Hepatitis C seroprevalence among a tertiary hospital based general population in Northern India

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By

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CERTIFICATE

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LIST OF ABBREVIATIONS

ALT	Alanine aminotransferase
ANOVA	Analysis of variance
ART	Antiretroviral therapy
AST	Aspartate aminotransferase
BIA	Bioelectrical impedance analysis
C	Control region
CDC	Centers for Disease Control and Prevention
CMIA	Chemiluminescent microparticle immunoassay
CRF	Chronic renal failure
E_1, E_2	Envelope glycoproteins
EIA	Enzyme immunoassay
ELISA	Enzyme Linked Immunosorbent Assay
ER	Endoplasmic reticulum
HbsAg	Hepatitis B surface antigen
HCC	Hepatocellular carcinoma
HCV	Hepatitis C Virus
HCWs	Health care workers
HD	Haemodialysis
HIV	Human immunodeficiency virus
ICT	Immunochromatography test
ICU	Intensive Care Unit
IDU	Injecting drug use
IgM	Immunoglobulin M
IPD	Indoor patient
NANBH	Non-A, non-B hepatitis
NAT	Nucleic acid testing
NS	Non-structural proteins
OPD	Outdoor patient
ORF	Open reading frame
PA	Phase angle
r.p.m.	Revolutions per minute
RIBA	Recombinant immunoblot assay
RNA	Ribonucleic acid
RT-PCR	Reverse Transcription-Polymerase Chain Reaction
SLE	Systemic lupus erythematosus
STD	Sexually transmitted disease
T	Test region
UTRs	Untranslated regions
VAP-A	Vesicle-associated membrane protein-associated protein-subtype A
WHO	World Health Organization

ABSTRACT

INTRODUCTION: Hepatitis C is a disease which is caused by a virus that infects the liver. It causes inflammation of the liver that lead to liver cirrhosis and finally hepatocellular carcinoma. It is an enveloped, positive stranded RNA virus which is a member of Flaviviridae family. Its genome consists of open reading frame (ORF). N-terminal region of ORF codes for structural proteins and rest of region codes for non-structural proteins. The ORF have 5´ and 3´ untranslated regions (UTR). Hepatitis C virus is cause of post transfusion hepatitis. According to World Health Organization (WHO), 10-24 million people are HCV infected in India. Symptoms of HCV include fatigue, dark urine, belly pain, joint pain, itchy skin, sore muscles and jaundice. It is a blood-borne transmitted agent. Use of unsafe therapeutic injections also leads to HCV infection. Diagnosis of HCV infection can be done by various methods like Enzyme Immunoassay (EIA) and Recombinant Immunoblot Assay (RIBA). It is confirmed by HCV-RNA. It can be treated by antiviral therapy .i.e. ribavirin and interferon.

METHODS AND MATERIALS: A prospective study was conducted for four months (January-April) at Tertiary Hospital in Northern India. Total numbers of 1643 blood samples were screened for the presence of anti-HCV antibodies in patient's serum. Samples were tested by HCV TRI-DOT rapid test. Positive samples were retested by SD BIOLINE HCV rapid test. Finally, all positive samples were confirmed by ELISA.

RESULT: A total of 1643 patients were screened in the present study. Out of 1643, 102 (6.2%) samples were HCV positive. Among seropositive samples, 48 were males (2.9%) and 54 were females (3.2%). HCV seropositivity was shown by 40 IPD patients (2.4%), 52 OPD patients (3.1%) and 10 ICU patients (0.6%). Among departments, patients from Recovery showed (0.6%), General ward (1.0%), Private room (0.2%), Neurology lab (0.1%) and Emergency (0.3%) showed HCV seropositivity. On analyzing age-wise seropositivity, it was found that maximum seropositivity was seen in 30-40 years (2.9%) followed by >55 years (1.4%), 40-55 years (1.2%) and <30 years (0.5%). Analysis of patients on the basis of risk factors showed that 17 had history of surgical operation (1.0%), 18 had history of blood transfusion (1.0%), 6 had history of dental procedure (0.3%), 25 showed history of injecting drug use (1.5%), 21 used contaminated syringes (1.2%) and 15 patients was under haemodialysis (0.9%).

CONCLUSION: Hepatitis C is an enveloped and positive stranded RNA virus that causes inflammation of liver and ultimately damage to the liver that lead to cirrhosis and hepatocellular carcinoma. Primary prevention activities should be adopted such as counseling, screening and infection-control practices to decrease HCV infection. Secondary prevention activities should be adopted such as medical management of infected persons and screening of persons at high-risk to reduce chronic liver diseases. Sharing of personal items should be avoided. Professional people should protect themselves while handling infected blood. Counseling and testing should be done for those who are at risk for infection.

CHAPTER-1

INTRODUCTION

INTRODUCTION

The term 'Hepatitis' refers to damage to the liver by causing inflammation, cirrhosis and hepatocellular carcinoma. Inflammation is the local reaction of the body. It leads to accumulation of inflammatory cells, swelling to tissue and cells and eventually death of cells.

A number of different agents can cause hepatitis including infective agents (virus, bacteria, other organisms, chemical poisons, drugs and alcohol or an immune response towards the organ itself (autoimmune hepatitis).

Viral hepatitis refers to a set of at least five viruses that are known to cause hepatitis:

- Hepatitis A (HAV)
- Hepatitis B (HBV)
- **Hepatitis C (HCV)**
- Hepatitis D (HDV)
- Hepatitis E (HEV)[1]

Hepatitis C virus is an enveloped, spherical, positive stranded RNA virus which is a member of Flaviviridae family. It is 50 to 60 nm virus. It is surrounded by an envelope having glycoprotein spikes[2].

Hepatitis C is a disease which is caused by a virus that infects the liver. It can cause infection throughout the life. It is blood-borne infection. This virus passes from blood of an infected person to blood of an uninfected person[3].

Humans are only natural hosts of HCV. Hepatitis C is known to be as a "silent disease" because it is difficult to detect it in early stage and that is why early treatment of HCV is difficult. In most of the people, virus infection is not resolved naturally. The virus mutates to escape surveillance. When the liver is not able to remove the virus, the person becomes chronically infected. Chronic infection further causes cirrhosis followed by hepatocellular carcinoma and finally death[4].

DISCOVERY OF HEPATITIS C:

Harvey J. Alter (Mid 1970s) was first person who worked on virus. Michael Houghton first cloned and identified the virus. Initially, virus was called as non-A, non-B hepatitis (NANBH).Medical researches took decade to identify virus. It was collaboration of Chiron Corporation, Centers for Disease Control and Prevention of America and American multinational biotechnology firm. Molecular cloning was used to identify an unknown organism. In 1988, NANBH specimen was found in unknown organism which was later on found that it was virus. In April 1989, the name was changed from NANBH to Hepatitis C virus[5].

GENOME OF HEPATITIS- C VIRUS:

HCV is a member of Flaviviridae family. It is an enveloped virus having two or more envelope proteins (E). There is nucleocapsid composed of small basic protein (core or C) and consist of RNA genome. Its genome is a positive strand RNA molecule which consists of 9.6 to 12.3 thousand nucleotides with an open reading frame (ORF). The ORF codes for polyprotein of 3000 amino acids or more.

N-terminal region of ORF codes for structural proteins and rest of region codes for non-structural proteins. The ORF have 5´ and 3´ untranslated regions (UTR). These regions have role in RNA replication and polyprotein translation (Thurner et al., 2004).

5´ Untranslated Region- It is most conserved region. It consists of four structured domains, I to IV having a pseudoknot and stem-loops (Brown et al., 1992). It consists of 341 nucleotides. First 12 to 30 nucleotides along with domains II, III and IV make internal ribosome entry site (IRES) (Honda et al., 1996).

3´ Untranslated Region- This region consist of 225 nucleotides. It is divided into three regions, a variable region having 30-40 nucleotides, a long poly (U)- poly (U/UC) tract and a 3´ terminal stretch of 98 nucleotides having three stem-loop structures SL1, SL2 and SL3 (Kolykhalov et al.,1996). The 3´ UTR interacts with NS5B and also with two of four stem-loop structures. The 3´ X region and 52 nucleotides of poly (U/C) tract have

role in RNA replication and rest of sequence of 3´ UTR have role in viral replication (Ito and Lai., 1997).

GENOTYPE OF HEPATITIS-C VIRUS:

The hepatitis C genotype is strain of hepatitis C virus.

Following are six genotypes of hepatitis C:

- Genotype 1: It is most common genotype in the Europe and United States. In US, 80% of HCV infections are genotype 1. Treatment to this genotype is peginterferon and ribavirin which should be taken for 48 weeks.
- Genotype 2: It is considered as the second most common genotype in US. Approximately, 10% of HCV infections are genotype 2. Recent treatment includes combination of peginterferon and ribavirin for 24 weeks.
- Genotype 3: It is common in Southeast Asia. It has uneven distribution in India and Australia. In America, 6% of HCV infections are genotype 3. Its treatment is similar as the treatment for genotype 2.
- Genotype 4: It is common in European countries and Africa. Genotype 4 is most prevalent in Egypt. Its treatment is similar as treatment for genotype 1.
- Genotype 5: South Africa has high number of HCV infections with genotype 5. Treatment is combination therapy for 48 weeks.
- Genotype 6: It is common in Hong Kong, Southeast Asian countries and South China. Treatment is combination therapy for 48 weeks[6]

In HCV infected patient, HCV genotype does not change. The treatment of HCV depends upon strain of genotype present[7].

HCV PROTEINS:

HCV encodes a single polyprotein. There is release of structural proteins (core, E_1 and E_2) and p7 protein from polyprotein. The non-structural proteins are NS2, NS3, NS4A, NS4B, NS5A and NS5B are used for replication of viral genome.

STRUCTURAL PROTEINS:

1) CORE: It is a multi-functional protein. It is produced from polyprotein which is encoded by viral genome. This protein is able to self-assemble in HCV-like particles in endoplasmic reticulum (ER) membranes[8]. Core proteins pass from ER to mitochondria and help in Ca^{+2} regulation and apoptotic signals[9].
2) ENVELOPE GLYCOPROTEINS: E_1 and E_2 are envelope glycoproteins which form the structural component of virion. They are present on outer coat as spike-like projections of HCV. The function of E_1 and E_2 is host-cell entry through receptor binding.

