NEGATIVE RESULTS**

The presence of only control band within the result window indicates the negative results.

**POSITIVE RESULTS**

The presence of both bands Test band and Control band ("T "band and "C" band) within the result window.

**INVALID RESULTS**

If the control band is not visible within the result window after performing the test, the result is considered invalid. The directions may not have been following correctly for the test may have detoriated. It is recommended to retest the specimen.

## 4.2.2 SD BIOLINE HCV ONE STEP HCV TEST FOR QUALITAIVE DETECTION OF ANTI-HCV ANTIBODIES IN HUMAN SERUM

SD BIOLINE is a visuals, sensitive and accurate one step immunoassay for the qualitative detection of anti-HCV antibodies in human serum or plasma. The assay is intended to be used as an aid in the recognition and diagnosis of acute infectious and chronic carriers of the hepatitis C virus.

## PRINCIPLE

The test cassette contains a membrane strip, which is pre-coated with the 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 SD BIOLINE HCV 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 the Control line in the result window are not visible before applying any samples. The Control Line is used for the procedural control. The control line should always appear if the test procedure is performed properly and the reagents of the control line are working. The SD BIOLINE HCV can identify anti-HCV antibodies in plasma and serum specimens with a high degree of sensitivity.

## PROCEDURE

1. Bring the required number of SD BIOLINE foil pouches and specimen to room temperature prior to testing.

2. Remove the test device from foil pouch, and place it on flat, dry surface.

3. Using micropipette add 100ul of specimen into the sample well (S) or using disposable dropper add 3-4 drops of serum or plasma in the sample well.

4. As the test began to work, you will see purple color moves across the result window in the centre of the test device.

5. Interpret the test results at 20 minutes.

6. A positive result will not change once it has been established at 20 minutes. However, in order to prevent any incorrect results, the test results should be interpreted after 30 minutes.

## INTERPRETATION OF THE RESULT

1. A color band will appear at the left section of the result window to show that the test is working properly. This band is the "Control Band".

2. The right section of the result window indicates the test results. If another color band appears at the right section of the result window, this band is the "Test Band".

## NEGATIVE RESULTS

The presence of only control band within the result window indicates the negative results.

## POSITIVE RESULTS

The presence of both bands Test band and Control band ("T "band and "C" band) within the result window.

## INVALID RESULTS

If the control band is not visible within the result window after performing the test, the result is considered invalid. The directions may not have been following correctly for the test may have detoriated. It is recommended to retest the specimen.

## 4.2.3 ErbaLisa SEN HBsAg

## PRINCIPLE

The ErbaLisa SEN HBsAg is based upon the use of a solid phase prepared with polyclonal anti-HBsAg. Detection is carried out using monoclonal anti-HBsAg. This system of using

poly-mono blend of antibodies aims at achieving high assay sensitivity and specificity respectively.

The performance of the test includes the following steps:

1. The specimen to be tested along with the controls are added to the wells. If the surface antigen to Hepatitis B virus is present in the specimen, it will bind to the polyclonal antibodies coated on the wells.

2. Subsequently, peroxidase labelled monoclonal anti- HBsAg (conjugate) is added to the well which in turn binds to the HBsAg captured on the solid phase.

3. After removal of unbound conjugate, the antigen-antibody complex is identified by the addition of substrate.

4. After the reaction has been stopped, the absorbance values are read. The intensity of the color developed is proportional to the amount of HBsAg bound on the solid phase..

## RECONSTITUTION OF REAGENTS

Dilute washing solution 1:20 in distilled or deionized water. Homogenize. Washing solution may form crystals under cold storage condition. If so, use it after thawing at $37^0$C in water bath.

