Training Report

Internship Training Report

Submitted to

Lovely Professional University, Punjab

In partial fulfillment of the requirements

For the degree of

Master of Science in Clinical Microbiology

Submitted by:
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(Reg. No. 11405772)

SCHOOL OF PHYSIOTHERAPY AND PARAMEDICAL SCIENCES
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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. Saurabh Saxena (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: Rohini Kumari

Date: ……

Place:

CERTIFICATE

This is to certify that ***Ms. Rohini Kumari*** bearing **Registration Number** 11405772 has completed her Master of Science in Clinical Microbiology internship under our guidance and supervision. This report is record of the candidate's 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

Background & Objectives: - Co-infection with hepatitis B virus (HBV) and hepatitis C virus (HCV) in human immunodeficiency virus (HIV) infected individual's results in increased hepatic complications. This study was to evaluate the presence of HBV and HCV Co-infection in HIV-positive patients.

Methods: -A total of 386 cases with HIV infection and 386 non-HIV subjects were included in the study. The samples were analyzed for the presence of HBV and HCV on the basis of presence of HBsAg and anti-HCV (IgG) initially by Rapid method and then were further confirmed by Enzyme Linked Immunosorbent assay (ELISA) method.

Results: -A total of 386 HIV-infected patients (242 males, 144 females), and 386 non-HIV subjects were included in the study. Overall results showed that 10 (2.59%) patients were co-infected with HBV and/ or HCV in the HIV-positive patients. HBsAg was detected in 9(2.33%) patients, gender wise 7(1.81%) males and 2(0.51%) females) among which one patient was having HCV also. The detection of HCV-antibodies was found in 2 patients (0.51%) gender wise 1(0.25%) male and 1(0.25%) female, out of which one patient was having HBV also. Triple infection with HBV, HCV and HIV was seen in 1(0.25%) patient. In non-HIV subjects, HBsAg was found in 9(2.33%) patients and no patient was found with HCV-positive.

Conclusion: - The findings of our study showed presence of co-infection in (2.59%) in HIV-positive patients and there were 9 patients found with HBV-positive and no infection with HCV was found in non-HIV patients. Co-infection with HBV and HCV is a common problem in HIV-positive patients in India. Hence, all HIV patients need to be routinely tested for markers of HBV and HCV infection.

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LIST OF ABBREVATIONS

HBV	Hepatitis B virus
HIV	Human immunodeficiency virus
HBsAg	Hepatitis B surface antigen
ELISA	Enzyme Linked Immunosorbent Assay
ART	Anti-retroviral therapy
HCV	Hepatitis C virus
HAART	Highly Active Anti-retroviral Therapy

CHAPTER-1

INTRODUCTION

Co-infection is the state of infection with more than one disease at the same time. Hepatitis B virus (HBV) & Hepatitis C virus (HCV) co-infections are commonly seen in people infected with HIV-infected patients. HIV weakens the immune system, so other infections are more likely to advance in a person infected with Human immunodeficiency virus (HIV). Co-infections can become serious more rapidly in people infected with HIV than in people who are not infected with the virus. Human immunodeficiency virus (HIV) and Hepatitis B & Hepatitis C virus are major health concerns. The co-infection of Hepatitis B and Hepatitis C in HIV-patients has not been studied yet in Jammu region of India; therefore, present study was designed to evaluate the co-infection among HIV patients. As these viruses share the same transmission routes, so the HIV-positive patients are more prevalent towards HBV and HCV co-infection.

Human immunodeficiency virus (HIV) and Hepatitis B & C virus are the three most common chronic viral pathogens of major public health concerns, all over the world. Their routes of transmission are similar including sexual, blood-contact, and injecting drug usage (1-3). In the last few decades, Human immunodeficiency virus (HIV) and hepatitis B virus (HBV) and hepatitis C virus (HCV) have emerged as a leading cause of morbidity due to liver disease throughout the world (4,5). The diseases of hepatobiliary system are a major problem in patients with human immunodeficiency (HIV) infection. It has been estimated that one-third of deaths in HIV patients are directly or indirectly related to liver disease. Liver diseases in HIV-infected persons can occur due to hepatitis B virus (HBV) and hepatitis C virus (HCV) co-infections, hepatic tuberculosis, chronic alcoholism, or due to the effects of anti-retroviral therapy (ART) (6,7). Worldwide, HIV is responsible for 38.6 million infections as estimated at the end of 2005 (8), while HBV and HCV account for around 370 million and 130 million chronic infections respectively. Moreover, among the HIV-infected patients, 2-4 million are estimated to have chronic HBV co-infection while 4-5 million are co-infected with HCV (8). In co-infection, the presence of one virus impacts the natural history of other virus. Among the HIV infected patients, HBV and HCV co-infections are more prevalent due to overlapping transmission routes. HIV accelerates the natural course of HBV infection and facilities faster progression of liver disease to cirrhosis and hepatocellular carcinoma. Disease progression to cirrhosis in HIV

