"Seroprevalence of Hepatitis C Virus and Human Immunodeficiency Virus in HBsAg positive patients at KLE'S Dr. Prabhakar Kore Charitable Hospital and Medical Research Center, Belagavi"- A One Year Cross Sectional Study."

By

REG NO:BI0116002

Dissertation

Submitted to the KAHER, Belagavi, Karnataka
In partial fulfillment of the requirements
for the degree of

DOCTOR OF MEDICINE (M.D)
IN
MICROBIOLOGY

DEPARTMENT OF MICROBIOLOGY,
JAWAHARLALNEHRUMEDICALCOLLEGE, KAHER,
BELAGAVI– 590010

APRIL- 2018

KAHER, BELAGAVI

Endorsement by the Head of Department of Microbiology/ Principal of the Institution

This is to certify that the dissertation entitled **"Seroprevalence of Hepatitis C Virus and Human Immunodeficiency Virus in HBsAg positive patients at KLE'S Dr.Prabhakar Kore Charitable Hospital and Medical Research Center, Belagavi"-A One Year Cross Sectional Study."** is a bonafide research work done by **REG NO:BI0116002.**

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LIST OF ABBREVIATIONS

HBsAg	Hepatitis B surface Antigen
HBV	Hepatitis B Virus
HIV	Human Immunodeficiency Virus
HCV	Hepatitis C Virus
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
AIDS	Acquired Immunodeficiency Syndrome
ELISA	Enzyme Linked Immunosorbant Assay
WHO	World Health Organisation
NACO	National AIDS Control Programme
ALT	Alanine aminotransferase
AST	Aspartate transaminase
SGOT	Serum glutamatic oxaloacetic transaminase
SGPT	Serum glutamatic pyruvate transaminase
NANBH	“nonA,nonB”hepatitis
PCR	Polymerase Chain Reaction
PLHIV	People living with HIV

ABSTRACT

INTRODUCTION: Worldwide,An estimated 257 million people are living with hepatitis B virus infection (defined as hepatitis B surface antigen positive). In 2015 hepatitis B resulted in 8,87,000 deathsmostly from complications due such as cirrhosis and Hepatocellular Carcinoma.Hepatitis B virus (HBV), hepatitis C virus (HCV) and Human immunodeficiency virus (HIV) co-infection has emerged as a leading cause of morbidity due to liver disease throughout the world in the last two decades. Approximately, 10 % of all HIV infected patients worldwide are estimated to have chronic HBV co-infection. In general, approximately 2–10% of anti-HCV-positive patients are found to be HBsAg positive. Co-infections are more prevalent due to overlapping transmission routes. In co-infection the presence of one virus impacts the natural history of the other virus

OBJECTIVES:

1. To Study The Prevalance of Co-infection of HCV and HIV among HBsAg positive patients

MATERIALS AND METHODS:

The study was carried out from January 2017 to December 2017. All the sera which arer ecieved for HBsAg Screening to central lab of KLES DR Prabhakar Kore charitable Hospital & MRC, Belagavi were taken.

Total 75 HBsAg positive serums were taken which were tested by using immunochromatography test and further confirmed by ELISA. These HBsAg positive serum samples were further tested for HIV and HCV by ELISA method.

RESULTS: Out of 75 HBsAg positive patients maximum patients were between age group of 41 to 60 yrs (40%), followed by 21 to 40 yrs (34.46%), Out of 75 HBsAg positive patients male were more predominant 56 (75%)Most common occupation among HBsAg Positive patients was Farmer 33 (44%) followed by Driver 10 (13.33%), maximum patients were illiterate 42(56%) ,Most common symptoms were Fever 40 (53.33 %) followed by Loss of appetite18 (24 %),46 (61.33%) showed increase in Liver enzymes, coinfection with HIV was seen in 4(5%) patients and 7 1 (95%) were negative.,out of HIV Postive patients 4 (7%) male and females were zero (0%),HBsAg and HCV coinfection was zero (0%) as well as HBsAg and HCV and HIV triple infection was zero (0%).

CONCLUSION: In current study 3278 patients screened for HBsAg out of which 75 were positive and prevalence was 2%.There was high prevalence of HIV and HBsAg coinfection among patients attending hospital which was 5%. There is no coinfection of HBsAg and HCV seen. The routine screening of HIV and HCV should be mandatory for HBsAg positive patients, as there is more chance of co infection with these viruses due to similar routes of transmission. Clear national policies should be established which should include clear economic and health care strategies test for HBsAg and HIV and HCV to improve quality of living conditions, and easy access to health care facilities.People should be educated about taking preventive measures, prophylaxis for Hepatitis B virus and avoid high risk activities to prevent for transmission of these viruses.

Key words: Prevalence, Hepatitis B virus, Human Immunodeficiency virus, Hepatitis C virus, ELISA

CONTENTS

LIST OF TABLES

LIST OF GRAPHS

LIST OF FIGURES

LIST OF PHOTOGRAPHS

INTRODUCTION

Hepatitis B virus (HBV) is a 42 nm double stranded DNA virus belonging to Hepadanaviridae family. Worldwide, estimated 257 million people are living with hepatitis B virus infection (defined as hepatitis B surface antigen positive). In 2015 hepatitis B resulted in 8,87,000 deaths mostly from complications due such as cirrhosis and Hepatocellular Carcinoma.The Hepatitis B Virus prevalence is highest in sub-Saharan Africa, East Asia, Amazon and the southern parts of eastern and central Europe and the prevalence is estimated between 5 to 10%. In the Middle East estimated prevalence is 2–5%. In Western Europe and North America estimated prevalence is less than 1%. In India the estimated Hepatitis B Virus prevalence is 2-5%.[1,2]

Hepatitis C Virus (HCV) is a 50 -60 nm virus with linear single stranded RNA genome belonging to Flaviviridae family under genus Hepacivirus.Worldwide, Every year130–170 million people are infected with Hepatitis C Virus (2 to 3% world population) and 3,50,000 people (approximately) die from complications due to HCV per year. The hepatitis C virus prevalence is highest in Egypt and it is estimated to be 10%. Prevalnce in Eastern Europe and Latin America and certain countries in Africa, Middle East, and South Asia estimated to be 3%. Prevalence in Developed countries like Australia, United States and most countries in Western Europe is estimated to be to be <2. [3] In India the estimated prevalence of Hepatitis C virus infection ranges from 0.1-8% .[1,3,4]

Human immunodeficiency virus (HIV) is the causative agent of AIDS, belongs to the Lentivirus subgroup of the family Retroviride.Worldwide No of people living with Human Immunodeficiency Virus in 2017 were 36.9 million and People

newly infected with HIV were 1.8 million in year 2017. HIV continues to be a major global public health issue, having claimed more than 35 million lives so far. In 2017, 940 000 people died from HIV-related causes globally. Now India has the third largest number of individuals with human immunodeficiency virus after South Africa and Nigeria. The national HIV prevalence is 0.8% and there are certain areas such as Maharashtra, Andhra Pradesh, Tamil Nadu, Karnataka, Manipur and Nagaland that account for over 80% of all the reported AIDS cases in the country. [1,5,6]

Hepatitis B virus (HBV), hepatitis C virus (HCV) and Human immunodeficiency virus (HIV) co-infection has emerged as a leading cause of morbidity due to liver disease throughout the world in the last two decades. Co-infections are more prevalent due to overlapping transmission routes. In co-infection the presence of one virus impacts the natural history of the other virus. HIV/HCV accelerates the natural course of HBV infection and facilitates faster progression of liver disease to cirrhosis and hepatocellular carcinoma.[7]

Prevalence of HBV and HCV co-infection worldwide among pateints has been reported to be 7% to 14% in Italy, in Spain it is 10% to13%,in Taiwan 3.4% to 18%,Japan it is 13% to 22%,China 11% to 15%,Thiland it is 2.7%. In India, the prevalence of HBV and HCV co-infection among patients has been estimated to be upto3%.[8]

Approximately, 10 % of all HIV infected patients worldwide are estimated to have chronic HBV co-infection . Highest prevalence is 20% to 30% in Sub-Saharan Africa and Asia, 5–10 % in areas such as North America, Europe and Australia.[9] HIV and HBV Co-infection prevalence is 10% in India.[9]

HBV/HCV/HIV co-infection causes severe liver disease than as mono-infection. Hence early diagnosis and treatment is important.

OBJECTIVES OF THE STUDY

1. To Study the Prevalence of Co-infection of HCV and HIV among HBs Ag positive patients.

REVIEW OF LITERATURE

HEPATITIS B VIRUS

Hepatitis B virus (HBV) is a DNA virus. It was discovered by Blumberg in 1963. It belongs to the family Hepadnaviridae, under the genus Orthohepadnavirus. HBV the most widespread and the most important type among hepatitis viruses. Though it commonly produces an acute self-limiting hepatitis which may be subclinical or symptomatic, it is also capable of causing a range of hepatic complications including chronic hepatitis, fulminant hepatitis, cirrhosis of liver and liver cancer.[14]

History

The discovery of HBV was through an indirect route. To identify and track genetic differences in human populations, Blumberg and colleagues were using sera from multiple blood transfused individuals as sources of antibody to human serum proteins. The idea was that, these sera would contain antibodies that bound to proteins differing in sequence from those of the transfusion recipients. During the course of these studies a new antigen, named "Australia antigen," was identified in serum from an Australian Aborigine.[10]

It was subsequently shown to be the surface component of HBV and the name Australia antigen was changed to hepatitis B surface antigen (HBsAg).[13]

Morphology:

Electron microscopy of serum of the patients infected with HBV reveals three morphological forms. They are

1. Spherical forms it is most numerous, small forms measuring 22 nm in diameter. These particles are exclusively made up of HBsAg.
2. Tubular or filamentous forms which have the same diameter,200 nm long and are also exclusively made up of HBsAg.
3. Complete form or Dane particles, these are less frequently observed and are larger, 42 nm size spherical virions and are made up of Outer surface envelope i.e. HBsAg (Hepatitis B surface antigen).Inner 27nm size nucleocapsid consists of core antigen (HBcAg) and pre-core antigen(HBeAg) and partially double stranded DNA.[14]

HBsAg is antigenically complex and contains two components common group reactive antigen'a' epitope and two pairs of type specific antigens d/y and w/ r; only one member of each pair being present at a time.Thus, four subtypes of HBsAg have been observed: adw, ayw, adr, and ayr.

Hepatitis B core antigen (HBcAg): HBcAg forms the intracellular core protein. It is not secreted and does not circulate in blood, but can be demonstrated in hepatocytes by immunoflorescence. Hepatitis B precore antigen (HBeAg)is a non particulate soluble antigen possessing a signal protein which enables it to be secreted.[14]

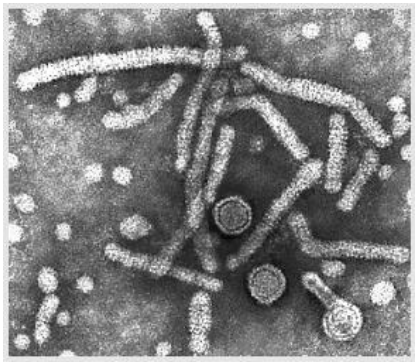


Figure 1: Electron microscope image showing Tubular and Spherical form and Dane form

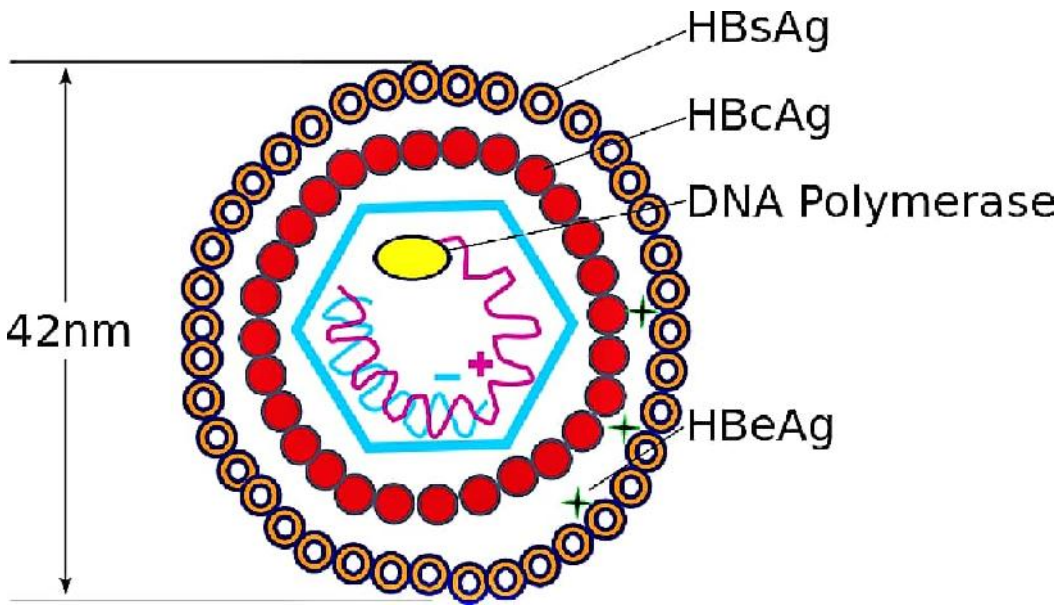


Figure 2: Schematic Diagram Of Hepatitis B Virus

Typing of HBV

HBV has four major serotypes (adr, adw, ayr, ayw) based on antigenic epitopes present on its envelope protein HBsAg. The immunity is serotype specific as the dominant 'a' antigen is shared by all. But they are useful for epidemiological investigations, as all the cases during an epidemic would likely to have the same subtype. Serotypes exhibit distinct geographical distribution. adw is the predominanat subtype in Europe, Australia and America. In India adr is prevalent subtype in South and East India; whereas ayw is prevalent in Northern and Western India.

HBV is divided into eight genotypes (A-H) according to nucleotide sequence variation of the genome. Genotypes A and D are prevalent in India. The HBV genome consists of partially circular dsDNA of3200 bp in length. The minus strand of DNA is complete and full-length and is identical in all HBV isolates. The positive strand is incomplete and of variable length (50-80%).

Hepatitis B virus genome is compact and consists of four overlapping genes S gene, it has three regions- S gene,pre-S1and pre-S2. They code for surface antigen (HBsAg).S region codes for the major protein and product of S region combines with that of adjacent Pre-S2 region to form the middle (M) protein. Pre-S1, Pre-S2 combines with S to code for large protein and present only in the virion, while the M and S proteins are found in the circulating HBsAg particles as well. C gene consists of pre-C and C-regions, which code for two nucleocapsid proteins. The Pre-C region codes for HBeAg where region codes for HBcAg. X gene, it codes for HBxAg, which can activate the transcription of cellular and viral genes. It may contribute to carcinogenesis by binding to p53.HBxAg and its antibody are elevated in patients with severe chronic hepatitis and hepatocellular carcinoma.Pgene, it is the largest gene and codes for polymerase protein which has three enzymatic activilies-1. DNA polymerase activity 2.Reverse transcriptase activity 3. RNase H activity.[14]

Hepatitis B Replication

Replication of HBV is different from other DNA viruses. The HBV attaches to host cells by Pre-S region of HBsAg , penetrates into cytoplasm and gets uncoated to release viral DNA and polymerase. In the host cell nucleus, the partially dsDNA of HBV gets converted into covalently closed circular dsDNA mediated by host enzymes. The cDNA serves as the template for the production of HBV mRNAs and pregenomic RNA by undergoing transciption.HBV mRNAs translate to form various components of viral proteins. The pregenomic RNA comes to cytoplasm and gets encapsidated by newly synthesized HBcAg.Within the core particle, the pregenomic RNA serves as template to form the minus strand of the DNA; mediated by the reverse transcriptase activity of the polymerase gene. Next the RNA template is

removed from the negative-strand DNA; mediated by the RNase H activity of the polymerase gene. Then, the polymerase starts to synthesize the positive DNA strand, but the process is not completed. As a result the dsDNA which is formed has a full-length minus-strand DNA and a variable-length **(**50-80%) positive strand DNA. Core particles containing these dsDNA bud from pre-Golgi membranes and may either exit the cell or re-enter the intracellular infection cycle.[14]

Transmission

Hepatitis B virus transmission occurs via multiple routes. The most common mode of transmission is via blood and blood products and needle prick injuries. Transmission also occurs by inoculation during surgical or dental procedures or percutaneous inoculation via shared razors and tooth brushes.HBV is more infectious than HIV and HCV. As little as 0.00001 mL of blood can be infectious. Chance of transmission of HBV following a contaminated needle prick injury is nearly 30% as compared to 3% and 0.3% with HCV and HIV respectively. Sexual transmissions found to be the most common route in most developed countries; particularly homosexual males being at higher risk. Vertical transmission i.e. the spread of infection from HBV carrier mothers to babies appears to be an important mode of transmission particularly in China and South East Asia. Transmission occurs at any stage like in utero, during delivery and during breast feeding. Risk is maximum if the mother is HBeAg positive. Direct skin contact with infected open skin lesions may transmit the virus, e.g. impetigo. High risk groups which are more prone for acquiring infection are Surgeons, Paramedical workers, Sex workers especially homosexual males, Recipients of blood transfusion and organ transplantation and drug addicts.[14]

Epidemiology

Global Situation

In 2015, the worldwide prevalence of HBV infection in the general population was 3.5%. Among those born before the hepatitis B vaccine became available, the proportion of persons living with chronic HBV infection remains high. Prevalence was the highest in the Africa (6.1%) and Western Pacific countries (6.2%). Overall, about 257 million people were living with HBV infection Assuming that women of reproductive age constitute 25.3% of the world's population according to United Nations data, Adults chronically infected may include 65 million women of childbearing age who can potentially transmit HBV to their babies. In community-based studies, worldwide reports range from less than 5% to about 10%. In health-care facility-based studies, the proportion is higher.[42]

The epidemiology of hepatitis B can be described in terms of the prevalence of hepatitis B surface antigen (HBsAg) in a population, broadly classified into high- (.8% HBsAg prevalence), intermediate- (2%–7%) and low prevalence (2%) areas. High-HBV prevalence is common in much of the Asia Pacific and sub-Saharan African regions of the world. Globally, it has been estimated that 45% of the world's population lives in an area of high prevalence .There is evidence to suggest that vertical transmission is more common in Asia than in Africa, where a greater proportion of women are highly infectious at childbearing age.[87]

Intermediate-Prevalence Populations Regions of the world in which HBV prevalence is classified as intermediate (2%–7%) include North Africa and the Middle East, parts of Eastern and Southern Europe, parts of Latin America, and South Asia.

These represent a similar proportion of the global population to high prevalence areas (slightly more than 40%).In these regions, transmission occurs either perinatally or horizontally. Although the predominant mode of transmission varies according to country, perinatal acquisition is thought to be less common in intermediate compared with high prevalence countries, owing to a lower prevalence of high infectivity among women of childbearing age. India falls in the intermediate endemicity zone .[67,87]

Low-Prevalence population (<2%). People living in low-HBV-prevalence countries make up the minority of the global population (12%), and include Australia, Asia, Northern and Western Europe, Japan, North America, and some countries in South America. In low-prevalence areas, the incidence of vertical and horizontal transmission in childhood is low, with most incident infections occurringin adolescence and adulthood through sexual contact, injecting drug use, and otherblood-related exposures. [87]

India has one-fifth of the world's population, which accounts for a large proportion of the worldwide HBV burden. India harbors 10–15% of the entire pool of HBV carriers of the world. It has been estimated that India has about 40 million HBV carriers. About 15–25% of HBsAg carriers are most likely to suffer from cirrhosis and liver cancer and may die prematurely. Infections occurring during infancy and childhood have the more risk of becoming chronic. Of the 2.6 Crore (26 million) infants born every year in India, approximately 10 Lakhs (1 million) run the life-time risk of developing chronic HBV infection.[88]

Human Immunodeficiency Virus (HIV).

Human immunodeficiency virus (HIV) is the etiologic agent of Acquired Immunodeficiency Syndrome (AlDS) the biggest threat to mankind in last three decades.

History/Origin of AIDS

The first case of AIDS was described from New York (USA) in 1981; which was soon followed by the discovery of HIV-I from Pasteur Institute, Paris in 1983. HIV in humans was believed to be acquired from chimpanzee by the cross species infections of simian counter part of HIV in rural Africa. It has been postulated that though such zoonotic transmission *to* humans was going on repeatedly over many years in the past, only by the late 20thcentury. The virus underwent changes which enabled it to adapt to human environment and to reach the epidemic level.[10]

Morphology:

HIV and other lentiviruses have a unique structure. They are spherical and 80-110 nm in size, and envelope. The envelope is made up of lipid part. It is host cell membrane derived and Protein part. It has two components l. Glycoprotein-120 (gp120). These are projected as knob like spikes on the surface and 2. Glycoprolein-41 (gp 41).They form anchoring transmembrane pedicles. It consists of which is icosahedral in symmetry and made up of core protein. Inside, there is a dense cylindrical inner core which encloses RNA. Two identical copies of single-stranded positive sense linear RNA. Viral enzymes such as reverse transcriptase, integrase and proteases which are closely associated with HIV RNA.[14]

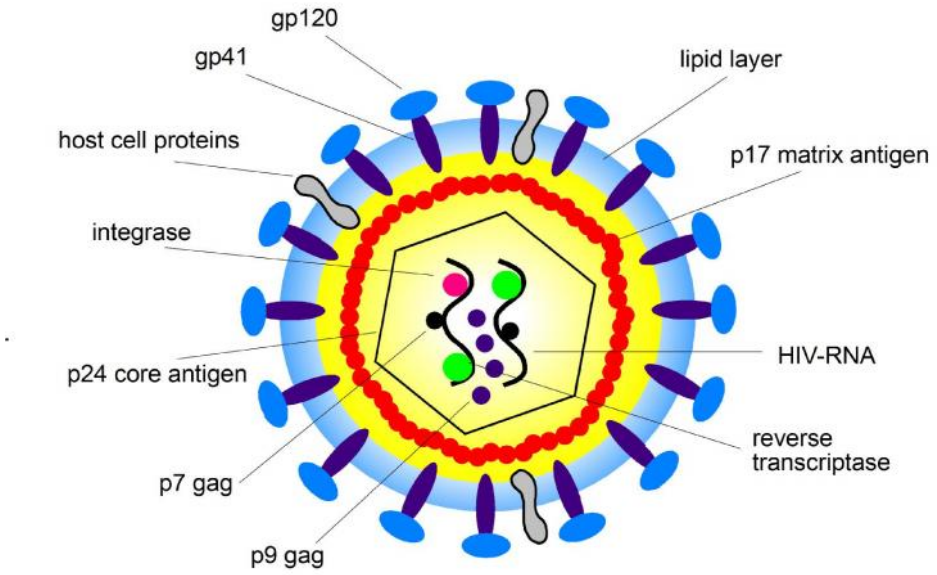


Figure 3: Human Immunodeficiency Virus structure.

Mode of Transmission:

Sexual mode is by far the most common mode of transmission, accounts for 75% of total cases in the world. Heterosexual route male to female via vaginal coitus is the commonest mode. However, the risk of transmission through sexualroute is minimal i.e.0.1- 1 % per coitus. Anal intercourse among homosexual males or even male to female has higher risk of transmission than vaginal intercourse.