## PROCEDURE

1. Bring all reagents and test specimen at room temperature and shake well before use.
2. Define the sample / control distribution and identification plan. In each run, assign one well for the blank (A1), 3 wells for the HBsAg negative controls (B1, C1 and D1) and 1 well for the HBsAg positive control (E1).
3. Break the number of required wells for the run. Wrap the balance unused wells tightly in a zip-lock bag with desiccant and return it to 2-$8^0$C immediately.
4. Add 100ul of the sample diluent in a well A1 (blank).
5. Add 25ul of the sample diluent to the rest of the wells.
6. Add 75ul of the HBsAg negative control in wells B1, C1, and D1.
7. Add 75ul of the positive control in well E1.
8. Add 75ul of the first sample in well F1, second sample in well G1 and so on...
9. Add 50ul conjugate into all the wells including well A1 (blank). Mix well, cover the wells with the strip sealers and incubate for 60 minutes at $37^0$C.
10. Remove the sealer. Discard / aspirate the contents of the well into the waste disposal

container. Add a minimum of 350ul of washing solution to each well. Aspirate again after 30 seconds of soak time. Repeat the washing step 5 times (invert the plate and tap it on absorbent pad to remove the remaining washing solution).

11. Add 50ul of the color reagent to all the wells including well A1 (blank). Cover the plate with the black cover provided and allow the reaction to develop in the dark for 15 minutes at room temperature (20-300$^0$C).

12. Add 100ul stopping solution to all wells. Homogenize. After the solution of stopping solution the blue color of the substrate turns yellow (for positive samples) or remain colorless (for negative samples).

13. Carefully wipe the plate bottom.

14. Read the optical density at 450 nm (using 620/630/650 nm as the reference wavelength) within 15 minutes after pipetting of stop solution.

## CALCULATION AND INTERPRETATION OF THE RESULTS

**BLANK VALUE**: Absorbance value of the blank should be less than 0.2.

**POSITIVE CONTROL**: Absorbance value of the positive control should be greater than 1.0

**NEGATIVE CONTROL**: Absorbance value of the individual negative controls should be less than 0.1

Calculate the men of the measured absorbance values for the hepatitis B Negative Control (NCx)

Calculation of the Cut-Off Value (COV)

COV =0.15+NCx

## INTERPRETATION OF THE RESULT

**NON-REACTIVE**: Samples with an optical density less than the cut-off value are considered non-reactive.

**REACTIVE**: Samples with an optical density equal to or greater than the cut-off value are considered initial reactive. These samples should be retested duplicate.

If the optical density of duplicates is less than the cut-off value, the specimen is considered non-reactive.

If the test result of the duplicate found reactive, the specimen considered repeatedly reactive.

### 4.2.4 ErbaLisa Hepatitis C

ErbaLisa Hepatitis C is an in-vitro diagnostic kit for qualitative detection of antibodies against Hepatitis C virus in patient serum/plasma.

## PRINCIPLE

The ErbaLisa Hepatitis C is based on indirect ELISA using a solid phase prepared with the mixture of synthetic peptides and recombinant proteins of HCV i.e., CORE, NS3, NS4 and NS5. Detection is carried out using anti-human IgG antibodies conjugated with horseradish peroxidase (HRPO).

The performance of the test includes the following reaction steps:

- The specimen to be tested along with the controls are added to the wells. If antibodies to the Hepatitis C virus are present in the specimen, they will bind to the antigens coated on the wells.
- After a wash, peroxidase labelled anti-human IgG (conjugate) is added to the wells which in turn binds to the specific antibodies captured on the solid phase.
- After removal of unbound conjugate, the antigen-antibody complex is identified by addition of a substrate.
- After the reaction has been stopped, the absorbance values re read. The intensity of the color developed is proportional to the amount of anti-Hepatitis C antibody bound to the solid phase.

