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EVALUATING THE TRANSMISSION OF HEPATITIS C VIRUS IN ADULT POPULATION IN SWASTIK DIAGNOSTIC LABORATORY, JAMMU

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ABSTRACT.

Testing for anti-HCV antibodies with a serological test identifies people who have been infected with the virus. If the test is positive for anti-HCV antibodies, a nucleic acid test for HCV ribonucleic acid [RNA] is needed to confirm chronic infection and the need for treatment. This test is important because about 30% of people infected with HCV spontaneously clear the infection by strong immune response without the need for treatment. Although, no longer infected, they will still test positive for anti-HCV antibodies. This nucleic acid for HCV RNA can either be done in a lab or using a simple point of care machine in the clinic.

After a person has been diagnosed with chronic HCV infection, an assessment should be conducted to determine the degree of liver damage [Fibrosis and cirrhosis]. This can be done by liver biopsy or through a variety of non-invasive test. The degree of liver damage is used to guide treatment discussion and management of the disease. Early diagnosis can prevent health problems that may result from infection and prevent transmission of virus. WHO recommended testing people who may be at increased risk of infection.Some of the objectives of my study were also fulfilled and it was observed and concluded that risk factors involved in HCV infection were injected drugs, patients who had a history of STD/ born to a woman with HCV, needle stick injuries caused due to usage of tattoo unsterile equipment, blood transfusion and organ transplant from an infected donor, and patients already infected with HIV, HBV.

After screening, it was also observed that 26 patients were positive for both Anti HCV and HCV RNA. Although, after treatment, no longer infected, they will still test positive for anti-HCV antibodies.

INTRODUCTION

Blood-borne hepatitis C virus (HCV) was first discovered in 1989 (2). HCV has an impact on the liver, and some those with the virus develop carcinoma of the liver and cirrhosis (17). Globally, an estimated 71 million persons had HCV infection in 2015 (46), and 1.75 million occurrence infections happen each year (32). Every

year, it is estimated that approximately 400,000 individuals pass away from problems spurred on by HCV (30). The World Health Organisation (WHO) set goals in 2016 to make HCV no longer be a public health issue by 2030(30). These goals include an 80% decrease in penetration infections and a 65% decrease in HCV-related premature death from 2015 levels (56). Following the creation of direct-acting acyclovir (DAA) cure for HCV, the WHO published those goals (56). DAAs produce sustained virologic responses with a mean (an effective cure) of 95%, which is a significant improvement above the effectiveness of earlier interferon-based treatments (5). The epidemiological study of HCV must be fully understood, nevertheless, in order to eradicate it. When there are many participating healthcare priorities asking investment, having the knowledge will enable policy-makers to decide which populations to focus on for HCV testing and treatment. This might boost the yield of people with the virus from testing and the prevention advantages associated with treatment.

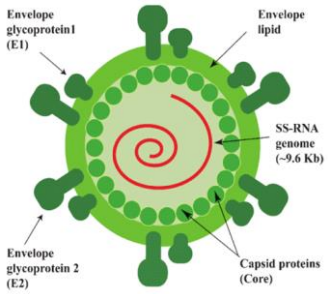

Fig.

As more nations adopt DAAs to treat HCV, progress is currently monitored by the United Nations World Health Organisation in its 2018 Having access to HCV Management Report(57). Egypt, resulting in one of the greatest rates of HIV infection in the world and was projected to be 10.0% in 2015, is an example of a nation that has made significant progress (44). In contrast to most other nations, Egypt's microbiology of HCV has received the greatest attention due to its large burden. The WHO's extinction targets have received significant backing from politicians, in part because of knowing of the the epidemiological discipline of HCV in Egyptians and the serious toll it will place on the health system; commencing in October 2018, approximately 30-million individuals in Egypt had HCV tests throughout the course of four months (44).The majority of nations, however, have progressed with far less progress than Egypt, in part because of a dearth to understanding about the the epidemiology topic and medical assistance load of HCV in those nations (57). The significance of several HCV transmission pathways has grown (55, 11). In the past, unsafe blood transfusions from others were the most common method of transmission worldwide. However, this has significantly decreased as a result of the implementation that contains blood screening at various intervals and under various circumstances in various nations (47). Following this, it is believed that a greater percentage of new infections have been brought about by the use of non-sterile medical shots and other operations, as well as by the sharing of IDU-related equipment (55).Infections have also been linked to childbirth (49), sexual transmission (26), and going to a barber who doesn't sanitise their blades (10). The primary risk factors for HCV infection are unclear for many nations, and it is unclear how important the various modes of