NON-STRUCTURAL PROTEINS:

1) HCV NS2: It is a transmembrane hydrophobic protein. It is membrane-associated cysteine protease. NS2 interact with other HCV non-structural proteins. NS2 help in particle assembly step.
2) HCV NS3: It is crystal structure protein. It is tri-functional protein having serine protease, NTPase activities and an RNA helicase. It is required for post-translational modifications like phosphorylation and N-terminal acetylation.
3) HCV NS4A: It is a cofactor for NS3 serine protease activity. It is present on the ER and also on mitochondria. One of its functions is viral pathogenesis. By this activity, it affects cellular functions.
4) HCV NS4B: It is hydrophobic ER membrane protein. It consists of four transmembrane domains. Egger et al reported that when NS4B is expressed in cultured human osteosarcoma cells, it induced membrane alteration. It is present within membranous web along with other structural and NS proteins.
5) HCV NS5A: This protein is having RNA-binding activity. It is present in basally phosphorylated and hyper-phosphorylated cell forms. Interaction of NS5A with VAP-A is possible due to phosphorylation property of NS5A. It is divided into three domains: Domain I, Domain II and Domain III. In recent studies, NS5A is going to be important target for HCV infection therapy.
6) HCV NS5B: It is a RNA dependent RNA polymerase. Its structure consists of typical fingers, palm and thumb sub-domains. It does not have "proofreading"

function. NS5B nucleoside inhibitors are considered as functional chain terminators.

EPIDEMIOLOGY OF HCV:

Hepatitis C virus is a cause of post transfusion hepatitis[10]. HCV is compared with 'viral time bomb' as it is leading hepatotropic virus. It is cause of acute hepatitis, hepatocellular carcinoma and chronic liver diseases[11]. Worldwide, 200 million people are infected with HCV[12]. About 170 million have chronic HCV with 500,000 deaths per year[13]. According to World Health Organization, 10-24 million people are HCV infected in India[14]. In healthy people, HCV seroprevalence ranges from 1.5% to 4%[15]. In blood donors, it varies from 0.48% in Vellore to 1.85% in New Delhi[16]. In patients suspected for acute viral hepatitis, seroprevalence ranges from 3% to 12%[17]. Region-specific estimates range from <1.0% in Northern-Europe to >2.9% in Northern Africa. In HCV infected people, 27% of cirrhosis occurs worldwide. In last 20-40 years, most HCV transmission occurs in young adults and similar rate was found in Australia. The prevalence of HCV infection (age-wise) increase with age in Turkey, Spain, Italy, Japan and China. In Italy, Japan and China, there are hyper endemic areas of country in which HCV prevalence in older persons is 20 fold greater as comparison to overall persons in other areas of country. In Taiwan, the mean age with newly acquired HCV infection was 50 years and in Japan, it was 40 years (Miriam J Alter et al.)[18]

STATUS OF HCV INFECTION WORLDWIDE:

Hepatocellular carcinoma (HCC) is one of the most frequent malignancies in Asia. Cancer deaths exceeds in number and is ranked 3rd in Japan and Korea. In Korea, the number of deaths from liver cancer increased from 5,789 in 1983 to 9,966 in 1994. After that, it remains constant at 9,500/100,000 in 2003. In Japan, 80% of HCC cases are caused by HCV infection[19]. The rate of HCC mortality in Iran increased from 1999 to 2004[20]. In United States, incidence of acute hepatitis C has declined phase in 1992 but, since 2003, rates have plateaued[21].

In UK, chronic HCV infection and end-stage liver disease have been identified[22]. World Health Organization (WHO) found that immigrants to the UK may be at risk[23].

Country	Number of people living in UK	HCV prevalence estimated by WHO (%)	Number of people living in UK with HCV infection
India	467, 634	1.8	8, 417
China	149, 010	3	4, 470
Pakistan	321, 164	2.4	7, 708
Kenya	129, 635	0.9	1, 167
South Africa	141, 404	1.7	2, 404
Nigeria	88, 378	1.4	1, 237
Bangladesh	154, 354	2.4	3, 704
Zimbabwe	49, 529	7.7	3, 814
Ghana	56, 113	2.8	1, 571
Uganda	55, 207	1.2	662
Egypt	24, 705	18.1	4, 472
Philippines	40, 123	3.6	1, 444
Total infected			**41, 071**

Table 1: Immigrants to the UK with HCV infection

STATUS OF HCV INFECTION IN INDIA:

Hepatitis C is an important pathogen in India which is causing liver disease[24]. In India, there is very high frequency of HCV (60-90%). HCV infection was found to be more common in injecting drug users (IDU) [25]. In Delhi, HCV prevalence was 0.78% in voluntary blood donors and 1.33% in pregnant women[26]. Chadha et al., reported 0.09% HCV prevalence in rural Maharashtra[27]. In Hyderabad, it was found to be 1.4% in gastroenterology camps[28]. 7.89% HCV seroprevalence was found in Arunachal

0Pradesh[29]. In pregnant women, HCV seroprevalence varies from 0.6% to 1.4%[30-32]. Chandra et al and Khaja et al reported HCV prevalence as 1.4% and 2.02% in two studies from Andhra Pradesh. Another study conducted in West Bengal showed 0.71% positivity by PCR (Chowdhery et al). Study conducted in Lucknow and Chennai showed co-infection of HIV-HCV as 1.61% and 2.2%[33].

STATUS OF HCV INFECTION IN PUNJAB:

Studies related to seroprevalence of HCV become "eye openers" as it is causing chronic infections[34,35]. Study conducted by Sood et al. reported 5.2% HCV seroprevalence among families of Punjab. Age-group 40-60 years were highest seroprevalent[36.] Presence of anti-HCV antibodies among injecting drug users was found to be 49%[37].Paramdeep Singh et al. reported highest frequency of HCV (30.04%) in Ludhiana and lowest frequency (0.39% each) Tarn Taran and Ropar. Rural locality of Punjab was more HCV seroprevalent (67.25%) than urban locality (32.75%).

HCV distribution in different districts of Punjab is as follows[38]:

District of Punjab	Percentage (%)
Amritsar	1.16
Barnala	3.88
Bathinda	3.88
Faridkot	1.55
Fatehgarh Sahib	0.97
Ferozpur	6.98
Gurdaspur	1.36
Hoshiarpur	1.74
Jalandhar	2.71
Kapurthala	2.71
Ludhiana	30.04
Mansa	4.46
Moga	17.83
Muktsar	4.46
Nawanshehar	2.71
Patiala	1.16
Ropar	0.39
Sangrur	11.63
Tarn Taran	0.39

Table 2: Distribution of HCV positive patients according to districts of Punjab

SYMPTOMS OF HCV:

The symptoms of hepatitis C include:

- Fatigue
- Dark urine
- Belly pain
- Joint pain
- Itchy skin
- Sore muscles
- Jaundice[39]

According to Centers for Disease Control and Prevention (CDC), 70-80% of people having acute hepatitis C do not develop symptoms until the liver get damage[40]. People with chronic hepatitis C will have cirrhosis in which tissue of liver changes to fibrous tissue and ultimately scar-like hardening and liver stops functioning. The symptoms of cirrhosis are:

- Shrinkage of muscles
- Bleeding from enlarged veins in digestive tract
- Redness of palms of hands
- Clusters of blood vessels
- Damage to brain and nervous system[41]

TRANSMISSION OF HCV:

BLOOD TRANSFUSION: This type of transfusion is rare in most developed countries because of screening of blood before transfusion. It is rarely transmitted because of proper screening for presence of virus and inactivation procedures which kill blood borne viruses. In newly-infected patients having Hepatitis C antibody negative, presence of virus is detected by utilizing nucleic acid amplification of HCV. This technique minimizes cases of transfusion-associated HCV infections[42].

VERTICAL TRANSMISSION: Babies born to Hepatitis C women will test antibody positive at birth because they get mother's antibodies. The risk of this type of transmission is 5%. At the age of 18 months, most of the babies cleared mother's antibodies and will test negative for Hepatitis C.

Following factors increase risk of vertical transmission during pregnancy:

- Increased time period between membrane rupture and delivery
- HIV and Hepatitis C co-infection in mother
- The use of device which penetrates inside body
- High concentration of Hepatitis C virus in blood[43]

DRUG PREPARATION AND INJECTING EQUIPMENT: In Australia, mostly HCV transmission occurs by sharing or re-using contaminated needles during drug injection. Transmission can also occur by sharing of contaminated items such as swabs, water, syringes, plungers, mixing spoons. During preparation, hands and surfaces can also become contaminated. A small amount of blood which is not visible to naked eye can also transmit Hepatitis C. There is need to adopt cleanliness and hand washing. HCV transmission in injecting drug users is four times higher than HIV infection. The incidence of new infections is also high (Hahn JA.et al) [44].

SEXUAL CONTACT: HCV infection increases with number of lifetime sexual partners. History of sexually transmitted disease, more than five sexual partners per year is responsible for positive HCV. The frequency of HCV transmission between monogamous partners is low (Vandelli C et al.)[45]. HCV is not included in sexually transmissible infections (STI).This transmission is rare and occurs when there is blood to blood contact during sex. Sexual transmission occurs through use of sex toys, during menstruation or presence of STI which involves sores or ulcers. Some reports showed that there is increased risk of Hepatitis C transmission between men who have sex with men and who are also HIV positive.

OCCUPATIONAL EXPOSURES: Health care workers who have contact with blood or blood-borne pathogens are at risk of HCV transmission. According to CDC, rate of anti-HCV seroconversion from HCV positive source by unintentional needles or sharps

exposure is 1.8%. Italian study found 14 seroconversions using 4,403 needle sticks among health care workers. Health care workers after dealing with patient having chronic HCV must follow interferon and ribavirin therapy (De Carli G et al.)[46]

DIAGNOSIS OF HCV:

The diagnosis of HCV infection can be done by enzyme immunoassay (EIA) or enzyme linked immunosorbent assay (ELISA) and recombinant immunoblot assay (RIBA). It is confirmed by HCV RNA through reverse transcription-polymerase chain reaction (RT-PCR) [47].