Blood transfusion though is the least common mode of transmission (5%) but the risk of transmission is maximum (90- 95%).Percutaneous/ mucosal transmission modes such as needle stick injury, injection drug abuse and sharing razors or tattooing or splashes of infected blood on eyes etc. are among the less effective modes of transmission.

Perinatal mode in the absence of any intervention, the risk of transmission from mother to fetus is about 20-40%.Transmission may occur at any time during

pregnancy and breast feeding but the risk is maximum during delivery. Risk is maximum if mother is recently infected or has already developed AIDS. There isno evidence of HIV transmission by casual contact or kissing or insect bite.Viral load is maximum in blood, genital secretions, and CSF; variable in breast milk and saliva zero to minimal in other body fluids or urine.[14]

Replication :

Following attachment to receptor and co-receptorto gp l20, fusion of HIV to host cell takes place and it is mediated by gp4l. After fusion, HIV nucleocapsid enters into the host cell cytoplasm and which is followed by uncoating and release of two copies of ssRNA and viral enzymes. The Viral reverse transcriptase mediates transcription of it sssRNA into ssDNA so that DNA-RNA hybrid is formed. The RNA is degraded by viral endonuclease and ssDNA replicates *to* form ds DNA. Transcription of the DNA occurs to form the components of viral proteins.

The nucleoprotein complex formed consists of linear dsDNA, gag matrix protein, accessory vpr protein and viral integrase this is called as pre-integration complex, which is then transported into the host cell nucleus. The viral dsDNA gets integrated into the host cell chromosome which is mediated by viral integrase and the integrated virus is called as provirus. In the integrated state, HIV establishes a latent infection for variable period.[14]

Epidemiology

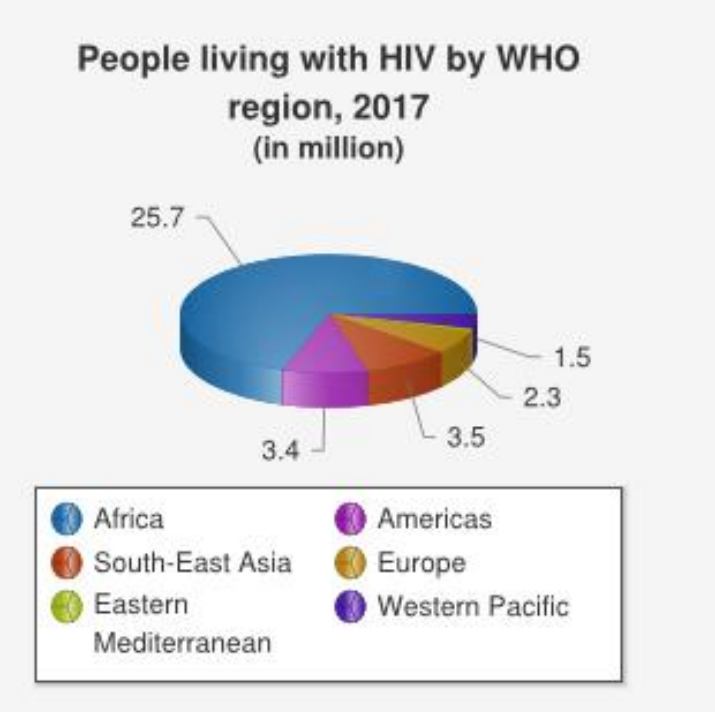
Global Situation

- "Globally 36.9 million [31.1 million–43.9 million] people were living with HIV in 2017.
- 21.7 million [19.1 million–22.6 million] million people approximately were accessing antiretroviral therapy in 2017.
- 1.8 million [1.4 million–2.4 million] people became newly infected with HIV in 2017.
- 940 000 [670 000–1.3 million] people died from AIDS-related illnesses in 2017.
- 77.3 million [59.9 million–100 million] people have become infected with HIV since the start of the epidemic.
- 35.4 million [25.0 million–49.9 million] people have died from AIDS-related illnesses since the start of the epidemic.

  People living with HIV ▪ In 2017: 36.9 million [31.1 million–43.9 million] people living with HIV which includes:

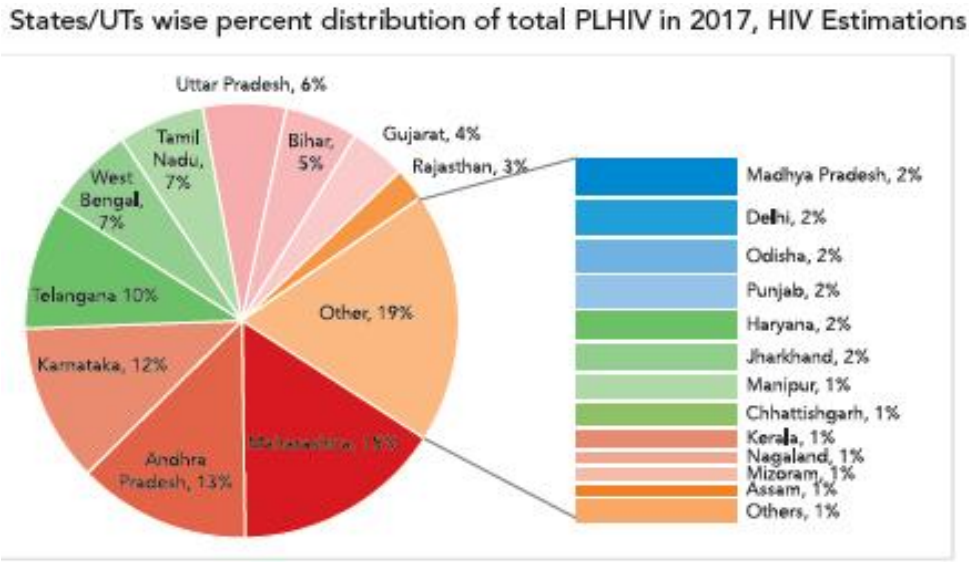
  - 35.1 million [29.6 million–41.7 million] adults
  - 1.8 million [1.3 million–2.4 million] children

75% [55–92%] of all people living with HIV knew their HIV status in 2017." [122]



Source: http://www.who.int/hiv/data/en/ date of download 2/10/18

According to WHO people living with HIV by WHO in 2017 were in Africa 25.7 million , which accounts for maximum number of people followed by South East Asia 3.5 million America 3.4 million, Europe 2.3 million, Western Pacific 1.5 million.



Source: GOI/NACO/MES/HIV Estimations 2017/100818 date of download 2/10/18

"In India according to NACO in 2017 people living with HIV (PLHIV)/AIDS The estimation show that India had around 21.40 lakh people. With 3.30 lakh [2.531-4.353] PLHIV, Maharashtra had the highest number of PLHIV contributing 15% to the total PLHIV size in the country. Andhra Pradesh [2.70 lakh, 2.005-3.581], Karnataka [2.47 lakh, 1.914-3.235] and Telangana [2.04 lakh, 1.495-2.773] are other States with PLHIV number in the range of 2 to 3 lakh. West Bengal had 1.44 lakh [1.038-1.914], Tamil Nadu 1.42 lakh [0.932-1.975], Uttar Pradesh 1.34 lakh [1.018-1.776] and Bihar 1.15 lakh [0.838-1.587] PLHIV in 2017. Together these 8 States contribute almost three fourths of total PLHIV in the country."[123]

Hepatitis C virus (HCV)

Hepatitis C virus (HCV) is the common cause of post transfusion hepatitis in developing countries

History

By the mid1970s,it was apparent that at least one viral hepatitis agent other than hepatitis A virus (HAV) or hepatitis B virus(HBV) was the primary agent of post transfusion hepatitis, a syndrome termed "nonA,nonB"hepatitis (NANBH).

Studies of transfusion recipients revealed that NANBH tended to be milder in its acute form than HBV but could cause severe complications including cirrhosis and liver failure. Inoculation of chimpanzees with blood components from humans having both acute and chronic NANBH resulted in characteristic elevations of hepatic transaminases, providing a valuable animal model for NANBH and establishing the chronic nature of NANBH. By the mid1980s, physicochemical studies of infectious inoculahadrevealed that the NANBH agent was a small (less than 80 nm), enveloped

virus; however, the agent efforts directed at conventional viral cultivation and immunological detection.

Serial passage of NANBH in chimpanzees provided key pathologic, physiologic, and biochemical insights, as well as a well-characterized pool of specimens in which the agent was known to be present. A team led by Michael Houghton assembled lambda phage library of complementary DNA (cDNA) derived from one such high-titer chimpanzee plasma specimen and then screened more than 1 million expression clones using serum from a chronic NANBH patient to and a single positive DNA clone called 5-1-1.116 This discovery led to initial assays for detection of antibodies to the newly named hepatitis C virus.[10]

Morphology

Hepatitis C virus is classified under family Flaviviridae, genus *Hepacivirus.* It is spherical, 60 nm size and enveloped. Nucleic acid contains a positive sense ssRNA. And proteins of HCV possesses three structural proteins such as nucleocapsid core protein C and two envelope glycoproreins El and E2 and Six non structural (NS) proteins. NS1, NS2, NS3, NS4A, NS4B, NS5A and NS5B.[14]

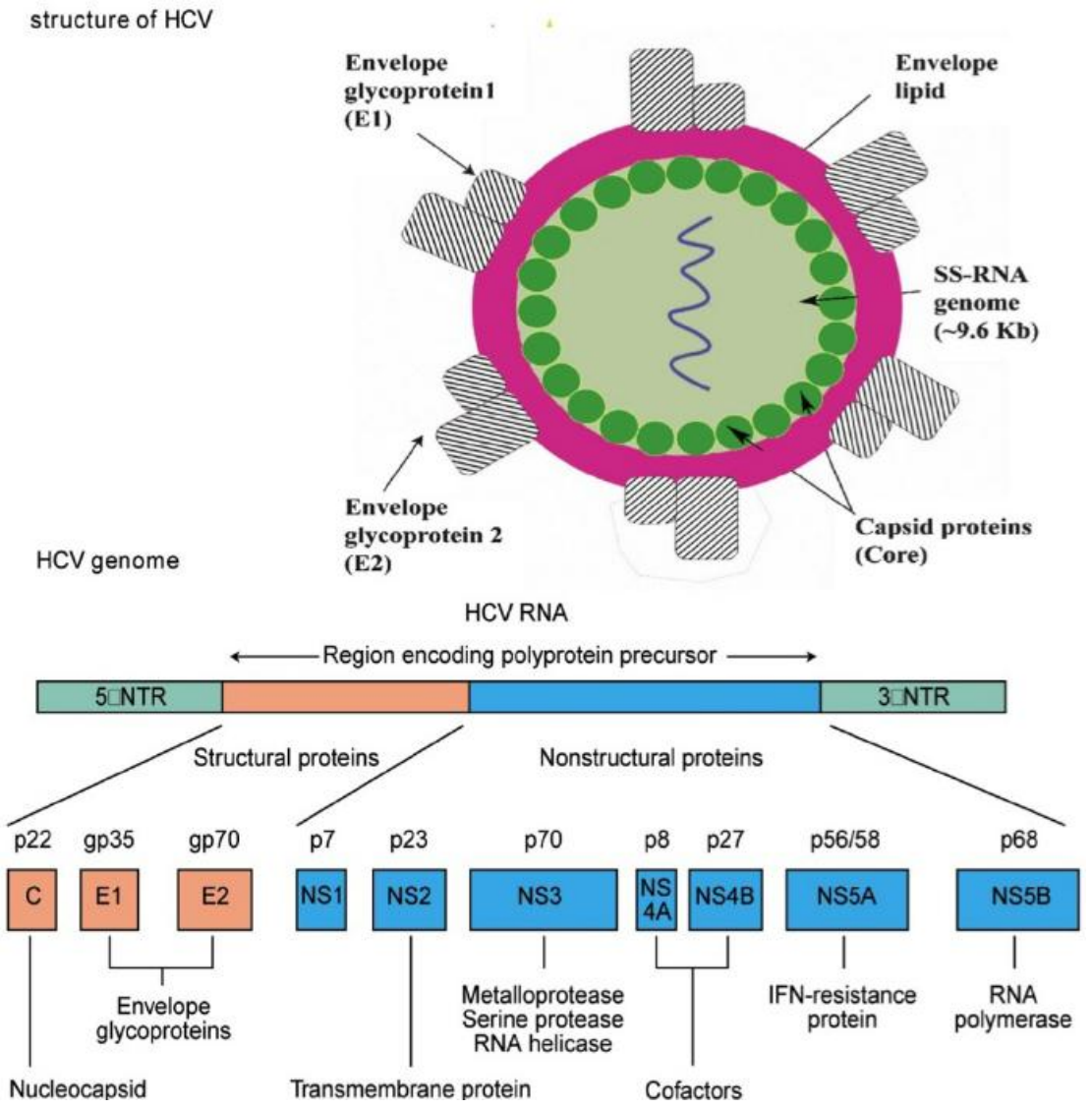


Figure 4: Hepatitis C virus structure

Genetic Diversity of HCV

Hepatitis C virus displays diversity in the RNA genome that occurs because of high rates of mutations seen in the virus. Genotypes of HCV is divided into six major genotypes or clades, which differ from each other by 25- 35% in their RNA sequence and Genotypes are further divided into more than 100subtypes, which differ from each other by 15- 25% in their RNA nucleotide sequence. Within any given patient, the subtypes of HCV circulate as complex closely related viral population known as

quasispecies. The E2 envelope proteinis the most variable region of the entire HCV genome followed by the non-structural proteins especially, NS5B encoded RNA polymerase, hence they are more prone to undergo mutations. Unfortunately, E2 protein happens to be the target against which most of the neutralizing antibodies are produced. Thus, diversity in the gene coding E2 protein enables the emergent mutant virus strains to escape from host's humoral immunity, which in turn can result in Establishment of chronic infection, Failure of development of effective vaccine, HCV genotypes do not vary in clinical severity but they vary in their epidemiological distribution: Genotype 1 is the most common type, distributed worldwide. Other genotypes are geographically restricted, e.g. genotype 4 (Egypt); genotype 5 (South Africa) and genotype 6 (Hong Kong).In India, genotypes 1 and 3 are more prevalent. The genotypes also vary in their susceptibility to antiviral drugs. Patients with genotype1b respond poorly to therapy than other genotypes.[14]

Transmission :

Various modes of transmission of HCV are such as Parenteral ,Vertical transmission and Sexual transmission. Parenteral is the most common it is most commonly transmitted through exposure to infectious blood. Recipients of contaminated blood transfusions, blood products or organ transplantations, Contaminated needles and sharps pricks Injection drug users. Vertical transmission from infected mother to fetus may occur but at much lower rate (6%) than that of HBV (20%). Sexual transmission is rare, Hepatitis C virus doesn't spread through breast milk, food or casual contacts including hugging or kissing.[14]

Epidemiology:

Hepatitis C virus infection occurs worldwide. Every year,3-4 million people are infected with HCV with more than 3.5 lakhs deaths.The most affected regions are WHO Eastern Mediterranean and European Regions, with the prevalence of 2.3% and 1.5% respectively. Prevalence of HCV infection in other WHO regions varies from 0.5% to 1.0%. Depending on the country, hepatitis C virus infection can be concentrated in certain populations (for example, among people who inject drugs) and/or in general populations. There are multiple strains (or genotypes) of the HCV virus and their distribution varies by region.Higher prevalence rates have been documented from Africa (up to10%) followed by South America and Asia, In India, the prevalence is about 1 % .[14]

HBV/ HIV coinfection

Due to the shared route of transmission, coinfection with HBV and human immunodeficiency virus (HIV) is common. The prevalence of HBV infection among HIV-infected patients may vary widely with risks for HIV and HBV transmission, different levels of endemicity of HBV infection general population, implementation of HBV vaccination programs, and also with the geographic regions. HBV and HIV have a impact on each other, HIV infection accelerates HBV-related liver damage, leading to earlier cirrhosis and end-stage liver disease,and the presence of HBV infection complicates the management of HIV infection, impairs CD4 recovery, accelerates immunologic progression, and increases the morbidity and mortality of HIV-infected patients.[69]

Worldwide , Prevalence of HBV–HIV coinfection About 2.7 million of the 36.7 million living with HIV are also infected with HBV. The worldwide prevalence of HBV infection in HIV-infected persons is 7.4%. The prevalence of HBV infection across different groups of HIV-infected persons is different according to risk factors , and in person with high risk activity it is (6.6%)or with higher risk behaviours, such as persons who inject drugs (7.0%) and men who have sex with men is (6.1%). Most HIV–HBV-coinfected persons live in sub-Saharan Africa (71%; 1.96 million). As HIV infected persons continue to live longer due to increased uptake of antiretroviral therapy, HBV coinfection is associated with accelerated progression of chronic hepatitis and higher liver-related mortality. From 2015, WHO has recommended treatment for everyone diagnosed with HIV infection, regardless of the stage of disease. However, by the end of 2016, only about50% of people with HIV were receiving treatment. WHO now recommends the use of tenofovir as part of first-line treatment for HIV and for the treatment of chronic HBV infection, including among pregnant HIV–HBV-coinfected women. Therefore, expansion of ten of over based treatment for HIV will give effective treatment for HBV infection for those who are coinfected with HIV and HBV, and will help prevent transmission of HBV from mother to child. But , data are lacking onthe actual coverage of tenofovir-based treatment for people who are coinfected with HIV and HBV.[42]

Prevelanace of HIV and HBV coinfection worldwide and India:

Study done in Nepal showed prevalence of 19%,Nigeria 12.1%, Phillipine 4.2% In India, the coinfection rate has been found to be 0.2–8% in peripheral HIV/sexually transmitted disease clinics and tuberculosis clinics. In the major metropolis areas, the rates are15% in Hyderabad, 9% in Chennai, and 5.3% in Delhi,

16.7% in Mumbai, 3% in Kolkata. Patients are higher with sexually transmitted HIV infection than compared to that of parent rally acquired HIV infection. [29,36, 49, 67,89, 90, 91, 92, 93, 94.]

HBV /HCV coinfection

The hepatitis B virus (HBV) and the hepatitis C virus (HCV) share common mode of transmission. Therefore, co-infection is common.In cases of HBV and HCV co-infection, the replication of either virus can be inhibited, virus can be dominantor the dominance can alternate between the two. It is more commonly seen for HBV to appear to be suppressed by HCV. [58]

Co-infections can appear in various manners:

a) Simultaneous acute infection with HBV and HCV: same source and transmission pathway, but indicates that the interaction between the two viruses is similar to that which occurs in chronic infections. There are descriptions of cases in which there is a delay in the identification of the Hepatitis B surface antigen (HBsAg), lower levels of alanine aminotransferase (ALT), and lower HBV antigenemia, which can be attributed to suppression of HBV activity by HCV.

b) Superinfection by one virus, the other virus beingc hronically present it should be suspected above all in individuals with risk factors, such as the use of illicit intravenous drugs, multi-transfused individuals, and those living in areas of high HBV prevalence.[58]

Prevalence of HBV and HCV worldwide and India

Prevalence in United States in ranges from1.3% to 11%,[95,96,97,98], In China 14.47%[99]and11.39%[100,] Mongolia in Children 1.2%,[101] and in Chronic liver disease patients 7.7%,[102]in Taiwan in HBsAg positive pateints- 3.4%-18%,[103-105]

Japan HBsAg+ 23%,[106]8%,[107] Saravanan et al [108] in India among the Chronic liver disease patients - 5.9 -16%% [109,110], in Iran among HBsAg positive 12.3% [111], in Yemen among Pregnant women 0%[112] and in Italy among HbsAg postive is 7%-15%[113,115]

C. Siva Kalyani et. al., at a tertiary care hospital at Visakhapatnam, Andhra Pradesh, India. found that12 (12%) were co infected with HBV in 100 HIV positive patients. Out of these 7(58.3%) were females and 5(41.7%) were males. Co infection was most commonly seen in the age group of 31-40 years and followed by 21–39 years. They used rapid test and ELISA methods to screen and HIV status was confirmed by three rapid tests.[117]

Naval Chandra et al at a tertiary care centre Hyderabad India, found that, of the 120 cases of HIV infected patients, coinfectiom with HBV was seen in 18 (15%). HIV was confirmed by Western Blot, HBsAg by ELISA method. [118]

Jutang B et al. in Mangalore India, found that, among 137 HIV patients, coinfection with HBV was seen 6.6%. 31 to 40 years were most common age group affected. Males (66.7%) were dominantly infected than females (33.3%).HIV confirmed as per National AIDS Control Organization guidelines. HBsAg by commercially available rapid tests and by ELISA. [9]

Gupta S et al. at north India, studied 837 HIV positive patients for coinfection of HBV and was seen in 61(7.28%). Of these, 54(88.4%) were males and 7(11.7%) females. The most common age group was 21 to 40 years. HBsAg screened by ELISA. [119]

Sureena Chouhan et al. of Ajmer, India found that ,Out of 256 HIV positive patients, HBV coinfection was seen in 12(4.68%). The highest prevalence was found among age group of 36-45 years (50%) and male were predominant i.e.9 (75%) and female were 3(25%). [12]

Goyal S et al. at Patiala North India, found that among 200 HIV patients coinfection with HBV was seen in 4.5% (9/200). Among them, major route of transmission was being heterosexual in 6/9 and unsafe injection in 2/9. The prevalence of both HBV and HCV infection was 0.5% (1/200). Coinfection was predominantly seen in males.[120]

Khunte P et al. at Chattisgarh India, found that ,Coinfection of HIV and HBV was 6 (6%) of cases and it was more in female patients and age group 30 to 40 years.[15]

Bhaumik P A (Tripura India)[6], found that, Among 453 HIV-positive patients, the co-infecion HBV was 17(3.75%). Males were predominant (88.23%) as compare to females (11.76%), The source of HBV infection in all the patients were heterosexual mode and majority of them infected from female sex worker through unprotected sex. Maximum patients were in age group of 29 to 40 years. They used enzyme-linked immune-sorbent assay to screen.[6]

G. Ionita et al. in Nepal, studied 677 People Leaving with HIV for HBsAg using, Rapid test and ELISA and found HBV co-infection is 4.4%. The age group with the highest rates of co-infection was between 30–39 years. In this study males were more predominant than females.[29]

In Mangalore, presence of HIV and HBsAg was screened by ELISA. Of the 24,576 sera from the patients were HIV positives, 285 (7%)were Hepatitis B surface antigen positive. Among the positive cases, a majority were between the age group of 21 to 40 years and males were predominant.[32]

Ishaku A et al. in Nigeria, Studied 200 sero- positive HIV patients and found 11% (22/200) were positive for HBV.[33]

Sinha SK et al. in eastern part of India screened 44,173 healthy voluntary donors. All blood samples were screened for HIV I & II, Hepatitis B surface Antigen . It was observed that 283 tested positive for HIV (0. 64%), 1001 (2.27%) were positive for HbsAg .[35]

Ekanem U.S. in Nigeria, studied 239 HIV patients were screened for the presence of HbsAg. The prevalence of HBV among HIV –infected patients in this study was 12.1%. [36]

Roshan Nikbakht in south west Iran, the screened HBsAg and HIV antibodies among infertile couple for HIV and HBV. Among 712 couple, 6 (0. 8) women and 5 (0. 7%) men were HBsAg positive and HIV was zero percent.[40]

A study done by Hamid Kalantari et al. in Iran, screened One thousand one hundred and sixty beta-thalassemia (545) and haemophilia (615) and were tested for

HBs Ag and HIV 10 (2%) had HBsAg positive. None of the thalassemia and hemophilia patients were positive for HIV Ab.[41]