transmission are for each nation's epidemic. The primary prevention for transmitting through improved prevention of infections and injection safety in health care facilities and in the community, all screening of blood and its derivatives, harm prevention programmes, and increased public awareness of risk factors for HCV infection are all necessary components of the comprehensive approach needed to control HCV infection. However, in nations with middle and low incomes, this is less certain.Furthermore, little research has been done on the entire epidemiological investigation benefits of this therapy, including how curing HCV infection affected people can also stop the spread of the infection to others. evaluating the epidemiology of HCV in that area is crucial for evaluating the impact of therapy because this protective benefit could be impacted by the unique characteristics of the catastrophic in each country.

Most people who spontaneously recover from an infection do so to support the first six months, and those who are still sick after a year are not feasible to (48). It might be challenging to estimate how long HCV RNA needs to persist in the body after the first infection in order for it to be considered a chronic infection. This is because the acute period of infection is typically asymptomatic and the dissemination date is frequently unknown (21).The flavivridae subfamily is the natural home of the hepatitis C virus (HCV). It is a 50–60 nm pathogen with a linear, positively polarised single-stranded RNA that is contained within a core and encased in an envelope that contains spikes of glycoprotein. It has been placed in the family Flaviviridae under a different genus called Hepacivirus.

The virus demonstrates a diverse set of genomic and epitope traits. Many subtypes and six distinct genetic variants suggest to significant mutability. While HCV genotype 1 is more difficult to treat, genotypes 2 and 3 cause more severe liver damage. Due to this diversity, there is little complementary or even analogous post-infection antigen reaction in hepatitis C infection.

There are two forms of HCV: acute and chronic. Young-onset hepatitis C is a temporary infection. There may be a six-month interval between symptoms. Chronic hepatitis C is a long-lasting infection. Although the hepatitis C virus has been cloned using a strain of Escherichia coli, a viral infection has not yet been created in culture.When someone contracts HCV, they are at the acute stage of the condition. 15% of persons initially suffer symptoms (48). Although these often mild symptoms occasionally come with jaundice (48), a very uncommon but serious incident of scan may also result in mortality (81). Gender, age, genotype, and the presence of certain conditions are only a few of the variables that may affect the spontaneous clearance of an unexpected infection.

After spontaneous clearance, there are are no longer any HCV-related negative consequences in that person (21). It is yet unknown if the person will be resistant to re-infection with HCV or whether they have a larger genetic propensity to recuperate from the virus (4, 42).

People have a higher likelihood of quickly recovering from an infection (4). The majority of persons who recover on their own from a disease do so within the first six months, while individuals who are still sick after

a year are unlikely to do so (48). Estimating how long DNA from H must remain in the body during the first infection for it to be categorised as a chronic infection may be difficult. This is because the acute period of infection is typically asymptomatic, which makes it harder to identify the exact date that it spreads in many circumstances (21).

Testing for HCV antibodies, such as the enzyme-linked immunosorbent assay (ELISA), might reveal a person's history of acute infection (39). A positive HCV antibody test result, however, does not reveal whether the subject has already eradicated their infection on their own or is still infected (39). To determine whether an active infection is present, HCV RNA assays, such as a nucleic acid test (NAT), or an HCV core epitope test, such as the chemiluminescence the immunoassay (CLIA), are necessary(39). The 2017 revision of the World Health Organization's testing guidance for hepatitis B and C identified NAT testing for HCV RNA as the best method for determining the presence of a chronic infection (31).

Hepatocellular carcinoma can occur in people with the compensated cir as compared to uncompensated cirrhosis at a rate of 1–5% on a yearly (21). The annual liver-related death rate for neglected, chronically infected patients with cirrhosis is around 7%; the severity of cirrhosis and the presence of cancer of the liver are the key prognostic indicators for mortality associated with HIV infection (38). For decompensated cirrhosis, the likelihood of surviving five years is 50% (36).

Fig. **Chronic HCV infection liver cirrhosis and hepatocellular carcinom**

MATERIAL AND METHODS

1 Methods:

The study was conducted from 1 January 2023 to 31 March 2022 in the Swastik diagnostic laboratory and Department of Biochemistry and Molecular Biology.