patients is almost three-times faster as compared to HIV negative patients (9-11). It has been reported that the world prevalence of HIV-HCV co-infection among intravenous drug users (IDU's) can surpass 90% in certain populations (12, 13). It has been observed that HBV/HIV co-infection leads to increased morbidity and mortality as compared to HIV or HBV mono-infections (14). With increased assess to antiretroviral drugs for HIV patients, migrating populations and social networking by intravenous drug use, cases of HBV and HCV co-infections have been on the rise (15). Co-infections of HBV/HCV with HIV has been associated with reduced survival, increased risk of progression to liver disease and increased risk of hepatotoxicity associated with anti-retroviral therapy (16). It has been reported that HIV can negatively affect the natural history, physiology, diagnosis and therapeutic responses to HBV (8). As a result of liver related diseases, patients co-infected with HIV and HBV have a high mortality rate, up to 19-fold higher than those infected with HBV alone, and are up to 6-fold more likely to develop chronic hepatitis B than are HIV-negative individuals. The patient infected with both HBV and HIV is more likely to develop chronic hepatitis B infection compared to those patients infected with HBV only (17). If liver disease emerges in the presence of anti-HBs in HIV-infected individuals, it is recommended that tests for HBV serological markers and HBV DNA be repeated (14). In addition HIV decreases the rate of seroconversion of HBeAg to anti-HBe and increases the level of HBV DNA replication (14, 18). Co-infection with HBV and HCV is a common problem in HIV infected patients in India. Hence, all HIV patients need to be routinely tested for markers of HBV and HCV infection. (19).

1.1 Hepatitis B virus:-It is a hepatotropic virus; a specie of the genus *Orthohepadenavirus*, which is likewise a part of the *Hepadenaviridae* family viruses (20). This virus is responsible for causing Hepatitis. In addition to causing hepatitis, infection with HBV can lead to cirrhosis and hepatocellular carcinoma (21). The virus called Dane particle (virion) consists of an outer lipid envelope which contains hepatitis B surface antigen (HBsAg), and an icosahedral nucleocapsid core composed of protein. This core contains hepatitis B core antigen (HBcAg).Inside the core is the genome, a circular double stranded DNA and a DNA polymerase (22).

1.2 Hepatitis C virus: - Hepatitis C Virus (HCV) is a major cause of hepatocellular carcinoma (HCC). (23) It belongs to the family Flaviviridiae and genus Hepacivirus. (24) It is a 50-60 nm virus with a linear single stranded RNA of positive polarity, enclosed within a core and surrounded by an envelope, carrying glycoprotein spikes. (22)

1.3 Human Immunodeficiency Virus (HIV): - HIV is a spherical enveloped virus, about 90-120nm in diameter. It contains two identical copies of single stranded, positive sense RNA genome. The virus core is surrounded by a nucleocapsid composed of protein. The virus contains a lipoprotein envelope. The major virus encoded envelope glycoproteins are the projecting spikes on the surface and the anchoring transmembrane pellicles (22).

CHAPTER -2

REVIEW OF LITERATURE

Diseases of the hepatobiliary system are a major problem in patients with HIV infection (16). Viral hepatitis is an inflammation of the liver due to viral infections HBV/HCV viruses is responsible for the most common chronic viral infections worldwide. The prevalence of HBV and HCV infection is higher among people living with the human immunodeficiency virus (HIV) compared to the general population due to the common transmission routes and the overlap of behavioral risk factors for this viruses (8). It has been observed that HBV/HIV co-infection leads to increased morbidity and mortality as compared to HIV or HBV mono-infections (14).With increased assess to antiretroviral drugs for HIV patients, migrating populations and social networking by intravenous drug use, cases of HBV and HCV co-infections have been on the rise (15).

In a report, Badridge *et al.* (2008) studied the prevalence of HBV and HCV co-infection among HIV positive patients. Study participants were voluntary individuals 18yrs of age or older recruited from AIDS center in Tbsilis, Georgia. Total 175patients were taken for the study. Most patients were male (71.4%) are age range of HIV positives varied from 20-77 yrs. old. According to their study, prevalence HCV among HIV positive patients was high. Almost half (48.57%) HIV positive patients are co-infected with HCV. Men were more likely than women co-infected with HCV (60.80% and 18%) accordingly. Major risk of male co-infection was s related to drug use, needle and injection equipment sharing. Prevalence of HCV among infecting drug users was (73.40%). Prevalence of being infected with HBV (Anti-HBc) among HIV positive patients was 43.42% (76/175) and the prevalence of Chronic HBV (HBsAg positive) was 6.86%

Moreover, Mahmoud *et al.* (2009) conducted a study in which one hundred ninety-one anti-HBc positive sera were taken from Belgian patients co-infected with HIV and HBV were collects during 1998-2008. Full-length HBV genomes as well as large S or partial S genes were amplified and their molecular evolutionary history was analyzed. Clinically, 30(65.8%) patients were categorized as “overt infection” and 16 (34.7%) cases were categorized as “occult infection”. Five distinct HBV genotypes comprising A (69.9%), E (19.6%), followed by D, C, and G were detected.