S. K. Mustapha and Y.B. Jibrin in Nigeria. Found that among 200 HIV patients comprising 97 males and 103 females who were screened for HBsAg using ELISA. Prevalence of coinfection was 53(26.5%). Co-infection rate in males (24.7%) did not differ significantly from than that of females (28.2%). Co-infection was highest in the 40-49 years age group (41.60%), while no case of co-infection was recorded in the ≤ 19 years. Among the different occupational groups businessmen had the highest co-infection rate (44%) followed by long distance drivers (39.5%).[43]

Olanisun Olufemi Adewole et al. in Nigeria, found that out of 260 HIV patients 30(11.5%) had coinfection with HBsAg. Individuals younger than 40 years of age were more affected, male and female were equally affected that is50%.[44]

Joy Baseke in Uganda, included 89 HIV positive patients and 15 showed positive for HBsAg (16.9%). Hepatitis B was more prevalent among women 22.8% than men 6.2.[46]

Yumiko Yanase et al. in Philippine, studied over 1,44, 000 blood-screening results, the HIV prevalence was 0.006% in blood donars and 0.001% in overseas Filipino worker Candidates that of HBV was 4.2% in both groups. [49]

Mwumvaneza Mutagoma et al. at Rwanda, did a study in pregnant woman prevalence of HBsAg among 13,121 pregnant women was 3.7%.Coinfection with HIV-infection among HBsAg-positive pregnant women was 4.1% and co-infection was higher among women aged 15-24 years compared to those women aged 25–49 years.[52]

George Mondinde Ikomey in Buea, Cameroon, found that , among 97 HIV positive pregnant woman patients 14(14.43%) women were co-infected with HBV.[53]

Abioye et al. Karu, Nasarawa State at Nigeria, Found that among 250 HIV patients who were screened for HBsAg by using rapid test, 35 (14%) were positive for HBsAg. The highest prevalence rate 19.4% was recorded among patients within the age group of 51-60 years, while the lowest prevalence of 12.3% was seen among patients within the age group of 21-30 years. The prevalence was higher in the female than (14.3%) male (13.6%). [55]

Agarwal L et al. at Uttar Pradesh , included 3750 patients whose serum was tested for HBV and HCV for screening before any invasive/surgical procedure. Screening was done by rapid card test followed by the confirmation of all samples by enzyme immunoassay. Seroprevalence of HBV infection was found to be 147(3.9%). The prevalence for HBV infection among males and females were 5.5% and 2.4%, respectively. Sexual contact is found to be significantly associated with HBV infection. The highest seroprevalence of HBsAg was found in the age group of 35–44 years and 6(0.16%) were found to be coinfected with HCV. [7]

Mahajan S et al. at tanda ,included 5032 sera of patients in which HBsAg test was done by using Immunochromatography. Out of 5032 samples 71(1.41%) samples were positive for HBsAg. The males were most predominant i.e. 56.33% than females i.e.43.66%. Most common age group for HBV infection was between 15 to 60 years. Co-infection with HCV was seen in 6(8.45%) cases.[51]

In a study done by Supriya L. et al at Myanmar, consisted of 21358 serum samples who were screened for HBsAg and HCV antibodies by using rapid tests and

3rd generation ELISA. 75(1.57%) out of 4790 males were positive for HBsAg and whereas 132(0.8%) of 16568 females were positive for HBsAg. 21(0.44%) males and 5(0.03%) females were positive for both HBV and HCV infections. HBsAg seropositivity rates were maximum at 41- 50 years among positive males and at 21-30 years in females.[11]

Malhotra R. did a study in a patient who were on hemodialysis at Faridkot (Punjab), India. In this study patients of all age groups were tested for anti-HCV antibodies by fourth Generation HCV Tridot ELISA and for HBsAg by Hepalisa was used. Out of 262 patients 4 (1.5%) were found to be positive for HBsAg. Co-infection with HBV/HCV was observed in 2 (0.8%) patients. The majority of the patients were found to be age group of 41-60 years i.e.(41.3%) followed by 21-40 years (31.5%) and thereafter in 61-80 years (23.9%) and the lowest prevalence was observed in the age group of <20 years (2.2%) and >80 years (1.1%).[18]

Roy A. et al. at Northeast India, Included patients who inject drugs and other high risk groups. A total of 103 individuals of high risk groups comprised of PWID, unprotected sex with female sex worker, multiple partners, spouse of high risk people, men having sex with men and needle prick injuries. Serum were tested both for HBV and HCV by ElISA. Out of 103 cases, 87.4% were males and 12.6% females. Seroprevalence of HBsAg was 17.4%. The rate of HBV and HCV coinfection was 9.7%.[19]

Prakash S. et al. at lucknow included patients who were on hemodialysis. Out of 186 participants 6 (3.23%) patients were positive for HBsAg, comprising 3 (50%) males and 3 (50%) females. One(0.5%) patient had coinfection of HBV & HCV which was confirmed by both ELISA and PCR. Significant correlation was found

between number of blood transfusion and anti HCV antibody and HBsAg positivity. [20].

Ahmadi-Ghezeldasht S et al. in Iran, carried out HBsAg and anti-HCV antibody tests using ELISA. Total 284 HBsAg-positive individuals were taken, out of which 158 (55.6%) were male and 126 (44.4%)were female with mean age of 43 and 40 respectively. HBV and HCV coinfection was seen 1.41%.[28]

Tesfa H. et al. at Gondar university, Studied 2,684 clinically suspected hepatitis patients,14.4% were seropositive for HBV .The coinfection of HBV and HCV were found to be 36 (6.39%). And most common age group affected was 25–34 years old.[30]

METHODOLOGY

Study Centre: The present study was conducted at the Department of Microbiology, Jawaharlal Nehru Medical College, KAHER, Belagavi.

Source of data: All the sera which are received for HBsAg Screening at central lab of KLE'S Dr Prabhakar Kore charitable Hospital & MRC, Belagavi.

Study design: Cross-sectional study.

Study period: Period of one year from January 2017 to December 2017

Sample calculation: Average of last three years HBsAg positive sample at KLEs Dr Prabhakar Kore Charitable Hospital Lab is taken.

Average of last three years HBsAg positive sample is :

$$\frac{59+61+107}{3} = 75$$

Sample size: 75

Sampling Procedure: Universal sampling method

Inclusion Criteria: All the sera which are received for HBsAg screening and are found positive for HBsAg by Rapid test, of patients attending KLE's Dr. Prabhakar Kore Charitable Hospital and MRC, Belagavi.

Exclusion Criteria: Patients who are not ready to give consent to participate in HIV and HCV testing.

Statistical Analysis: Association of isolation and other variable will be done using Chi square test and categorical outcome will be studied using percentage.

Specimen collection: The first step which was used before collection was to positively identify the patient by asking name. Checked these against the requisition.. Later 2 cc syringe ,gloves and vacutainer were gatherd to withdraw blood.

Position the patient in a chair, or sitting or lying on a bed as required was done.

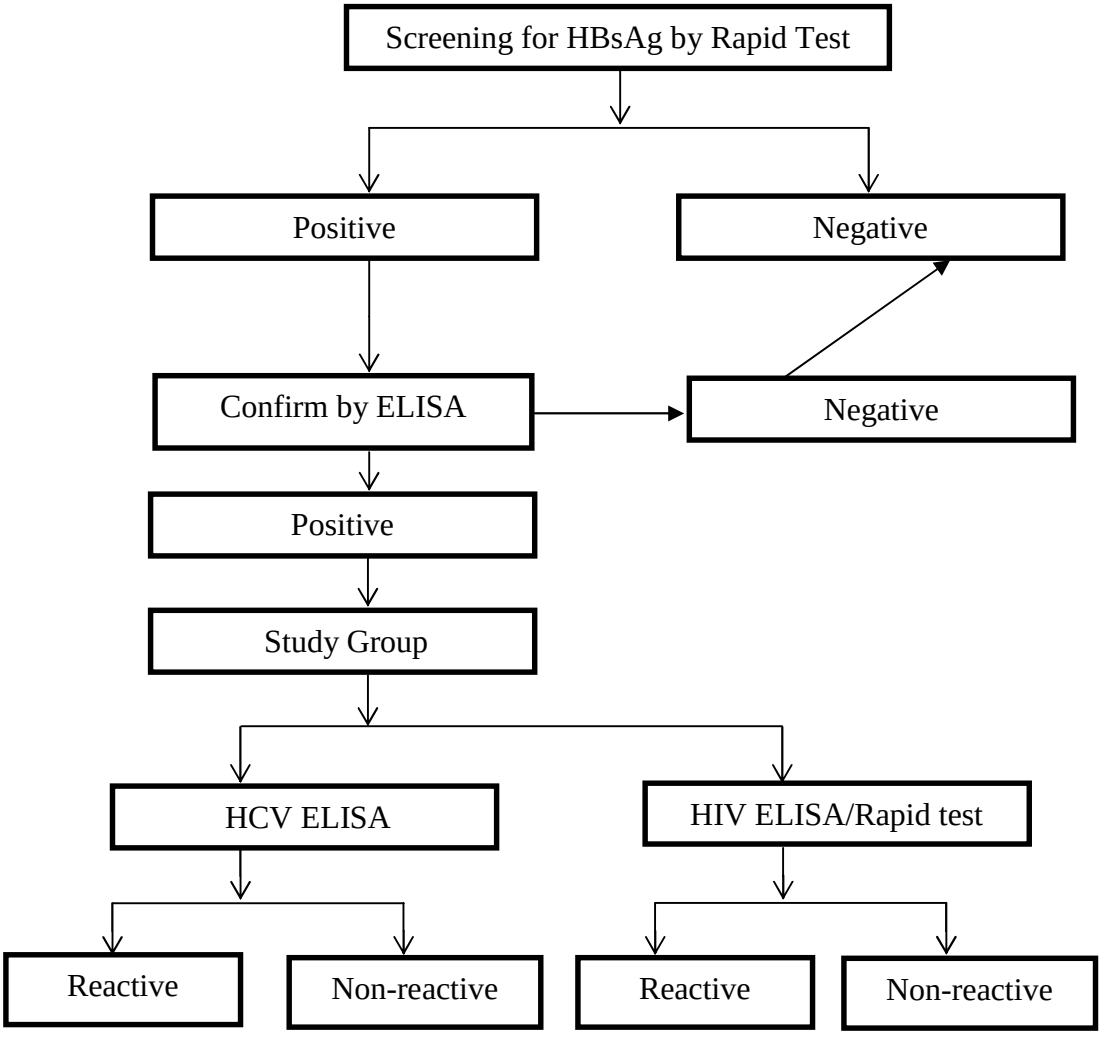
1. First hands were wash by me before collecting.
2. A suitable site was selected for venipuncture, and preferably left hand of patient was selected by placing the tourniquet 3 to 4 inches above the selected puncture site on the patient.
3. Tourniquet was not too tightly placed.
4. Next, non-latex gloves, were put and palpated for a vein.
5. When a vein was selected, area was cleansed in a circular motion with 70% ethyl alcohol, beginning at the site and working outward. Allowed the area to air dry.
6. Asked the patient to make a fist. And the patient's arm was grasped firmly using your thumb to draw the skin taut and anchor the vein.
7. Swiftly inserted the needle through the skin into the lumen of the vein. The needle was kept approximately in form a 15-30 degree angle with the arm surface. And excess probing was avoided.
8. Slowly the tourniquet was removed.
9. And 2 ml of blood was collected in syringe and later later transferd to vacutainer.

10. Removed the needle from the patient's arm using a swift backward motion.

11. Placed gauze immediately on the puncture site. Applied and held adequate pressure to avoid formation of a hematoma. After holding pressure for 1-2 minutes, taped a fresh piece of gauze or Band-Aid to the puncture site.

12. Disposed of contaminated materials/supplies in designated containers.

Overview of sample processing:



NAME OF THE TEST: Hepa (HBsAg) card an rapid immunochromatographic assay.

MANUFACTURER: Reckon Diagnostics P limited. 3/7, Industrial Estate, Gorwa, Baroda - 390 016

PRINCIPLE :

HEPACARD is a one step immunoassay based on the antigen capture, or "sandwich" principle. The method uses monoclonal antibodies conjugated to colloidal gold and polyclonal antibodies immobilized on a nitrocellulose strip in a thin line. The test sample is introduced to and flows laterally through an absorbent pad where it mixes with the signal reagent. If the sample contains HBsAg, the colloidal gold-antibody conjugate binds to the antigen, forming an antigen-antibody-colloidal gold complex .The complex then migrates through the nitrocellulose strip by capillary action. When the complex meets the line of immobilized antibody (Test line) "T", the complex is trapped forming an antibody-antigen-antibody colloidal gold complex. An additional line of anti-mouse antibody (Control line) "C", has been immobilized at a distance from the test line on the strip. If the test is performed correctly, this will result in the formation of a pink band upon contact with the conjugate.

STORAGE AND SHELF LIFE:

HEPACARD is stored at 2-30ºC in the coolest and driest area available. It has a shelf life of 24 months from the date of manufacturing. The HEPACARD should not be frozen and must be protected from exposure to humidity.

TEST PROCEDURE

1. Bring the HEPACARD foil pouches and specimen to room temperature prior to testing.
2. Take out HEPACARD device from the foil pouch.
3. Label the test card with patient's name or identification number.
4. Add 2 drops (70 µl) of human serum/plasma specimen into the sample well using the dropper.
5. Allow reaction to occur during the next 20 minutes.
6. Read results at 20 minutes.
7. Discard the HEPACARD immediately after reading result at 20 minutes, considering it to be potentially infectious.

INTERPRETATION OF RESULT

Positive:

Appearance of pink colored line, one each in test region "T" and control region "C" indicates that the sample is POSITIVE.

Negative:

Appearance of one distinct pink line in the control region "C" only, indicates that the sample is "NEGATIVE" for HBsAg.

INVALID :

When neither control line nor the test line appears on the membrane the test should be treated as invalid.

NAME OF THE TEST: Hepalisa Microwell ELISA Test for the Detection of Hepatitis B Surface Antigen (HBsAg) in Human Serum/ Plasma.

MANUFACTURER: J. Mitra & Co. Pvt. Ltd. A 180-181, Okhla Ind. Area, Ph-1, New Delhi-110 020, INDIA

PRINCIPLE :

HEPALISA is a solid phase enzyme linked immunosorbent assay (ELISA) based on the "Direct Sandwich" method. The microwells are coated with Monoclonal antibodies with high reactivity for HBsAg. The samples are added in the wells followed by addition of enzyme conjugate (polyclonal antibodies linked to Horseradish Peroxidase (HRPO)). A sandwich complex is formed in the well wherein HBsAg (from serum sample) is "trapped" or "sandwiched" between the antibody and antibody HRPO conjugate. Unbound conjugate is then washed off with wash buffer.

The amount of bound peroxidase is proportional to the concentration of HBsAg present in the sample. Upon addition of the substrate buffer and chromogen, a blue colour develops. The intensity of developed blue colour is proportional to the concentration of HBsAg in sample. To limit the enzyme-substrate reaction, stop solution is added and a yellow colour develops which is finally read at 450nm spectrophotometrically.

SPECIMEN COLLECTION & HANDLING:

1. Human serum or plasma samples should be used for the test. Fresh serum/plasma samples are preferred.

2. Specimens should be free from microbial contamination and may be stored at 2-8^{0}C for one week, or frozen at -20^{0}C or lower. Repeated freezing and thawing should be avoided.

3. Do not use Sodium Azide as preservative because it inactivates enzyme Horseradish peroxidase.

STORAGE AND STABILITY:

Store all components at 2-8^{0}Cwhen not in use.

ADDITIONAL MATERIAL AND INSTRUMENTS REQUIRED:

1. Micropipettes and microtips.
2. Timer.
3. Elisa reader.
4. Elisa washer
5. Distilled water.
6. Incubator 37^{0}C
7. Graduated Cylinders, for reagent preparation
8. Disposable gloves
9. Absorbent tissue

TEST PROCEDURE:

Fit the strip holder with the required number of strips. Arrange the assay control wells in a horizontal or vertical configuration and Configuration is dependent upon reader software.

1. Add 100 µl Negative Control in each well No. A-1 and B-1 respectively.
2. Add 100 µl Positive Control in C-1 & D-1 wells.
3. Add 100 µl of sample in each well, starting from E1.
4. Add 50µI of working Enzyme conjugate to each well. Gently shake the plate for 2-3 seconds to mix the sample & conjugate.
5. Cover the plate and incubate in an incubator at $37^{o}C + 1^{o}C$ for 60 minutes.
6. Dilute the wash buffer concentrate with distilled water to 1:25 dilution.
7. At the end of incubation period, take out the plate from incubator and wash with working wash buffer.

WASHING:

Washing can be performed either with ELISA WASHER as follows:

a) Empty the wells

b) Add 300-350µl of working washing solution into each well and give a soak time of 30 seconds.

c) Empty the wells.

d) Wash each well six times.

e) After the sixth wash, tap dry the Microwells a few times on an absorbent tissue.

f) Tap dry the wells after washing and add 100µI of working substrate solution in all the wells.

8. Cover the plate with an aluminium foil and incubate at room temperature (20-25°C) for 30 minutes in dark.

9. Stop the reaction by adding 100µl of stop solution to each well, mix gently.

10. Read absorbance at 450 nm. within 30 minutes in ELISA READER.

CALCULATION OF RESULTS:

Compute mean of negative and positive control absorbance.

Test Validity :

Positive Control Acceptance Criteria:

PC or PCx must be >0.5. If it is not so, the run is invalid and must be repeated.

1.430 C1 Well

PC 1.500 D1 Well

Total 2.930

Mean absorbance, PCx = 2.930/2 = 1.465

Negative Control Acceptance Criteria:

NC must be < 0.150

0.012 A1 Well

NC 0.010 B1 Well

Total 0.022

Mean absorbance **NCx**= 0.022/2 = 0.011

Cut-off Value

Cut-off value can be determined by using the following formula:

Cut-off Value = NCx + 0.1

Where NCx is mean absorbance (O.D) of Negative Control.

e.g. 0.011 +0.1 = 0.111

INTERPRETATION OF RESULTS :

a) Test specimens with absorbance (O.D.) value less than cut-off value are non reactive and may be considered as negative for HBsAg.

b) Test specimens with absorbance (O.D.) value greater than or equal to cut off value are positive for HBsAg by HEPALISA.

c) Test specimens with absorbance value within 10% below the cut off should be considered suspect for the presence of HBsAg and should be retested in

duplicate. Further confirmation by other EIA assays or confirmatory assays are recommended.

NAME OF THE TEST: MERISCREEN HIV 1-2 WB.HIV rapid wholw blood finger prick test

MANUFACUTURE: Meril Diagnostics.

PRINCIPLE:

Micro screen HIV 1-2 WB, The strips/cards incorporate both the antigen and signal reagent into the nitrocellulose strip. The specimen (usually followed by a buffer) is applied to the absorbent pad on the kit. The specimen migrates through the strip and combines with the signal reagent. A positive reaction results in a visual line on the membrane where the HIV antigen has been incorporated. A procedural control is usually incorporated into the strip The test device is incorporated with distinct bands of purified gp120 and gp41 synthetic peptides, specific to HIV-1 at test region '1' and gp36 synthetic peptide specific to HIV-2 at test region '2.' The third band incorporated at region 'C,' corresponds to the assay performance control. If present, antibodies to HIV-1 and/or 2 are captured by the respective antigens. After washing with a buffer, the Protein A conjugated reagent is added to reveal the presence/absence of bound antibodies. Post-final wash, a positive reaction is visualized by the appearance of colored bands at specific sites. The absence of bands at test region '1' & '2' is a negative test result. The appearance of a control band validates the test.

TEST PROCEDURE

1. Bring the card and specimen to room temperature prior to testing.
2. Label the test card with patient's name or identification number.
3. Add 2 drops (70 µl) of human serum/plasma specimen into the sample well using the dropper.
4. Allow reaction to occur during the next 20 minutes.
5. Read results at 20 minutes.
6. Discard the card immediately after reading result at 20 minutes, considering it to be potentially infectious

INTERPRETATION OF RESULTS:

Negative : Appearance of only the control band, correspondingtocontrolregion'C'

Positive : In addition to the control band 'C,' appearance of reactive band at test region '1' Specimen positive for antibodies to HIV-1. In addition to the control band 'C,' appearance of reactive band in test region '2' Specimen positive for antibodies to HIV- 2. In addition to the control band 'C,' appearance of reactive bands at test region '1' and test region '2' Specimen positive for antibodies to HIV-1 and HIV-2. Invalid test result:

Invalid: The test should be considered invalid if neither the test band nor the control band appears. In case of invalid test, repeat the test using a new device.

NAME OF THE TEST: Microlisa - HIV Microwell ELISA Test for the Detection of Antibodies to HIV-1 (Including Group O & Subtype C) and HIV-2 in Human Serum/ Plasma

MANUFACTURER: J. Mitra & Co. Pvt. Ltd. A 180-181, Okhla Ind. Area, Ph-1, New Delhi-110 020, INDIA

PRINCIPLE:

Microlisa HIV test is an enzyme immunoassay based on Indirect ELISA.HIV envelope proteins gp41, C terminus of gp 120 for HIV-1, and gp 36 for HIV-2 representing immune dominant epitopes are coated onto microtiter wells. Specimens and controls are added to the microtiter wells and incubated. Antibodies to HIV-1 and HIV-2 if present in the specimen, will bind to the specific antigens absorbed onto the surface of the wells. Horseradish peroxidase (HRP) conjugated antihuman IgG is added to each well. This conjugate will bind to HIV antigen antibody complex present. When substrate solution containing chromogen and hydrogen peroxide is added a blue colour will develop in proportion to the amount of HIV-1 and / or HIV-2 antibodies present in the specimen. The colour reaction is stopped by a stop solution. The enzyme substrate reaction is read by EIA reader for absorbance at a wavelength of 450 nm.

REQUIREMENT:

1. Micropipettes and microtips.
2. Timer
3. Elisa reader

4. Elisa washer
5. Distilled or deionized water
6. Incubator 37^0C
7. Graduated Cylinders, for reagent dilution
8. Vortex Mixer
9. Sodium hypochlorite solution
10. Disposable gloves
11. Absorbent tissue
12. Glassware

SPECIMEN COLLECTION&PREPARATION:

1. Only human serum or plasma samples should be used for the test. While preparing Serum samples, remove the serum form the clot as soon as possible to avoid hemolysis, Fresh serum/plasma samples are preferred.
2. Specimens should be free of microbial contamination and may be stored at 2-8ºC for one weeks, or frozen at -20ºC or lower. Avoid repeated freezing and thawing.
3. Use of heat inactivated, icteric hyperlipemic and hemolyzed and Icteric hyper lipemic samples should be avoided as may give erroneous results.

TEST PROCEDURE

Fit the strip holder with the required number of Microlisa-HIV strips. Arrange the assay control wells so that well A-1 is the reagent blank. From well A-1 arrange all controls in a horizontal or vertical configuration. Configuration is dependent upon reader software.