EIA: Enzyme immunoassay (EIA) or ELISA is a technique for detection or quantification of antigens or antibodies in samples. In this process, there is addition of colored substrate and a visible product is seen. The product is determined by spectrophotometer. The intensity of color is proportional to presence of either antigens or antibodies in samples[48].

RIBA: HCV EIA is confirmed with RIBA. It detects HCV antibodies to HCV proteins[49]. It is needed to be done for low risk patients or if high risk patient is HCV RNA negative. RIBA is not useful for detection of weakly positive samples. The person with indeterminate RIBA should be retested by HCV RNA.

HCV RNA: It is used to measure amount of virus in the person. Active infection is detected if plasma contains HCV RNA. It can be detected 1 to 3 weeks after exposure to HCV infection. Reverse transcriptase (RT-PCR) and transcription-mediated amplification (TMA) are the methods to be used[50].

CHEMILUMINESCENT MICROPARTICLE IMMUNOASSAY (CMIA): It is advanced diagnostic method for the detection of HCV. It is a rapid and costly method. It is modified form of ELISA technique. It is having architect system which detects structural and non-structural proteins of HCV genome. In this method, bound acridinylated conjugates generate chemiluminescent signals. In the final step, chemiluminescent signals are compared with signal cut-off value to obtain result[51].

BIOELECTRICAL IMPEDANCE ANALYSIS (BIA): It is a technique which is used to calculate hepatic fibrosis in patients having chronic hepatitis C infection. BIA is a method

used to measure electrical resistance ® and reactance (X_c). This is further used to calculate phase angle (PA) [52].

IMMUNOHISTOCHEMICAL TECHNIQUE: This technique is used for detection of hepatitis C virus in liver biopsies of HCV infected patients. Haematoxylin and Eosin stain is used to examine histological activity and fibrosis. Immunohistochemical technique is used to detect HCV NS3 antigen by using monoclonal antibody against HCV NS3 protein[53].

TREATMENT OF HCV:

Treatment for Hepatitis C is different for different individual depending upon patient's age, medical history, type and stage of disease. The first step for treatment is prevention which includes avoiding unprotected sex or use of unsterilized needles or sharing of needles for drug use. Hepatitis C can be treated by giving antiviral therapy .i.e. ribavirin and interferon. Liver transplantation is also done in case of fulminant liver failure[54.] Recently, with the use of direct-acting HCV protease inhibitors, its treatment has been improved[55]. People having mild infection are suggested to take medical care and monitoring of liver. Antibiotics are not referred by doctors because it is caused by virus and antibiotics are not effective for viral infection.

The therapy for HCV depends upon viral genotype. Genotype 1 infection requires 48 weeks course. Genotype 2 or 3 infection requires 24 weeks course[56]. HCV therapy will be suggested by direct antiviral agents within next five years. A viral protease inhibitor named telaprevir is in phase 3 trials[57]. There is combination of telaprevir with pegylated interferon and ribavirin for three months. After that, three months of consolidation therapy is followed. There was 65% of response rate shown at international meetings. Other drugs are in developments which are also to be used in novel combinations[58].

CHAPTER-2

REVIEW OF LITERATURE

AIMS AND OBJECTIVES

- The aim of this study is to estimate seroprevalence of Hepatitis-C in both sexes and different age groups in hospital based general population.
- To evaluate knowledge about Hepatitis and risk factors amongst tertiary hospital patients.
- To study trends of HCV infections in a tertiary hospital located at Northern India.

CHAPTER-3

METHODS AND MATERIALS

REVIEW OF LITERATURE

When the term infectious diseases are applied then hepatitis C is considered as one of the most important new disease. Reverse transcription and PCR amplification of sub genomic fraction of HCV genome lead to genetic characterization of HCV. Initial studies showed that 40% of HCV infections in U.S. were 'community acquired' having no known risk factors. But, later on, it was found that most of cases were linked with risk factors for HCV like intravenous drug use. Further there was identification of many risk factors so term 'community acquired' was no longer in use. Molecular epidemiology indicated that there were six different genotypes evolved throughout the world. Pharmacodynamic studies showed rate of HCV production exceeds one trillion new virions each day (David R. Gretch et al.)[59]. HCV is an enveloped positive strand RNA virus. It belongs to family Flaviviridae (Snawar Hussain et al.)[60]. HCV is most common cause of chronic disease, cirrhosis and hepatocellular carcinoma. It infects 170 million people worldwide (Afdhal NH et al.)[61].

The study was conducted from 7th November 1995-6th November 2000 to determine susceptibility of patients with type-2 diabetes to Hepatitis C infection during long term haemodialysis. The prevalence of HCV infection in patients on haemodialysis having end stage renal disease is higher than general population (Sangiorgio et al.)[62] The study is comprised of 196 patients. Factors like period on dialysis, surgical interventions, invasive procedures, blood transfusions were determined and compared statistically between diabetic and non-diabetic cohorts. Serum samples were examined for HCV positive cases. Statistical analyses were performed. The Chi-square test was done to determine difference between proportions of anti-HCV positive patients in two groups (with type-2 diabetes and non-diabetics).The Student t-test was performed to compare means of two quantitative variables. Mantel-Haenszel odds ratio and 95% confidence-intervals were find out to determine association between seroconversion rates and selected risk factors. The cumulative survival curves were obtained. The conclusion was males were more anti-HCV positive than females. Out of 196 patients, 54 were type-2 diabetics and 142 were non-diabetics. Patients with type-2 diabetes mellitus were more anti-HCV positive than non-diabetics. In type-2 diabetics, higher annual seroconversion rate was detected

than non-diabetics on haemodialysis. The sharing of haemodialysis machines and reuse of dialysers is considered to play role in HCV transmission (Anil K. Saxena et al.)[63]

Seroprevalence of Hepatitis C virus was determined for two years in a hospital based general population in South India between October 1997 and July 1999. There was detection of IgG antibodies in serum samples from patients attending JIPMER Hospital, Pondicherry, India. The antibodies were tested against HCV proteins c200, c22-3 and RNA polymerase by enzyme immunoassay kit. The tests were done with adequate controls and absorbance of solution in wells by ELISA reader. The Chi-square test was also done. The result was out of 661 patients, 32 were HCV positive. Seroprevalence among males and females were 5.9% and 3.3%.Highest seroprevalence was among males of age-group 40-49 years and females of 30-39 years age-group. Out of 36 health care workers, males were 20 and females were 16. None was HCV positive (Bhattacharya S et al.)[64]. The seroprevalence of HCV in hospital based population was found to be 4.8% and it is similar to study conducted in Mauritius 1994 i.e.5.9 %(Schwarz TF et al.) [65]

Investigation of prevalence of human immunodeficiency virus, hepatitis B and hepatitis C virus antibodies and hepatitis B antigen among commercial sex workers in Japan in 2001 was determined. Sera of 308 women and 241 control subjects who attended sexually transmitted disease (STD) in Tokyo were screened for HCV antibody. Screening test for HCV was done by chemiluminescent enzyme immunoassay (EIA) by use of HCV recombinant antigen. Analysis of data was done statistically by Fisher's direct test. Subjects found to be positive for HCV antibody were further examined for liver function and other STD complications. The result of this study was HCV antibody positive rate was 3.2% in commercial sex workers and 0.4% in control group. In 10 HCV positive commercial sex workers, four had impaired liver function (>32 IU/L aspartate aminotransferase) and three cases were having other STD (one case positive for syphilis, one case positive for *Chlamydia trachomatis* and *Neisseria gonorrhoeae)*. HCV positive rate is 8-10 times higher in CSW than in women in general population (Kazuhisa Ishi et al.)[66]

Seroprevalence of hepatitis C virus antibodies, hepatitis B surface antigen and antibody in high risk groups of Egyptian children and its effect on liver functions was detected in

December 2004. The examination was done on 100 children. 44 were males and 56 were females. Children were divided in four groups:

Group 1 comprised of 34 children having Insulin Dependent Diabetes Mellitus. All children were on insulin regimen.

Group 2 comprised of 31 children with Chronic Renal Failure (CRF) undergoing haemodialysis at Cairo University Children's Hospital. Dialysis sessions were done three days per week. Infection control measures were followed according to Center for Disease Control and Prevention in haemodialysis unit.

Group 3 comprised of 15 patients suffering from Systemic Lupus Erythematosus (SLE). Antimalarial drugs and steroids were given to patients and 14 were given immunosuppressive drugs.

Group 4 comprised of 20 healthy children. These children were not having history of diabetes mellitus or connective tissue disease.

All children were examined with full history and clinical examination. Laboratory investigations were performed. Hepatitis C antibodies were detected by ELISA. Data were analyzed by statistical methods. The seroprevalence of anti-HCV antibodies comes out to be 38%. The mean serum levels of AST and ALT were higher in anti-HCV seropositive than seronegative children. 15 diabetic children, 16 CRF cases, 6 SLE patients and 1 healthy child were anti-HCV seropositive. The conclusion was parenteral transmission which is a major risk factor for infection. Nosocomial HCV transmission in diabetic patients is not due to medical practices (Manal E. Kandil et al.)[6]

A Study was conducted in which seroprevalence of Hepatitis C and correlation between viral load and viral genotype is determined among primary care clients in Mexico from January 2006 to December 2009. The main motive of this study was to explain importance of early identification of HCV for treatment. Samples were collected from regions of Mexico: North, Center, Pacific/South and Disrito Federal Mexico. ELISA test was done. HCV sub typing was done by sequencing 339bp amplicon of NS5b region. PCR was performed with NS5-1 and NS5-2 primers for primary amplification and NS5-1 and NS5-3 for secondary amplification. Statistical analysis was calculated to obtain

frequency of HCV antibodies by ELISA and frequency of presence of viral RNA by RT-PCR test. A direct correlation was obtained between viral load and viral genotype. Seroprevalence of HCV was found 1.5%. HCV distribution according to regions was North (1.65%), Center (1.55%), Pacific/South (0.81%) and Distrito Federal Mexico (1.59%). Males were more HCV infected. HCV RNA positive group consist of most of families having history of cirrhosis. Viral genotype among Mexico population comes out to be that most frequent genotype was genotype 1/subtype 1A (33%) followed by genotype1/subtype 1B (21.4%) and genotype2/subtype 2A (8.50%). A linear positive correlation was observed for highest viral load and viral genotype 1 with a R^2 of 80%. This study was conducted to prevent people with chronic infection towards cirrhosis or hepatocellular carcinoma (Ana I Burguete-Garcia et al.) [68]

