Original Article

DETECTION AND GENOTYPING OF HCV IN PATIENTS OF SHEIKH ZAYED HOSPITAL, LAHORE, PAKISTAN

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ABSTRACT

BACKGROUND: A vast majority of chronically infected HCV individuals remain undiagnosed and unaware of their infection status for several years. This study investigated the prevalence of HCV patients in a tertiary-care hospital.

METHODS: Patients attending out-patient department of Sheikh Zayed hospital Lahore were examined during January-March 2012. Diagnosis of Hepatitis C was based on the occurrence of anti-HCV antibodies in serum. HCV RNA infection was confirmed by Real time PCR. Genotypic detection of amplified product was performed on cytoflour. Liver function test (LFT) of ALT was also performed to assess the liver function.

RESULTS: Four hundred and fifty four males and 546 females aged 12-80 years were examined for detection and genotyping of HCV. Sixty nine percent of study population belonged to 30-59 yrs, 26% to ≤29 years, and 5% to ≥60 year old age-groups. Fifty five percent were females. Seventy one percent subjects were found with ALT levels >35. 63% subjects were RNA positive. Forty two percent of males and 58% of females showed genotype 3; In age-groups of ≤19 years, 20-29 years, 30-39 years, 40-49 years, 50-59 years and ≥60 years, genotype 3 was detected in 44%; 51%, 60%, 58%, 58% and 43% respectively. Statistical difference of study variables was non-significant (p≥0214) both for age-groups and genders.

CONCLUSION: HCV, genotype 3 highly detected, was equally prevalent in both males and females irrespective of demographic factors. Middle-aged (30-50 years) study population was mostly affected by HCV.

KEY WORDS: HCV, Genotype, RNA, Prevalence, Population, Lahore

INTRODUCTION:

Chronic hepatitis C virus (HCV) infection is one of the most important known causes of chronic liver diseases, together with cirrhosis and hepatocellular carcinoma.[1] Hepatitis C virus is a blood borne pathogen; high-risk infection is noted in individuals who have frequent transfusions (of blood or blood products), prisoners, intravenous drug abusers, and patients on hemodialysis, healthcare workers exposed to needle and sharp objects. No obvious risk factor in about 50% of infected (sporadic cases) patients.[2] Risk factors associated with transmission of HCV are low socioeconomic status, drug use, infected blood

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product transfusions, patient care or clinical laboratory employees, exposure by household member or infected sex partner, exposure to numerous sex partners.[3] Most important categories considered as potential correlates of HIV and HCV included are: (a) behaviors related to drug use such as the regularity and duration of drug use and sharing of syringes; (b) percutaneous exposures (medical and other), such as dental surgery, medical injection and barber shave; and (c) sexual behaviors.[4] 70% of notified patients acquire disease in the hospitals through reuse of syringes and major/ minor surgery in Pakistan.[5] Due to the lack of symptoms, a vast majority of chronically infected individuals remain undiagnosed and unaware of their infection status for several years until secondary complications decompensate liver function and disease eventually develops. In addition, these people may serve as a reservoir of HCV transmission to others. Still, at the present time, the most important goal to achieve is early diagnosis, in order to potentially improve the survival of patients with chronic hepatitis C. .[6] Apart from quantification of viral loads of quantitative PCR, Genotyping of HCV is very significant for regular laboratory diagnosis. Indeed, viral genotype must be given weight when prescribing a therapy. The duration and sustained response to existing standard therapy regimens are linked with HCV genotypes.[7] Evidence suggests that in type 2 and type 3 patients, HCV infections show a constant response to therapy as compared to type 1 patients.[5] This study was designed in order to determine the prevalence of HCV in patients being reported in a general hospital setting located in the city of Lahore, and based on a group of individuals for whom HCV detection have yet not been carried out. Viral genotyping and ALT were also analyzed. The study may help the HCV patients to become aware of their disease status and seek treatment well in time to avoid morbidity and mortality.