1. Add 100 µl sample diluent to A-1 well as blank.
2. Add 100µl Negative Control in each well no. B-1 & C-1 respectively. Negative Control is ready to use and hence no dilution is required.
3. Add 100µl Positive Control in D-1, E-1 & F-1 wells. Positive Control is ready to use and hence no dilution is required.
4. Add 100 µl sample diluent in each well starting from G-1 followed by addition of 10µlsample.
5. Apply cover seal.
6. Incubate at 37ºC+2ºC for 30min.+2min.
7. While the samples are incubating, prepare Working Wash Solution and Working Conjugate as specified in Preparation of Reagents.
8. Take out the plate from the incubator after the incubation time is over and, wash the wells.
9. 5 times with Working Wash solution according to the wash procedure given in the previous section (wash procedure).

10. Add 100 µl of Working Conjugate Solution in each well including A-1.

11. Apply cover seal.

12. Incubate at 37ºC+2ºC for 30min.+2min.

13. Aspirate and wash as described in step no. 8.

14. Add 100 µl of working substrate solution in each well including A-1.

15. Incubate at room temperature (20 - 30ºC) for 30 min. in dark.

16. Add 100 µl of stop solution.

17. Read absorbance at 450 nm within 30 minutes in ELISA READER after blanking A-1 well.

WASHPROCEDURE:

1. Incomplete washing will adversely affect the test outcome.

2. Aspirate the well contents completely into a waste container. Then fill the wells completely with wash buffer avoiding overflow of buffer from one well to another and allow to soak (approx. 30 seconds). Aspirate completely and repeat the wash and soak procedure 4 additional times for a total of 5 washes.

3. Automated washer if used should be well adjusted to fill each well completely without over filling.

4. Tap upside down on absorbent sheet till no droplets appear on the sheet, taking care not to dislodge the wells.

CALCULATION OF RESULTS

Abbreviations

NC - Absorbance of the Negative Control

NCx - Mean Negative Control

PC - Absorbance of the Positive Control

PCx - Mean Positive Control

Blank acceptance Criteria

Blank must be <0.100 in case of differential filter being used. In case differential filter is not available in the reader the blank value may go higher.

Negative Control Acceptance Criteria:

NC must be<0.150. If it is not so, the run is invalid and must be repeated.

Positive Control Acceptance Criteria:

1. PC must be>0.50
2. Determine the mean (PCx) value If one of three positive control values is outside of these limits, recalculate PCx based upon the two acceptable positive control values.
3. If two of the three positive control values are outside the limits, the assay is invalid and the test must be repeated.

CUT OFF VALUE

Absorbance (O.D.)

NC - 0.042 B1Well PC - 1.412 D1Well

- 0.040 C1Well - 1.392 E1Well

Total : 0.082 2Wells - 1.407 F1Well

Total : 4.211 3Wells

NCx=0.082/2=0.041 PCx=4.211/3=1.403

The cut off value is calculated by adding Mean Negative Control (NCx) and Mean Positive

Control (PCx) as calculated above and the sum is divided by 6.

NCx+PCxNCx=0.041

Cut off Value= e.g.

6 PCx=1.403

Cut off Value=0.041+1.403=1.444=0.240

6 6

INTERPRETATION OF RESULTS

1. Test specimens with absorbance value less than the cut off value are non-reactive and may be considered as negative for anti-HIV.

2. Test specimens with absorbance value greater than or equal to the cut off value are reactive for anti-HIV by Microlisa-HIV.

Note: Test specimens with absorbance value within 10% below the cut off should be considered suspect for the presence of antibodies and should be retested in duplicate.

1. Specimens with absorbance value equal to or greater than the cut off value are considered initially reactive by the criteria of Microlisa-HIV. Original specimen should be retested in duplicate.

NAME OF THE TEST: HCV Microlisa Microwell ELISA Test for the Detection of Antibodies to Hepatitis C virus in Human Serum/ Plasma

MANUFACTURER: J. Mitra & Co. Pvt. Ltd. A 180-181, Okhla Ind. Area, Ph-1, New Delhi-110 020, INDIA

PRINCIPLE :

The 3rd generation HCV Microlisa is based on a highly sensitive technique, Enzyme LinkedImmunosorbent Assay which detects antibodies against HCV in human serum and plasma. The 3rd generation HCV Microlisa utilises a combination of antigen with the sequence of both HCV structural and non-structural antigen i.e. CORE, E1, E2, NS3, NS4 and NS5. The combination of antigens for the structural and non-structural HCVproteins are coated onto the microwells. Diluted sample and controls are then incubated. Antibodies to HCV, if present, bind to the immobilized HCV antigens on the micro well. An enzyme conjugate, anti-human IgG conjugated with HRPO is added. At this stage the microwellshold only the bound antigen-anti HCV-enzyme conjugate complex The enzyme substrate reaction leads to development of a blue colour which is indicative of the Ag-Ab reaction which has occurred in the microwell. In the final step the Stop Solution is added and the optical density of the developed colour is read photometrically.

STORAGE AND STABILITY

Store all components at 2-8ºC when not in use.

ADDITIONAL MATERIAL AND INSTRUMENTS REQUIRED

1. Micropipettes and microtips.
2. Timer
3. Elisa reader
4. Elisa washer
5. Distilled or deionized water
6. Incubator 37^0C
7. Graduated Cylinders, for reagent dilution
8. Vortex Mixer
9. Sodium hypochlorite solution
10. Disposable gloves
11. absorbent tissue
12. Glassware

SPECIMEN COLLECTION & PREPARATION

1. Human serum or plasma samples should be used for the test. Fresh serum/plasma samples are preferred.

2. Specimens should be free from microbial contamination and may be stored at 2-8^{0}C for oneweek, or frozen at -2^{0}C or lower. Repeated freezing and thawing should be avoided.

TEST PROCEDURE:.

Fit the stripholder with the required number of HCV Microlisa strips.

The instructions of the procedure must be strictly followed. Arrange the assay control wells in a horizontal or vertical configuration. Configuration is dependent upon ELISA reader software.

1. Add 100µl Negative Control in well No. A-1. Negative control is ready to use & hence no dilution is required.

2. Add 100µl Positive Control in B-1, C-1 & D-1 wells. Positive control is ready to use & hence no dilution is required.

3. Add 100µl Sample Diluent in each well, starting from E-1 well followed by addition of 10µlsample.

4. Apply cover seal and incubate at $37^{o}C + 2^{o}C$ for 30 mins. + 2 minutes.

5. While the samples are incubating, prepare working wash solution and working conjugate as specified in "preparation of reagents".

6. After the incubation is over, wash the wells 6 times with working wash solution according to the wash procedure given in the previous section.

7. Add 100 µl of Working Conjugate Solution in each well.

8. Apply cover seal & incubate at $37^{o}C + 2^{o}C$ for 30 mins. + 2 mins.

9. While conjugate is incubating, prepare substrate solution in last 5 minutes of incubation as specified in "preparation of reagents". **Protect from light.**

10. Aspirate and wash the wells after incubation as described in step no. 6.

11. Add 100 µl working substrate solution in each well.

12. Incubate at room temperature (20-30^{o}C) in dark for 30 mins.

13. Add 100 µl of stop solution.

14. Read absorbance at 450 nm in ELISA READER.

CALCULATION OF RESULTS:

Positive Control Acceptance Criteria:

PC or PCx must be > 0.5. If it is not so, the run is invalid and must be repeated.

- 1. 897 B1 Well

PC - 1. 855 C1 Well

- 1. 858 D1 Well

Total 5. 610

PCx = 5.610/3 = 1.870

Negative Control Acceptance Criteria:

NC must be < 0.150. If it is not so, the run is invalid and must be repeated.

CUT-OFF VALUE

The cut-off value is calculated as below:

Cut off Value = PCx X 0.23

e.g. PCx = 1.87 then

Cut off Value = 1.87 X 0.23 = 0.430

INTERPRETATION OF RESULTS :

1. Test specimens with absorbance value less than the cut-off value are non-reactive for Anti-HCV.

2. Test specimens with absorbance value greater than or equal to the cut-off value are reactive for Anti-HCV.

3. Test specimens with absorbance value within 10% below the cutoff should be considered Suspect for the presence of antibodies and should be retested in duplicate.

Photograph 1.Hepa (HBsAg) card Kit

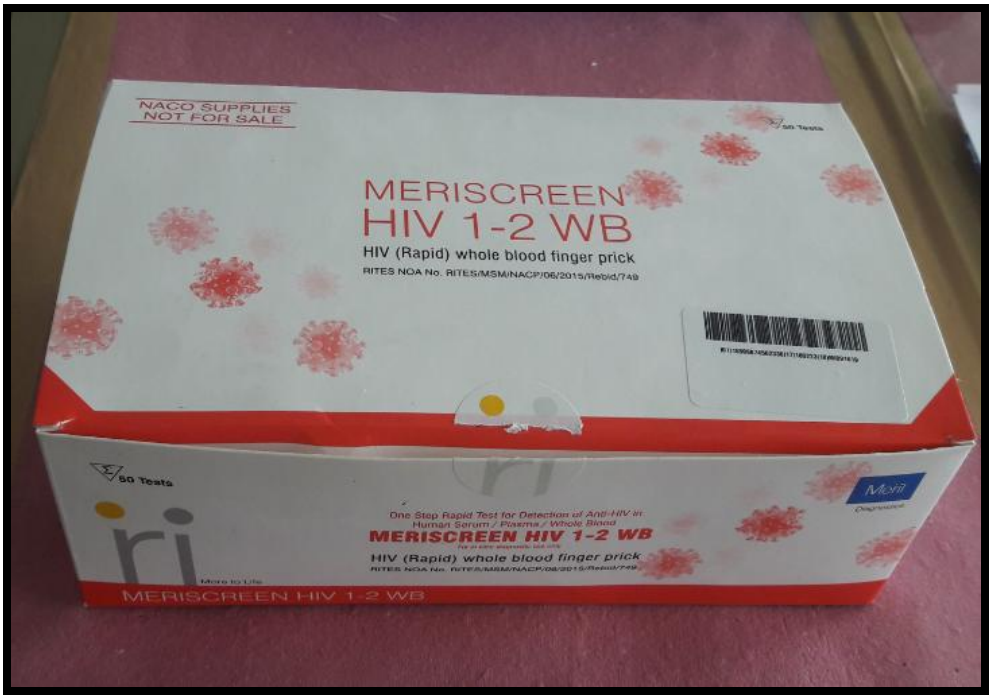
Photograph 2: Hepa (HBsAg) Card Test Result

Positive **Negative**

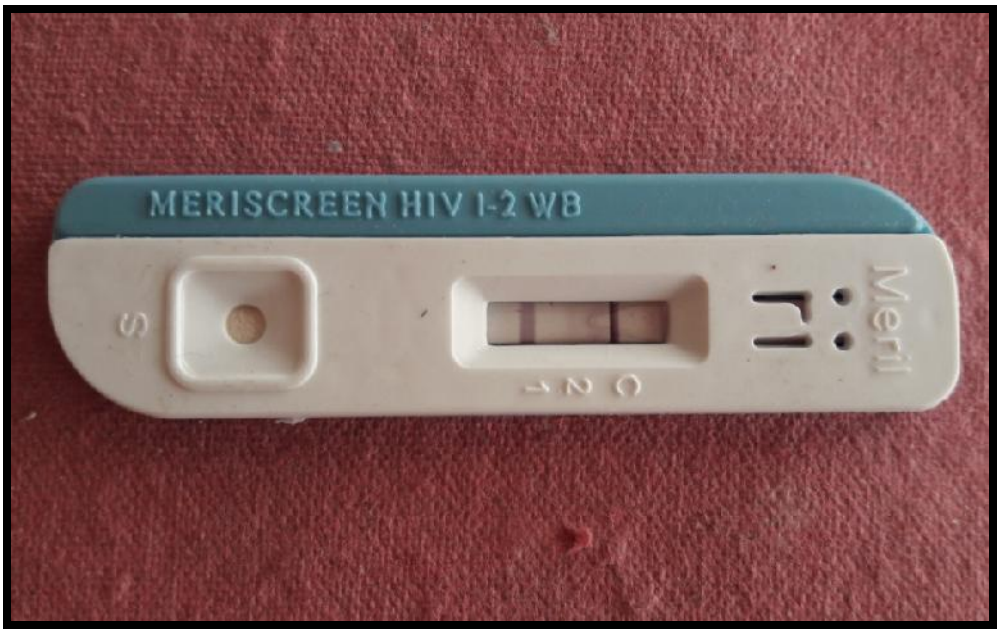
Photograph 3:Hepalisa (HBsAg ELISA Kit)

Photograph 4:Microlisa (HIV ELISA Kit)

Photograph 5: Meriscreen HIV 1-2 WB (Rapid Test kit)



Photograph 6: HIV 1 Meriscreen Rapid Test Reactive



Photograph 7:Microlisa(HCV ELISA Kit)

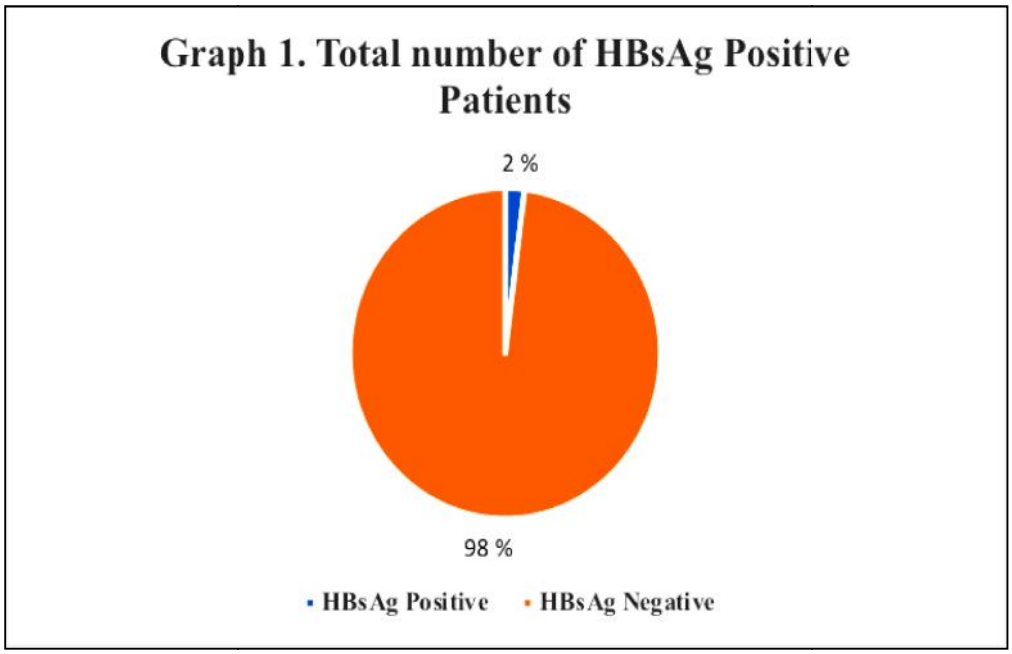
Photograph 8:Performing ELISA Test

RESULTS

All the sera which were received for HBsAg Screening at central lab of KLE'S Dr. Prabhakar Kore charitable Hospital & MRC, Belagavi, during period of January 2017 to December 2017 were taken. Total 3278 patients were screened for HBsAg, out of which 75(2%) HBsAg positive sera were further tested to study prevalence of coinfection with HIV and HCV by ELISA method.

Table 1. Total Number of HBsAg Positive Patients

	Patients	
	Number	**Percentage**
HBsAg Positive	75	2.00
HBsAg Negative	3203	98.00
Total	**3278**	**100**

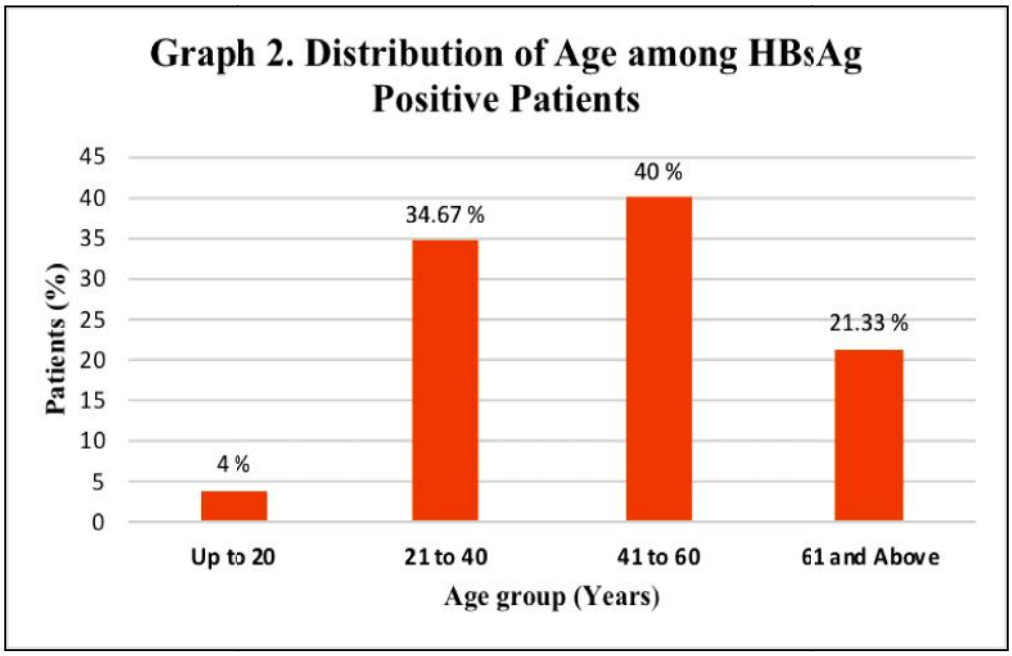


In present study -

3278 patients were screened for HBsAg and 75(2%) showed positive for HBsAg via card test which was later confirmed by ELISA method.

Table 2. Distribution of Age among HBsAg Positive Patients

Age (Years)	Patients	
	Number	Percentage
Up to 20	3	4.00
21 to 40	26	34.67
41 to 60	30	40.00
61 and Above	16	21.33
Total	**75**	**100**

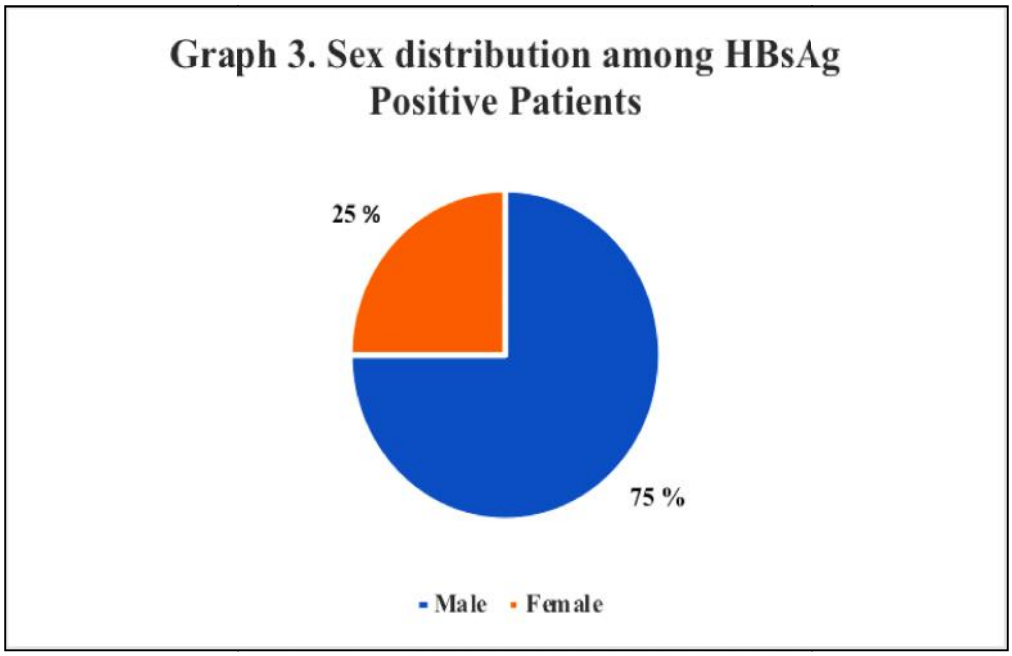


In present study-

Maximum patients were between age group of 41 to 60 yrs (40%), followed by 21 to 40 yrs (34.46%), 61 and above (21.33%), and upto 20 yrs (4%). Minimum age was 18 and maximum was 78 yr old.

Table 3. Sex distribution Among HBsAg Positive Patients

Sex	Patients	
	Number	Percentage
Male	56	75.00
Female	19	25.00
Total	**75**	**100**

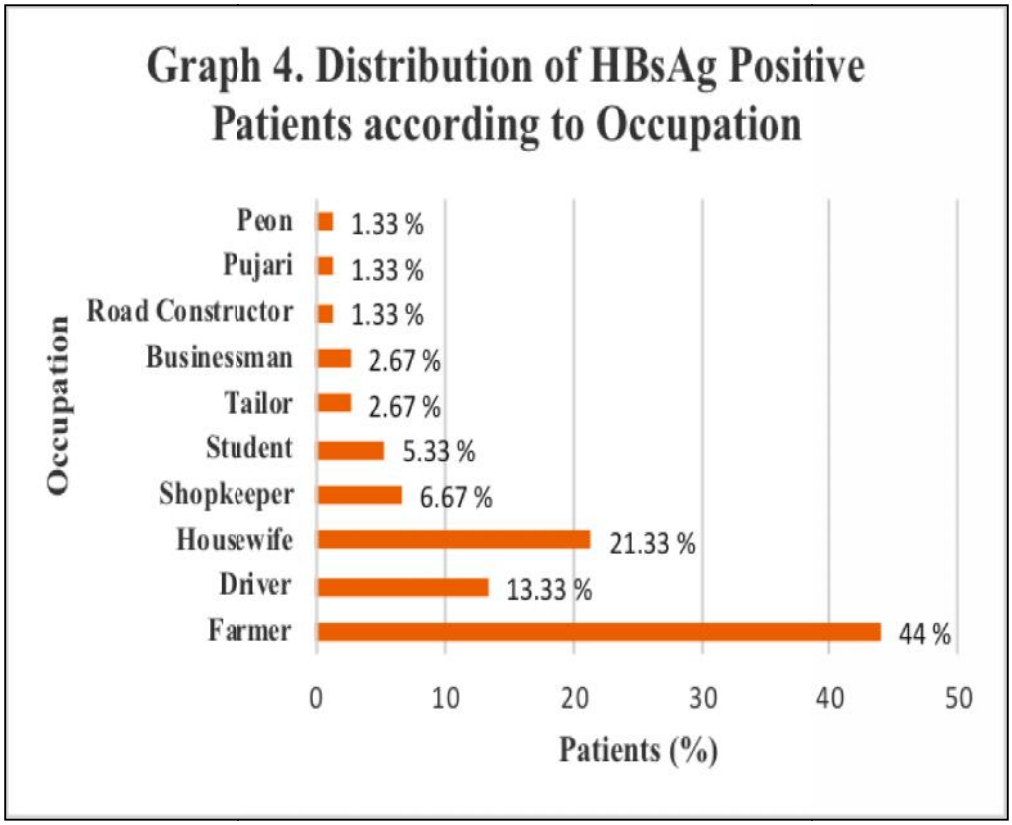


In present study-

Among 75 HBsAg positive patients male were more predominant 56 (75%) than female 19 (25%).