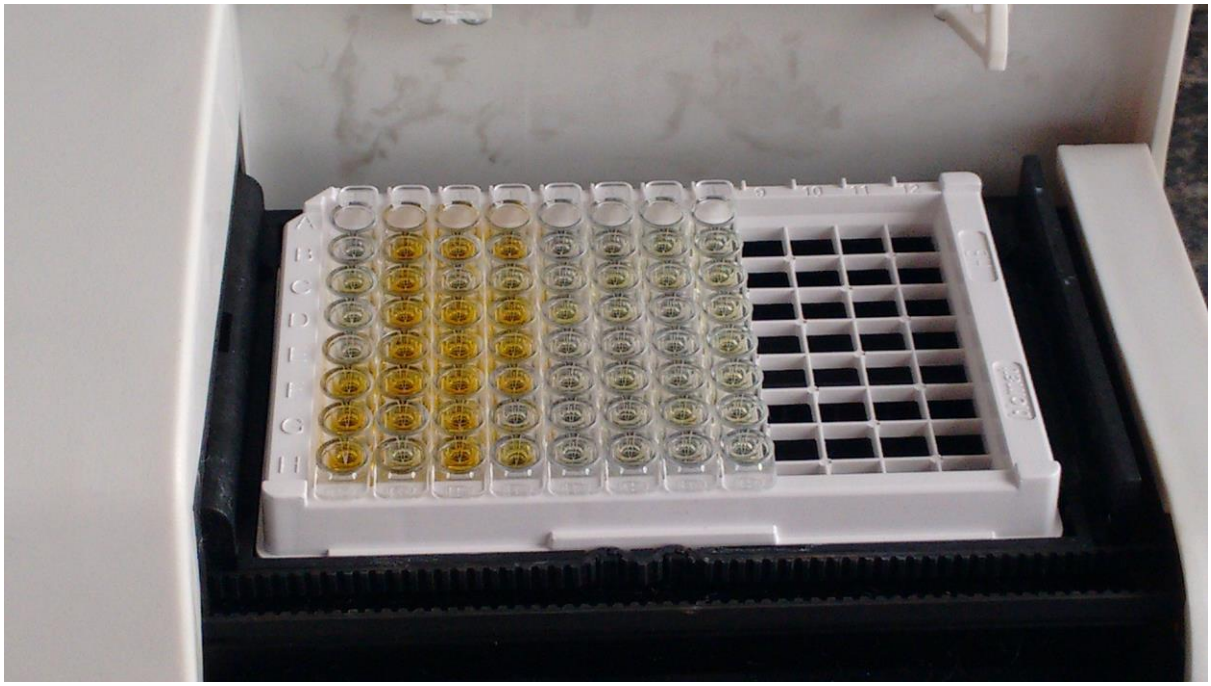
## RECONSTITUTION OF REAGENTS

Dilute washing solution 1:20 in distilled or deionized water. Homogenize. Washing solution may form crystals under cold storage condition. If so, use it after thawing at $37^0$C in water bath.

## PROCEDURE

1. Bring all reagents and test specimen at room temperature and shake well before use.
2. Define the sample / control distribution and identification plan. In each run, assign one well for the blank (A1), 3 wells for the HCV negative controls (B1, C1 and D1) and 1 well for the HCV positive control (E1).
3. Break the number of required wells for the run. Wrap the balance unused wells tightly in a zip-lock bag with desiccant and return it to 2-$8^0$C immediately.
4. Add 100ul of the sample diluent to all the wells **except in the well A1 (blank)**
5. Add 10ul of the HCV negative control in wells B1, C1, and D1.

6. Add 10ul of the HCV positive control in well E1.
7. Add 10ul of the first sample in well F1, second sample in well G1 and so on.
8. Mix well, cover the wells with the strip sealers and incubate for 45minutes at room temperature (20-30$^0$C).
9. Remove the sealer. Discard / aspirate the contents of the well into the waste disposal container. Add a minimum of 350ul of washing solution to each well. Aspirate again after 30 seconds of soak time. Repeat the washing step 5 times (invert the plate and tap it on absorbent pad to remove the remaining washing solution).
10. Add 50ul conjugate into all the wells ***except in well A1 (blank)***.Cover the wells with the strip sealers and incubate for 15 minutes at room temperature (20-30$^0$C).
11. Repeat the step 9.
12. Add 50ul of the color reagent to all the wells ***including well A1 (blank)***. Cover the plate with the black cover provided and allow the reaction to develop in the dark for 15 minutes at room temperature (20-30$^0$C).
13. Add 100ul stopping solution to all wells. Homogenize. After the solution of stopping solution the blue color of the substrate turns yellow (for positive samples) or remain colorless (for negative samples).
14. Carefully wipe the plate bottom.
15. Read the optical density at 450 nm (using 620/630/650 nm as the reference wavelength) within 15 minutes after pipetting of stop solution.