Chandra *et al.* (2012) included a total of 120 cases with HIV infection and 120 healthy control subjects in their study. The findings of their study showed the presence of HBV (15%) and HCV (8.3%) co-infection in HIV positive patients, which was higher than that seen in HIV negative controls. Triple infection with HBV/HCV and HIV was seen in three patients. CD4 T-lymphocyte count less than 200/µl was seen in 22 of 28 co-infected cases.

A study by Chole *et al. (*2013) on 2105 HIV-infected subjects from 11 countries, the median age was 34 years 63% were Black. In the co-infected subjects, 49.6% had HBeAg-negative HBV. 60.2% had genotype A HBV, and 13% were HDV positive. Of the HBsAg-negative subjects, 66% had HBV DNA less than or equal to 2000IU/ml compared to 5.2% of the HBeAg-positive subjects. Drug-resistant HBV was not detected. In their conclusion they found that HBsAg may be useful to assess the need for HBV treatment.

Pal *et al.* (2013) investigated the molecular diversity of Hepatitis B virus (HBV) among the HIV co-infected patients from eastern India. The study was done on 874 HIV-infected patients. Of these, 73 could be amplified for HBV DNA from the surface gene region. These 73 HIV-infected patients, positive for both HBsAg and HBV DNA were analyzed in this study. Three HBV genotypes [HBV/A (21/73; 28.77%), HBV/C 3/73; 4.11%) and HBV/D (49/73; 67.12%)] were detected in these HIV co-infected patients by RFLP. The results were underscored the need for proper monitoring in HIV co-infection.

Ahuja *et al.* (2013) conducted a study to evaluate the prevalence of HIV co-infection with HBV and HCV in Central Delhi and adjoining areas. A total of 877 patients enrolled in ART Centre were retrospectively analyzed for the presence of HBV and HCV on the basis of presence of HBsAg and anti-HCV (IgG). In 877 HIV seropositive patients, 43 (4.9%) were positive for HBV and 15 (1.7%) for HCV and no case was simultaneously positive for both HBV and HCV. It was concluded that patients have high probability of getting HBV/HCV infection due to enhanced immunodeficiency by HIV.

Edith *et al.* (2014) studied to check the seroprevalance of hepatitis B surface antigen (HBsAg) co-infections among HIV positive indiduals. About 188 HIV confirmed subjects on Highly Asctive Anti-retroviral Therapy HAART were enrolled for the study. Overall results showed that 66 (35%) were positive for hepatitis B surface antigen. Gender distribution showed that 18 (9.6%) were males compared to 48 (25.5%) females.

Moreover, Zhang *et al.* (2014) did a nationwide retrospective observational cohort study with data from the China National Free Antiretroviral Treatment Program from 2010-11. Patients older than 18yrs. Starting standard antiretroviral therapy for HIV who had tested positive for HBV and HCV were followed up to Dec 31, 2012. 33861 patients, with HIV met eligibility criteria. 2958 (8.7%) participants had HBV co-infection, 6149 (18.2%) had HCV co-infection and 1114 (3.3%) had triple infection. All-cause mortality was higher in participants with triple infection and HCV co-infection, than in those with HIV only, but not in those with HBV co-infection. People with triple infection were also more likely to have virological failure than were those with HIV only, whereas the difference was not significant for those with HBV co-infection or HCV co-infection. No co-infection was significantly associated with a difference in CD4 cell count after one year of treatment. Loss to follow up was more common among participants with triple infection and HCV co-infection, but not HBV co-infection, than among those with HIV only.

Bui Vu Huy, *et al.* (2014) examined prevalence and characterization of HBV and HCV co-infection among HIV/AIDS patients. The cross-sectional, retrospective study analyzed 724 HIV/AIDS patients in the HIV clinic at the National Hospital of Tropical Diseases, from 5/2005 to 4/2011. The results showed the prevalence of HBV, HCV, and HIV co-infection was 50.3% (364/724), of which HBsAg, HCV and both of HBsAg, and HCV positivity were 8.4%, 35.4% and 6.5%, respectively. They found the risk of co-infection with HIV and HCV in the age of 30-39 years.

CHAPTER-3

AIMS AND OBJECTIVES

3.1 AIM

The aim of the current study was to evaluate the co-infection of HBV and HCV in HIV-positive patients.

3.2 OBJECTIVES

The objectives of present study were:

1. Screening of HBV/HCV in HIV positive patients, by Rapid SD Bioline kit.
2. Confirmation of HBV/HCV, by Erba ELISA kit.
3. To determine the co-infection of HBV and/ or HCV in HIV positive patients, in Government Medical College and Hospital Jammu.