Seroprevalence of HIV, HBV and HCV among blood donors was determined from May 2006-December 2010 at Max Super Speciality Hospital, Saket, New Delhi, India. Blood is a source of transmission of infectious diseases such as HIV, HBV and HCV. HCV is considered to be main cause of chronic liver diseases like cirrhosis, fibrosis and hepatocellular carcinoma. Serological screening, nucleic acid testing (NAT) and quality control was performed. NAT was done by Roche Cobas TaqScreen MPX assay. Complete history and clinical examination was taken from donors. Sample that indicate 'pool reactive' were tested again. Positive samples were sent for discrimination. Quality controls were done by using positive and negative controls from manufacturers. A total of 41,207 units of blood from donors were screened for HIV, HBV and HCV infection. The conclusion of this study was prevalence of HCV in blood donors in different parts of world ranges from 0.5% to 1.85%. Prevalence of HCV infection have increasing trend from 0.18% in 2005 to 0.82% in 2009.The seroreactivity of HCV based on enhanced chemiluminescence assay comes out to be 0.7%. Dual testing strategy and NAT used in this study is useful to find infectious blood units with all phases of infection which further enhance safety for blood transfusion(Sangeeta Pathak et al.)[69]

Age-wise seroprevalence of hepatitis C virus infection in clinically suspected infectious hepatitis patients was determined for a period of 1 year from January 2008 to December 2008 at Lady Hardinge Medical College, New Delhi in the department of Microbiology. Hepatitis C virus is cause of post transfusion hepatitis. About 75% of infections are

indicated by abnormal liver function test or seropositivity. Patients were divided into two groups. Group 1 consists of 600 patients from out-patient department having clinically suspected acute infectious hepatitis. Group 2 consist of 200 patients with no acute infectious hepatitis. Their age and sex were also matched. ELISA was performed to detect immunoglobulin M(IgM) anti HCV in serum of patients(study and control group).Both groups were divided into 0-10 years,11-20 years,21-30 years,31-40 years and >40 years. In study group, 5.5% samples were positive for IgM anti HCV while in control group, 1.5% samples were positive. Maximum seropositivity was seen in 11-20 years (9%) followed by 21-30 years (6.1%), 0-10 years (5.1%) and >40 years (3.7%) in study group. Maximum seropositivity was seen in 0-10 years (2.8%) followed by 11-20 years (2.7%) in control group. There is a decline in HCV seropositivity from 1997 to 2002(12 to 3.3%) but now it is on increasing trend. Young adults are more HCV positive because of risk of exposure with age. Children less than 10 years are affected by mother to baby transmission. Hepatitis C is a major health problem due to unawareness of clinical status (Rajani M et al.)[70]

A Study of high prevalence of anti-HCV antibodies in two metropolitan emergency departments in Germany was conducted. Serum was taken from May 2008 to October 2009 in traumatology emergency department, Berlin. Then serum was taken from another department.i.e.J.W.Goethe university hospital emergency department, Frankfurt (between September 2009 and March 2010).Values for alanine aminotransferase and aspartate aminotransferase levels were defined as 35U/mL in females and 50U/mL in males. Blood samples were screened for anti-HCV antibodies by electrochemiluminescence immunoassay. Presence of HCV-RNA was tested in HCV positive samples by real-time PCR-based COBAS Ampliprep HCV-RNA assay. In Berlin, HCV genotyping was done with VERSANT HCV genotype 2.0 line probe assay. In Frankfurt, genotyping was analyzed from patient's chart. HCV positive patients were given guidelines about HCV awareness and the associated risk factors. Statistical analyses were performed by using chi-square test and Mann-Whitney U-test. Prevalence of anti-HCV antibodies comes out to be 2.4% at Berlin and 3.5% at Frankfurt. Patients visiting trauma departments were having high number of HCV-RNA positivity. Presence of HCV-RNA was detected in 67.9% anti-HCV positive patients. 26.8% patients from Berlin tested positive for anti-

HCV antibodies but negative for HCV-RNA. Most prevalent genotype was HCV genotype 1 followed by genotype 3 and 2. Increased levels of ALT and AST was observed in 52% of patients. Injecting drug users were reported as primary risk factor in 31% patients. The conclusion was seroprevalence of HCV in Germany differ by region. (Johannes Vermehren et al) [71]

A Study was conducted for two years from 1st May 2008 to 30th April 2010 at tertiary care hospital in northern India. The objective of this study was to determine seroprevalence of anti-hepatitis C virus antibody in injecting drug users (IDU) and non-injecting drug users with or without other HCV related risk behavior. Non-IDUs are vulnerable to HCV infection through non-injecting routes. HCV infection is more common in IDU than HBV and HIV infections. Serum from each subject was screened by enzyme-linked immunosorbent assay. Sample absorbance was detected by use of filter (450nm) with 620-690nm wavelength. Samples which come within grey zone absorbance were tested again by anti-HCV ELISA kit. The subjects were divided into three groups: injecting drug users, non-injecting drug users with high risk behaviour and non-injecting drug users without high risk behaviour. The subjects were compared by ANOVA. The Chi square test/Fisher exact test was performed for group comparisons. The conclusion was non-IDUs without risk behaviour were the oldest than non-IDUs with risk behaviour and the IDUs. 46% of IDUs, 8.1% non-IDUs with HCV-related risk and 3.7% non-IDUs without HCV related risk were found to be seropositive for anti-HCV antibody. Seroprevalence of HCV is more in IDUs as compared to non-IDUs and it is related to injecting risk behaviour. Duration of drug use and active use of drug is not responsible for HCV seropositivity (Debasish Basu et al) [72].

The Study was conducted between 1st January 2010 and 31st December 2010.The objective of this study was to determine seroprevalence and risk factors for HCV infection in pregnant women in tertiary care hospital, Hyderabad, Sindh. HCV infection is associated with cholestasis of pregnancy, preterm delivery, fetal infection and intrauterine growth. The risk factors for HCV infection in pregnant women are history of blood transfusion, intravenous drug abuse, HBV and HIV infection, sexually transmitted infections. Sera from women were tested by ELISA in Laboratory of Liaquat University of Medical and Health Sciences, Jamshoro. Detailed history was evaluated on cases and

controls. Further it was analyzed by SPSS version 16 statistical package. Odds ratio and 95% confidence interval were calculated to measure association between cases and controls with risk factors by multivariate logistic regression analysis. The conclusion was HCV pregnant women come out to be 4.7% out of 3078 women. The infected women have history of blood transfusion, surgery, sharing of household articles and therapeutic injection use. In logistic regression, it has been shown that there was no association between all variables and HCV positivity. Unsafe injection use is considered as major factor for HCV prevalence. Lack of expert staff, standard operating procedures, lack of organized infrastructure and people who can't afford for screening contribute spread of disease (Seema Bibi et al) [73].

A Study was conducted for 11 months (from March 2010 to February 2011) at Krishna Hospital and Medical Research Center, Karad, Maharashtra. Seroprevalence of HCV in both sexes and different age groups was estimated. Patients from OPD and IPD were tested for HCV antibody testing. HCV serology was performed by taking serum samples. Sera were tested by third generation enzyme immunoassay kit (ELISA). Manufacturer's instructions were followed. Test was performed with adequate controls and absorbance of solutions in wells was at 450nm within 15 minutes in ELISA. The positive samples were tested again in duplicates. Results were analyzed by chi-square test. Out of 7373 serum samples, 3238(43.92%) were males and 4135(56.08%) were females. Seroprevalence of HCV was found to be 0.38%. Males were more HCV seroprevalent (0.62%) than females (0.19%). No statistically significant difference was found in males and females. Age-group (41-50 years) in males were more HCV infected (2.05%) and age-group (51-60 years) in females were more HCV infected (0.87%). But age-group (11-19 years) in both sexes was not found to be HCV infected. HCV infection is important cause of chronic hepatitis. Treatment for HCV depends upon genotype so genotype is studied for further treatment (Patil Satish R et al) [74]

Seropositivity and co-infection of Hepatitis B and C among patients seeking hospital care in Islamabad, Pakistan was determined from 1st July to 31st August 2011at Pakistan Institute of Medical Sciences. HCV infection can cause liver disease such as liver failure, cirrhosis and hepatocellular carcinoma. Co-infection of HBV and HCV occur because of same mode of transmission. Total numbers of 845 blood samples were screened with

proper detail of age, sex, areas and date of collection for detection of HBV and HCV by use of immunochromatography test. Further samples were analyzed by ELISA. HCV ELISA kit is used for qualitative detection of antibodies of HCV. Indirect ELISA was performed. Analysis of data was done. The result was found to be 23.5% samples were HCV positive. Co-infection of HBV and HCV was found in 1.3% samples. Female patients had more HCV positivity. Married patient had high frequency of HCV. The conclusion was HBV and HCV prevalence is at increasing rate in Pakistan (Jafar Khan et al) [75]

Seroprevalence of hepatitis C virus among health care workers were determined at rural teaching hospital in Tamil Nadu from June to July, 2012. Blood samples were collected from 85 health care workers. Questionnaire form was filled. Sera samples were tested by third generation ELISA kit. HCV seropositivity was found to be 1.17%. Lab technicians were found to be 25% HCV seropositive. HCV viral load was examined by Taqman PCR. Viral load was within normal limits. Liver function tests were normal (Vallab Ganesh Bharadwaj B) [76]