ELISA Test plate

## CALCULATION AND INTERPRETATION OF THE RESULTS

**BLANK VALUE**: Absorbance value of the blank should be less than 0.1.

**POSITIVE CONTROL**: Absorbance value of the positive control should be greater than 1.0

**NEGATIVE CONTROL**: Absorbance value of the individual negative controls should be less than 0.2

Calculate the men of the measured absorbance values for the hepatitis B Negative Control (NCx)

Calculation of the Cut-Off Value (COV)

COV =0.3+NCx

## INTERPRETATION OF THE RESULT

**NON-REACTIVE**: Samples with an optical density less than the cut-off value are considered non-reactive.

**REACTIVE**: Samples with an optical density equal to or greater than the cut-off value are considered initial reactive. These samples should be retested duplicate.

If the optical density of duplicates is less than the cut-off value, the specimen is considered non-reactive.

If the test result of the duplicate found reactive, the specimen considered repeatedly reactive.

# CHAPTER- 5

## RESULT

A total of 155 positive samples was identified from 4390 serum of different patient and were identified and characterized.

## 5.1 TOTAL NUMBER OF POSITIVE SAMPLES

| Total number of clinical specimens | Number of positive patients | Percentage |
|---|---|---|
| 4390 | 155 | 3.53% |

Table no 1: total number of positive samples

## 5.2 TOTAL NUMBER OF POSITIVE MALE AND FEMALE

| Gender | No. of positive patients | Percentage (%) |
|---|---|---|
| Male | 102 | 77.41% |
| Female | 53 | 34.19% |
| Total | 155 | 100% |

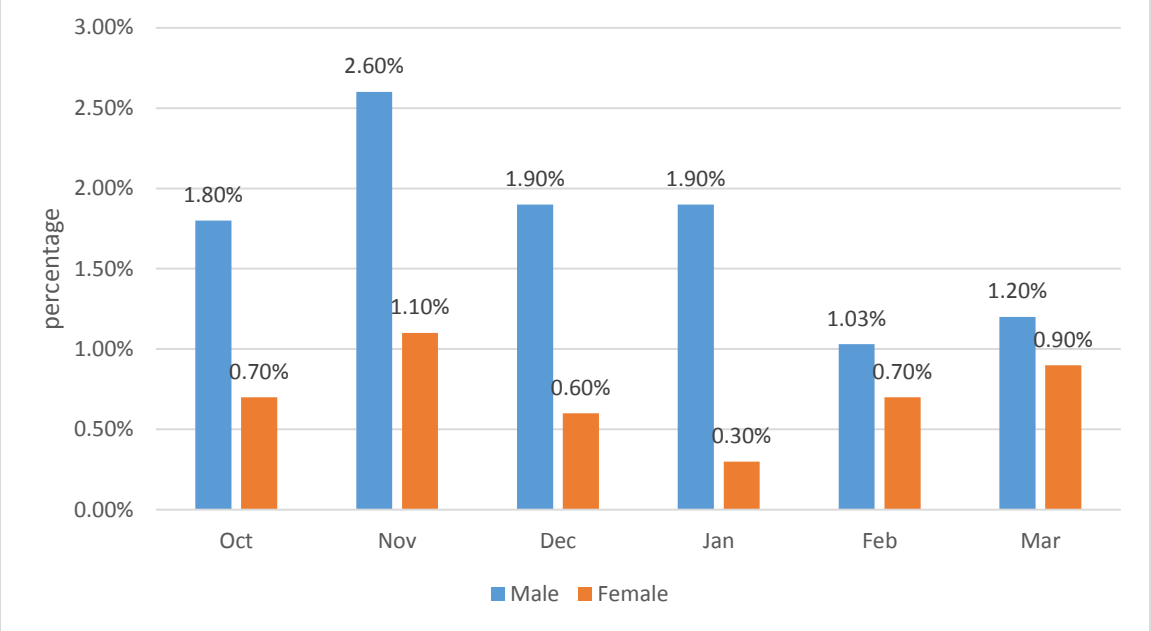
Table no.2: total number of positive male and female