CHAPTER-4

MATERIAL AND METHODS

4.1 Study design

This was a retrospective study carried out in the Viral Research and Diagnostic Laboratory (VRDL) under Indian Council of Medical Research (ICMR) in the department of microbiology, Govt. Medical College and Hospital, Jammu which is a tertiary care hospital. It was a one year study (April 2015 - March 2016) for the detection of co-infection with HBV and HCV in HIV positive patients attending the Integrated Counselling and Training Centre (ICTC) department of the hospital.

4.2 Inclusion Criteria

HIV infected positive patients were taken for the analysis.

4.3 Exclusion Criteria

Patients having HBV or HCV infection, from non HIV-patients were excluded from the study.

4.4 Samples

Antiretroviral therapy (ART) referred blood samples were collected for analysis of HBV and/or HCV co-infection in the HIV positive individuals, as follows:

1. Samples were collected from the patients.
2. Blood samples 5ml each, was collected and serum was separated.
3. The sera were diagnosed for HBsAg and HCV by immunochromatography method.
4. All sera were stored in aliquots each at -20^0 for further use. The positive samples for were further confirmed for HBsAg and HCV by ELISA.

Specimen collection, storage and precautions for rapid test

Serum collection: Collect the whole blood in the collection tube (NOT containing anticoagulants) by venipuncture, leave to settle for 30 minutes for blood coagulation and then centrifuge to get serum specimen of supernatant.

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 (Transasia Bio-medicals Ltd.) is recommended to be used only for testing of human serum. Collect the specimens aseptically. 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, hemolyzed and icteric hyperglycemic samples may yield erroneous results.

Procedure

1. Each patient was screened for HBsAg and anti-HCV antibodies by immunochromatography method SD BIOLINE HBsAg One Step HBsAg test and SD BIOLINE HCV One Step anti-HCV test (Alere Medical Pvt. Ltd).
2. Positive sera were 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 3rd generation ErbaLisa SEN HBsAg and ErbaLisa Hepatitis C (Transasia Bio-Medicals Ltd).
4. The entire test was performed with the manufacturer's instructions.

4.5 SD BIOLINE HBsAg one step test for qualitative detection of HBsAg in human serum

SD BIOLINE is a visual, sensitive and accurate one step immunoassay for the qualitative detection of Hepatitis B surface Antigen (HBsAg) in human serum. The assay is done for the recognition and diagnosis hepatitis B virus infection.

Procedure

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

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

3. As the test began to work, you will see purple color moves across the result window in the center 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.

4.6 SD BIOLINE HCV one step test for qualitative detection of anti-HCV antibodies in human serum

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

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 100µl of specimen into the sample well (S) or using disposable

dropper add 3-4 drops of serum in the sample well.

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

4.7 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 is 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 labeled 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.

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 100µl of the sample diluent in a well A1 (blank).
5. Add 25µl of the sample diluent to the rest of the wells.
6. Add 75µl of the HBsAg negative control in wells B1, C1, and D1.
7. Add 75µl of the positive control in well E1.
8. Add 75µl of the first sample in well F1, second sample in well G1 and so on...
9. Add 50µl 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 350µl of washing solution to each well. Aspirate again after 30 seconds of soak time. Repeat the washing steps 5 times (invert the plate and tap it on absorbent pad to remove the remaining washing solution).
11. Add 50µl 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).
12. Add 100µl 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.

4.8 Erba Lisa Hepatitis C

Erba Lisa Hepatitis C is an in-vitro diagnostic kit for qualitative detection of antibodies against Hepatitis C virus in patient serum.

Principle

The Erba Lisa 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 is 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 labeled 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 by using distilled or deionized water. Homogenize. Washing solution may form crystals under cold storage condition. If so, use it after thawing at 37^0C 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°C immediately.
4. Add 100µl of the sample diluent to all the wells **except in the well A1 (blank)**
5. Add 10µl of the HCV negative control in wells B1, C1, and D1.
6. Add 10µl 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 45 minutes at room temperature (20-30^0C).
9. Remove the sealer. Discard / aspirate the contents of the well into the waste disposal container. Add a minimum of 350µl of washing solution to each well. Aspirate again

after 30 seconds of soak time. Repeat the washing step 5 times (inverts the plate and tap it on absorbent pad to remove the remaining washing solution).

10. Add 50µl 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 50µl 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 100µl 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.