A cross sectional study was conducted at tertiary hospital in Rwanda between October 2013 and December 2013 among health care workers. Seroprevalence of hepatitis B and C were determined. A total number of 378 health care workers were examined. A questionnaire was given to health care workers in order to know about their risk factors and knowledge of HCV infection prevention. Diagnosis was done by using 'Cypress Diagnostics' anti-HCV rapid diagnostic test. The dipstick test was also done. Positive samples were confirmed by an electro-chemiluminescence immunoassay. Statistical analysis was performed. HCV seroprevalence comes out to be 1.3%. Out of 378 health care workers, 57.1% were found to have contaminated blood exposure, 3.7% had blood transfusion, 55.6% had surgery, 52.7% had dental procedures, 20.1% had body piercing and 96% had visits to salons for hair and nail cutting (Fredrick Kateera) [77]

A Cross-sectional study was conducted in May 2014.The aim of this study was seroprevalence and risk factors of HBV and HCV and its effect on liver enzyme among HIV positive children at Felgehiwot referral hospital, Ethiopia. Co-infection of HIV infected patients with HBV and HCV is problem which occurs due to shared routes of transmission. HBV and HCV infections are causes of morbidity and mortality in HIV

positive patients which lead to liver cirrhosis and hepatocellular carcinoma. A questionnaire was given to children's parents in order to collect data on full history and associated risk factors. Sera were screened for presence for presence of hepatitis B surface antigens (HBsAg) and anti-HCV antibody. ALT level was determined by Beckman Coulter Synchron Clinical Systems auto lab analyzer. Along with the test, internal positive and negative controls were also run. Data was analyzed by statistical methods. Odds ratio and 95% confidence intervals were calculated. Out of 253 HIV infected children, 52.5% were boys and 47.5% were girls. 87.7% children were sent to antiretroviral therapy (ART) clinic. Seroprevalence of HCV was found to be 5.5%. Children from urban areas were more HCV seroprevalent than children from rural areas. 20.8% of girls had female genital mutilation ad 21% had uvulectomy. 3.5% had history of blood transfusion. The elevated ALT level in anti-HCV antibody positive children was 31.5%. In this study, seroprevalence of HCV was 5.5% is almost similar with HCV prevalence in Africa and it is highest in world. Co-infection of hepatitis in HIV positive children were linked with elevated ALT (Bayeh Abera et al) [78]

CHAPTER-4

RESULTS

METHODS AND MATERIALS

AREA OF STUDY: A prospective study was conducted for four months (January-April) in the Department of Clinical Microbiology at Tertiary Hospital in Northern India.

COLLECTION OF SAMPLE: Total numbers of 1643 blood samples were collected from individuals who attended outdoor patient and indoor patient departments of Tertiary hospital. Samples were kept in clean, dry and sterile red vials. Proper labeling was done with patient's full name, sex, age, areas and date of collection.

CENTRIFUGATION: Vials containing sample were centrifuged at 10,000 r.p.m. for 15 minutes. Sera were used for screening of anti-HCV antibodies.

SCREENING OF SAMPLE: A total of 1643 blood samples were screened for the presence of anti-HCV antibodies in the patient's serum.

There were two methods used for screening of HCV seropositivity which are discussed as follows:

- Rapid test methods
- Enzyme linked immunosorbent assay (ELISA)

RAPID TEST METHODS:

1) HCV TRI-DOT
2) SD-BIOLINE

1) HCV TRI-DOT RAPID TEST:

   The 4th Generation HCV TRI-DOT kit is used for qualitative detection of antibodies to Hepatitis C Virus in serum. It has increased sensitivity for core and NS3 antibodies. It uses modified HCV antigens which are for core (structural), NS3 (non-structural), NS4 (non-structural) and NS5 (non-structural) regions of virus. The device contain two test dots 'T_1' and 'T_2' and a Built in

Quality Control Dot "C". The function of control dot is to develop color which confirms that the procedure is done well.

PRINCIPLE:

There is immunofiltration membrane on which immobilized HCV antigens are present. Sample and reagents get pass through membrane. They are absorbed by absorbent pad. When patient's sample is passed through membrane then if HCV antibodies are present in serum, get bind to antigens. Protein-A conjugate is added and it binds to F_c portion of HCV antibodies. Pinkish purple dot is observed at regions 'T_1' and/or 'T_2'. At region 'C' a "built-in Quality Control Dot" is formed which confirms proper functioning of procedure.

KIT COMPONENTS:

COMPONENTS	CONTENTS	PREPARATION
1. HCV TRI-DOT test device	It is packed individually. There are dots "T_1" and "T_2" for test dots and "C" for Control dot	Cut and open the pouch before use
2. Buffer solution	It contain BSA and sodium azide	Ready to use
3. Protein-A conjugate	It is in liquid form and contain sodium azide	Ready to use
4. Sample Dropper	It is plastic dropper used to add the sample	Ready to use

STORAGE OF THE KIT:

The kit was kept at 2-8ºC in dry area. The kit components were brought to room temperature at 20-25ºC before running the test.

COLLECTION OF SAMPLE AND STORAGE:

The sample was collected in clean and dry vial. It was allowed to clot. By centrifugation, serum was separated. If serum was not be used immediately, it could be stored at 2-8ºC. Human serum or plasma could be used for the test. Specimens which were haemolysed or contaminated with microbes were discarded and fresh aliquot were collected.

PROCEDURE:

1. Pouch was opened and device kit taken out for performing the test.
2. Three drops of Buffer solution were added to centre of device.
3. One drop of 50µl of serum was added with the help of dropper.
4. Five drops of Buffer solution were added.
5. Two drops of protein-A conjugate were added.
6. Five drops of Buffer solution were added.
7. The device was discarded immediately because it was infectious[79]

Each reagent was added after soaking of previous reagent

BEFORE PROCESSING HCV PROCEDURE:

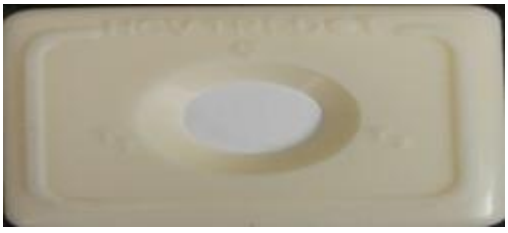

Figure 1: Before processing

AFTER PROCESSING HCV PROCEDURE:

Interpretation of result	Picture
Reactive result: • If two dots at 'C' and 'T_1' region appears • If two dots at 'C' and 'T_2' region appears • If three dots at 'C', 'T_1' and 'T_2' region appears	
Non-reactive result: • If one dot at 'C' region appears	
Invalid result: • If no dot appears	

Figure 2: Results of HCV TRI-DOT rapid test

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2) SD BIOLINE HCV RAPID TEST:

This test is immunochromatographic test for the qualitative detection of antibodies to HCV in serum, plasma and whole blood.

PRINCIPLE:

This test contains a membrane strip and it is pre-coated with recombinant HCV capture antigen (core, NS3, NS4 and NS5) on test band region. When antigen-antibody protein A gold particle complex is formed, protein A colloid gold conjugate and serum sample starts moving along membrane chromatographically to test region 'T'. There are two regions labeled as 'T' for test line and 'C' for control line. The control line appears when the test is done correctly.

MATERIALS PROVIDED:

This kit contains following materials:

- The test device is pouched with a desiccant
- Capillary pipette
- Assay diluent
- Package insert

COLLECTION OF SAMPLE, STORAGE AND PRECAUTION:

- The whole blood was collected into collection tube containing anticoagulants. It is allowed to clot for 30 minutes. It was centrifuged to separate serum.
- If serum was not tested immediately, it was refrigerated at 2-8ºC. They were brought to room temperature before use.
- If result comes inconsistent then sample was clarified before assaying.

PROCEDURE:

1. The pouch was opened and took out the device.
2. 10µl of serum was added into the sample well.

3. Four drops of assay diluent were added into sample well.
4. Result was interpreted in 5-20 minutes.

INTERPRETATION OF RESULTS:

- At control line 'C', the color band was appeared that shows test was performed well.
- The color band was observed on the right side which was the test line[80]

NON-REACTIVE RESULT:

The presence of color band on line "C" indicated non-reactive result.

REACTIVE RESULT:

The presence of both color bands "T" and "C" indicated reactive result.

Figure 3: Reactive Result

INVALID RESULT:

If control band "C" was not observed then the test was considered invalid. The sample was tested again with the use of new test device.

3) HCV MICROLISA: **The third generation HCV Microlisa is enzyme linked immunosorbent assay which is used for qualitative detection of anti-HCV antibodies in serum or plasma.** Before performing ELISA, blood is screened to eliminate infected units of blood.

PRINCIPLE:

Microlisa is highly sensitive technique which detects anti-HCV antibodies in serum. This technique uses combination of antigen with HCV structural and non-structural antigen. i.e. CORE, E1, E2, NS3, NS4 and NS5. It is having increased sensitivity and specificity.

The combinations of antigens are coated on the microwells for structural and non-structural HCV proteins. Incubation of diluted sample and controls is done. If HCV antibodies are present then they will bind to immobilized antigens on the microwell during incubation period.

Microwells are properly washed with diluted wash buffer in order to eliminate unbound anti-HCV or other human IgGs. Add enzyme conjugate, anti-human IgG conjugated with HRPO. The excess amount of enzyme conjugate is removed with diluted wash buffer. Microwells will hold bound antigen-anti HCV-enzyme conjugate complex.

Freshly prepared substrate solution is incubated with complex in microwells. There is antigen-antibody reaction which is indicated by blue color with enzyme substrate reaction. This reaction occurs in microwells. Finally, stop solution is added. Optical density of color developed is read photometrically.