Table 4. Distribution of HBsAg Positive patients according to Occupation

Occupation	Patients	
	Number	**Percentage**
Farmer	33	44.00
Driver	10	13.33
Housewife	16	21.33
Shopkeeper	5	6.67
Student	4	5.33
Tailor	2	2.67
Businessman	2	2.67
Road Constructor	1	1.33
Pujari	1	1.33
Peon	1	1.33
Total	**75**	**100**

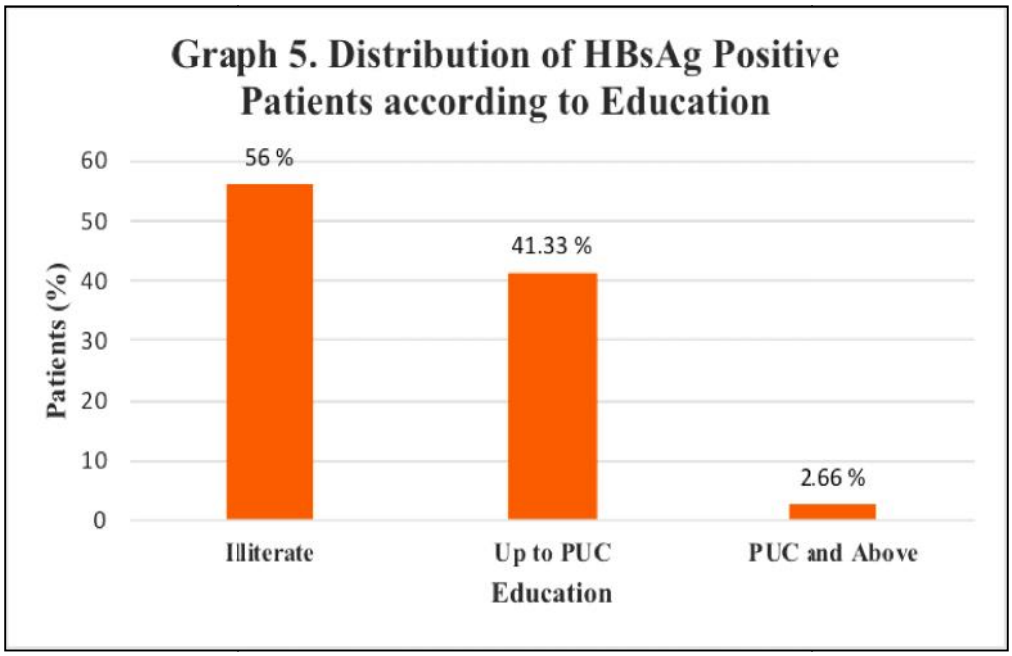


In present study-

Most common occupation among HBsAg Positive patients was Farmer 33 (44%) followed by Driver 10 (13.33%), Housewife 16 (21.33%), Shopkeeper 5 (6.67%) Student 4 (5.33%) ,Tailor 2 (2.67%) , Businessman 2 (2.67%) , Road Constructor 1 (1.33%) , Pujari 1 (1.33%), Peon 1(1.33%).

Table 5. Distribution of HBsAg Positive patients according to Education

Education	Patients	
	Number	Percentage
Illiterate	42	56.00
Up to PUC	31	41.33
PUC and Above	2	2.66
Total	**75**	**100**

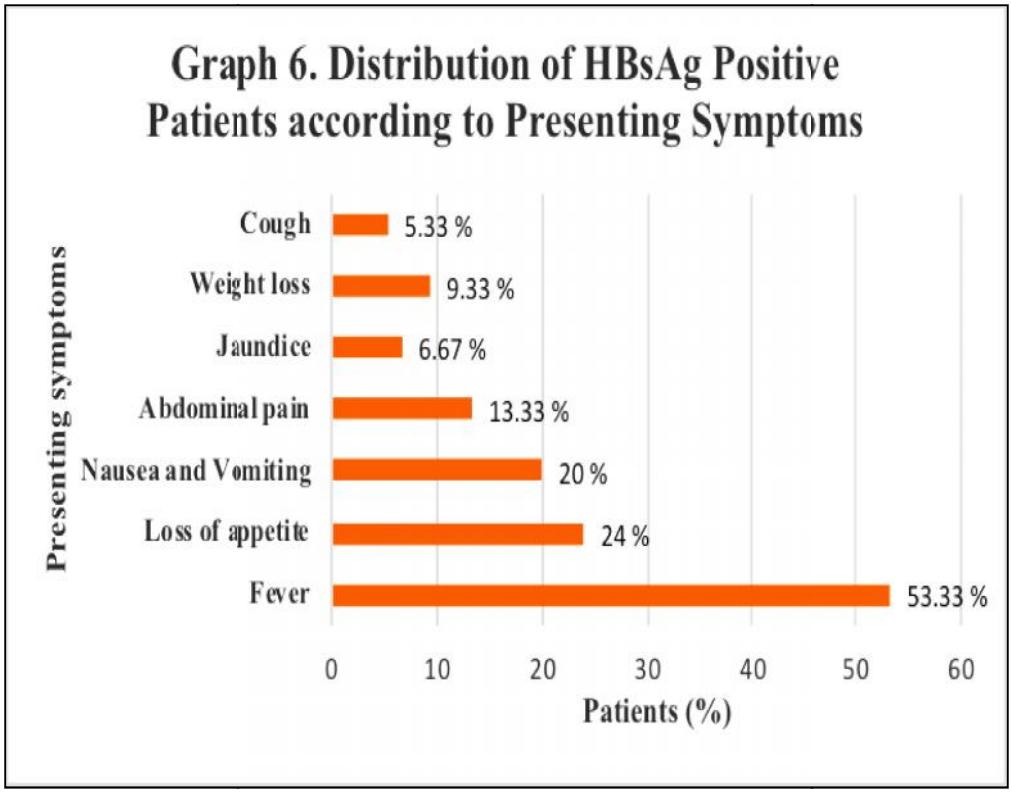


In present study-

Out of 75 HBsAg positive patients maximum patients were illiterate 42(56%) , upto PUC 31(41.33%) and PUC and above 2 (2.66%).

Table 6. Distribution of HBsAg Positive Patients according to Presenting symptoms

Symptoms	Patients	
	Number	**Percentage**
Fever	40	53.33
Loss of appetite	18	24.00
Nausea and Vomiting	15	20.00
Abdominal pain	10	13.33
Jaundice	5	6.67
Weight loss	7	9.33
Cough	4	5.33

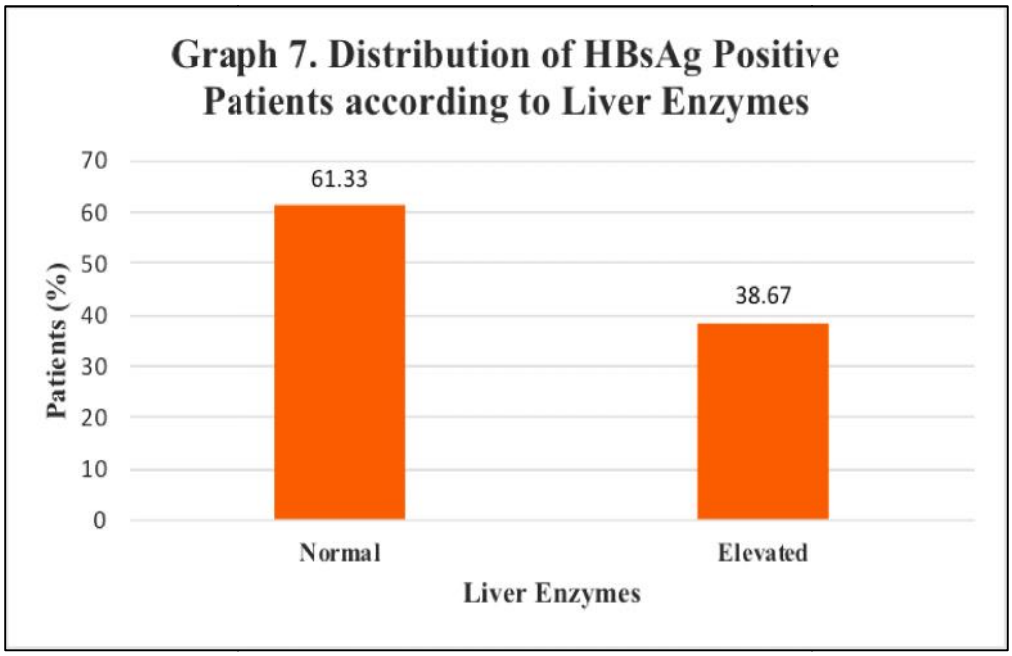


In present study-

Most common symptoms were Fever 40 (53.33 %) followed by Loss of appetite18 (24 %) , Nausea and Vomiting 15 (20%) , Abdominal pain 10 (13.33%), Jaundice 5(6.67%) , Weight loss 7 (9.335%) ,Cough 4 (5.33%).

Table 7. Distribution of HBsAg Positive patients according to Liver Enzymes

Liver Enzymes	Patients	
	Number	**Percentage**
Normal	46	61.33
Elevated	29	38.67
Total	**75**	**100**

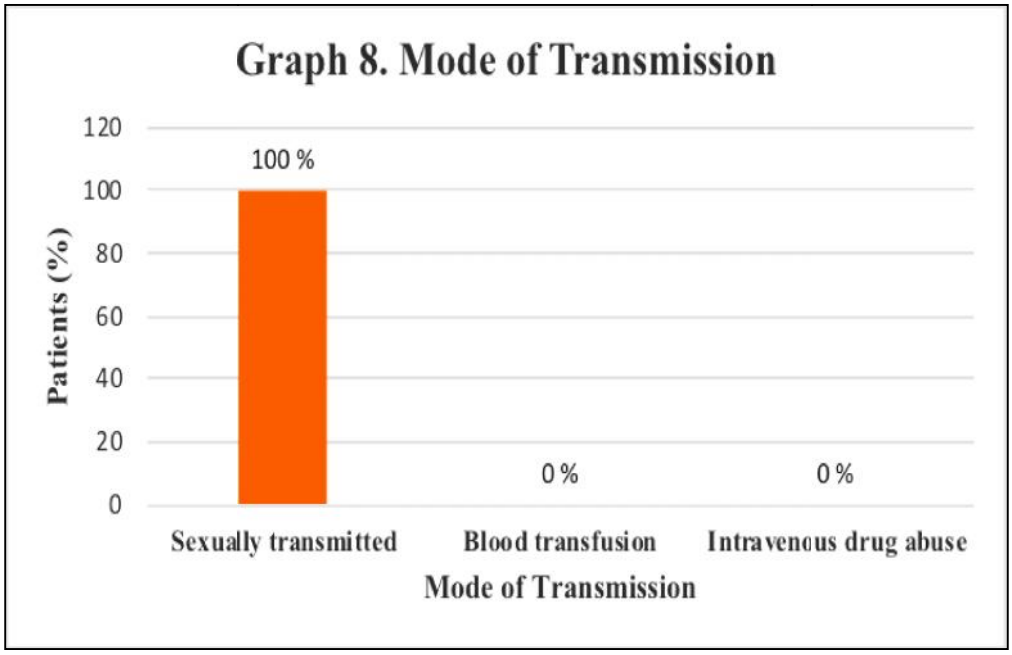


In present study-

Out of 75 HBsAg positive patients 46 (61.33%) showed increase in Liver enzymes.

Table 8. Mode of Transmission

Risk factors	Patients	
	Number	**Percentage**
Sexually transmitted	75	100
Blood transfusion	0	0.00
Intravenous drug abuse	0	0.00
Total	**75**	**100**

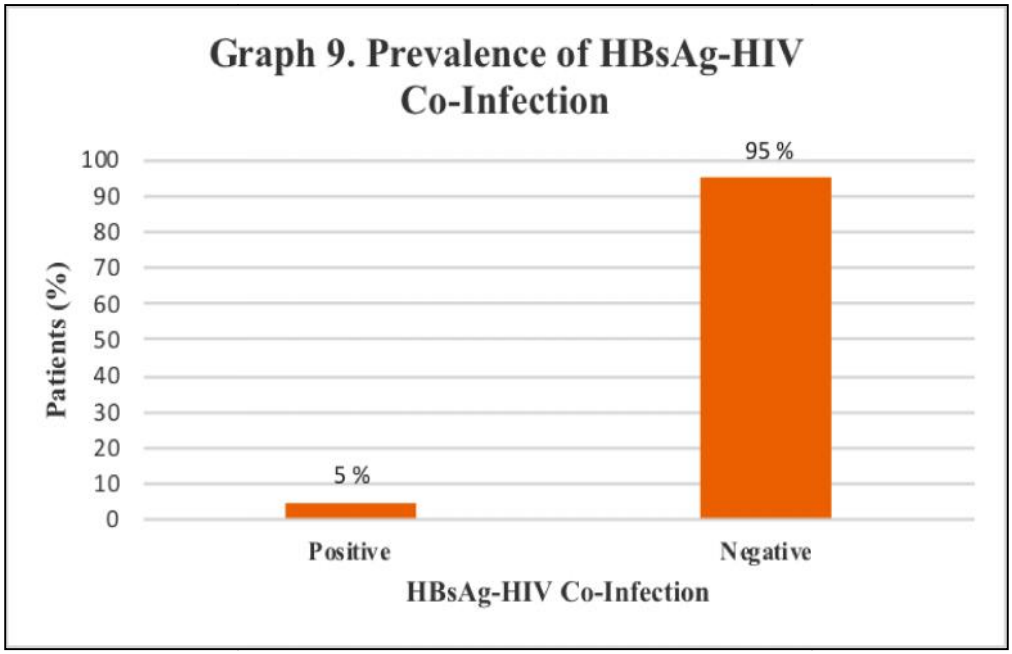


In present study-

Mode of transmission most common was sexually transmitted 75(100%)

Table 9. Prevalence of HBsAg-HIV Co-Infection

HIV-HBsAg Co-Infection	Patients	
	Number	**Percentage**
Positive	4	5.00
Negative	71	95.00
Total	**75**	**100**

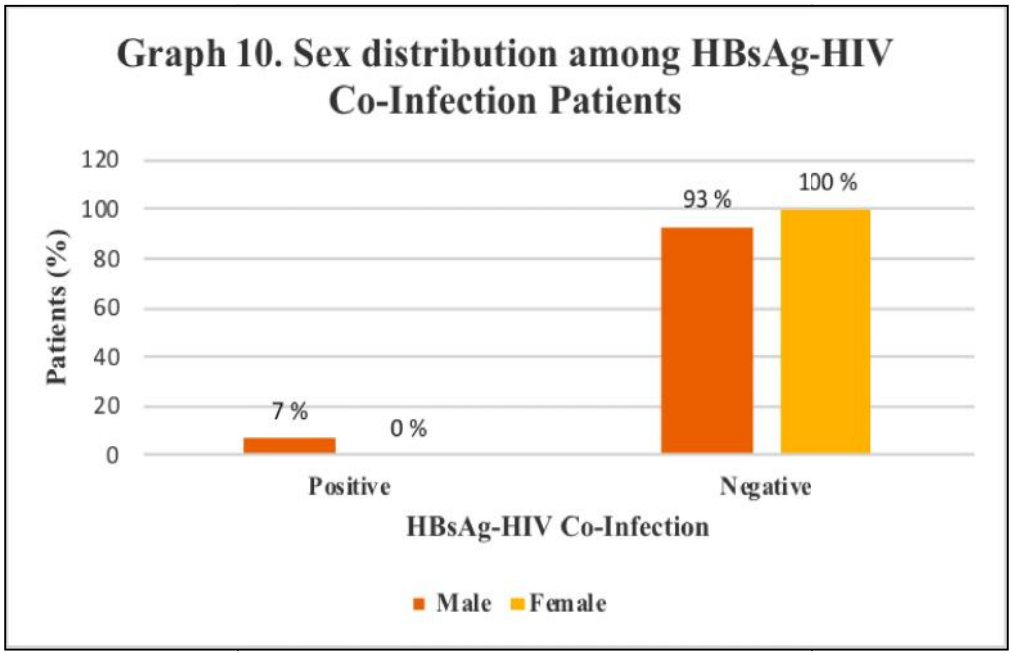


In present study-

Among 75 HBsAg positive patients, coinfection with HIV was seen in 4(5%) patients and 7 1 (95%) were negative.

Table 10. Sex distribution Among HBsAg-HIV Co-Infection Patients

Sex	Male		Female	
	Number	**Percentage**	**Number**	**Percentage**
Positive	4	7.00	0	0.00
Negative	52	93.00	19	100
Total	**57**	**100**	**19**	**100**

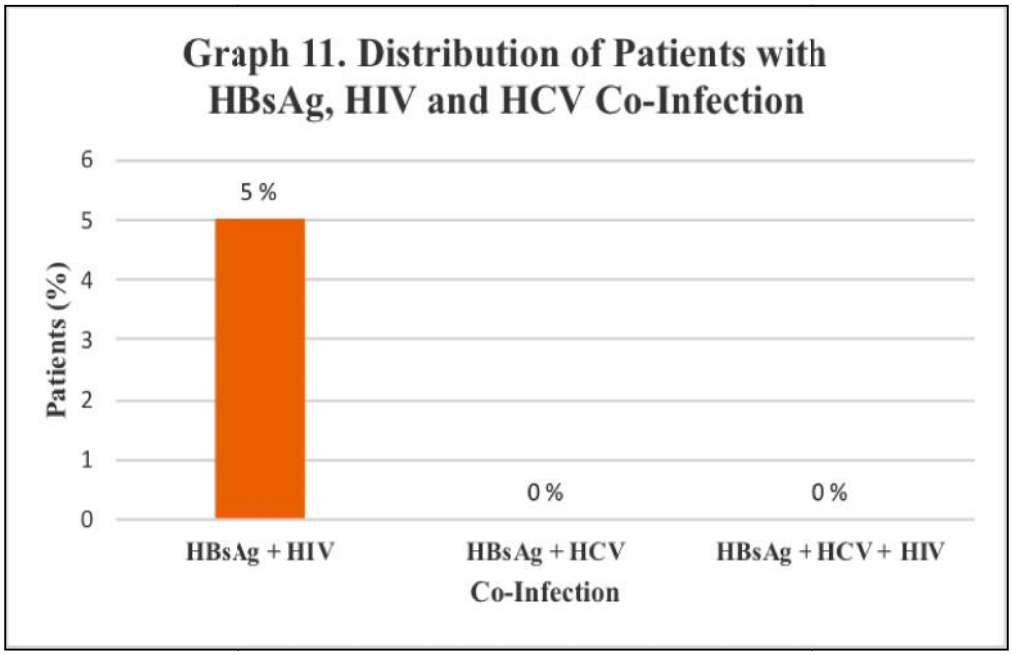


In present study-

Out of 57 HBsAg positive male patients HIV coinfection was seen 4 (7%) male and females were zero (0%).

Table 11. Distribution of Patients with HBsAg, HIV and HCV Co-Infection

Co-Infection	Patients	
	Number	Percentage
HBsAg + HIV	4	5.00
HBsAg + HCV	0	0.00
HBsAg + HCV + HIV	0	0.00



In present study-

HBsAg and HCV coinfection was zero (0%) as well as HBsAg and HCV and HIV triple infection was zero (0%).

DISCUSSION

India falls into the intermediate endemicity zone i.e. prevalence of 2–7%, with an average of 4% with a disease burden of about 50 million. The epidemiology of hepatitis in India has not been studied systematically. Several studies have been noted that prevalence of hepatitis were < 2%. But there is wide variation in social economic and health factor in India which explains the variation in carriers rate of HBV infection in different parts of the country.[8,67]

The prevalence of hepatitis B varies not only indifferent regions of our country but also shows inter country variation, and it depends on as behavioural , environmental, and host factors. In general, it is high in countries with low socioeconomic level and vice versa.[118]

1) Prevalence of Hepatitis B Virus

In our study out of 3278 patients who were screened for HBsAg prevelance of Hepatitiss B virus, who were HBsAg positive was 2%(75). Which was similar to study done by Mahajan S. et al. [51] 1.41% at Tanda (H.P.), Supriya L. et al.[11] 1.32% at Imphal India, Quadri SA et al.[82]1.63% at Bijapur Kanataka, Malhotra R et al.[18]1.5% at Punjab this study was done in patients who were on hemodialysis , study done by Agarwal L et al. [118] at UP showed prevalence of 3.9%. All these studies were hospital based study showing similar prevalence and study done by Tesfa H et al. [30] Gondar university Ethiopia showed higher prevalence i.e. 14.6%, this may due to differences in geographical distribution, Roy A et al.[19] at Imphal India, in patients who IV drug users showed higher prevalence i. e. 17.6% this may be due to this study was done in

high risk group of IV drug users who are highly vulnerable for HBV transmission through shared infected needles.

	HBsAg positive
Current study	75 (2%)
Mahajan S et al . Tanda(H. P) A Hospital Based Study.	1.41%
Supriya L et al . Imphal india .A hospital-based study.	1.32%
Malhotra R et al. Punjab patients on Hemodialysis, study from a tertiary care.	1.5%
Quadri SA et al. hospital based population in Bijapur.	1.63%
Agarwal L et al. UP , hospital-based general population in tertiary care hospital.	3.9%
Tesfa H et al. Gondar university Ethiopia patients attending serology laboratory.	14.6%
Roy A et al. Imphal India in IV drug users.	17.6%

2) Gender variation in HBsAg positive pateints

In our study showed more male predominance i.e. 56 (75%) compared to females 19 (25%). Which was in correspondance to other studies. A study done by Mahajan S. et al. [51]at Tanda (H.P.) there was slight more prevalence in male 56.33% than female 43.66%. and as well in study done by Supriya L et al.[11] at Imphal India, , Agarwal L et al. [118] at UP , Tesfa H et al. [30] at Gondar university Ethiopia, Roy A et

al.[19] at Imphal India, in all the studies there was male predominance seen, No plausible explanation has been given for the higher prevalence in males this may be due to consensus possible reason could be that females clear the HBV infection more efficiently as compared to males.[118]

	Male	Female
Current study	**56 (75%)**	19(25%)
Mahajan S et al . Tanda(H. P) A Hospital Based Study.	**56.33%**	43.66%
Supriya L et al . Imphal India .A hospital-based study.	**1.55 to 2.38%**	0.66 to 1%
Quadri SA et al. hospital based population in Bijapur.	**1.86%**	1.37%
Agarwal L et al. UP, hospital-based general population in tertiary care hospital.	**5.5%**	2.4%
Tesfa H et al. Gondar university Ethiopia patients attending serology laboratory.	**7.9%**	6.3%
Roy A et al. Imphal India in IV drug users.	**15.4%**	1.9%

3) Age distribution among HBsAg positive patients

In our study HBsAg positive patients most common age group was between 41 to 60 yrs (40%) followed by 21 to 40 yrs(34.67%). Which was similar to study done by Quadri SA et al [82] in Bijapur where more prevalence was seen in age group of 51 to 60 (2.66%) followed by age group of 21 to 30 (1.94%) as Belagavi and Bijapur being north Karnataka cities have shown similar results. Study done by Smita

Sood and Shirish Malvankar [121] at Rajasthan showed more prevalence in 2nd, 5th and elderly patients above 61 years. Agarwal L.et al [118] at UP showed more prevalence in age group of 35 to 44yrs. Tesfa H et al [30]. at Gondar university Ethiopia showed prevalence in lower age group 25 to 34 yrs (30%) this may be due indulgence Of high risk activities inn younger age group.