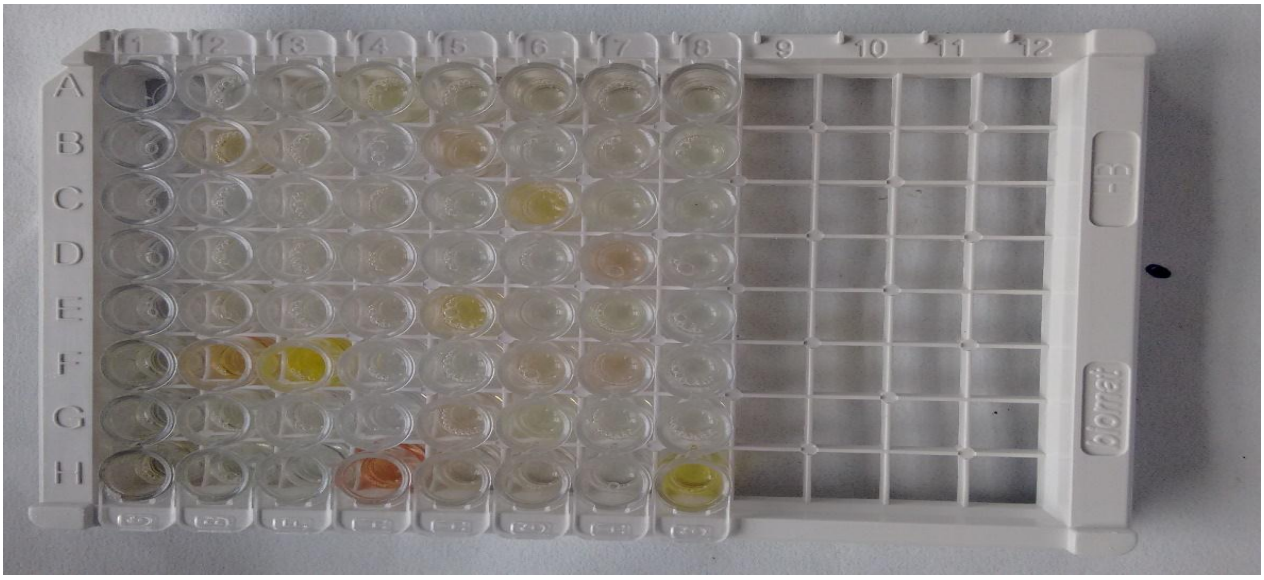

Fig- 1: ELISA Test Plate

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 (NC)

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

RESULTS

A total of 386 HIV-infected patients (242 males, 144 females), and 386 non-HIV subjects were included in the study. Overall results showed that 10 (2.59%) patients were co-infected with HBV and/ or HCV in the HIV-positive patients. HBsAg was detected in 9(2.33%) patients, gender wise 7(1.81%) males and 2(0.51%) females) among which one patient was having HCV also. The detection of HCV-antibodies was found in 2 patients (0.51%) gender wise 1(0.25%) male and 1(0.25%) female, out of which one patient was having HBV also. Triple infection with HBV, HCV and HIV was seen in 1(0.25%) patient. There were 9(2.33%) patients found with HBV-positive and no infection with HCV was found in non-HIV patients

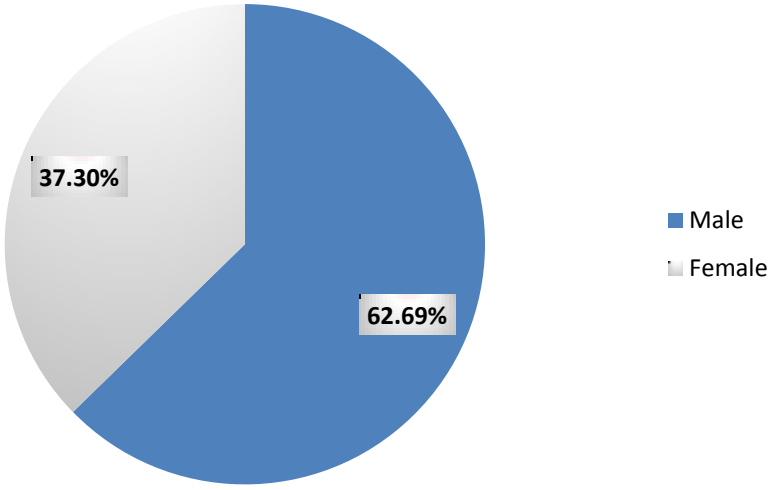

Fig- 2: Distribution of HIV-positive patients

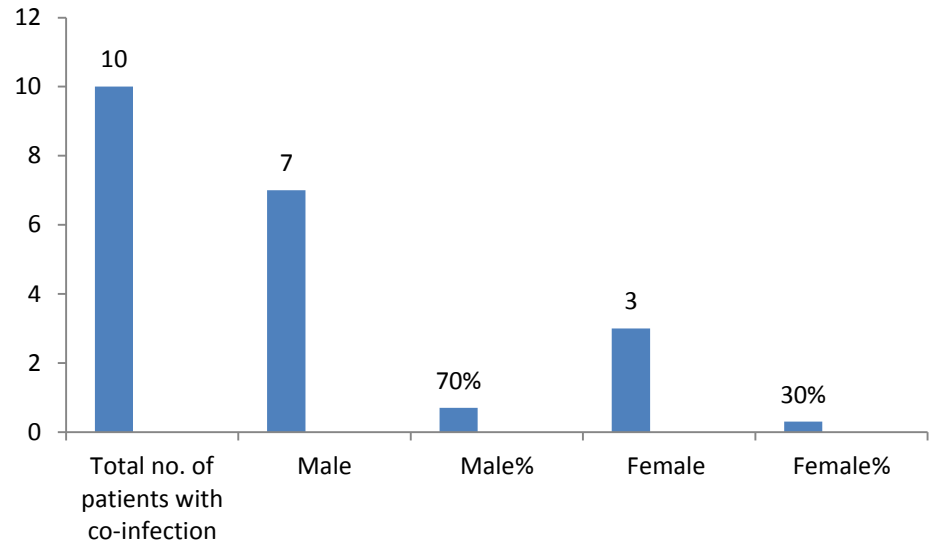

Fig- 3: Number of patients with co-infection of HBV and/or HCV in HIV positive patients