## 5.3 MONTH WISE DISTRIBUTION OF SEROPREVALNCE OF HBsAg AND HCV

| Month | Total no of patient | Total number of positive case | HBsAg positive | | HCV positive | |
|---|---|---|---|---|---|---|
| | | | Number | % | Number | % |
| Oct | 635 | 31 | 17 | 2.6 | 14 | 2.2 |
| Nov | 722 | 31 | 27 | 3.7 | 4 | 0.5 |
| Dec | 735 | 29 | 19 | 2.5 | 10 | 1.3 |
| Jan | 609 | 26 | 20 | 3.2 | 6 | 0.9 |
| Feb | 1065 | 23 | 19 | 1.7 | 4 | 0.3 |
| Mar | 624 | 15 | 13 | 2.0 | 2 | 0.3 |

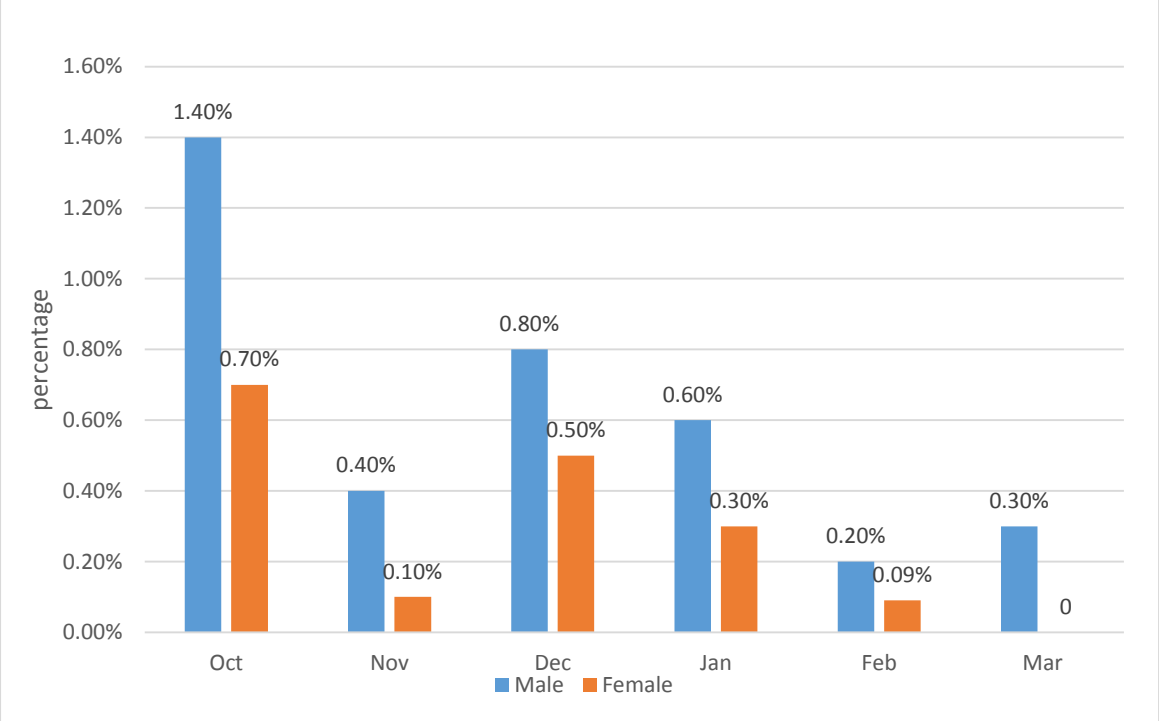
Table no. 3: month wise distribution of seroprevelance of HBsAg and HCV

## 5.4.1 GENDER WISE SEROPREVALANCE OF HBsAg




Graph no.1: gender wise seroprevelance of HBsAg and HCV

## 5.4.2 GENDER WISE SEROPREVALANCE OF HCV




Graph no.2: gender wise seroprevelance of HCV

## 5.5 TOTAL NO.OF PATIENTS HAVING HBV AND HCV

| Total number of clinical samples | Number of patient having HBV+HCV | Percentage |
|---|---|---|
| 4390 | 2 | 0.04% |