COMPONENTS OF THE KIT:

COMPONENTS	CONTENTS	PREPARATION
1. Microwells	12 strips (12 X 8 wells) Microwells coated with HCV antigens	Ready to use
2. Sample Diluent	1 bottle (20 ml) Buffer consist of protein stabilizers and antimicrobial agents	Ready to use
3. Enzyme Conjugate Concentrate (100 X)	1 Vial (0.25 ml) Anti-human IgGs conjugated with horseradish peroxidase with protein stabilizers	Ready to use
4. Conjugate Diluent	1 bottle (15 ml) Buffer contain protein	Ready to use

	stabilizers	
5. Wash buffer Concentrate (25 X)	1 bottle (50 ml) PBS with surfactant	1:25 was diluted with distilled water before use
6. TMB Substrate	1 bottle (10 ml)	It was diluted with TMB diluents before use
7. TMB Diluent	1 bottle (10 ml) Buffer solution contain H_2O_2 with preservative	Ready to use
8. Control(-)	1 vial (2.0 ml) Normal human serum. Sodium azide as preservative	Ready to use
9. Control (+)	1 vial (2.0 ml) Reactive for HCV antibodies. Sodium azide as preservative	Ready to use
10. Stop solution	1 vial (15ml) 1 N sulphuric acid	Ready to use
11. Plate sealers	Adhesive sheets to seal microwell plates	Ready to use

Figure 4: Components of the kit (Sample diluent, Conjugate diluent, Stop solution and Wash buffer concentrate)

ADDITIONAL MATERIALS REQUIRED:

- Distilled water
- Micropipettes or microtips
- Sodium hydroxide solution
- Elisa reader
- Absorbent tissue
- Graduated cylinders
- Incubator 37ºC
- Glassware
- Elisa washer
- Timer
- Vortex mixer
- Disposable gloves

Figure 5: Components of the kit (Enzyme conjugate concentrate, negative control, positive control, TMB diluent and TMB substrate)

STORAGE OF KIT:

The kit was stored at 2-8ºC.

COLLECTION AND PREPARATION OF SAMPLE:

- The serum was removed from the clot to avoid haemolysis. Fresh samples were preferred.
- Samples were stored at 2-8ºC.

PROCEDURE:

1. 100µl of sample diluent was added to A-1 well as blank.
2. 100µl negative control was added in each well number B-1 and C-1.
3. 100µl positive control was added in D-1, E-1 and F-1 wells.
4. 100µl sample diluent was added in each well, starting from G-1 well.
5. Then in the same wells starting from G-1 well, 10µl sample was added. Sample got diluted in sample diluent.
6. 100µl each sample starting from G-1 well was transferred in each well.
7. The wells were covered with seal and incubated at 37ºC ± 2ºC for 30 minutes ± 2 minutes.

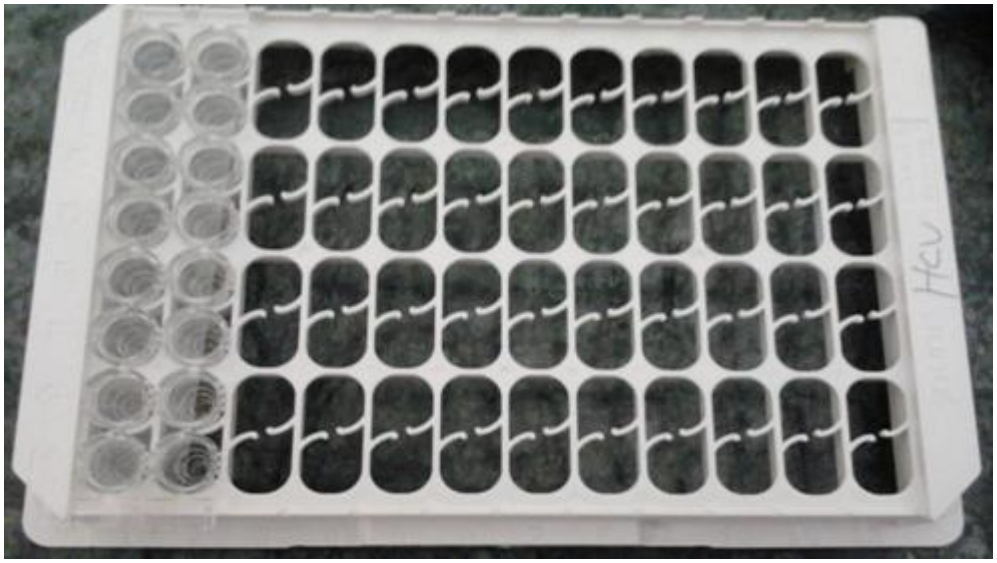


Figure 6: Wells covered with seal

8. Working wash solution and working conjugate was prepared.
9. After incubation period, wells were washed five times with working solution.
10. 100µl working conjugate solution was added in each well.
11. Again the wells were cover sealed and incubated at 37ºC ± 2ºC for 30 minutes ± 2 minutes.
12. Substrate solution was prepared when five minutes were left of incubation.
13. The wells were aspirated and washed five times with working wash solution.

Figure 7: Washing of wells

14. 100µl working substrate solution was added in each well.
15. Incubated for 30 minutes in dark at room temperature (20-30ºC).
16. 100µl stop solution was added.
17. After blanking A-1 well, absorbance was read in ELISA READER at 450nm.

INTERPRETATION OF RESULTS:

The cut-off value was calculated according to manufacturer's instructions.

- Samples with absorbance value less than cut-off value were non-reactive for HCV antibodies.
- Samples with absorbance value greater than or equal to cut-off value were reactive for HCV antibodies.
- Samples with absorbance value less than cut-off value.i.e. within 10% were suspected for HCV antibodies. Such samples were tested again.
- Samples with absorbance value equal to and greater than cut-off value were supposed to be initially reactive. Original sample was tested again in duplicate.
- If absorbance value of both duplicate retested sample was less than cut-off value, the sample was non-reactive for HCV antibodies.
- If absorbance value of any one of the duplicate retested sample or both duplicate retested comes equal to or greater than cut-off, the sample were reactive.

Some samples were not reactive in repeat. They were showing colors due to following reasons:

- Contamination of substrate
- Pipette tips contamination
- Improper washing
- Aspiration while washing[81]

All the samples were tested by **HCV TRI-DOT** kit method. Further, HCV positive samples were retested by another rapid method i.e. **SD-BIOLINE HCV** method. Finally, all positive samples were confirmed by **ELISA**.

CHAPTER-5

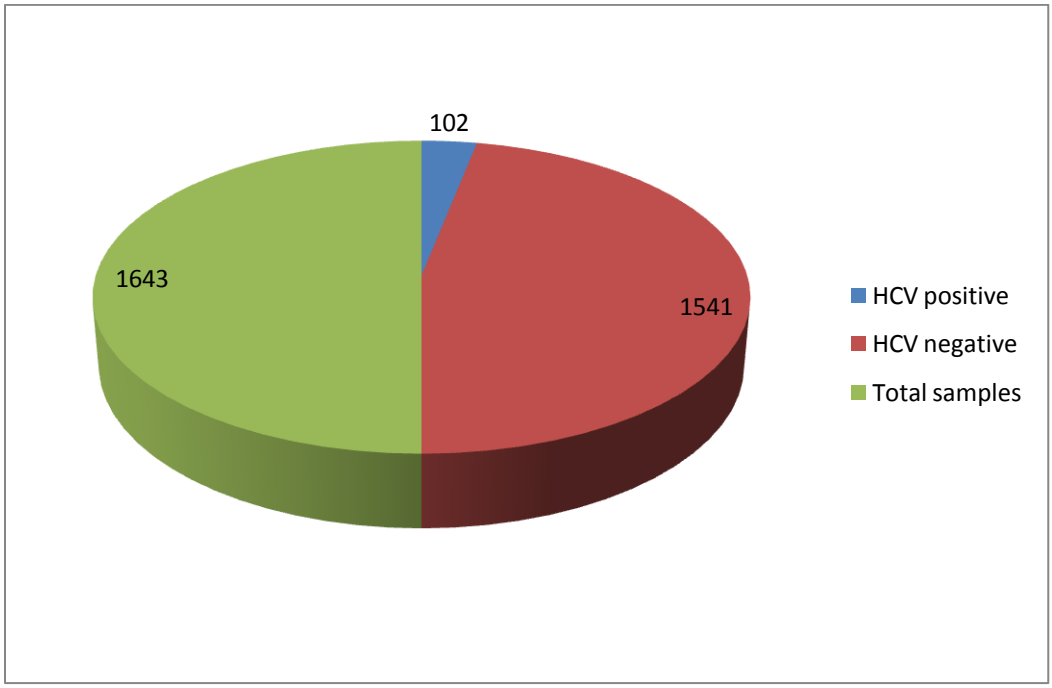
DISCUSSION

RESULTS

A total of 1,643 patients were screened in the present study which was conducted in a tertiary hospital in Northern India for four months. Sera were screened for the presence of anti-HCV antibodies. Out of 1,643 samples, 102 (6.2%) were HCV positive.

TABLE 3: SEROPOSITIVITY AND SERONEGATIVITY OF HCV:

	Number of samples	Percentage (%)
HCV positive	102	6.2%
HCV negative	1541	93.7%
Total samples	**1643**	

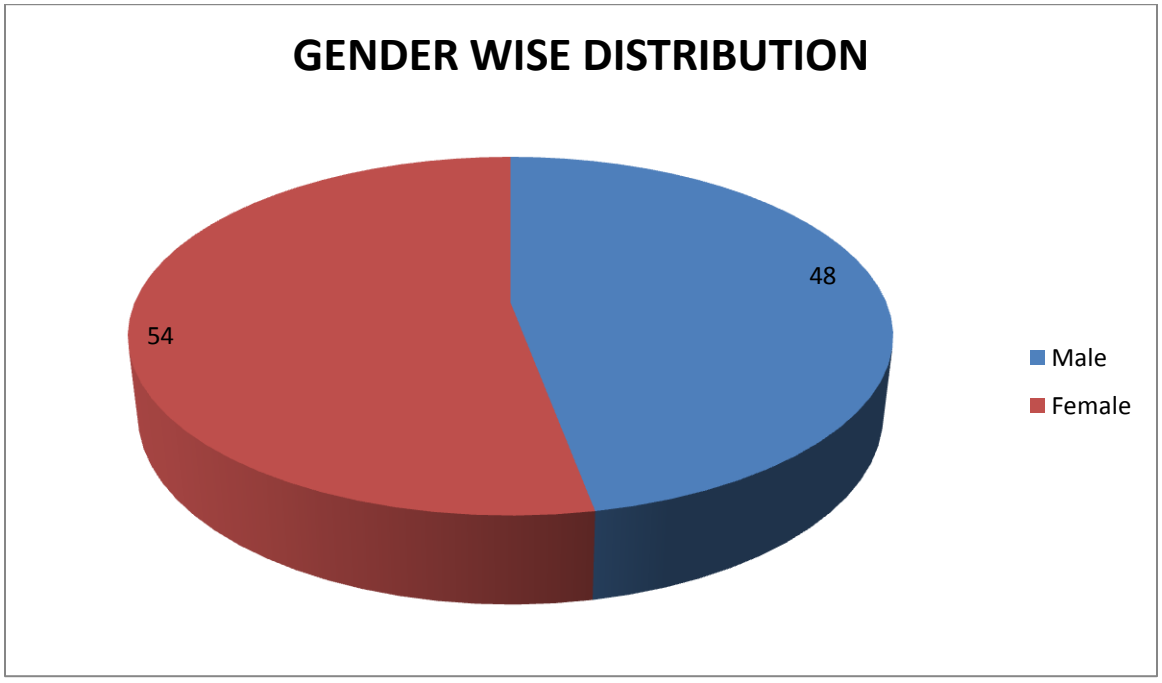


Graph 1: Distribution of HCV positive and negative samples

All HCV positive samples (102) were tested with HCV TRI-DOT kit and retested with SD-BIOLINE HCV kit. All positive samples were confirmed by ELISA. Among seropositive samples, 48 were males (2.9%) and 54 were females (3.2%). Females were more HCV prevalent.