Table- 1: Gender wise distribution of HBV co-infected patients in HIV-positive patients. Total = 9 patients (7 males, 2 females)

Gender	Number of patients
Male	7
Female	2
total	9

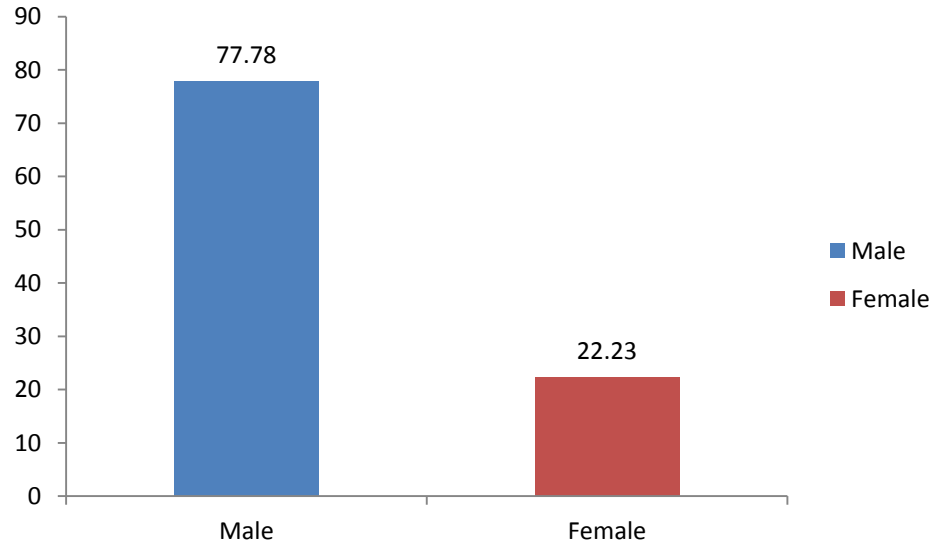

Fig. 4: Percentage wise distribution of HBV co-infected patients in HIV-positive patients.

Table- 2: Month wise distribution of HBV co-infected patients with HIV-positive patients

Month	**Total no. of ART patients**	**Male**	**Female**	**HBV+HIV**
April	36	21	15	0
May	39	26	13	1
June	23	19	4	1
July	29	18	11	0
August	41	22	19	1
September	30	19	11	1
October	27	20	7	6
November	26	16	10	0
December	27	17	10	0
January	31	17	14	0
February	38	25	12	0
March	39	22	17	0

Table- 3: Prevalence and gender-wise distribution of HCV co-infection in HIV patients.

Gender	Number of patients
Male	1
Female	1
Total	2

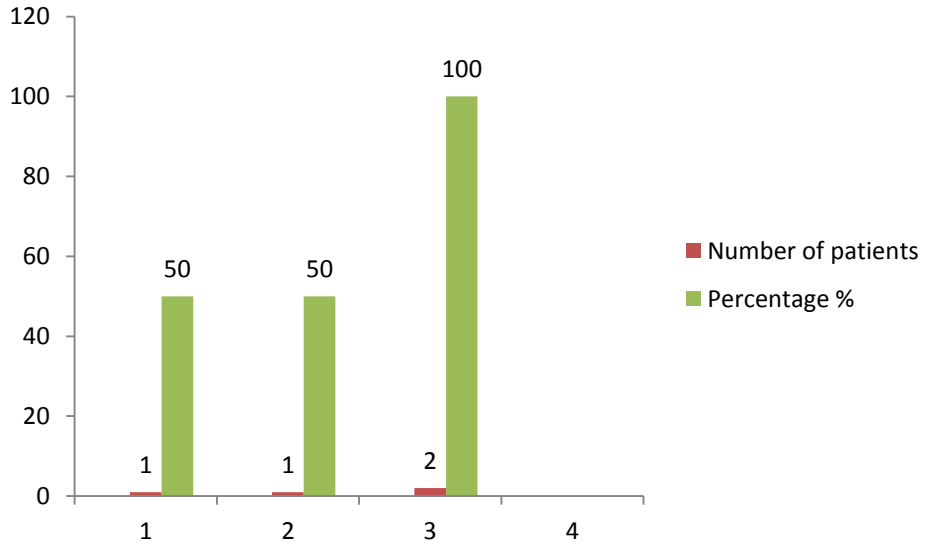

Fig 5: Percentage wise distribution of patients of HCV co-infected patients

Table- 4: Month wise distribution of HCV co-infected patients with HIV-positive patients