4) Mode of transmission in HBsAg positive patients

In our study most common mode of transmission for HBV was sexual route. Which was similar to study done by Agarwal L.et al UP.[118]

HIV, HBV, and HCV are the three most common chronic viral infections documented worldwide. These viruses have similar routes of transmission, namely through blood and blood products, sharing of needles to inject drugs and sexual activity, leading to co-infection with these viruses.[6]

Co-infection with HIV and HBV complicates the clinical course of HIV in infected HIV patients, and may also affect treatment of HIV infection.

The prevalence of HBV co-infection in HIV has been variably reported in different studies. The prevalence of HBV varies markedly among different HIV infected population and geographical location is one of the major determinants of prevalence. The prevalence of HBV co-infection varies from 5-7 percent in low endemicity areas. In intermediate and high endemicity, it varies from 6-20 per cent. The prevalence of HCV co-infection varies from 9-16 percent.[7]

5) Prevalence of coinfection of HBsAg and HIV

In our study showed coinfection of HIV among HBsAg positive patients was 4(5%). Which was similar to study done by Ahuja S.et al. [16] 4.9% from Delhi ,B. Pradip et al. [6] 3.75% at Tripura , at Nepal by G. Ionita et al.[29] showed prevalence to be 4.4%, Tiewsoh JB et al [27] at Mangalore showed prevalence to be 6.6%, but study done at south India by Chandra N et al. [7] at Hyderabad and Kalyani CS et al. [117] at Vishakhapatnam showed higher prevalence rate of 15% and 12% respectively this may be due HIV in India being more prevalent in Andhra Pradesh and Tamil nadu. As well as study done by AKYALA Ishaku A et al.[33] in Nigeria showed higher prevalence 11% this may be due to variation in endemicity of these viruses according to geographical distribution.

6) Gender distribution of HIV and HBsAg coinfection patients

In our study HIV-HBV coinfection was seen predominantly in male patients which was similar to study done by Ahuja S.et al. [16] from Delhi , B. Pradip et al [6] at Tripura , G. Ionita et al.[29] in Nepal , Tiewsoh JB et al [27] Mangalore, where male were more predominant supporting the fact that male subjects are significantly at high risk of developing HBV HIV co-infection,3than female, except for study done by Kalyani CS et al. [117] at Vishakhapatnam showed female predominance.

7) Age distribution among HIV and HBsAg coinfection patients

HIV-HBV coinfection was seen in age group of 30 to 50. which was similar to study done by Ahuja S.et al. [16] from Delhi, B. Pradip et al. [6] at Tripura , at Nepal by G. Ionita et al.[29], Tiewsoh JB et al [27] at Mangalore, Kalyani CS et al. [117] at Vishakhaptnam , AKYALA Ishaku A et al.[33] at Nigeria .

	HIV-HBV coinfection prevalence	Gender predominance in HIV -HBV coinfection	Most common age group in HIV HBV coinfection
Current study	5%	Male	31 to 50 yrs
Ahuja S. Delhi from from a Tertiary Care Hospital.	4.90%	Male	31 to 40 yrs
Tiewsoh JB tertiary care teaching hospital in Mangalore, South India.	6.60%	Male	31 to 40 yrs
Kalyani CS Vishakhaptnam at a tertiary care hospital.	12%	Female	31 to 40 yrs
Chandra N et al Hyderabad from tertiary care centre.	15%	-	-
AKYALA Ishaku A et al Nigeria,in patients accessing healthcare.	11%	-	31 to 40 yrs
B. Pradip Tripura hospital based study in HIV pateints.	3.75%	Male	20 -40 yrs
G. Ionita Nepal patients visiting antiretroviral therapy centres.	4.40%	Male	25 to 49 yrs

HCV was initially identified as a major cause of post transfusion hepatitis intravenous users and patients on haemodialysis or tattooing is also leading cause of HCV infection. [51]

8) Prevalence of HBsAg and HCV coinfection

HBV and HCV coinfection was 0 (0%) but other studies showed higher prevalence such as study done by Mahajan S et al [51] 1.41% at Tanda, Malhotra R et

al.[18] 0.8% at Punjab in patients on Hemodialysis, Agarwal L et al [118] 6 (0.16%) at UP, Tesfa H et al.[30] 2% [at] Gondar university Ethiopia ,Roy A et al.[19] 9.7% at Imphal India, Ahmadi-Ghezeldasht S et al. [28] 1.41% at Iran.

	HBV and HCV coinfection
Current study	0 (0%)
Mahajan S et al . Tanda(H. P) A Hospital Based Study.	1.41%
Malhotra R et al. Punjab patients on Hemodialysis, study from a tertiary care.	0.8%
Agarwal L et al. UP , hospital-based general population in tertiary care hospital.	6 (0.16%)
Tesfa H et al. Gondar university Ethiopia patients attending serology laboratory.	2%
Roy A et al. Imphal India in IV drug users.	9.7%
Ahmadi-Ghezeldasht S et al. in a HBsAg positive patients Iran.	1.41%

CONCLUSION

- In current study 3278 patients who were screened for HBsAg out of which 75 were positive and prevalence was 2%.
- There was high prevalence of HIV and HBsAg coinfection among patients attending hospital which was 5%.
- There is no coinfection of HBsAg and HCV seen.
- The routine screening of HIV and HCV should be mandatory for HBsAg positive patients, as there is more chance of co infection with these viruses due to similar routes of transmission.
- Clear national policies should be established which should include clear economic and health care strategies to test for HBsAg and HIV and HCV to improve quality of living conditions, and easy access to health care facilities.
- People should be educated about taking preventive measures, prophylaxis

  for Hepatitis B virus and avoid high risk activities to prevent for transmission of these viruses.

SUMMARY

- In the present study, 3278 patients serum sample were received for HBsAg screening from January 2017 to December 2017 at central lab of KLE'S DR Prabhakar Kore charitable Hospital & MRC, Belagavi.
- The prevalence of HBsAg was 75 (2%).
- Out of 75 HBsAg positive patients maximum patients were between age group of 41 to 60 yrs (40%), followed by 21 to 40 yrs (34.46%), 61 and above (21.33%) and upto 20 yrs (4%). Minimum age was 18 and maximum was 78 yr old.
- Out of 75 HBsAg positive patients males were more predominant 56 (75%) than female 19 (25%).
- Most common occupation among HBsAg Positive patients was Farmer 33 (44%) followed by Driver 10 (13.33%), Housewife 16 (21.33%), Shopkeeper 5 (6.67%) Student 4 (5.33%) ,Tailor 2 (2.67%) ,Businessman 2 (2.67%) , Road Constructor 1 (1.33%) , Pujari 1 (1.33%), Peon 1(1.33%).
- Out of 75 HBsAg positive patients maximum patients were illiterate 42(56%) , upto PUC 31 (41.33%) and PUC and above 2 (2.66%)
- Most common symptoms were Fever 40 (53.33 %) followed by Loss of appetite18 (24 %) , Nausea and Vomiting 15 (20%) , Abdominal pain 10 (13.33%),Jaundice 5(6.67%) , Weight loss 7 (9.335%) ,Cough 4 (5.33%).
- Out of 75 HBsAg positive patients 46 (61.33%) showed increase in Liver enzymes.

- Out of 75 HBsAg positive patients, coinfection with HIV was seen in 4(5%) patients and 7 1 (95%) were negative.
- Out of 57 HBsAg positive male patients HIV coinfection was seen 4 (7%) male and females were zero (0%).
- HBsAg and HCV coinfection was zero (0%) as well as HBsAg and HCV and HIV triple infection was zero (0%).

BIBLIOGRAPHY

1. Winn W,AllenS,Janda W, Koneman E, Procop G, Schreckenberger P, et al. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th edition. Baltimore:Lippincott Williams ;2006,p 1339 ,1364-1365.
2. World Health Organisation ,Hepatitis B Virus(18 july 2018)(fact sheet).Retrivedfrom,http://www.who.int/news-room/fact-sheets/detail/ hepatitis-b
3. Averhoff F, Glass N, and Holtzman D. Global Burden of Hepatitis C: Considerations for Healthcare Providers in the United States. OxfordJMed& HealthClin Infect Dis 2012; 55: Issue suppl 1Pp. S10-S15.
4. Bharadwaj B, Vazhavandal G, Uma A and Chitra p, rajalakshmil. Seroprevalence of Hepatitis C Virus Infection among Patients attending a rural teaching hospital in South India:A Three Year Study. Int.J.Curr. Microbiol.App.Sci 2014 ; 3(2): 123-27.
5. World Health Organisation (July 2018).Human Immunodeficiency Virus (fact sheeth) Retrived from ,http://www.who.int/mediacentre/ factsheets/ fs360/en/
6. Bhaumik P, Bhattacharjee P, Sil S. Hepatitis B and Hepatitis C Virus Co-infectionamong Human Immunodeficiency Virus Infected Patients of Tripura.*IJSS*2015 ;3: 6
7. Chandra N, Joshi N, Y.S.N. R, Kumar A, and Teja V. Hepatitis B and/or C co-infection in HIV infected patients: A study in a tertiary care centre from south India. Indian J Med Res. 2013 Dec; 138(6): 950–954.
8. Chu C, Lee S. Hepatitis B virus/hepatitis C virus coinfection: Epidemiology,

clinical features, viral interactions and treatment. *J* Gastroenterol *Hepatol*2008; 23(4):512-520.

9. Agyeman A ,Ofori-AsensoR. Prevalence of HIV and hepatitis B coinfection in Ghana: a systematic review and meta-analysis. AIDS Res Ther 2016;13 :23.
10. FieldsBN,Knipe D M, Howley P M, andGriffin D E. Fields virology 6th edition;2001, Lippincott Williams &Wilkins ,Philadelphia :p 795,1502,2185.
11. Supriya L, Vyas K, Bishwalata R. Seroprevalence of hepatitis B surface antigen and antibodies to hepatitis C virus at an Indian State bordering Myanmar: A hospital-based study. IJIRD2014 Jul 12.
12. Chouhan S, Parihar G, Srivastava S. Significance of seroprevalence of hepatitis b in HIV positive patients.Int.J.Bioassays 2014 Mar 27;3(6):3079-83.
13. R Ananthnarayan,Panikar CKJ. Textbook of Microbiology 8th edition 2009, University press, ch 59 Hepatitis Viruses: 538-545.
14. ShastryAS,KSB . Essential of Medical Microbiology 1stedition:Jaypee Brothers Medical Publishers (P) Ltd. New Delhi; p503-509,530 -538,
15. KhunteP ,Khare L R,Beck P, Kumar S. Prevalence of hepatitis B virus and hepatitis C virus co-infection in human immunodeficiency virus positive patients: a study from tribal area of central India.Int J Res Med Sci. 2015 Sep;3(9):2311-2315
16. Ahuja S, Malhotra S, Chauhan A, Hans C. Seroprevalence of hepatitis B and C Co infection in HIV positive patients from a tertiary care hospital. JIMSA2013 Apr;26:91.
17. Tripathi AK, Khanna M, Gupta N, Chandra M, King GS. Low prevalence of hepatitis B virus and hepatitis C virus co-infection in patients with human immunodeficiency virus in Northern India. JAPI. 2007 Jun;55:429-31.

18. Malhotra R, Soin D, Grover P, Galhotra S, Khutan H, Kaur N. Hepatitis B virus and hepatitis C virus co-infection in hemodialysis patients: A retrospective study from a tertiary care hospital of North India. J Nat Sc Biol Med. 2016 Jan;7(1):72.
19. Roy A, Sh P, Devi KS, Haokip P, Laldinmawii G, Damrolien S. Seroprevalence of hepatitis B and hepatitis C in people who inject drugs and other high risk groups in a tertiary care hospital in Northeast India. *Int J Community Med Public Health.* 2017 Aug 23;4(9):3306-9.
20. Prakash S, Jain A, Sankhwar SN, Usman K, Prasad N, Saha D, Singh KP, Jain P, Singh DD. Prevalence of hepatitis B & C viruses among patients on hemodialysis in Lucknow, Uttar Pradesh.JEGH 2014 Apr 1;2(1):19-23.
21. Denue BA, Ajayi B, Abja AU, Bukar AA, Akawu C, Ekong E, Alkali MB. A survey of hepatitis B and C virus prevalence in human immunodeficiency virus positive patients in a tertiary health institution in North Eastern Nigeria. Int. J. Med. Med. Sci. 2012 Jan 26;4(1):13-8.
22. MacLachlan JH, Cowie BC. Hepatitis B virus epidemiology. Cold Spring HarbPerspect Med. 2015 May 1;5(5):a021410.
23. Askari A, Hakimi H, Ahmadabadi BN, Hassanshahi G, Arababadi MK. Prevalence of Hepatitis B Co-Infection among HIV Positive Patients: Narrative Review Article. IRAN J PUBLIC HEALTH.2014 Jun;43(6):705.
24. Kamenya T, Damian DJ, Ngocho JS, Philemon RN, Mahande MJ, Msuya SE. The prevalence of hepatitis B virus among HIV-positive patients at Kilimanjaro Christian Medical Centre Referral Hospital, Northern Tanzania. *Pan* Afr Med J. 2017;28.
25. Gaglio PJ, Sterling R, Daniels E, Tedaldi E, Terry Beirn Community

Programs for Clinical Research on AIDS Hepatitis Working Group. Hepatitis B virus and HIV coinfection: results of a survey on treatment practices and recommendations for therapy. Clin. Infect. Dis. 2007 Sep 1;45(5):618-23.

26. Shimelis T, Tassachew Y, Tadewos A, Hordofa MW, Amsalu A, Tadesse BT, Tadesse E. Coinfections with hepatitis B and C virus and syphilis among HIV-infected clients in Southern Ethiopia: a cross-sectional study. HIV/AIDS (Auckland, NZ). 2017;9:203.
27. Tiewsoh JB, Boloor R, Antony B. Seroprevalence of hepatitis B virus/hepatitis C virus among human immunodeficiency virus-infected patients at a tertiary care teaching hospital in Mangalore, South India. Ann Trop Med Public Health. 2017 Nov 1;10(6):1443.
28. Ahmadi-Ghezeldasht S, Hedayati-Moghaddam MR, Akbarin MM. The Prevalence of Hepatitis B and C Virus Co-Infection Among a Group of Iranian Population. Mod. Care. J. 2017;14(2).
29. Ionita G, Malviya A, Rajbhandari R, Schluter WW, Sharma G, Kakchapati S, Rijal S, Dixit S. Seroprevalence of hepatitis B virus and hepatitis C virus co-infection among people living with HIV/AIDS visiting antiretroviral therapy centres in Nepal: a first nationally representative study. Int J Infect Dis. 2017 Jul 1;60:64-9.
30. Tesfa H, Biadgo B, Getachew F, Tegegne K, Yismaw G, Muluye D. Seroprevalence of hepatitis B and C virus infection among patients attending serology laboratory of Gondar University Hospital. BMC research notes. 2013 Dec;6(1):164.
31. Owolabi LF, Ibrahim A, Musa BM, Gwaram BA, Dutse AI, Hamza M, Yakasai AM, Habib AG, Borodo MM. Prevalence and Burden of Human

Immunodeficiency Virus and Hepatitis B Virus Co-infection in Nigeria: A Systematic Review and Meta-Analysis. J AIDS Clin Res. 2014;5(308):2.

32. Roche R, Amrita S, Leslie RN. Prevalence of the Human Immunodeficiency Virus, the Hepatitis B Virus and the Hepatitis C Virus among the Patients at a Tertiary Health Care Centre: A Five Year Study. J. clin. diagn. 2012 May;6(4):623-26.
33. A I A,GodwinO ,Ishaleku D.Seroprevalence of hepatitis b and c co-infection among cohort seropositive hiv patients accessing healthcare in nasarawa state north central, nigeria. brJPsychol res. 2013 dec;1(1):15-24.
34. Okeke KN, Egbuonu I, Ugochukwu EF, Ulasi TO, Okani CO. Seroprevalence of HBV and HIV Co-Infection in Children in Nnewi, South-East Nigeria. Ped Health Res. 2017;2(3):20.
35. Sinha SK, Roychoudhury S, Biswas K, Biswas P, Bandopadhyay R. Prevalence of HIV, Hepatitis B, Hepatitis C and Syphilis in donor's blood: A study from eastern part of India. OpenJ Hematol. 2012 Jan 30;3(1).
36. Ekanem US, Eyoh JE, Esubok NU. Prevalence of hepatitis-B viru s infection among HIV patients seen in university of UYO teaching hospita l (UUTH), UYO. Int J Res Biosciences. 2013;2:92-8.
37. Firnhaber C, Chen CY, Evans D, Maskew M, Schulz D, Reyneke A, Kramvis A. Prevalence of hepatitis B virus (HBV) co-infection in HBV serologically-negative South African HIV patients and retrospective evaluation of the clinical course of mono-and co-infection. Int J Infect Dis. 2012 Apr 1;16(4):e268-72.
38. Abumwais JQ, Idris OF. Prevalence of hepatitis C, hepatitis B, and HIV infection among hemodialysis patients in Jenin District (Palestine). Iran J

Virol.2010 Oct 15;4(2):38-44.

39. Sari SY, Rachmawati A, Alisjahbana B, Susanto H. Seroprevalence of HIV, HBV, HCV and syphilis in obstetrics and gynaecology patients in West Java, Indonesia. Int J Infect Control. 2015 Sep 1;11(3).
40. Roshan N, Nasrin S, Farzam F. Prevalence of HBsAG, HCV and HIV antibodies among infertile couples in Ahvaz, South-West Iran. Jundishapur J Microbiology. 2012 Apr;2012(2, Spring):393-7.
41. Kalantari H, Mirzabaghi A, Akbari M, Shahshahan Z. Prevalence of hepatitis C virus, hepatitis B virus, human immunodeficiency virus and related risk factors among hemophilia and thalassemia patients In Iran. Iran J Clin Infect Dis. 2011;6(2):82-4.
42. Global Hepatitis Report 2017. Geneva: World Health Organization; 2017 retrived from apps.who.int/iris/bitstream/10665/255017/1/WHO-HIV-2017. 06-eng.pdf.
43. Mustapha SK, Jibrin YB. The prevalence of hepatitis B surface antigenaemia in patients with human immunodeficiency virus (HIV) infection in Gombe, Nigeria.
44. Adewole OO, Anteyi E, Ajuwon Z, Wada I, Elegba F, Ahmed P, Betiku Y, Okpe A, Eze S, Ogbeche T, Erhabor GE. Hepatitis B and C virus co-infection in Nigerian patients with HIV infection. J Infect Dev Ctries.2009 Jun 1;3(05):369-75.
45. Marceline DN, Cyriaque AA, Marlene GT, Paul MF. Human immunodeficiency virus and hepatitis B virus (HIV/HBV) co-infection in people living with HIV/AIDs identified in Yaound Central Hospital, Cameroon: Seroprevalence and impact on the disease progression. J AIDS and

HIV Res. 2017 Jun 30;9(6):123-8.

46. Baseke J, Musenero M, Mayanja-Kizza H. Prevalence of hepatitis B and C and relationship to liver damage in HIV infected patients attending Joint Clinical Research Centre Clinic (JCRC), Kampala,Uganda. Afr health Sci.2015;15(2):322-7.
47. Chen JJ, Yu CB, Du WB, Li LJ. Prevalence of hepatitis B and C in HIV-infected patients: a meta-analysis. Hepatobiliary Pancreat Dis Int. 2011 Apr 15;10(2):122-7.
48. Chou SL, Chou MY, Wang YH, Kuo FC, Kao WF. The impact of chronic carrier of hepatitis B virus on liver function in a 7-day ultramarathon race. Journal of the Chinese Medical Association. 2016 Apr 1;79(4):179-84.
49. Yanase Y, Ohida T, Kaneita Y, Agdamag DM, Leaño PS, Gill CJ. The prevalence of HIV, HBV and HCV among Filipino blood donors and overseas work visa applicants. Bulletin of the World Health Organization. 2007;85:131-7.
50. Nikolopoulo GK, Paraskevis D, Hatzitheodorou E, Moschidis Z, Sypsa V, Zavitsanos X, Kalapothaki V, Hatzakis A. Impact of hepatitis B virus infection on the progression of AIDS and mortality in HIV-infected individuals: a cohort study and meta-analysis. Clin. Infect. Dis.2009 Jun 15;48(12):1763-71.
51. Mahajan S, Chauhan M, Manish S, Abrol RK. Preliminary Screening for Prevalence of Hepatitis B, C Viruses and their co-infection: A Hospital Based Study of North India. *Indian J Microbiol Res.* 2015;2(3):189-91.
52. Mutagoma M, Balisanga H, Malamba SS, Sebuhoro D, Remera E, Riedel DJ, Kanters S, Nsanzimana S. Hepatitis B virus and HIV co-infection among pregnant women in Rwanda. BMC infectious diseases. 2017 Dec;17(1):618.

53. Ikomey GM, Jacobs GB, Tanjong B, Mesembe MT, Eyoh A, Lyonga E, Mfoataw E, Ngoh R, Tamandjou CR, Ikomey G, Assoumou MC. Evidence of Co and triple infections of Hepatitis B and C amongst HIV infected pregnant women in Buea, Cameroon. Health sci. dis.2016 May 15;17(2).
54. Malhotra P, Nanda S, Malhotra V, Chauhan M, Malhotra N, Chugh A, Chaturvedi A, Chandrika P, Singh I. Prevalence of HIV, Hepatitis B, Hepatitis C in Pregnancy at Tertiary Care Center of Northeren India.
55. Abioye JO, Inemesit A, Adogo LY. Prevalence of Hepatitis B Surface Antigen among HIV Patients Attending Limi Hospital, Abuja.
56. Jaroszewicz J, Reiberger T, Meyer-Olson D, Mauss S, Vogel M, Ingiliz P, Payer BA, Stoll M, Manns MP, Schmidt RE, Flisiak R. Hepatitis B surface antigen concentrations in patients with HIV/HBV co-infection. PloS one. 2012 Aug 15;7(8):e43143.
57. Konstantinou D, Deutsch M. The spectrum of HBV/HCV coinfection: epidemiology, clinical characteristics, viralinteractions and management. Ann Gastroenterol:quarterly publication of the Hellenic Society of Gastroenterology. 2015 Apr;28(2):221.
58. Mitre HP, Mendonça JS. Co-infection with hepatitis B virus and hepatitis C virus. Braz J Infect Dis, 2007 Oct;11:33-5.
59. Chen F, Zhang J, Wen B, Luo S, Lin Y, Ou W, Guo F, Tang P, Liu W, Qu X. HBV/HCV dual infection impacts viral load, antibody response, and cytokine expression differently from HBV or HCV single infection. Scientific reports. 2016 Dec 23;6:39409.
60. Kourtis AP, Bulterys M, Hu DJ, Jamieson DJ. HIV–HBV coinfection—A global challenge. N Engl J Med, 2012 May 10;366(19):1749-52.

61. Chen M, Wong WW, Law MG, Kiertiburanakul S, Yunihastuti E, Merati TP, Lim PL, Chaiwarith R, Phanuphak P, Lee MP, Kumarasamy N. Hepatitis B and C co-infection in HIV patients from the TREAT Asia HIV observational database: analysis of risk factors and survival. PLoS One. 2016 Mar 2;11(3):e0150512.

62. Saha D, Pal A, Sarkar N, Das D, Blackard JT, Guha SK, Saha B, Chakravarty R. Occult hepatitis B virus infection in HIV positive patients at a tertiary healthcare unit in eastern India. PloS one. 2017 Jun 7;12(6):e0179035.