MATERIAL AND METHODS:

Study type and Setting

Cross-sectional study, conducted at Sheikh Zayed Hospital Lahore, Pakistan

Study Population

All Patients referred to the molecular biology department of Sheikh Zayed Hospital from Jan to Mar 2012 were enrolled for hepatitis C detection. Patients of any age, gender, race, ethnic origin, residence (rural/urban), educational status, occupation were included in the study. Patient's general and medical history including symptoms and risk factors that act as major transferable routes are blood transfusions, tattoos or acupuncture, hemodialysis are noted. In this study, 1000 cases, whose complete data was available, were evaluated for hepatitis C infection. Sheikh Zayed hospital is one of the biggest tertiary care hospitals of Pakistan and proportionally serves a large population from Lahore and other areas of country.

Diagnostic Criteria

Hepatitis C was diagnosed by anti-HCV antibodies in serum and detection was done by third-generation commercially available enzyme-linked immunosorbent assay (ELISA) kits. HCV infection was confirmed through the detection of viral RNA by real time PCR (using Rotorgene 3000, Corbett research, Australia) and the Qiagen extraction and amplification kit. Genotypic assessment of amplified product was performed on Cytoflour (third wave technology USA). Liver function test (LFT) based on alanine aminotransferase (ALT) was also carried out to assess the liver normal function.

Blood Sampling and Plasma Separation

Four millimeters of peripheral blood was collected in a labeled EDTA tube after applying tourniquet and cleaning the forearm with alcohol swab. Blood was centrifuged at 3000rpm for 10 minutes, and plasma was transferred into two epondrof tubes (one for RNA extraction and viral genome quantification by real time PCR reaction and second one for LFT).

Blood analysis

A. Detection of HCV viral RNA and Genotyping

HCV viral RNA was isolated using Qiagen mini extraction kit according to protocol suggested by manufacturer (supplier). HCV genotyping was carried out on Cytoflour using the third wave technology USA. This system uses Cleavase enzymes to recognize and cleave specific structures formed by the addition of two oligonucleotides (an Invader Oligo and a Primary Probe) to a nucleic acid. The detection methodology was adopted from other studies. (8, 9)

B. Liver Function Test

ALT:

One hundred microliters (100µL) of serum sample was added to 1000µL ALT reagent and after 1 minute incubation at 37°C, absorbance readings were taken on spectrophotometer (Microlab 200).[10]

Statistical Analysis

Outcome observations were analyzed among age-groups and between genders. Chi-square test was applied to assess statistical difference between categorical variables, using a significance level of $p \leq 0.05$. Ethics committee of the Sheikh Zayed Hospital, Lahore approved the study.

RESULTS:

Four hundred and fifty four males and 546 females were examined in the study for detection and genotyping of HCV. Age range was 18-76 years. Of the total subjects 74.5% were reported from Lahore and 25.5% from other cities. Figures 1-4 provides details of the subjects' age range, gender distribution, viral RNA subtype, ALT levels and HCV genotype. Age-wise distribution showed that 69% population belonged to middle-age group (30-60 yrs) and 26% were below 30 yrs and 5% above 60 yrs. Majority of both males and females belonged to age-groups (30-39 / 40-49 years), 23% were 20-29 yrs old and 8-20% was either ≤19 yrs or ≥50 yrs. Statistical difference was insignificant (p=0.947).

ALT categories were defined at cut-off level of ≤35 IU and >35 IU and were found insignificant (age groups: p=0.547; genders: 0.533). Seventy two percent (72%) of age-group ≤19 years, 64% of 20-29 years, 74% of 30-39 years, 72% of 40-49 years, 68% of 50-59 years and 70% from ≥60 years were in ALT category of >35IU. Sixty nine percent (69%) males and 73 females were with ALT levels of >35IU. Sixty three percent (63%) in age-group ≤19 years, 58% in 20-29 years, 68% in 30-39 years, 60% in 40-49 years, 64% in 50-59 years and 63% in ≥60 years were RNA positive cases. Sixty two percent (62%) males and 63% females were RNA positive in this study. Statistical difference among age groups and between genders was insignificant (p=0.498 and p=0.884 respectively)