Month	**Total no. of ART patients**	**Male**	**Female**	**HCV+HIV**
April	36	21	15	0
May	39	26	13	0
June	23	19	4	2
July	29	18	11	0
August	41	22	19	0
September	30	19	11	0
October	27	20	7	0
November	26	16	10	0
December	27	17	10	0
January	31	17	14	0
February	38	25	12	0
March	39	22	17	0

Table- 5: Prevalance of HBV and HCV in HIV-positive patients

Month	**Total no. of ART patients**	**Male**	**Female**	**HBV+HCV+HIV**
April	36	21	15	0
May	39	26	13	0
June	23	19	4	1
July	29	18	11	0
August	41	22	19	0
September	30	19	11	0
October	27	20	7	0
November	26	16	10	0
December	27	17	10	0
January	31	17	14	0
February	38	25	12	0
March	39	22	17	0

Table 6: Age wise distribution of Co-infected patients of HBV and HCV in HIV patients

Age group in years	HBV+HIV	HCV+HIV	HBV+HCV+HIV
0-10 yrs.	0	0	0
10-20yrs.	0	0	0
20-30yrs.	4	1	1
30-40yrs.	3	0	0
40-50yrs.	0	0	0
50-60yrs.	1	0	0
60-70yrs.	0	0	0

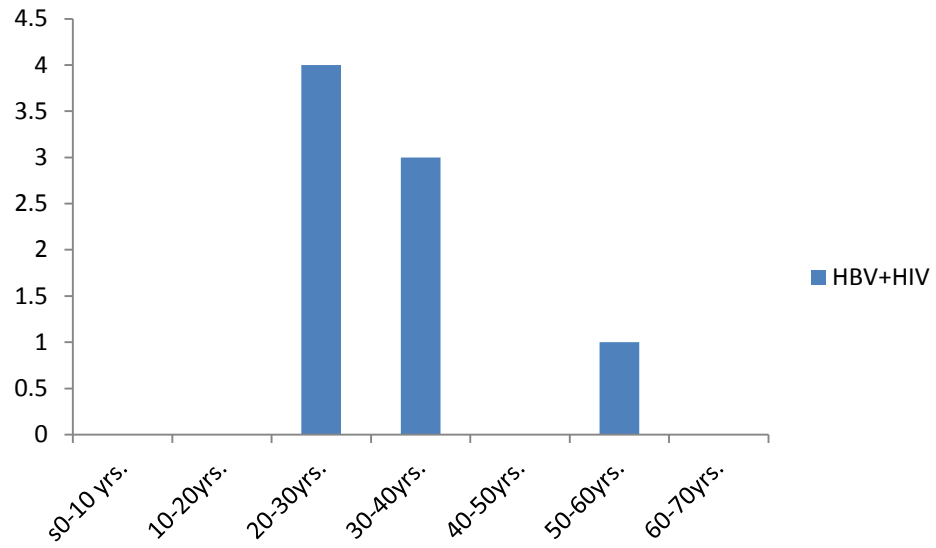

Fig 6: Age wise distribution of Co-infected patients of HBV in HIV patients.

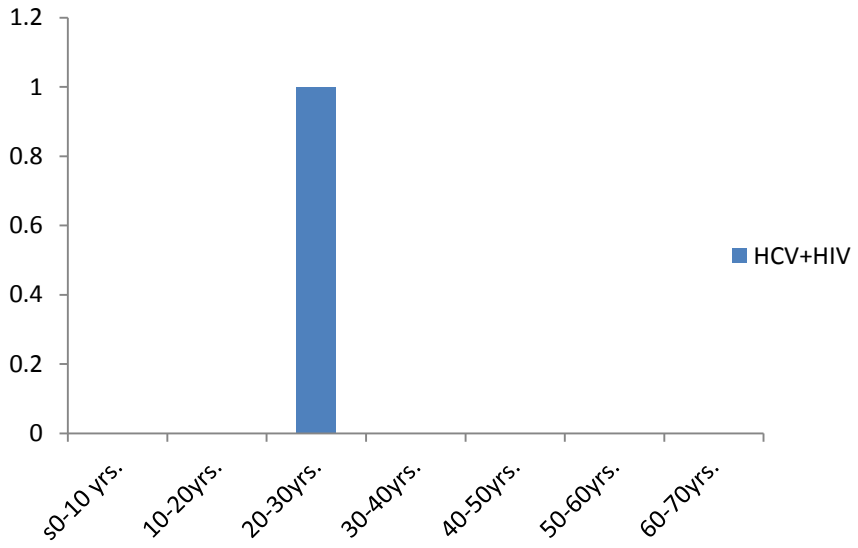

Fig 7: Age wise distribution of Co-infected patients of HCV in HIV patients.