TABLE 4: GENDER WISE DISTRIBUTION:

Gender	Number of reactive patients	Percentage (%)
Male	48	2.9%
Female	54	3.2%
Total number of patients	**102**	

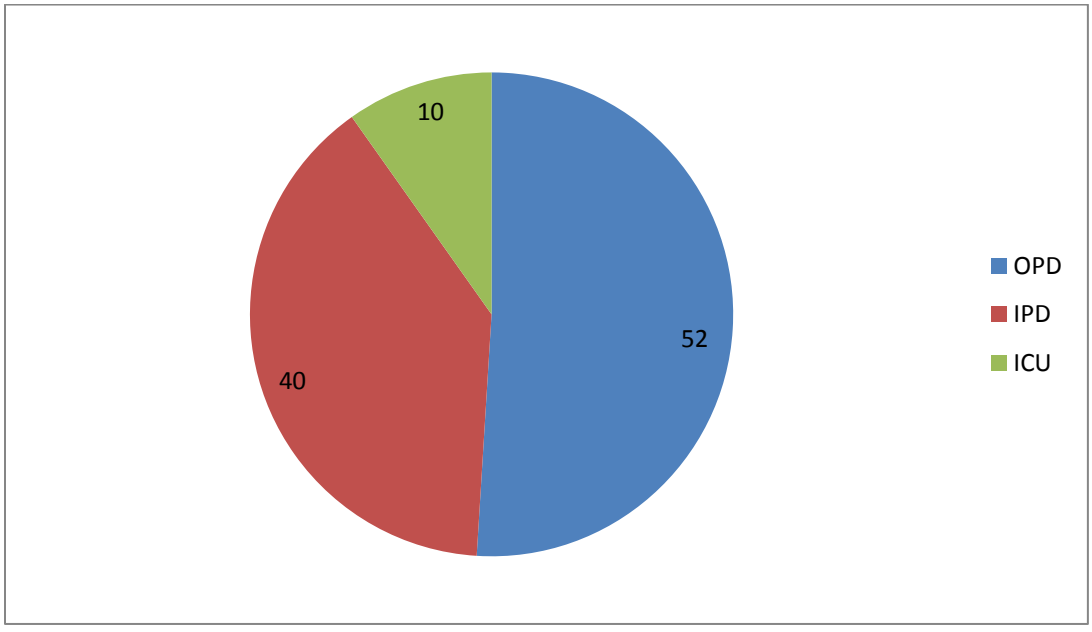


Graph 2: Distribution of HCV positive patients according to gender

Samples were collected from different wards .i.e. IPD (indoor patient department), OPD (outdoor patient department) and ICU (intensive care unit). HCV seropositivity was shown by 52 OPD patients (3.1%), 40 IPD patients (2.4%) and 10 ICU patients (0.6%). Samples collected from OPD showed increased HCV infection rates (3.1%) while samples collected from ICU showed less HCV infection rates (0.6%).

TABLE 5: PATIENT DISTRIBUTION:

Ward	Positive	Percentage (%)
OPD	52	3.1%
IPD	40	2.4%
ICU	10	0.6%

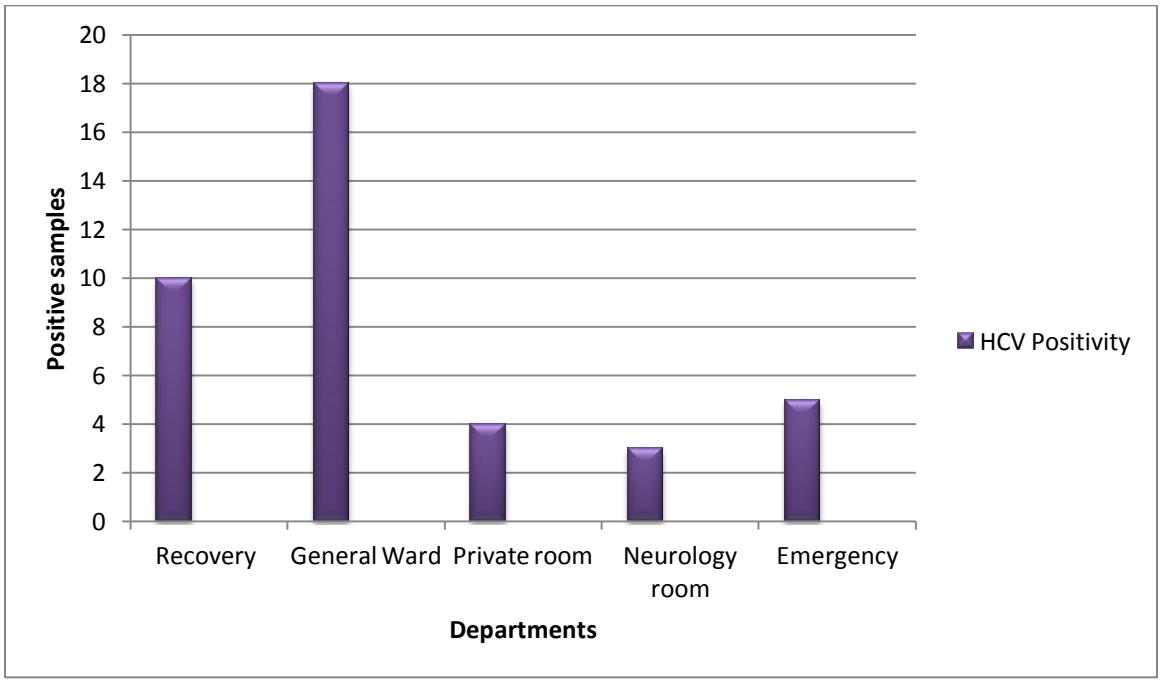


Graph 3: Distribution of patients according to wards

There were many departments in hospital named as Recovery, General ward, Private room, Neurology lab and Emergency. The seropositivity of HCV was higher in patients of General ward (1.0%) and lower in Neurology lab (0.1%).

TABLE 6: DEPARTMENT WISE DISTRIBUTION:

Department	Positive	Percentage (%)
Recovery	10	0.6%
General Ward	18	1.0%
Private room	4	0.2%
Neurology room	3	0.1%
Emergency	5	0.3%

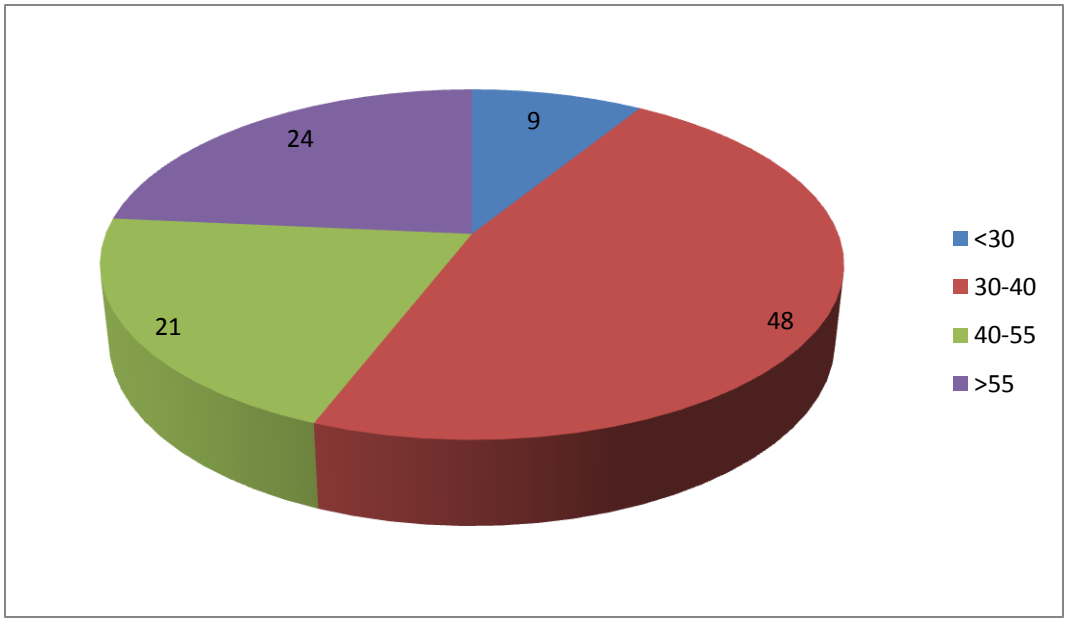


Graph 4: HCV distribution according to departments

TABLE 7: AGE-WISE DISTRIBUTION:

The present study was divided age-wise into <30 years, 30-40 years, 40-55 years and >55 years. Maximum seropositivity for HCV was seen in 30-40 years (2.9%) followed by >55 years (1.4%), 40-55 years (1.2%) and <30 years (0.5%). HCV found to be more frequent in age-group 30-40 years (2.9%) and less frequent in age-group <30 years (0.5%).