Gender distribution was noted for genotype of HCV patients and observed insignificant (p=0.446). Fifty one percent (51%) males and 60% females showed genotype 3; 19% males and 19% females were type 1; 15% males and 10% females were detected type 2. Eight percent (8%) of both genders had type 4 and 7% untypable genotype. In age-group ≤19 years, 22% subjects showed genotype type 1, 22% type 2, 44% type 3, 5.5% type 4 and 5.5% type untypable. In 20-29 years old subjects, 26% subjects showed genotype type 1, 11% type 2, 51% type 3, 8% type 4 and 4% type untypable. Thirteen percent (13%) subjects showed genotype type 1, 12.5% type 2, 60% type 3, 10.5% type 4 and 4% untypable in age-group 30-39 years. In 40-49 years old sample, 19% subjects showed genotype type 1, 12 type 2, 58% type 3, 6% type 4 and 5% untypable. Nineteen (19%) subjects with genotype type 1, 9.5% with type 2, 58% with type 3, 6.5% with type 4 and with 7% untypable subjects were in age-group of 50-59 years. In age-group ≥60 year old, 21% were genotype 1, 21% type 2, 51% type 3, 8% type 4 and 7% untypable. Statistical difference was noted as p=0.214 for age-groups and p=0.799 for genders.

DISCUSSION

This study showed that majority of patients

referred to Sheikh Zayed hospital in Lahore was infected with HCV genotype 3 that augment other studies from Pakistan.[11] RNA was also detectable in both males and females at high rate. A developing country like Pakistan is ranked high for incidence of HCV carriers who are undiagnosed and may pose potential danger to public health as they allow the sustained and silent circulation of HCV. HCV possesses a positive-sense and consist of single-stranded RNA genome that is classified into six (1 to 6) different genotypes.[12] Determination of genotype is a applicable experimental practice that helps to predict the chance of continued virological response that is 40%-45% in genotype 1 and 70%-80% in genotypes 2 and 3), and is also used in practice to decide treatment duration (48 weeks for genotypes 1 and 4 and 24 weeks for genotypes 2 and 3).[13]

This study shows that genotype 3 is the most common type in Lahore, which is in agreement with reports from other regional countries, as well it corresponds with data from Nepal, Bangladesh and Southern and Northern India.[14] Multiple studies provide evidence that type 3 is the principal HCV genotype in Pakistan, with prevalence of 75–90%.[13,15] Other types are 1, 2, and 4 with little evidence of rare types. Studies[16,17] provide evidence of high incidence of genotype 3 and very low occurrence of genotype 2. Occurrence of un-typed samples indicates that either the genotyping method of third wave technology fails to detect the genotypes or new genotypes are found in this region. In this relationship, sequencing of such samples can be supportive. Findings of our study advocate that additional studies are required to explore the obscurity of occurrence of HCV genotypes in Pakistani population with more latest and optimized technique.

Primary prevention of hepatitis C is supposed to decrease spread of the virus by targeting those who are at risk of the virus. Prevention program may consist of health education, risk reduction counseling, and screening of HCV and treatment for substance abuse. In United States, the Centers for Disease Control (CDC)[18] recommend screening of the following groups of population: 1) individuals who have ever injected illegal drugs once or few times. 2) individuals who got a transfusion of blood or organ transplant before July 1992; 3) individuals who got clotting factor concentrates before 1987; 4) persons who has ever been on continuing dialysis; 5) children born to HCV-positive women; 6) emergency medical, healthcare and workers from public safety after needle sticks, sharps, or having a mucosal exposures to HCV positive blood; 7) individuals with chronic liver disease.

Population groups living in precise settings/localities such as correctional institutions, programs for drug treatment, and high risk youth programs, HIV counseling and testing sites, and sexually transmitted diseases clinics need additional attention. In such localities, physicians ought to perform screen for HCV serostatus. In contrast to HIV, HCV is in high concentrations in rinsing liquids, spoons, and filters that are applied in connection with needles. Patients need to be advised on infected equipment as a source of contamination. Care from addiction and attention should be given to those with possible referrals for psychotherapy and detoxification.[19]

Prevention of HCV transmission in health-care settings must be emphasized by doing improved sterilization, safer injections, dropping percutaneous exposures to blood.