2 Sample collection: In the present study 100 HCV samples were taken from patients who registered in Swastik Diagnostic Laboratory Jammu for HCV test. HCV samples collected from the venous blood in Clot Activator vials [RED TOP]. Wait for 30 minutes at room temperature. Then sample centrifuged at 3000 rpm for 15 min. The sample should be checked for haemolysis, if sample is haemolysed then repeat the test with fresh sample.

The pathologist did Anti-HCV /HCV RNA examination. Any abnormal findings on Anti-HCV /HCV RNA will be selected for further analysis.

3.Anti-Hepatitis C Virus [HCV]

The Elecsys Anti-HCV II assay is an in vitro diagnostic test for the qualitative detection of antibodies to hepatitis C virus [HCV] in human serum and plasma.

The electrochemiluminescence immunoassay "ECLIA" is intended for use on cobas-pro [cobas-e 801] immunoassay analyzers.

SAMPLE COLLECTION:

HCV RNA requires purified nucleic acids from whole blood/ plasma collected in EDTA anticoagulant specimens that are extracted using the Universal Cartridge based Sample Prep Device and Universal Cartridge based Sample Prep kit.

Storage and Stability:

- Sample should be Store at 2-8 ºC for two days.
- Extracted RNA should be store at -70ºC to -80ºC.

3.4.2 PRINCIPAL:

HCV RNA works on the principle of Real Time Reverse Transcription Polymerase Chain Reaction (RT PCR) based on Taqman chemistry. The RNA from the patient sample is first extracted using Universal Cartridge Based Sample Prep Device and Universal Cartridge Based Sample Prep Kit. The HCV RNA chip is placed on the chip tray of the Real Time micro PCR Analyzer. Six (6) µL of the purified RNA is then dispensed using the provided micropipette and tip into the micro tube containing freeze dried RT PCR reagents and allowed to stand for 30-60 seconds to Get a clear solution.

HCV RNA Extractions

PROCEDURAL STEPS OF EXTRACTION

- Take 250 µl of whole blood in Trueprep AUTO Universal Sample Pre-Treatment [USPT] Lysis buffer bottle using 1 ml disposable pipette provided.
- Vortex it well and keep it for incubation for 10 minutes at room temperature or 37ºC.
- After incubation, open Trueprep AUTO UNIVERSAL CARTRIDGE based sample prep kit pack and place the cartridge on cartridge stand.
- Open BLACK CAP of cartridge; transfer all the content of USPT using 3 ml of disposable pipette and close the cap.
- Press POWER button of Trueprep AUTO Extraction device.
- Press EJECT button which makes the lid open.
- After completion of extraction process, the device beeps and ejects the lid.
- Collect all ELUTE IMMEDIATELY using sterile disposable pipette provided with cartridge pack in sterile empty collection tube.
- Label Elute Collection Tube with patient name and ID.
- Discard the empty cartridge and disposable pipette in 1 % Hypo solution

RESULTS.

200 subjects were analyzed for 3rd generation of HCV and HCV RNA. The table shows the age-wise distribution of patients coming for RNA screening. Patients were divided into six age groups 20-30, 31-40, 41-50, 51-60, 61-70, and 71-80. The highest number of patients were seen in the age group 20-30 (63), followed by 31-40 (47), followed by age group 41-50[25], followed by 51-60 (30), followed by age group61-70 [19] and followed by age group 71-80 [15]. Out of 50 patients, 16 patients were infected due to Injected drugs, 13 patients were infected who had a history of STD/ born to a woman with HCV, 11 patients were infected due to needle stick injuries caused due to usage of tattoo unsterile equipment, 6 patients were infected due to blood transfusion and organ transplant, 4 patients were infected due to have HIV, HBV.

4.1Primary Screening

200 patients were subjected to primary screening by HCV ELISA testing. Out of the total Patients screened by HCV ELISA, 150 were to be negative. Rest 50 patients were HCV positive. It was observed that highest frequency of patients were seen in age group 20-30 while lowest frequency of patients lied in age group 71-80. 47 patients were screened in age group 31-40, 25 patients were screened in age group 41-50, 30 patients were screened in age group 51-60, and 19 patients were screened in age group 61-70.

