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ANTI HCV ANTIBODY TEST FALSE POSITIVITY AND HEPATITIS C VIRUS (HCV) INFECTION IN AN UNDERPRIVILEGED COMMUNITY OF LAHORE

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ABSTRACT

Objectives: The population was screened for hepatitis C virus infection. The false positivity of Anti HCV antibody test was determined by PCR HCV RNA to differentiate the active infection from false positive results having no infection. **Study Design:** Cross sectional study. **Setting:** Population of a suburban village Dera Chahal approx. 25 km from Lahore. **Period:** 2016 to 2018. **Methods:** 930 subjects both male and female were included in this study. A doctor took an informed consent and asked demographic information. Clinical examination was done for any hepatic infection. Blood specimen was collected at random state, for detection of hepatitis C Virus and related biochemical parameters i.e. LFT's. Screening devices and ELISA Anti HCV antibody test for detection of Hepatitis C virus infection were used. Real-time RT-PCR was used to examine the HCV RNA levels and to confirm hepatitis C infection in anti HCV antibody test positive cases. HCV RNA levels distinguished the active infection from false positive results having no infection. **Results:** A total 930 adult subjects (age17-90yrs) were tested for HCV infection. Anti HCV antibody was found positive in 520 cases whereas 410 cases were anti HCV antibody negative. PCR test was performed on 462 anti HCV antibody positive cases. PCR test was positive in 313 (67.7%) cases, whereas PCR was negative 149 (32.25%) cases. These 149 PCR negative were anti HCV antibody false positive cases. High percentage of anti HCV antibody positive and PCR test positive cases were found in lower age groups 13% (10-20yrs), 42% (21-30yrs) and 45% (31-40yrs). Whereas upper age groups had low percentage of anti HCV antibody positive and PCR test positive cases i.e. 27% (41-50yrs), 13% (51-60yrs) and 2% (61-70yrs). Prevalence of hepatitis C infection was 68% and anti HCV antibody false positivity was 32%. Medication of hepatitis C virus infection was done in 173 anti HCV antibody positive and PCR test positive cases. PCR test was repeated after two months, in which hepatitis-C virus was not detected in all treated cases. **Conclusion:** High prevalence of hepatitis C virus infection and anti HCV antibody false positivity was found in the community. Anti HCV antibody test false positivity was resolved with PCR HCV RNA test to differentiate the active infection from false positive results having no infection.

Key words: Hepatitis C virus infection, Anti-HCV antibody test false positivity, PCR HCV RNA

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INTRODUCTION

Pakistan is having Hepatitis C virus burden second largest in the world, WHO commented on an outcome report on the Prevention and Control of Hepatitis Programme (2017-2021) in the Punjab province. In this progamme suspected population was to be screened for HCV and HBV infection and given antiviral treatment for eradication of the infection. Antiviral therapy was covered in few thousand patients and left many without treatment, which has increased the risk of transmission of HCV and HBV to healthy population. Twelve million people in Pakistan are infected from hepatic viruses, and approximately 150,000 new cases are added every year. It has been imperative for Pakistan to fight against the

HCV epidemic[1].

It has been studied that antiviral drugs have more than 95% cure rate in active hepatitis C infection cases. Therefore accurate diagnosis of infection and regular surveillance programs are required[2]. Primarily HCV infection is diagnosed by a preliminary test for detection of anti HCV antibodies using chemiluminescence or enzyme immunoassay, positivity of this assay is reassessed with HCV RNA testing[3]. False results of Anti HCV antibody test appeared if the patient had ongoing, resolved or chronic infection. In addition poor sensitivity of the Anti HCV antibody test was seen during the first four to six weeks of infection[4].Some studies have reported a reasonable percentage of false positive results using third-generation anti HCV test kits[5,6].

Clinically positive anti HCV antibody test is considered for active virus infection. However due to high rate of false positive results of anti HCV antibody assay, it has been mandatory to confirm it with HCV ribonucleic acid (HCV RNA) assay by reverse transcriptase polymerase chain reaction (RT PCR) for the diagnosis and treatment of infection[7,8].This two way testing is required in the population with high percentage of anti HCV antibody positive subjects before any antiviral treatment[9].

This study was conducted by Zeenat Hussain Foundation in an underprivileged community of Dera-Chahal a village near Lahore to identify the patients of active infection of hepatitis C virus with anti HCV antibody screening and its confirmation with PCR HCV RNA detection, and to distinguish the active infection from false positive results having no infection. To abolish the disease in the community free medication was done of PCR positive cases and confirmed the cure with repeating PCR test after two months.

MATERIAL AND METHODS

This cross sectional study was conducted in a village Dera Chahal 25 km from Lahore. This village has approximately total 12000 population, 930 adult subjects of both genders aged >17ys (children under 17yrs age not included), were included in this study using convenient random sampling technique. These subjects were screened for hepatitis C virus infection. Dr. Mubashir Hassan of Zeenat Hussain Foundation had a meeting with the elite of the community to explain the objectives of this research that it will diagnose the viral hepatic infection which is curable with medication, medicine will be provided free of cost to the hepatitis C patients. A doctor took an informed consent from the person to include in the study and asked demographic information. Clinical examination was done to find possible hepatic infection. For minor infection ill health patients, medicines were dispensed free of cost.