63. Saeed U, Waheed Y, Ashraf M. Hepatitis B and hepatitis C viruses: a review of viral genomes, viral induced host immune responses, genotypic distributions and worldwide epidemiology. *Asian* Pac *J* Trop Dis,2014 Apr;4(2):88.

64. Raza SA, Clifford GM, Franceschi S. Worldwide variation in the relative importance of hepatitis B and hepatitis C viruses in hepatocellular carcinoma: a systematic review. Br. J. Cancer. 2007 Apr;96(7):1127.

65. Crockett SD, Keeffe EB. Natural history and treatment of hepatitis B virus and hepatitis C virus coinfection. Ann Clin Microbiol Antimicrob. 2005 Dec;4(1):13.

66. E.Ameeta Wong T. Background document: HIV and hepatitis B co-infection.

67. Ray G. Current Scenario of Hepatitis B and Its Treatment in India. J Clin Transl Hepatol.2017 Sep 28;5(3):277.

68. Caccamo G, Saffioti F, Raimondo G. Hepatitis B virus and hepatitis C virus dual infection.WJG. 2014 Oct 28;20(40):14559.

69. Sun HY, Sheng WH, Tsai MS, Lee KY, Chang SY, Hung CC. Hepatitis B virus coinfection in human immunodeficiency virus-infected patients: a

review. WJG. 2014 Oct 28;20(40):14598.

70. Chakravarty R, Pal A. Insights into human immunodeficiency virus-hepatitis B virus co-infection in India. *World J* Virol.2015 Aug 12;4(3):255.
71. Jamma S, Hussain G, Lau DT. Current concepts of HBV/HCV coinfection: coexistence, but not necessarily in harmony. Current hepatitis reports. 2010 Nov 1;9(4):260-9.
72. Premalatha E. Seroprevalence of Hepatitis-B and Hepatitis-C Infection among HIV Positive and HIV Negative Individuals in a Tertiary Care Hospital.
73. Liu CJ, Chen PJ, Chen DS, Tseng TC, Kao JH. Perspectives on dual hepatitis B and C infection in Taiwan. J FORMOS MED ASSOC. 2016 May 1;115(5):298-305.
74. Jahanbakhsh A, Farzinepoor Z, Mansouri F, Vaziri S, Afsharian M. Prevalence of occult hepatitis B in HIV positive patients (adolescents and adults) in Kermanshah-Iran. Int J Pediatr. 2017;5(9):5797-803.
75. Larsen C, Salmon D, Pialoux G, Antona D, Piroth L, Pol S, Le Strat Y, Rosenthal E, Neau D, Delarocque-Astagneau E, Desenclos JC. Prevalence of hepatitis C and hepatitis B virus infections in HIV infected persons (France, 2004). AIDS. 2006 Jan 1;33:30-.
76. Padmapriyadarsini C, Chandrabose J, Victor L, Hanna LE, Arunkumar N, Swaminathan S. Hepatitis B or hepatitis C co-infection in individuals infected with human immunodeficiency virus and effect of anti-tuberculosis drugs on liver function. J. Postgrad. Med. 2006 Apr 1;52(2):92.
77. Khokar N, Jethwa D, Lunagaria R, Panchal N. Seroprevalence of Hepatitis B, Hepatitis C, Syphilis and HIV in pregnant women in a tertiary care hospital, Gujrat, India. Int J CurrMicrobiol. 2015;4(9):188-94.

78. Rezvani H, Mokhtari A, Esmailzade A, Ghafarzadegan K, Ahmadlou M. Prevalence of HIV Co-infection with Hepatitis B and C. Zahedan. J. Res. Med. Sci. 2014 Nov 1;16(11):60-.

79. Tankhiwale SS, Khadase RK, Jalgoankar SV. Seroprevalence of anti-HCV and hepatitis B surface antigen in HIV infected patients. INDIAN J MED MICROBI 2003 Oct 1;21(4):268.

80. Tandon BN, Acharya SK, Tandon A. Epidemiology of hepatitis B virus infection in India. Gut. 1996 Jan 1;38(Suppl 2):S56-9.

81. Sood S, Malvankar S. Seroprevalence of Hepatitis B surface antigen, antibodies to the Hepatitis C virus, and human immunodeficiency virus in a hospital-based population in Jaipur, Rajasthan. Indian J Community Med : official publication of Indian Association of Preventive & Social Medicine. 2010 Jan;35(1):165.

82. Quadri SA, Dadapeer HJ, Arifulla KM, Khan N. Prevalence of Hepatitis B Surface Antigen in hospital based population in Bijapur, Karnataka. Al Ameen J Med Sci. 2013;6(2):180-2.

83. Bhattacharya S, Badrinath S, Hamide A, Sujatha S. Seroprevalence of hepatitis C virus in a hospital based general population in South India. Indian J Med Microbiol. 2003 Jan 1;21(1):43.

84. Arif SH, Afrose R, Khan AI, Akram M, Singh SK. Seropositivity Rates for Hepatitis B and C viruses in indoor patients of a tertiary care centre of northern India. Int J Curr Microbiol Appl Sci. 2015;1:243-47.

85. Hofer M. et al.Frequent chronic hepatitis B virus infection in HIV-infected patients positive for antibody to hepatitis B core antigen only.Eur. J. Clin. Microbiol. Infect. Dis., 1998, 17:6-13

86. Waite J, Gilson RJ, Weller IV, Lacey CJ, Hambling MH, Hawkins A, Briggs M, Tedder RS. Hepatitis B virus reactivation or reinfection associated with HIV-1 infection. AIDS (London, England). 1988 Dec;2(6):443-8.

87. MacLachlan JH, Cowie BC. Hepatitis B virus epidemiology. Cold Spring Harbor perspectives in medicine. 2015 May 1;5(5):a021410.

88. Puri P. Tackling the hepatitis B disease burden in India. J Clin Exp Hepatol.2014 Dec 1;4(4):312-9.

89. Kumar R, Singla V, Kacharya Sk. Impact and management of hepatitis B and hepatitis C virus co-infection in HIV patients. Trop Gastroenterol 2008;29:136–147.

90. Pal A, Sarkar N, Saha D, Guha SK, Saha B, Chakrabarti S, et al. High incidence of lamivudine-resistance-associated vaccine-escape HBV mutantsamong HIV-coinfected patients on prolonged antiretroviral therapy. AntivirTher2015;20:545–554. doi: 10.3851/IMP2942.

91. Saravanan S, Velu V, Kumarasamy N, Nandakumar S, Murugavel KG, Balakrishnan P, et al. Coinfection of hepatitis B and hepatitis C virus in HIVinfectedpatients in south India. World J Gastroenterol 2007;13:5015–5020. doi: 10.3748/wjg.v13.i37.5015

92. Gupta S, Singh S. Hepatitis B and C virus co-infections in human immunodeficiencyvirus positive North Indian patients. World J Gastroenterol 2006;12:6879–6883. doi: 10.3748/wjg.v12.i42.6879.

93. Sawant S, Agrawal S, Shastri J. Seroprevalence of hepatitis B and hepatitisC virus infection among HIV infected patients in Mumbai. Indian J SexTransm Dis 2010;31:126. doi: 10.4103/0253-7184.75025.

94. Saha D, Pal A, Biswas A, Panigrahi R, Sarkar N, Sarkar J, et al.

Characterizationof treatment-naive HIV/HBV co-infected patients attending ARTclinic of a tertiary healthcare centre in eastern India. PLoS One 2013;8:e73613. doi: 10.1371/journal.pone.0073613.

95. Tyson GL, Kramer JR, Duan Z, Davila JA, Richardson PA, El-Serag HB. Prevalence and predictors of hepatitis B virus coinfection in a United States cohort of hepatitis C virus-infected patients. Hepatology 2013; 58: 538-545 [PMID: 23505059 DOI: 10.1002/hep.26400.
96. Bini EJ, Perumalswami PV. Hepatitis B virus infection among American patients with chronic hepatitis C virus infection: prevalence, racial/ethnic differences, and viral interactions. Hepatology 2010; 51: 759-766 [PMID: 20140950 DOI: 10.1002/hep.23461.
97. Siddiqui F, Mutchnick M, Kinzie J, Peleman R, Naylor P, Ehrinpreis M. Prevalence of hepatitis A virus and hepatitis B virus immunity in patients with polymerase chain reactionconfirmed hepatitis C: implications for vaccination strategy. Am J Gastroenterol 2001; 96: 858-863 [PMID: 11280565 DOI: 10.1111/j.1572-0241.2001.03633.x]
98. Fong TL, Di Bisceglie AM, Waggoner JG, Banks SM, Hoofnagle JH. The significance of antibody to hepatitis C virus in patients with chronic hepatitis B. Hepatology 1991; 14: 64-67 [PMID: 1648540 DOI: 10.1002/hep.1840140111].
99. Chen X, Xuan M, Wu D. [Study of superinfection of HBV and HCV]. ZhonghuaLiu Xing Bing XueZazhi1999; 20: 141-143 [PMID: 10682522].
100. Li W, Zhu Y, Hua Z. [Exploration on the association between the pattern of HBV markers and infection of HCV among population]. Zhonghua Liu Xing Bing XueZazhi1994; 15:212-214 [PMID: 7530602].

101. Tsatsralt-Od B, Takahashi M, Endo K, Agiimaa D, Buyankhuu O, Ninomiya M, Lorenzo FR, Okamoto H. Prevalence of hepatitis B, C, and delta virus infections among children in Mongolia: progress in childhood immunization. JMed Virol2007; 79: 1064-1074 [PMID: 17596839 DOI: 10.1002/jmv.20867].
102. Tsatsralt-Od B, Takahashi M, Nishizawa T, Endo K, Inoue J, Okamoto H. High prevalence of dual or triple infection of hepatitis B, C, and delta viruses among patients with chronic liver disease in Mongolia. J Med Virol2005; 77: 491-499 [PMID: 16254981].
103. Liaw YF. Role of hepatitis C virus in dual and triple hepatitis virus infection. Hepatology 1995; 22: 1101-1108 [PMID: 7557857 DOI: 10.1002/hep.1840220413].
104. Dai CY, Yu ML, Chuang WL, Lin ZY, Chen SC, Hsieh MY, Wang LY, Tsai JF, Chang WY. Influence of hepatitis C virus on the profiles of patients with chronic hepatitis B virus infection. J Gastroenterol Hepatol2001; 16: 636-640 [PMID: 11422616 DOI: 10.1046/j.1440-1746.2001.02494.x].
105. Chan CY, Lee SD, Wu JC, Hwang SJ, Wang YJ, Huang YS, Lo KJ. Superinfection with hepatitis C virus in patients with symptomatic chronic hepatitis B. Scand J Infect Dis 1991; 23:421-424 [PMID: 1659736 DOI: 10.3109/00365549109075089].
106. Sato S, Fujiyama S, Tanaka M, Yamasaki K, Kuramoto I, Kawano S, Sato T, Mizuno K, Nonaka S. Coinfection of hepatitis C virus in patients with chronic hepatitis B infection. J Hepatol1994; 21: 159-166 [PMID: 7527435 DOI: 10.1016/ S0168-8278(05)80389-7].
107. Ohkawa K, Hayashi N, Yuki N, Hagiwara H, Kato M, Yamamoto K, Eguchi H, Fusamoto H, Masuzawa M, Kamada T. Hepatitis C virus antibody and

hepatitis C virus replication in chronic hepatitis B patients. J Hepatol1994; 21: 509-514 [PMID: 7529271 DOI: 10.1016/S0168-8278(94)80094-4].

108. Saravanan S, Velu V, Nandakumar S, Madhavan V, Shanmugasundaram U, Murugavel KG, Balakrishnan P, Kumarasamy N, Solomon S, Thyagarajan SP. Hepatitis B virus and hepatitis C virus dual infection among patients with chronic liver disease. J Microbiol Immunol Infect 2009; 42: 122-128 [PMID: 19597643].
109. Chakravarti A, Verma V, Jain M, Kar P. Characteristics of dual infection of hepatitis B and C viruses among patients with chronic liver disease: a study from tertiary care hospital. Trop Gastroenterol 2005; 26: 183-187 [PMID: 16737047].
110. Semnani S, Roshandel G, Abdolahi N, Besharat S, Keshtkar AA, Joshaghani H, Moradi A, Kalavi K, Jabbari A, Kabir MJ, Hosseini SA, Sedaqat SM, Danesh A, Roshandel D. Hepatitis B/C virus co-infection in Iran: a seroepidemiological study. Turk J Gastroenterol 2007; 18: 20-21 [PMID: 17450490].
111. Murad EA, Babiker SM, Gasim GI, Rayis DA, Adam I. Epidemiology of hepatitis B and hepatitis C virus infections in pregnant women in Sana'a, Yemen. BMC Pregnancy Childbirth 2013; 13: 127 [PMID: 23758990 DOI: 10.1186/1471-2393-13-127].
112. Gaeta GB, Stornaiuolo G, Precone DF, Lobello S, Chiaramonte M, Stroffolini T, Colucci G, Rizzetto M. Epidemiological and clinical burden of chronic hepatitis B virus/ hepatitis C virus infection. A multicenter Italian study. J Hepatol 2003; 39: 1036-1041 [PMID: 14642623 DOI: 10.1016/ S0168-8278(03)00470-7].

113. Di Marco V, Lo Iacono O, Cammà C, Vaccaro A, Giunta M, Martorana G, Fuschi P, Almasio PL, Craxì A. The long-term course of chronic hepatitis B. Hepatol 1999; 30: 257-264 [PMID: 10385664 DOI: 10.1002/hep.510300109].
114. Fattovich G, Tagger A, Brollo L, Giustina G, Pontisso P, Realdi G, Alberti A, Ruol A. Hepatitis C virus infection in chronic hepatitis B virus carriers. J Infect Dis 1991; 163: 400-402 [PMID: 1846394 DOI: 10.1093/infdis/163.2.400].
115. Crespo J, Lozano JL, de la Cruz F, Rodrigo L, Rodríguez M, San Miguel G, Artiñano E, Pons-Romero F. Prevalence and significance of hepatitis C viremia in chronic active hepatitis B. Am J Gastroenterol 1994; 89: 1147-1151 [PMID: 8053425].
116. Voiculescu M, Iliescu L, Ionescu C, Micu L, Ismail G, Zilisteanu D, Radasan A, Micu G, Pertache I. A cross-sectional epidemiological study of HBV, HCV, HDV and HEV prevalence in the SubCarpathian and South-Eastern regions of Romania. J Gastrointestin Liver Dis 2010; 19: 43-48 [PMID: 20361074].
117. Kalyani CS, Lakshmi N, Lakshmi KR, Kumar VA, Cheemala SS. Seroprevalence of hepatitis virus and hepatitis C virus co-infection in human immunodeficiency virus infected patients at a tertiary care hospital in South India. Int. J Res Med Sci . 2017 Jan 10;3(8):2041-5.
118. Agarwal L, Singh AK, Agarwal A, Singh RP. Incidental detection of hepatitis B and C viruses and their coinfection in a hospital-based general population in tertiary care hospital of Uttar Pradesh. J *Family Med* Prim Care. 2018 Jan;7(1):157.
119. Gupta S, Singh S. Occult hepatitis B virus infection in ART-naive HIV-

infected patients seen at a tertiary care centre in north India. BMC infectious diseases. 2010 Dec;10(1):53.

120. GoyatS .Prevalence of Hepatitis B and Hepatitis C Virus Coinfection in HIV Positive Patientsfrom Tertiary Care Hospital in Patiala District of Northern India. J Young *Med* Res .2014;1: 2.
121. Sood S and Malvankar S. Seroprevalence ofHepatitis B Surface Antigen, Antibodies toHepatitis C virus and Human immunodeficiencyvirus in a Hospital Based Population in Jaipur,Rajasthan, Indian J Community Med 2010;35(1):165-169.
122. UNAIDS fact sheets 2016. Retrived fromhttp://www.unaids.org/sites/ default/ files/media_asset/UNAIDS_FactSheet_en.pdf

ANNEXURES

ANNEXURE I - ETHICAL CLEARANCE LETTER

K.L.E.UNIVERSITY'S
JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)
(Accredited 'A' Grade by NAAC)

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Fax No. +91 (0)831 – 2470759

Ref: MDC/DOME/ 08 **Date: 17/10/2016**

To,

BELAGAVI.

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"SEROPREVALENCE OF HEPATITIS C VIRUS AND HUMAN IMMUNDEFICIENCY VIRUS INFECTION IN HBsAg POSITIVE PATIENTS AT KLES DR. PRABHAKAR KORE CHARITABLE HOSPITAL AND MEDICAL RESEARCH CENTRE, BELAGAVI- A ONE YEAR CROSS SECTIONAL STUDY ",** is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

(Dr. Arathi Darshan)
Member Secretary
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

(Dr. Ganga Pilli)
Chairman,
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

6

ANNEXURE II - CONSENT FORM

CONSENT FOR PARTICIPATION IN RESEARCH

TITLE: Seroprevalence of Hepatitis C Virus and Human Immundeficiency Virus in HBsAg positive patients at KLE'S Dr. Prabhakar Kore Charitable Hospital and Medical Research Center, Belagavi"-A One Year Cross Sectional Study

Study Investigator

Guide

INTRODUCTION: Worldwide, Every year 240 million people are infected with Hepatitis B Virus. More than 6,86, 000 (approximately) die from complications due to HBV per year. In India the estimated Hepatitis B Virus prevalence is 2-5%. Worldwide, Every year130–170 million people are infected with Hepatitis C Virus(2 to 3% world population) and 3,50,000 people (approximately) die from complications due to HCV per year. In India, the estimated prevalence of Hepatitis C virus infection ranges from 0.1-8%. Worldwide No of people living with Human Immunodeficiency Virus in 2015 were 36.7 million and People newly infected with HIV were 2.1 million in year 2015. India now has the third largest number of individuals with human immunodeficiency virus after South Africa and Nigeria. In India, the prevalence of HBV and HCV co-infection among patients has been estimated to be upto3%. HIV and HBV Co-infection prevalence is 10% in India. Hepatitis B virus (HBV), hepatitis C virus (HCV) and Human immunodeficiency virus (HIV) co-infection has emerged as a

leading cause of morbidity due to liver disease throughout the world in the last two decades. Co-infections are more prevalent due to overlapping transmission routes. In co-infection, the presence of one virus impacts the natural history of the other virus. HIV/HCV accelerates the natural course of HBV infection and facilitates faster progression of liver disease to cirrhosis and hepatocellular carcinoma.

OBJECTIVE OF THE STUDY: The purpose of research is to To Study the Prevalance of Co-infection of HCV and HIV among HBsAg positive patients.

PROCEDURE INVOLVED: You are requested to participate in this study which will help to provide appropriate and effective treatment. During the study you will be asked some questions and you are supposed to answer to the best of your knowledge**.**

If you agree to be a part of this study, few examination will be done and blood is withdrawn and further processed..

RISKS AND BENEFITS: There are no risks involved and benefit is to know the weather positive for theses viruses and for further treatment accordingly.

ALTERNATIVES: Your participation in research is voluntary. Your decision whether or not to participate in the study will not affect your relationship with Jawaharlal Nehru Medical College. If you decide to participate you are free to withdraw at any time.

PRIVACY AND CONFIDENTIALITY: The only people to know that you are a research subject are members of the research team. No information about you or provided by you during research will be disclosed to others without your written permission, except in emergency to protect your rights and welfare.

AUTHORIZATION TO PUBLISH RESULTS: When the results of research are published or discussed in a conference, no information will be displaced that would disclose your identity. Any information that is obtained in connection with this study and that can be identified with you will remain confidential.

FINANCIAL INCENTIVES FOR PARTICIPATION: You will not be paid /offered any gifts/incentives for participating in the study. You will not be reimbursed for expenses.

In case you have any questions about your rights as a participant, you can contact Dr.Ganga S Pilli, Professor of Pathology and Chairman of Institutional Ethics Committee, JNMC (Office no. 0831-2471350 Ext: 4052)

CONSENT STATEMENT

I, the undersigned _____have been explained in my vernacular language about the study and my participation in the study is voluntary. If I want, I can withdraw at any time. Also I have been given enough time to clear my doubts and rights as study participant.

Signature or left hand thumb print of participant or legally authorized representative.

Participant's Namesignature

Witness Name signature

Experimenter's Name signature

Date:

Place:

ಒಪ್ಪಿಗಯನ್ನು ಸೂಚಸುವ ಹೇಳಿಕ

ನಾನು ರುಜುಮಾಡರುವ _____ ಅಧ್ಯಯನ ಬಗ್ಗ ನನ್ನ ದೇಶೀಯ ಭಾಷಯಿಲ್ಲ ವಿವರಸಲಾಗದ ಮತ್ತು ಅಧ್ಯಯನದಲ್ಲ ನನ್ನ ಭಾಗವಹಿಸುವಿಕ ವ್ಯಯಿಕ್ತಕವಾಗದ್ದು. ನಾನು ಬಯಸಿದರ, ನಾನು ಯಾವುದೇ ಸಮಯದಲ್ಲ ಹಿಂದಕ್ಕ. ನಾನು ಅಧ್ಯಯನ ಭಾಗಯಾಗ ನನ್ನ ಅನುಮಾನಗಳನ್ನು ಮತ್ತು ಹಕ್ಕುಗಳ ತರವುಗೂಳಿಸಲು ಸಾಕಷ್ಟು ಸಮಯ ನೀಡಲಾಗದ.

ಸಹ ಸ್ಪರ್ಧ ಎಡಗೈ ಹಬ್ಬರಳು ಮುದ್ರಣ ಕಾನೂನು ಬದ್ಧ ಪ್ರತಿನಧಿಯ .

ಭಾಗವಹಿಸುವವರ

ಎಟ್ನಿಸ್ ಹಸರು ಸಹಿ

ಪ್ರಯೋಗ ಹಸರು ಸಹಿ

ದನಾಂಕ:

ಸ್ಥಳ:

संमती विधान

मी निम्न _____ अभ्यास बद्दल माझ्या देशी भाषामध्ये स्पष्ट केला आणि अभ्यास माझा सहभाग ऐच्छिक आहे. मी इच्छित असल्यास, मी कोणत्याही वेळी काढू शकतात. मी अभ्यास सहभागी म्हणून माझे शंका आणि अधिकार साफ करण्यासाठी पुरेसा वेळ देण्यात आला आहे.

स्वाक्षरी किंवा सहभागी डाव्या हाताच्या अगठ्याच प्रिंट किंवा कायदेशीर अधिकृत प्रतिनिधी .