AGE GROUP	FREQUENCY
20-30	63
31-40	47
41-50	25
51-60	30
61-70	19
71-80	15
TOTAL	200 (100%)

Table 2: Frequency of patients in different age groups [Primary screening]

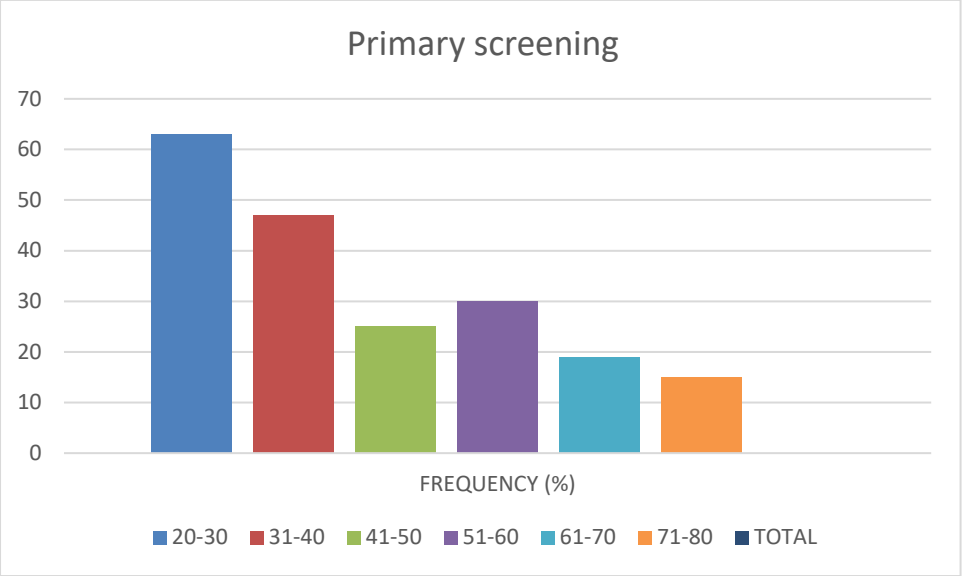

Fig 1: Graphical representation of frequency of patients in different age groups [Primary screening]

4.2 Secondary screening

50 patients who resulted positive in primary screening were then further subjected to secondary screening by HCV RNA quantitative [Viral Load] screening. It was then concluded that 52% of patients were positive for HCV RNA, while 48% of patients who were positive in primary screening tested negative for secondary screening. It was observed that highest frequency of patients were seen in age group 20-30 while lowest frequency of patients lied in age group 71-80. 10 patients were screened in age group 31-40, 3 patients were screened in age group 41-50, 4 patients were screened in age group 51-60, 7 patients were screened in age group 61-70.

AGE GROUP	FREQUENCY
20-30	25
31-40	10
41-50	3
51-60	4
61-70	7
71-80	1
TOTAL	50

Table 3 Frequency of patients in different age groups [Secondary screening]

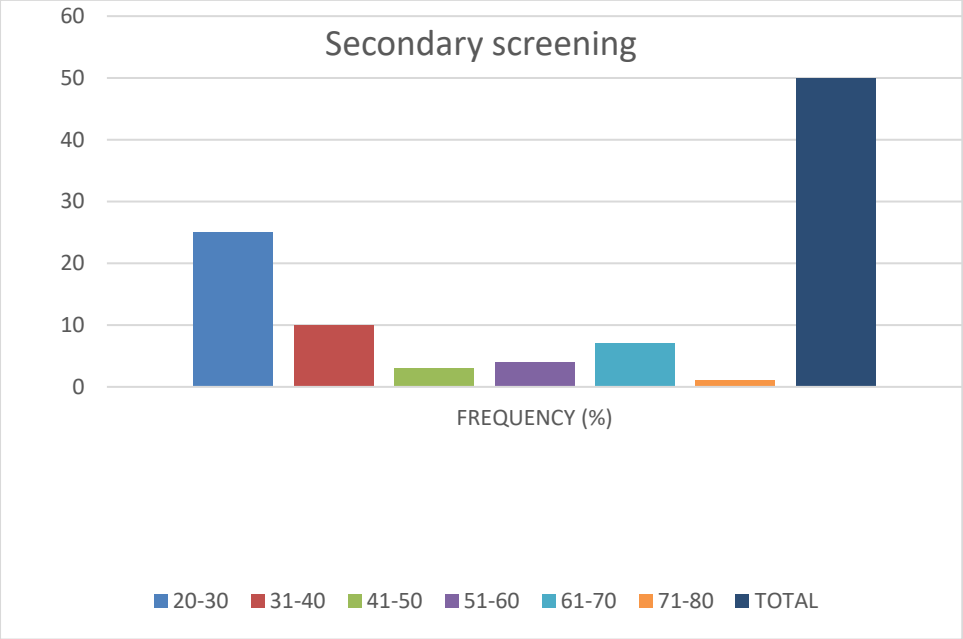

Fig 2: **Graphical representation of frequency of patients in different age groups [Secondary screening]**