Better procedures for blood screening and screening of donors should be done to reduce the number transmissions from transfusion in developing countries, if a patient is reported with hepatitis C, counseling needs to be provided to reduce the risk of HCV transmission to others. The physician is supposed to provide counseling regarding treatment, suggest reduction of alcohol use and do vaccination with hepatitis A and B, pneumococcal and influenza. Person without HCV having chances of continuing risk factors also want counseling and immunization with hepatitis A and B vaccines.[20]

CONCLUSION

HCV, genotype 3 was highly detected, and equally prevalent in both males and females irrespective of demographic factors. Middle-aged (30-50 years) population was mostly affected by HCV.

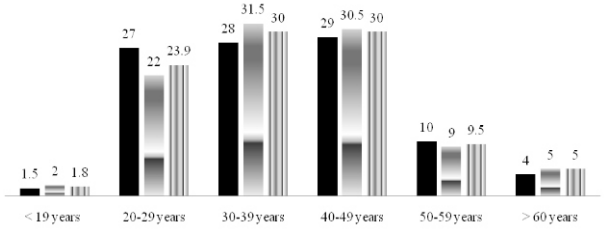

Figure 1: Gender distribution (%) in Age-Groups

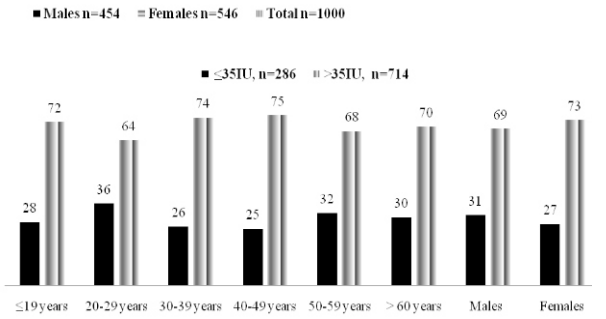

Fig 2: Comparison of individuals (%) with ALT levels (≤/> 35 IU) in Age-Groups and Genders

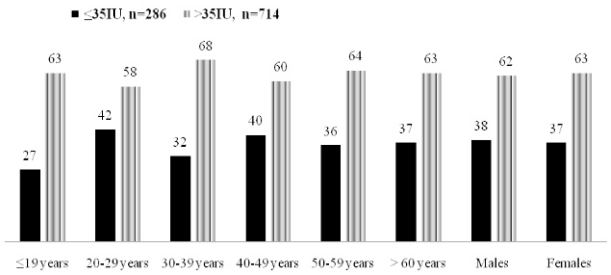

Fig 3: Comparison of individuals (%) with RNA Positivity in Age-Groups (years) and Genders

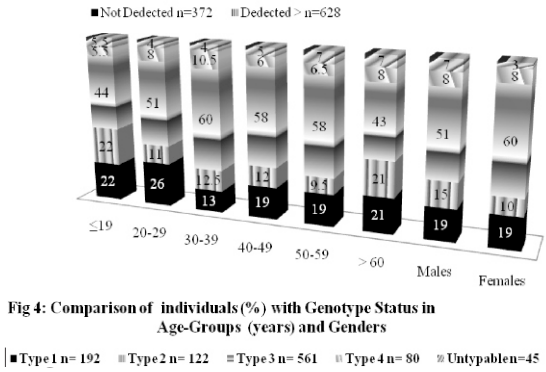

Fig 4: Comparison of individuals (%) with Genotype Status in Age-Groups (years) and Genders

Figure 1: Age and Gender wise distribution of study subjects, Statistical difference non-significant (p=0.947)

Figure 2: ALT levels in different age-groups and genders of study subjects, Statistical difference non-significant (Age groups: p=0.547, gender: p=0.533)

Figure 3: RNA positivity in different age-groups and genders of study subjects, Statistical difference was non-significant (Age groups: p=0.498, gender: p=0.884)

Figure 4: Genotype status of study subjects by age-groups and genders, Statistical difference was non-significant (Age groups: p=0.214, gender: p=0.799)

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Take warning! He has not exposed so many of your sinful activities that it appears as if He has forgiven you (it may be that He has given you time to repent).

Hazrat Ali (Karmulha Wajhay)