या सहभागी य च्या

साक्षीदार नाव स्वाक्षरी

EXpermenter नाव स्वाक्षरी

तारीख:

स्थान:

ANNEXURE - III – PROFORMA

Name: **Occupation:**

Age: **sex:**

OPD number: **Education:**

Marital status: **Monthly income:**

No of family memebrs: **Prasad 's clssification:**

Address: **Phone number:**

Presenting complaints:

H/o Fever: Yes / No

H/o Nausea or vomiting: Yes / No

H/o Abdominal pain: Yes / No

H/o Jaundice : Yes / No

H/o Loss of Appetitite: Yes / No

H/o Weight loss: Yes / No

H/o Cough: Yes /No

Past History:

H/o Similar Complaints in Past and if any treatment taken: Yes / No

H/o Blood transfusion: Yes / No

H/o High Risk sexual Activity: Yes /No

H/o Injectable drug abuse: Yes / No

H/o TB/DM: Yes/No

H/o Any STD:

Habits:

H/O Smoking:

H/O Alcohol intake:

Current treatment if any:

Local examination:

Abdominal examination:

Respiratory examination:

Laboratory investigation:

1. HBsAg test by Rapid Method:
   Kit used:
   Lot no :
   Expiry :
2. HBsAg confirmed by ELISA:
   Kit used:
   Lot no:
   Expiry:
3. HIV ELISA:
   Kit used:
   Lot no:
   Expiry:
4. HCV ELISA:
   Kit used:
   Lot no:
   Expiry:

5. Liver profile :
   SGOT
   SGPT
6. Liver biopsy:
7. Abdominal scan report
8. Chest X ray report:
9. Any other:

ANNEXURE – IV MASTER CHART

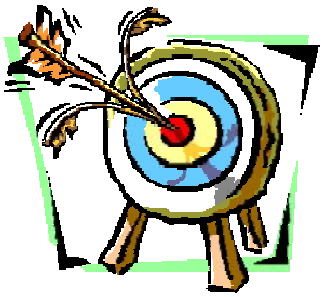
Serial Number	IP/OP Number	Age (Years)	Sex	EDUCATION	OCCUPATION	MONTHLY INCOME	mode of transmission	Address	Presenting Complaints							Past History							Habits		HBsAg Test by Rapid Method	HBsAg Confirmed by ELISA	HIV ELISA	HCV ELISA	Liver Enzymes									Abdominal Scan Report	Chest X ray Report	Local Examination	Abdominal Examination	Respiratory Examination
									Fever	Nausea or Vomiting	Abdominal Pain	Jaundice	Loss of Appetite	Weight Loss	cough	H/O Similar Complaints& If any Treatment	Blood Transfusion	High Risk Sexual Activity	Injectable Drug Abuse	TB	DM	Any STD	Smoking	Alcohol	Result	Result	Result	Result	SGOT	SGPT	TB	DB	IDB	TP	ALB	GLB	AG RATIO	ALK. P					
1	754341	60	M	NIL	FARMER	5000	sexualy	BGM	N	N	N	N	Y	N	N	N	N	N	N	N	N	N	N	Y	Y	P	P	NR	NR	53	40	0.8	0.2	0.6	7.6	4.2	3.4	1,2	311	NAD	NAD	NAD	NAD	NAD
2	760958	39	M	NIL	FARMER	4000	sexualy	BGM	Y	N	N	N	N	N	N	N	N	Y	N	N	N	N	N	Y	Y	P	P	NR	NR	23	17	0.4	0.3	0.1	8.2	5.6	2.6	2,1	382	NAD	NAD	NAD	NAD	NAD
3	761342	40	M	NIL	FARMER	5000	sexualy	BGM	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	Y	P	P	NR	NR	26	48	0.4	0.2	0.4	7.7	3.6	4.1	0,87	359	NAD	NAD	NAD	NAD	NAD
4	762367	55	M	10TH	FARMER	6000	sexualy	KAKATI	N	Y	N	N	N	N	N	N	N	Y	N	N	N	N	N	Y	Y	P	P	NR	NR	107	70	0.6	0.2	0.4	7	3	4	0.7	340	NAD	NAD	NAD	NAD	NAD
5	762404	54	M	5TH	DRIVER	7000	sexualy	KOPPAL	Y	Y	N	N	N	N	N	N	N	Y	N	N	N	N	N	Y	N	P	P	NR	NR	43	32	0.4	0.1	0.3	6.7	3.9	2.8	1.4	302	NAD	NAD	NAD	NAD	NAD
6	764842	55	M	10TH	BUISINES	20000	sexualy	MAHALINGPUR	Y	Y	N	N	Y	Y	N	Y	N	Y	N	N	N	N	N	Y	Y	P	P	R	NR	200	219	1.5	1	0.5	7.8	2.7	5.1	0.5	450	NAD	NAD	NAD	NAD	NAD
7	766841	56	M	12TH	SHOP	10000	sexualy	BGM	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	P	P	NR	NR	140	81	1.3	0.5	1.2	5.6	4	1.6	0.7	370	NAD	NAD	NAD	NAD	NAD
8	767745	36	M	NIL	ROAD CONSTRUCTION	4000	sexualy	BGM	N	Y	N	N	N	N	N	N	N	Y	N	N	N	N	N	N	N	P	P	NR	NR	100	60	0,6	0.2	0.4	7.0	3	4	0.7	200	NAD	NAD	NAD	NAD	NAD
9	768762	63	M	5TH	FARMER	6000	sexualy	MUDHOL	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	Y	Y	P	P	NR	NR	40	50	1	0.7	0.3	6.0	3.6	2.4	0.7	300	NAD	NAD	NAD	NAD	NAD
10	775320	42	F	10TH	HOUSE WIFE	NIL	sexualy	KAKATI	N	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	53	58	0.6	0.4	0.2	7.5	4.2	3.3	0.5	220	NAD	NAD	NAD	NAD	NAD
11	775407	18	M	12TH	STUDENT	NIL	sexualy	BGM	Y	N	N	N	N	N	Y	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	159	162	1.7	1.1	0.6	6	4	2	0.5	220	NAD	NAD	NAD	NAD	NAD
12	777832	42	M	NIL	FARMER	5000	sexualy	KALBURGI	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	38	35	0.6	0.2	0.4	7.5	3	4.5	0.9	100	NAD	NAD	NAD	NAD	NAD
13	778243	55	M	5TH	FARMER	4000	sexualy	HUDALI	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	Y	P	P	NR	NR	35	40	0.6	0.3	0.3	8	4	4	0.8	200	NAD	NAD	NAD	NAD	NAD
14	779301	47	M	B.COM	BUSINESS	10000	sexualy	HEBBAL	Y	N	N	N	Y	Y	Y	Y	N	Y	N	Y	N	N	Y	Y	P	P	R	NR	240	242	1.6	1	0.6	8	4	4	0.8	400	NAD	NAD	NAD	NAD	NAD
15	779337	50	M	10TH	FARMER	4000	sexualy	KALBURGI	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	33	42	0.4	0.2	0.2	7.5	3.5	4	0.8	218	NAD	NAD	NAD	NAD	NAD
16	778991	52	M	NIL	FARMER	5000	sexualy	BGM	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	Y	P	P	NR	NR	37	42	0.6	0.2	0.4	7	3	4	0.7	200	NAD	NAD	NAD	NAD	NAD
17	780453	38	M	10TH	POOJARI	8000	sexualy	BGM	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	40	42	1	0.5	0.5	8	3.5	4.5	0.7	180	NAD	NAD	NAD	NAD	NAD
18	781796	50	M	NIL	DRIVER	10000	sexualy	BAGALKOT	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	Y	P	P	NR	NR	241	243	1.9	1.4	0.5	7.5	3	4.5	0.6	270	NAD	NAD	NAD	NAD	NAD
19	783571	55	M	NIL	FARMER	10000	sexualy	NINDAL	Y	N	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	39	42	0.8	0.6	0.2	6	3	3	1	180	NAD	NAD	NAD	NAD	NAD
20	788961	38	M	NIL	FARMER	6000	sexualy	BAGALKOT	N	N	N	N	Y	N	N	N	N	N	N	N	Y	Y	Y	P	P	NR	NR	65	42	1.5	0.8	0.7	6.5	4.5	3	1.5	300	NAD	NAD	NAD	NAD	NAD
21	790137	65	M	NIL	SHOPKEEPER	15000	sexualy	INDI	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	N	P	P	NR	NR	45	42	1	0.5	0.5	7	4	3	1.3	150	NAD	NAD	NAD	NAD	NAD
22	786284	40	F	5TH	TAILOR	10000	sexualy	BGM	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	48	42	1	0.5	0.5	7	4	3	1.3	150	NAD	NAD	NAD	NAD	NAD
23	795280	30	F	4TH	HOUSE WIFE	NIL	sexualy	BILAGI	Y	N	N	N	Y	Y	N	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	145	142	1.8	1.3	0.5	7.7	4	3.7	1	400	NAD	NAD	NAD	NAD	NAD
24	796625	30	F	5TH	HOUSE WIFE	5000	sexualy	RAMDURG	Y	Y	Y	N	N	Y	Y	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	13	42	0.5	0.2	0.3	6.9	3.8	3.1	1.2	250	NAD	NAD	NAD	NAD	NAD
25	797572	22	M	NIL	FARMER	3000	sexualy	LINGASUR	N	N	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	Y	P	P	NR	NR	28	42	0.9	0.5	0.4	6	4	3	1.3	200	NAD	NAD	NAD	NAD	NAD
26	800286	42	M	NIL	FARMER	5000	sexualy	HUKKERI	Y	N	Y	N	Y	Y	N	N	N	Y	N	N	N	N	N	N	Y	P	P	R	NR	77	42	0.8	0.4	0.4	7	4	3	1.3	213	NAD	NAD	NAD	NAD	NAD

27	803120	36	F	NIL	HOUSE WIFE	5000	sexualy	BGM	N	N	N	Y	Y	N	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	55	42	1	0.6	0.48	8	4	4	1	265	NAD	NAD	NAD	NAD	NAD
28	803204	49	M	10	FARMER	6000	sexualy	BGM	Y	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	N	P	P	NR	NR	45	42	0.9	0.6	0.3	6.5	3.5	3	1.1	300	NAD	NAD	NAD	NAD	NAD
29	803658	60	F	NIL	HOUSE WIFE	5000	sexualy	BGM	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	54	42	1.3	0.5	0.8	7.5	4.5	3	1.5	270	NAD	NAD	NAD	NAD	NAD
30	804640	29	F	NIL	HOUSE WIFE	5000	sexualy	NAVNAGAR	N	Y	Y	N	Y	Y	N	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	42	42	0.3	0.1	0.2	6.5	3.4	3.1	1	155	NAD	NAD	NAD	NAD	NAD
31	804639	35	M	NIL	DRIVER	10000	sexualy	BGM	N	N	Y	N	Y	N	N	N	Y	N	N	N	N	Y	Y	P	P	NR	NR	52	42	0.5	0.3	0.2	5.5	2.5	3	0.8	165	NAD	NAD	NAD	NAD	NAD
32	806014	71	M	NIL	FARMER	10000	sexualy	MUDHOL	Y	N	N	N	Y	Y	Y	N	N	Y	N	N	N	N	Y	Y	P	P	NR	NR	34	42	0.5	0.2	0.3	6.8	3.2	3.6	0.9	211	NAD	NAD	NAD	NAD	NAD
33	810064	68	M	NIL	FARMER	6000	sexualy	BGM	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	45	42	0.3	0.2	0.1	6.5	3.4	3.1	1	234	NAD	NAD	NAD	NAD	NAD
34	810737	35	M	NIL	FARMER	7000	sexualy	INDI	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	28	42	0.6	0.2	0.4	7	4.2	2.8	1.4	300	NAD	NAD	NAD	NAD	NAD
35	811276	45	M	NIL	FARMER	5000	sexualy	JARAUAD	N	N	N	N	N	N	N	N	Y	N	N	N	N	Y	N	P	P	NR	NR	15	42	0.3	0.2	0.1	6.5	3	3.5	0.8	189	NAD	NAD	NAD	NAD	NAD
36	811274	68	M	NIL	FARMER	8000	sexualy	BAGALKOT	N	N	N	N	Y	N	N	N	N	N	N	N	N	N	Y	Y	P	P	NR	NR	27	42	0.5	0.4	0.1	5	3.5	2.5	1.4	250	NAD	NAD	NAD	NAD	NAD
37	813696	70	F	NIL	HOSE WIFE	NIL	sexualy	CHIKODI	N	N	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	20	42	0.7	0.2	0.5	6.3	3.3	3	1.1	280	NAD	NAD	NAD	NAD	NAD
38	813462	62	M	NIL	FARMER	5000	sexualy	BGM	Y	N	N	N	N	N	N	N	Y	N	N	N	N	Y	Y	P	P	NR	NR	40	42	1	0.5	0.5	6.4	3.3	3.1	1	300	NAD	NAD	NAD	NAD	NAD
39	814758	42	M	NIL	DRIVER	10000	sexualy	BGM	N	N	N	N	N	N	N	N	N	Y	N	N	N	Y	N	P	P	NR	NR	55	42	1	0.3	0.7	6	4	2	2	280	NAD	NAD	NAD	NAD	NAD
40	816602	42	M	NIL	DRIVER	15000	sexualy	BGM	N	N	N	N	N	N	N	N	N	N	Y	N	N	Y	Y	P	P	NR	NR	130	142	0.9	0.5	0.4	7	4	3	1.2	290	NAD	NAD	NAD	NAD	NAD
41	817848	70	M	NIL	FARMER	5000	sexualy	BGM	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	Y	P	P	NR	NR	57	42	1.4	0.8	0.6	5.8	2.3	3.5	0.6	170	NAD	NAD	NAD	NAD	NAD
42	820033	70	F	NIL	HOUSE WIFE	NIL	sexualy	BGM	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	34	42	1	0.6	0.4	6	4	2	2	290	NAD	NAD	NAD	NAD	NAD
43	820273	65	F	NIL	HOUSE WIFE	4000	sexualy	BGM	Y	N	N	N	N	N	N	N	Y	N	N	N	N	N	N	P	P	NR	NR	23	42	0.9	0.6	0.3	5.7	2.4	3.3	0.7	240	NAD	NAD	NAD	NAD	NAD
44	820267	60	F	NIL	FARMER	5000	sexualy	BGM	Y	N	N	N	Y	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	55	42	0.4	0.3	0.1	6.1	3.9	2.2	1.7	300	NAD	NAD	NAD	NAD	NAD
45	821774	63	M	NIL	DRIVER	12000	sexualy	BGM	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	N	P	P	NR	NR	120	43	0.5	0.2	0.3	5	3.2	1.8	1.7	200	NAD	NAD	NAD	NAD	NAD
46	822252	30	F	NIL	HOUSE WIFE	4000	sexualy	BGM	N	N	N	N	Y	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	35	40	0.5	0.3	0.2	7	4	3	1.1	110	NAD	NAD	NAD	NAD	NAD
47	8822848	55	F	NIL	HOUSE WIFE	4000	sexualy	BGM	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	29	30	0.4	0.3	0.1	5.7	3	2.7	1.1	150	NAD	NAD	NAD	NAD	NAD
48	821461	18	M	8TH	STUDENT	NIL	sexualy	BGM	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	P	P	NR	NR	10	12	0.3	0.2	0.1	6	3	3	1	110	NAD	NAD	NAD	NAD	NAD
49	823422	18	M	8TH	STUDENT	NIL	sexualy	BGM	N	N	N	N	Y	N	N	N	N	N	N	N	N	Y	N	P	P	NR	NR	30	40	0.7	0.4	0.3	6.3	4	2.3	1.7	200	NAD	NAD	NAD	NAD	NAD
50	824357	51	M	NIL	DRIVER	15000	sexualy	BGM	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	N	P	P	NR	NR	23	32	0.9	0.5	0.4	6.5	3.5	3	1.1	260	NAD	NAD	NAD	NAD	NAD
51	824762	35	M	NIL	FARMER	6000	sexualy	NAVNAGAR	Y	N	N	N	N	N	N	N	N	N	N	N	N	y	y	P	P	R	NR	15	17	0.8	0.4	0.4	5.6	2.6	3	0.8	234	NAD	NAD	NAD	NAD	NAD
52	826105	64	M	4TH	FARMER	5000	sexualy	KAKTI	N	N	N	Y	N	N	N	N	N	N	N	N	N	Y	N	P	P	NR	NR	34	27	0.3	0.2	0.1	7.8	3.8	4	0.9	265	NAD	NAD	NAD	NAD	NAD
53	826056	64	M	8TH	FARMER	6000	sexualy	BGM	N	Y	N	N	N	N	N	N	Y	N	N	N	N	N	Y	Y	P	P	NR	NR	23	33	0.6	0.3	0.3	8	4	4	1	287	NAD	NAD	NAD	NAD	NAD
54	826859	78	M	8TH	FARMER	7000	sexualy	CHIKODI	N	N	N	N	N	N	N	N	Y	N	N	N	N	N	Y	P	P	NR	NR	13	15	0.3	0.2	0.1	7	3	4	0.7	232	NAD	NAD	NAD	NAD	NAD
55	828271	45	M	NIL	DRIVER	8000	sexualy	BGM	Y	N	N	N	N	N	N	N	Y	N	N	N	N	Y	Y	P	P	NR	NR	19	16	0.6	0.4	0.2	6.5	3.5	3	1.1	269	NAD	NAD	NAD	NAD	NAD
56	828335	23	M	B.COM	STUDENT	NIL	sexualy	BGM	Y	N	N	N	N	N	N	N	Y	N	N	N	N	Y	Y	P	P	NR	NR	35	36	0.9	0.5	0.4	7	3	4	0.7	119	NAD	NAD	NAD	NAD	NAD
57	829612	65	M	NIL	FARMER	NIL	sexualy	BILAGI	N	N	N	Y	N	N	N	N	N	N	N	N	N	Y	Y	P	P	NR	NR	39	41	1.2	0.6	0.6	6.3	4	2.3	1.7	277	NAD	NAD	NAD	NAD	NAD
58	829652	40	M	6TH	FARMER	9800	sexualy	BGM	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	23	54	1	0.6	0.4	6	4.5	1.5	3	345	NAD	NAD	NAD	NAD	NAD
59	829455	40	F	8TH	HOUSE WIFE	NIL	sexualy	BGM	Y	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	56	60	1.5	0.6	0.9	7.6	4.5	3.1	1.4	278	NAD	NAD	NAD	NAD	NAD
60	831774	39	M	NIL	PEON	8000	sexualy	BGM	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	34	45	0.3	0.2	0.1	6.8	4.3	2.5	1.7	234	NAD	NAD	NAD	NAD	NAD
61	832408	25	F	NIL	HOUSE WIFE	NIL	sexualy	KAKATI	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	13	12	0.2	0.1	0.1	6.5	4.5	2	2.2	200	NAD	NAD	NAD	NAD	NAD
62	866391	45	M	NIL	FARMER	NIL	sexualy	YAMKANMARDI	Y	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	15	17	0.7	0.5	0.2	7.6	4.6	3	1.5	199	NAD	NAD	NAD	NAD	NAD
63	832959	65	M	9TH	SHOPKEEPER	25000	sexualy	JAMKHANDI	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	65	76	1.5	0.5	1	8	3	5	0.6	213	NAD	NAD	NAD	NAD	NAD
64	830480	40	M	5TH	DRIVER	12000	sexualy	BGM	Y	N	N	N	N	N	N	N	N	N	N	N	N	Y	Y	P	P	NR	NR	13	16	0.3	0.2	0.1	6	3	3	1	165	NAD	NAD	NAD	NAD	NAD
65	834171	31	F	10TH	HOUSE WIFE	NIL	sexualy	BGM	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	34	23	0.7	0.3	0.4	6.5	4.5	2	2.2	198	NAD	NAD	NAD	NAD	NAD
66	840843	58	M	8TH	FARMER	10000	sexualy	RAYBAG	Y	N	N	N	N	N	Y	N	N	N	N	N	N	Y	Y	P	P	NR	NR	65	61	1	0.5	0.5	6	3	3	1	239	NAD	NAD	NAD	NAD	NAD
67	845599	40	M	6TH	FARMER	10000	sexualy	SOUNDATTI	Y	N	Y	N	N	N	N	N	N	N	N	N	N	Y	Y	P	P	NR	NR	45	20	0.8	0.4	0.4	6.8	3.9	2.9	1.3	268	NAD	NAD	NAD	NAD	NAD
68	845164	38	M	8TH	FARMER	9000	sexualy	BILAGI	Y	N	N	N	N	N	N	N	N	N	N	N	N	Y	Y	P	P	NR	NR	18	25	0.8	0.2	0.6	7.5	4	3.5	1.1	136	NAD	NAD	NAD	NAD	NAD
69	848495	44	M	6TH	FARMER	7000	sexualy	KERUR	Y	Y	N	N	N	N	N	N	N	N	N	N	N	Y	Y	P	P	NR	NR	203	289	2.3	1.4	0.9	7	3.3	3.7	0.9	300	NAD	NAD	NAD	NAD	NAD
70	864033	50	M	10TH	SHOPKEEPER	7000	sexualy	BELGAVI	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	200	213	1.5	0.5	1	8	3	5	0.6	255	NAD	NAD	NAD	NAD	NAD
71	863345	45	M	12TH	DRIVER	8000	sexualy	BELGAVI	Y	Y	Y	N	N	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	120	100	0.9	0.5	0.4	6.5	3.5	3	1.1	100	NAD	NAD	NAD	NAD	NAD
72	864745	28	M	12TH	SHOPKEEPER	6000	sexualy	JAMKHANDI	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	40	42	1.2	0.6	0.6	6.3	4	2.3	1.7	110	NAD	NAD	NAD	NAD	NAD
73	864868	35	F	NIL	HOUSE WIFE	5000	sexualy	RAYBAG	Y	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	45	45	0.3	0.2	0.1	7	3	4	0.7	200	NAD	NAD	NAD	NAD	NAD
74	849226	50	F	NIL	HOUSE WIFE	5000	sexualy	BAGALKOT	Y	Y	Y	Y	Y	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	50	51	0.6	0.4	0.2	6.5	3.5	3	1.1	151	NAD	NAD	NAD	NAD	NAD
75	847952	42	F	NIL	TAILOR	7000	sexualy	BELGAVI	Y	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	NR	NR	48	45	1	0.6	0.4	6	4.5	1.5	3	122	NAD	NAD	NAD	NAD	NAD

ANNEXURE – V KEY TO MASTER CHART

Presenting complaints:	Y= Yes
	N= No
	TB= Tuberculosis
	DM= Diabetes Mellitus
HBsAg test by rapid method:	P= Positive
HBsAg test by ELISA method:	P= Positive
HIV ELISA:	NR= Non Reactive
	R= Reactive
HCV ELISA:	NR= Non Reactive
	R= Reactive
Liver profile:	
SGOT:	Serum glutamatic oxaloacetic transaminase
SGPT:	Serum glutamatic pyruvate transamin
ALK. P:	Alkaline Phosphtase
TB :	Total Bilirubin
DB:	Direct Bilirubin
ID :	Indirect Bilirubin
TP:	Total Protien
ALB:	Albumin
GLB:	Globulin
AG RATIO:	Albumin /Globulin ratio
NAD:	No Abnormality Detected

Introduction



Objectives

Review of Literature

Methodology

Results

Discussion

Conclusion

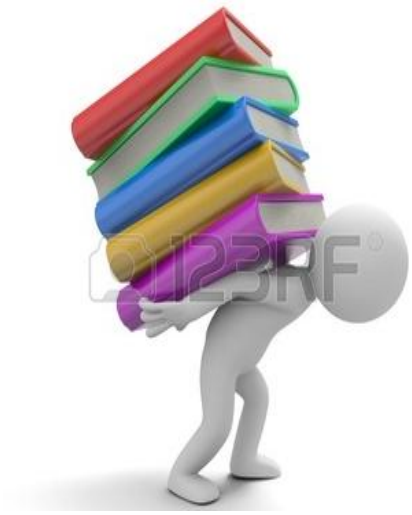
Summary

Bibliography

Annexure-I

Annexure-II

Annexure-III



Annexure-IV

Annexure-V