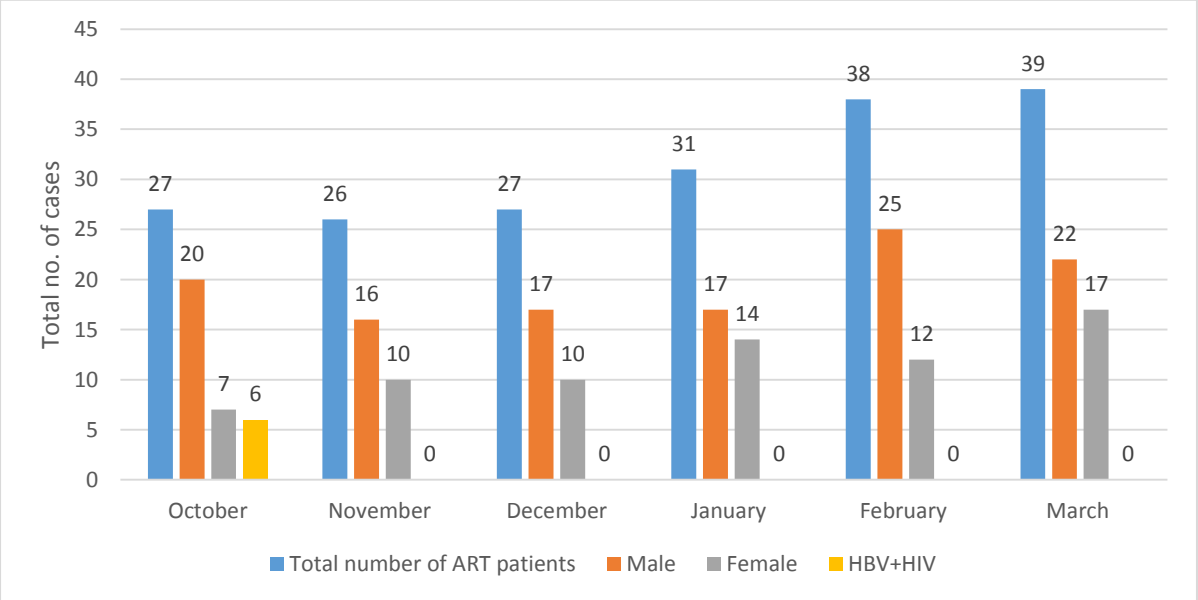
Table no. 4: total no. of patients having HBV and HCV

## 5.6 TOTAL NO. OF ART REFERRED PATIENTS

| Total number of clinical specimens | Number of ART referred patients | Percentage |
|---|---|---|
| 4390 | 188 | 4.28% |

Table no. 5: total number of ART referred patients

## 5.7 PREVALANCE OF HBsAg AND HCV IN HIV PATIENTS




Graph no3: prevalence of HBsAg and HCV in HIV patients

The present study is a short study conducted in Viral Research Diagnostic Laboratory, under ICMR Department of Microbiology, Government Medical College and Hospital, Jammu, for a period of six months from October 2015 - March 2016 from different units of hospital. A total of 4390 cases were screened in past six month, from which 155 (3.53%) were positive. Out of these positive patients 115 (2.61%) were positive for HBsAg and 40 (0.91%) were found positive for HCV. Positive male patients accounts for HBsAg were 75 (1.70%) and female positive patients accounts for HBsAg were 40 (0.91%), whereas Positive Male for HCV were 27 (0.61%) and 13 (0.29%) female were positive for HCV. The present study reveals that the seroprevelance of Hepatitis B is 2.61% which is higher than the Hepatitis C that is 0.91%. Hepatitis B virus is found to be more prevalent in males than in females. Hepatitis B was the most common infection followed by Hepatitis C. Age, marital status, history of the contact with Hepatitis B infected subject, extramarital sexual activity, intravenous drug use, major surgery, blood transfusion were found to be independent risk factors of being chronically infected with Hepatitis B virus and Hepatitis C virus. Hepatitis B and C were more prevalent among males than females.