Age groups (in years)	Positive	%age
<30	9	0.5%
30-40	48	2.9%
40-55	21	1.2%
>55	24	1.4%

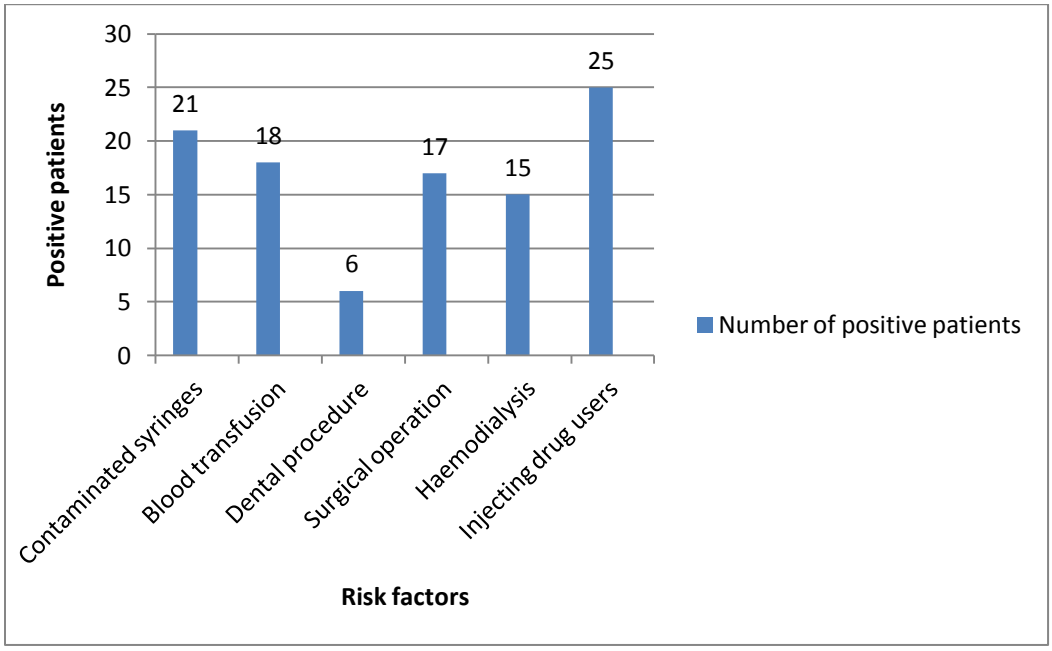


Graph 5: Distribution of patients according to age

Out of 102 HCV positive patients, 17 had history of surgical operation (1.0%), 18 had history of blood transfusion (1.0%), 6 gave history of dental procedure (0.3%), 25 showed history of injecting drug use (1.5%), 21 patients used contaminated syringes (1.2%) and 15 patients were under haemodialysis (0.9%).

TABLE 8: RISK FACTORS DISTRIBUTION:

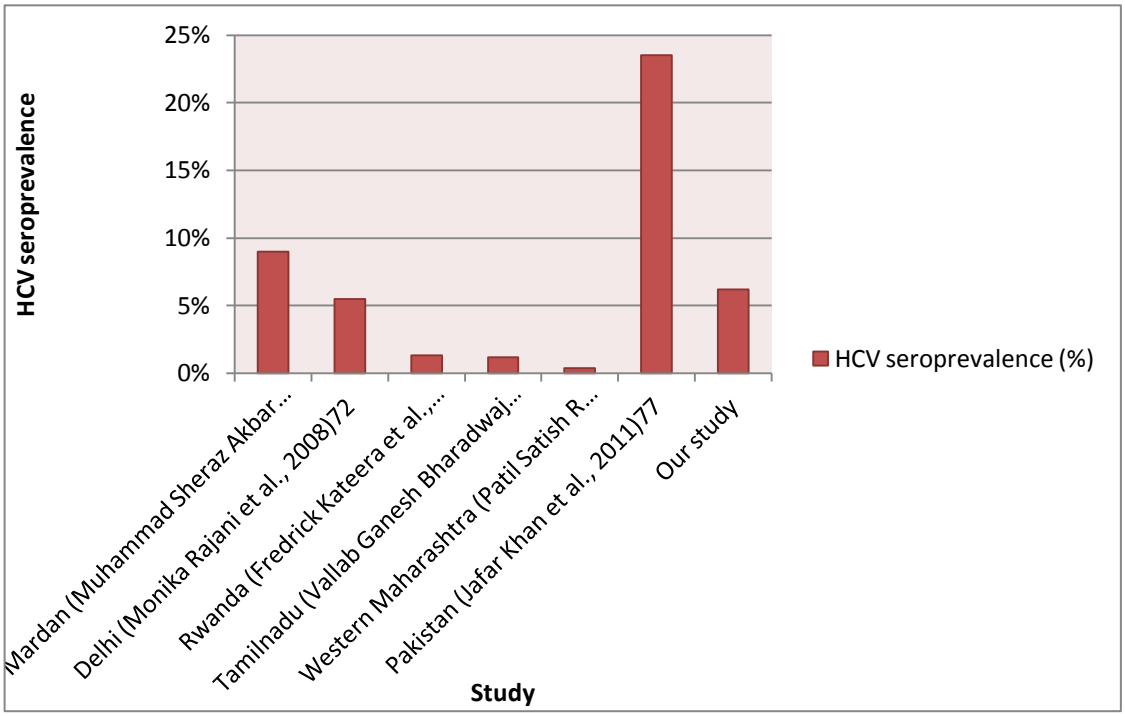
Risk factors	Number of positive patients	Percentage (%)
Contaminated syringes	21	1.2%
Blood transfusion	18	1.0%
Dental procedure	6	0.3%
Surgical operation	17	1.0%
Haemodialysis	15	0.9%
Injecting drug users	25	1.5%



Graph 6: Risk factors for HCV infection

TABLE 9: COMPARATIVE ANALYSIS OF SEROPREVALENCE OF HCV IN HOSPITAL-BASED POPULATION:

Studies conducted at various regions	HCV seroprevalence (%)
1. Mardan (Muhammad Sheraz Akbar Khan et al., 2004)[82]	9%
2. Delhi (Monika Rajani et al., 2008)[70]	5.5%
3. Rwanda (Fredrick Kateera et al., 2013)[77]	1.3%
4. Tamilnadu (Vallab Ganesh Bharadwaj B et al., 2012)[76]	1.17%
5. Western Maharashtra (Patil Satish R et al., 2011)[74]	0.38%
6. Pakistan (Jafar Khan et al., 2011)[75]	23.5%
7. Our study	6.2%



Graph 7: HCV seroprevalence in hospital-based population

CHAPTER-6

CONCLUSION

CONCLUSION

Hepatitis C is a virus that infects the liver by causing liver inflammation and damage. It is spread by exposure to contaminated blood, sharing of needles, sex with infected person and from mother to child during birth. According to Centers for Disease Control and Prevention (CDC), about 17,000 new hepatitis C cases were seen every year. Out of which 75 to 85% of cases become chronically infected.

The seroprevalence of HCV is 6.2% in our study. Such high HCV prevalence is due to increase in number of injecting drug users in Northern India. There should be counseling for young generation in order to reduce HCV infection.

Primary prevention activities and secondary prevention activities should be acquired to decrease the risk for HCV infection. Primary prevention activities include the steps taken to decrease the HCV infection and secondary prevention activities include the steps taken to reduce chronic liver diseases.

Following steps should be taken for primary prevention activities:

- Counseling for risk reduction
- Screening of blood, plasma, organ and tissue
- Infection-control practices
- Inactivation of virus from plasma-derived products

Following steps should be taken for secondary prevention activities:

- Medical management of infected persons
- Counseling and screening of persons at risk

Needles are used to inject drugs, cosmetics, steroids and other injections. As these activities can cause infection so sharing of needles should be avoided. Sharing of personal items such as razors, toothbrushes, nail-cutting equipments should be avoided so as to decrease the risk for hepatitis C. If personal items are shared with others then these can end up sharing diseases. Do not have sex without a condom. This can put a risk for

HCV infection. Tattoos and piercing should be done in licensed facility where artists use sterile equipments by taking all safety precautions.

Professional people should know the concepts of blood-borne pathogens and the risk associated in the healthcare settings. They should aware of how the infection can be caused. Wearing of gloves, gowns and face masks should be essential in healthcare settings as it can protect from the risk for hepatitis C. There should be proper disposal of sharp needles so as to decrease the risk of infection from someone else's blood.

Some important factors should be considered such as suitable diagnostic tests and public health awareness. Counseling and testing of persons should be done for those who are at risk for infection or who practice high-risk behaviors. Healthcare workers should carry out history about use of drugs or unprotected sex. Drug users should be advised to go for substance-abuse treatment, stop taking drugs, use sterilized syringes and dispose syringes in safely manner.

Patients taking home infusion therapy should be guided for the risk of blood-borne pathogens and to maintain infection-control practices.

Safety precautions are needed to be taken in haemodialysis settings. Staff should use gloves while handling blood, body fluids, excretions, secretions and contaminated equipments. Persons with high ALT (alanine aminotransferase) and AST (aspartate aminotransferase) levels should be checked daily for HCV infection. Patient with history of blood transfusion or organ transplantation should be screened for HCV infection.

Antiviral therapy is referred in case of early HCV infection and the person is guided to follow-up medical management. A child should be tested if the mother is HCV positive. Testing of such children should be done after 12 months of age.

Drugs like boceprevir and telaprevir should be used for HCV treatment. Along with that new direct-acting antiviral therapy and host-targeted drugs are in development. Protease inhibitors are likely to play effective role in future treatment. Others protease inhibitors under development include vaniprevir, simeprevir, danoprevir, BI-201335 and MK-5172. These inhibitors will improve tolerance and safety profiles. New interferons have been

explored. Immune system generates native human interferon lambda proteins which are directed against viral infection.

Information regarding transmission of HCV should be provided to public such as nature of hepatitis C, benefits of early detection, steps to be taken if HCV positive, screening tests and drug-treatment centers. Persons with HCV positive should be advised to prevent themselves from further liver disorders, stop drinking alcohol and not to take new medicines without consulting doctor.

The data of current study will be helpful in taking prevention and control measures against HCV infection.

CHAPTER-7

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