Blood specimen was taken at random state, kept in an icebox and shifted to the laboratory where stored in a refrigerator at 4°C. Biochemical tests i.e.LFTs were analyzed on Roche Cobas-600 Analyzer. Complete blood count was performed on Sysmex XE-5000 Analyzer. For detection of Hepatitis C Virus, third generation kit Cobas E411 (Roche) for ELISA Anti HCV antibody test was used. Screening devices from Roche were also used. Cut off value 1.0 was taken as anti HCV antibody positive/reactive result.

Quantitative PCR test was performed on Anti HCV positive cases to confirm hepatitis C virus infection. HCV RNA in serum from anti HCV antibody positive subjects were determined byReal-time RT-PCR. Reverse transcription PCR technique with real-time florescent detection for the quantification of HCV RNA was used. Abbott m2000sp automated nucleic acid extraction system was used to isolate HCV RNA from 0.5ml serum sample as described previously[10]. Detection of amplified samples and controls was done with the Abbott m2000rt

instrument[11]. Sensitivity: 12 IU/mL for the 0.5 mL sample. Linear range: 12 IU/mL (1.08 log IU/mL) to 100 million IU/mL (log 8.0 IU/mL).

PCR positive cases were given free of cost treatment of viral hepatitis C infection with medicine from Hilton Pharma or Global Pharma for two months. After medication of two months, quantitative PCR test was repeated to determine the effect of drug. This research was funded and conducted by Zeenat Hussain foundation.
Statistical analysis of data was done using Microsoft Office Excel 2007 program.

RESULTS

A total 930 adult subjects, 390 males and 540 females (age17-90yrs) were screened for HCV infection. Anti HCV antibody was found positive in 520 cases (217 males and 303 females) whereas 410 cases (173 males and 237 females) were Anti HCV negative.

PCR test was performed on 462 anti HCV antibody positive cases. PCR test was positive in 313 (67.7%) cases; these were 142 (45.36%) males and 171(54.63%) females. Females had HCV infection rate 10% higher than males. Whereas PCR test was negative 149 (32.25%) cases, these were 57 (38.25%) males and 92 (61.74%) females. These 149 PCR negative cases were anti HCV antibody false positive cases i.e. 149 cases (57 males and 92 females). False positivity of anti HCV antibody was 38% in males and 62% in females. High percentage of anti HCV antibody positive and PCR test positive cases were found in lower age groups 13% (10-20yrs). 42% (21-30yrs) and 45% (31-40yrs). Whereas upper age groups had low percentage of anti HCV antibody positive and PCR test positive cases i.e. 27% (41-50yrs), 13% (51-60yrs) and 2% (61-70yrs). Medication was done in 173 anti HCV antibody positive and PCR test positive cases. Medicine from Hilton or Global Pharma was given for two months. PCR test was repeated after two months, in which Hepatitis-C virus was not detected in all 173 cases. Anti HCV antibody assay was compared to the HCV RNA test, sensitivity, specificity, and positive and negative predictive values were 51%, 34%, 60%, and 27%, respectively.

DISCUSSION

There are not many reports on the prevalence of HCV infection between different age groups[12].A study in Liaoning Province China, reported anti HCV positive rate was significantly higher in patients above forty years age groups than in lower age groups[12]. Similar findings have been reported in other studies in Czech Republic, China and Taiwan[13-15]. In present study trend was reverse, HCV infection was higher in younger age groups than those in older age groups. Risk factors like sharing of syringe needles for drug abuse, injections with used syringes, body piercing and illicit sexual relations may be linked with high prevalence of hepatitis C infection[16-21].

False positive results at high percentage appeared with third generation anti HCV assay kits, which are considered more sensitive and specific than previous versions, therefore it is mandatory to confirm every reactive/positive result with PCR HCV RNA[5,6]. Yurongand Zhao et.al. reported that no concurrence found between anti HCV and HCV RNA positive cases as less than half of anti HCV patients were confirmed positive for HCV RNA[12]. Anti HCV antibody assay has a disadvantage, it showed false positive results at low titers. Studies have reported that in populations with a low HCV prevalence had false positive rate35% and another found 53%[3,22-24].Present study found false positivity 32% associated with screening for anti HCV antibody confirmed by PCR HCV RNA test. False positivity may occur due to interference of many auto antibodies, nonspecific immune responses, antibody cross reactions to other pathogens[25,26]. Furthermore inability of the anti HCV antibody assayto differentiate between ongoing, past and chronic infection and poor sensitivity in the initial stages of infection, successful treatment or body has cleared the

virus on its own[4]. A study in Lahore Pakistan found the HCV infection rate was almost the same in males and females[27], but present study revealed HCV infection rate approximately 10% higher in females than males, may be due to injections or blood transfusions during cesarean deliveries[16-21].

CONCLUSION

This study found high prevalence of hepatitis C virus and anti HCV antibody test false positivity. However, PCR test distinguished active HCV infection from false positive results having no infection. Therefore before declaring a person having hepatitis C infection, PCR HCV RNA test is recommended after anti HCV antibody test. Responsible factors for high prevalence of hepatitis C were possibly sharing of needles for drug injections, tattooing, ear and nose piercing, barber shaving many persons with one razor, in females blood transfusion during cesarean deliveries, in addition lack of education, poor socioeconomic status.

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