# CHAPTER- 6

## DISCUSSION

The present study was conducted in Viral Research Diagnostic Laboratory (VRDL) under ICMR, Government Medical Collage and Hospital Jammu, for a period of six months from October 2015 –March 2016. A total of 4390 cases were screened in past six month, from which 155 (3.53%) were positive. Out of these positive patients 115 (2.61%) were positive for HBsAg and 40 (0.91%) were found positive for HCV. Positive male patients accounts for HBsAg were 75 (1.70%) and female positive patients accounts for HBsAg were 40 (0.91%), whereas positive male for HCV were 27 (0.61%) and 13 (0.29%) female were positive for HCV. The present study reveals that the seroprevelance of Hepatitis B is 2.61% which is higher than the Hepatitis C that is 0.91. In the present study the seroprevelance of HCV among patients attending our hospital was found to be 0.91% which was similar to the study done in West Bengal(0.87%) (29). There were two patients having HBV and HCV co infection and their prevalence was found 0.04% respectively. This study is in agreement with the fact that the males population is more affected by HCV than females which was similar to the study done in South India (30). The seroprevelance of HBsAg among patients was found 2.61% which is similar to the study done in Kathmandu, Nepal (2.5%) (31). Hepatitis B virus is found to be more prevalent in males than in females ,the seroprevelance of Hepatitis B among males were more than females which was similar to the study conducted in Manipal hospital (32). This could be attributed to their exposure status to various HBsAg risk factors which was quite evident from their life style due to shaving at barber shop, unprotected sexual contact, and intravenous drug use. The seroprevelance of Hepatitis B is more as compared to Hepatitis C and males is more affected than females which is similar to the study conducted in Nepal (33).

The seropositivity rates are also found from the ART referred patients. Hepatitis B and Hepatitis C with HIV are also diagnosed because they share similar route of transmission. Number of patients screened for ART was 188 in six months out of which only 6 samples was positive for HBsAg and none for HCV. The seroprevelance of HBsAg and HCV in HIV patients was found to be 0.13% which is lower than the study done in Lucknow (2.25%), Mumbai (3.5%) and Chennai (7.7%) because there study was of 2 or more years (34).

There was increased incidence of HBsAg and HCV from Medicine unit (1.4%)followed by OPD(0.93%), Emergency(0.68%), Surgery(0.29%), ICU(0.09%), Orthopedic unit(0.13%) and Cardiology unit(0.04%) of Government Medical College and Hospital, Jammu.

# CHAPTER- 7

## CONCLUSION

Out of Hepatitis B and Hepatitis C, Hepatitis B is the more common viral Hepatitis, followed by Hepatitis C. Both are more common in males. From the study of six months the seroprevelance of Hepatitis B is found to be 2.61% and Hepatitis C is 0.91%, there is scope of retrospective analysis of data and also there is further scope of study for the detection of seroprevelance in patients attending Government Medical College and Hospital, Jammu. Both are common in males. Hepatitis B virus infection is a problem of public health, and a major cause of mortality and morbidity not only because of acute illness but also due to its chronic sequel like chronic hepatitis, cirrhosis, and hepatocellular carcinoma. The education of the people regarding infection, prevention and transmission, vaccinations the most effective way of infection control. Age, marital status, history of contact with hepatitis B infected subject, extramarital sexual activity, and intravenous drug use, major surgery and some jobs (dentist, driver, barber), blood transfusion, were found to be independent risk factor of being chronically infected with hepatitis B virus and Hepatitis C virus.

# CHAPTER- 8

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# APPENDIX

## APPENDIX- 1

## LIST OF ABBREVATIONS

| | |
|---|---|
| **HBV** | Hepatitis B virus |
| **HCV** | Hepatitis C virus |
| **HBsAg** | Hepatitis B surface antigen |
| **HCC** | Hepatocellular carcinoma |
| **RNA** | Ribonucleic acid |
| **DNA** | Deoxyribonucleic acid |
| **ELISA** | Enzyme linked immuno sorbent assay |

# APPENDIX- II

## ErbaLisa SEN HBsAg and ErbaLisa SEN HCV

## MATERIAL REQUIRED

- Absorbent paper
- disposable gloves
- Pipettes capable of delivering 25, 50, 75, and 100ul volumes.
- Disposable tips.
- Waste disposal cylinder
- Timer
- Elisa reader
- Automated / semi-automated washing system
- Distilled / deionized water.
- Sodium hypochlorite for disposal of waste.
- $37^0$C incubator.