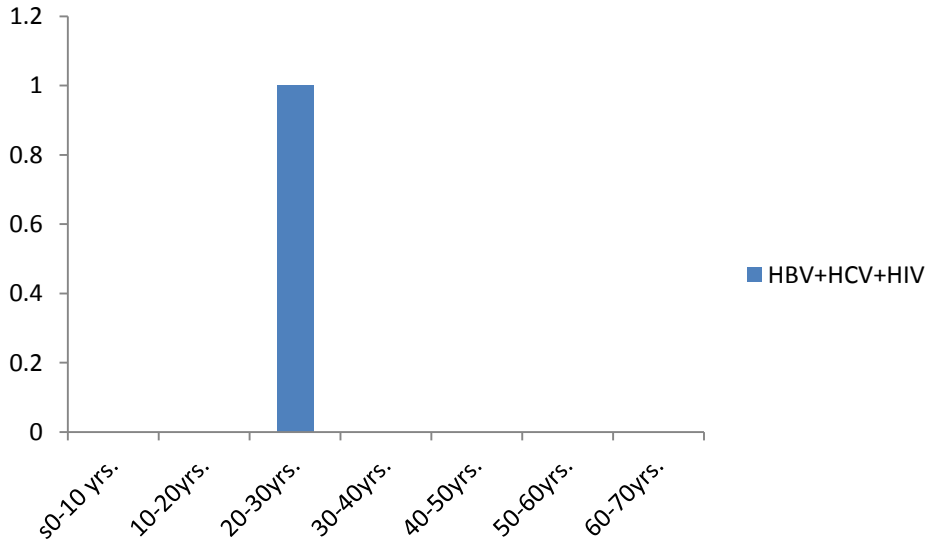

Fig 8: Age wise distribution of Co-infected patients of HBV and HCV in HIV patients.

CHAPTER- 6

DISCUSSION

HIV accounts for an estimated 40 million chronic infections while hepatitis C with and HBV cause 130 million and 370 million chronic infections respectively (25). The prevalence of HIV in India is quite high and it has the second highest number of people living with HIV (26). Hepatitis B virus in HIV infected individuals varies with the population studied. Co-infection with HBV complicates the clinical course of HIV-positive patients, and may also adversely affect the treatment of HIV infection (19). The prevalence of HBV varies markedly among different HIV infected population and geographical areas. The prevalence of HBV co-infection varies from 5-7% in low endemicity areas. In intermediate and high endemicity, it varies from 6-20 % (14).The prevalence of HCV co-infection varies from 9-16 % (27, 28). There are only a few reports from our country on prevalence of HBV/HCV co-infection in HIV patients and the observations have been highly variable (19). Co-infection observed in the previous studies was 30.4% from Nagpur, (29) 2.25% from Lucknow, (30) 7.7% from Chennai (1) and 3.5% from Mumbai (31). In Brazil, the results of a study showed the rates of (6.4and 5%) for HBsAg and HCV-antibodies co-infection in HIV positive patients (25).

In present study, a total of 386 HIV-infected patients (242 males, 144 females), and 386 non-HIV subjects were included. Overall results showed that 10 (2.59%) patients were co-infected with HBV and/ or HCV in the HIV-positive patients. HBsAg was detected in 9(2.33%) patients, gender wise 7 (1.81%) males and 2 (0.51%) females) among which one patient was having HCV also. The detection of HCV-antibodies was found in 2 patients (0.51%) gender wise 1 (0.25%) male and 1 (0.25%) female respectively, out of which one patient was having HBV also. Triple infection with HBV, HCV and HIV was seen in 1 (0.25%) patient. There were 9 patients found with HBV-positive and no infection with HCV was found in non-HIV patients. The route of transmission in the patients who were found to be co-infected with HBV and /or HCV in HIV-positive patients was sexual contact. Within India only, HBV and HCV co-infection among HIV-positive patients varies from one region to other as is evident from different studies. The co-infections with the other viruses may lead to early onset of advanced liver diseases.

CHAPTER- 7

CONCLUSION

It is clear from the study that apart from the other infections likes TB, HIV infected patients have high probability of getting HBV/HCV infection due to immunodeficiency caused by HIV. The findings of our study showed presence of co-infection in (2.59%) in HIV-positive patients. Co-infection with HBV and HCV is a common problem in HIV-positive patients in India. Hence, all HIV patients need to be routinely tested for markers of HBV and HCV infection. According to the present study the percentage of males having co-infection is more as compared to females and is seen mostly in age group ranging between 21-30yrs. This may be due to the highly active sexual state. In conclusion, it is evident from the present study the HIV-infected patients in this region have a high risk of acquiring HBV/HCV co-infections through the shared routes of transmission. HIV patients should routinely be tested for HBV and HCV markers. Screening the high-risk population for these infections would help in the diagnosis and treatment with improved outcomes in these patients which in turn may decrease the further spread of these chronic viral infections.

CHAPTER- 8

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