Co- relation of Anti- HCV and HCV RNA

After secondary screening, it was observed that 26 patients were positive for both Anti HCV and HCV RNA. Then co- relation of Anti- HCV and HCV RNA was found. The data related the above co relation is given below:

Age Group	**Anti HCV Positive**	**HCV RNA Positive**
20-30	25	10
31-40	10	6
41-50	3	1
51-60	4	4
61-70	7	5
71-80	**1**	0
Total	50	26

Table 4: Frequency of patients with Anti HCV positive and HCV RNA positive

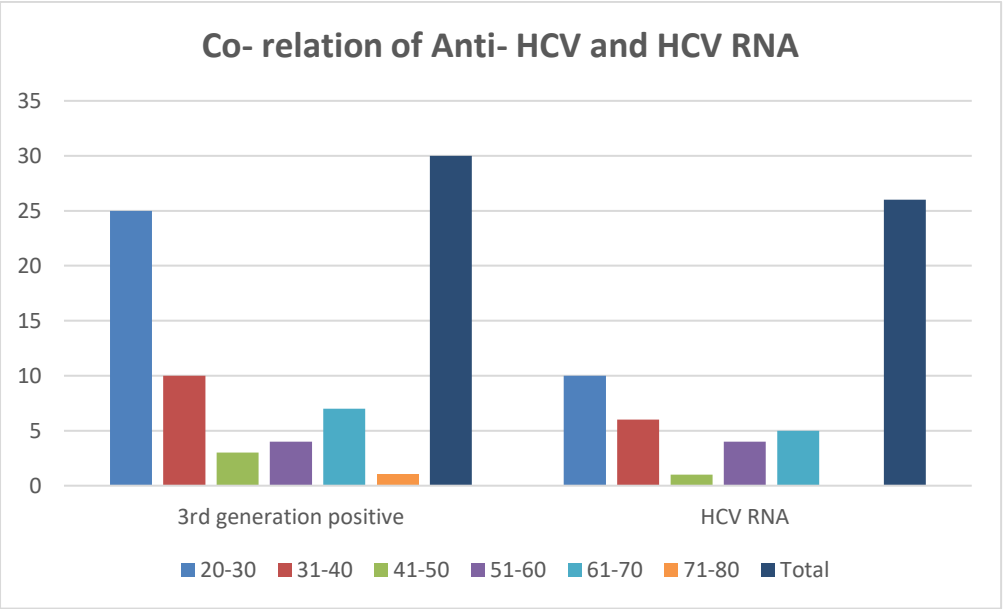

Fig 3: Graphical representation frequency of patients with Anti HCV positive and HCV RNA positive

CONCLUSION:

Hepatitis C is an inflammation of liver caused by hepatitis C virus. Hepatitis C can be a short-term illness, but for most people acute infection can lead to chronic infection chronic hepatitis C can be a lifelong infection if left untreated. Those who are acutely symptomatic may exhibit fever, fatigue, decreased appetite, nausea, vomiting, abdominal pain, dark urine, pale faeces, joint pain and jaundice. Chronic hepatitis C can cause serious health problems, including liver damage, cirrhosis [scarring of liver], liver cancer, and even death.

Hepatitis C is a virus that can infect the liver. If left untreated, it can sometimes cause serious and potentially life – threatening damage to the liver over many years. However, with modern treatments, its easily possible to cure the infection and have a normal life expectancy.

Control of HCV infection requires a comprehensive approach that incorporates primary prevention of transmission through enhanced infection control and injection safety in healthcare settings and in the community, universal screening of blood and blood products, harm reduction programs, and increased public awareness about risk factors for HCV infection.

It was observed that highest frequency of patients were seen in age group 20-30 while lowest frequency of patients lied in age group 71-80. 10 patients were screened in age group 31-40, 3 patients were screened in age group 41-50,4 patients were screened in age group 51-60, 7 patients were screened in age group 61-70. Out of 200 patients, 50 patients who were resulted positive were further investigated. Among 50 patients, 40 were males and 10 were females. Hence, it was concluded that HCV is mostly prevalent in males in comparison to females.

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