Original Research Article

Prevalence of HCV among Drug Addicts in Lahore and its Peripheries

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A b s t r a c t

HCV continues to be a major health threat globally; approximately 130 million people are infected around the world. The aim of the study was to describe the HCV prevalence in drug addicts the most deprived, isolated and most of the times poor part of our community. This was a cross sectional study focusing the drug addicts under treatment in rehabilitation centers. A total of 92 drug addicts were recruited from four different centers at Lahore including Silverlining Lahore, Silverlining Sheikhupura, Roshan Mustaqbil Lahore and Panah Lahore. All the subjects were tested for HCV RNA PCR, SGPT and SGOT. Out of 92 subjects 23 (25%) were HCV positive and remaining 69 (75%) were HCV negative concluding the drug addicts a higher risk group. Among the HCV positive IDU`s showed a greater percentage 78% (18) and non-IDU`s only 22% (05) proving the injection users the highest risk group. The HCV positive drug users also turned up with a much high SGPT and SGOT levels with a mean of 92 U/L and 72 U/L respectively as compared to that of HCV negative subjects which were 23 U/L and 27 U/L respectively. Drug addicts and specially IDU`s are the highest risk group for developing the HCV infection as concluded by the study and their massive awareness, focus on HCV treatment and also to check the other blood borne diseases are major suggestions advised by the research.

Keywords: HCV,   Prevalence,   IDU`s,   Pakistan.

Introduction

Hepatitis C virus, an RNA virus, belongs to the family *Flaviviridae* genus *Hepacivirus* [1]. the only known member in the genus [2]. In humans it causes pathogenesis in hepatocytes and possibly B lymphocytes [3] causing hepatitis, liver cirrhosis and hepatocellular carcinoma (Akbar *et al* 2009) and has emerged as the global public health problem [4] among the six different and distinct hepatitis viruses named as Hepatitis A, B, C, D, E and G viruses [5]. HCV was first discovered, in 1989, as non A, non B hepatitis causative agent [6].

Pakistan, a populous (about 170 million people) and developing country, stands at 134th position among 174 countries in respect to the health and educational standards according to the United Nation`s human development index. Consequently, having the terrible result of about 10 million people suffering from HCV. Among the common ones the other specific risk factors include, reuse of needle for ear and nose piercing, reuse of syringe, injecting drug users, shaving from barbers, unsterilized dental and surgical instruments and non implementation of international standards of blood transfusion [7]. Blood transfusion is still found to be the major route of HCV transmission in Pakistan [8]. On province wise distribution Punjab has 6.7% followed by Sindh 5%, Baluchistan 1.5% and Khyber Pakhtunkhwa 1.1% prevalence rate of HCV infection. Presence of anti HCV antibodies ranges between 38% to 55% among high risk group people including

health care workers, patients on hemodialysis, thalassemia and hemophilia patients [9]. Blood donors, considered to be the healthiest population, presents different prevalence in different areas of Pakistan. Highest rate in the interior regions of sindh which is 7.5% followed by 4.1% in Peshawar, 4.0% in Rawalpindi, 3.68% in Lahore and the least in Karachi which is 1.18% to 3.3% [10].

It is well documented and proved to be unsafe and hazardous the multiple use of sample needle, syringe, injecting equipment and also sharing the filters and "cooker" (including spoon, bottle and caps etc.) among the injection drug users (IDUs) [11].

Hepatitis C virus infection is a mega manic not only in Pakistan but also globally and it becomes much severe if we consider the drug addicts as being the most isolated, hated, neglected and poor community fellows. So, the major objectives of present study were to observe, prevalence rate of HCV infection among the addicts by HCV PCR a confirmatory test and also to determine the severity of infection and its effect on their liver by testing SGPT and SGOT.

Methods

Drug Addicts

Ninety two drug addicts, IDUs and oral drug addicts were recruited through four different rehabilitation centers of Lahore

and Sheikhupura including Silver Lining 8Lahore, Silver Lining Sheikhupura, Roshan Mustaqbil Shahdra Lahore and Panah Lahore.

Blood Testing

Blood samples of all ninety two drug addicts were drawn, using sterile BD (Becton Dickinson) vacutainer SST II advance get containing tubes for serum separation, and analyzed for HCV RNA by RT-PCR, SGPT (serum glutarate pyruvate transaminase) and SGOT (serum glutarate oxaloacetate transaminase). Serum was separated by centrifuging the blood containing BD gel tube at 5000 rpm for 05 minutes, soon after the blood collection, and was stored at -80 C in 1.5 ml RNA DNA free eppendorf serum collection vials.

RNA Extraction and RT-PCR

Kits used for both extraction and amplification was the Sacace Biotechnologies, Scace™ Italy. Extraction was achieved through spin column (silica membrane) extraction methodology according the recommended procedure using 150ul serum. HCV viruses were lysed quickly by RAV1 lytic buffer to reveal the RNA. Then ethanol was added which efficiently aided in binding the RNA to the silica membrane in the Ribo Virus columns. Addition of carrier RNA improves binding and recovery of the low concentrated viral RNA. Contaminations (potential PCR inhibitors) like salts, metabolites, soluble macromolecules and cellular components are removed in two washing steps with ehtanolic buffers RAW and RAV3. The nucleic acid RNA is now ready to be eluted from the silica membrane by low salt buffer or water and ready to be amplified. Master mix included 300ul of RT-PCR mix 1, 200ul of RT-PCR mix 2, 20ul of Hot Start Taq Polymerase and 10ul of M-MLV Revertase in DTT tube. To the SmartCycler PCR reaction tube added 12.5ul of master mix and 12.5ul of the extracted RNA of each test, three of the standards (QS1, QS2, QS3) and positive and negative controls. The thermal cycler was preprogrammed as stage 1 hold for 1800 seconds at 50 C, stage 2 hold for 900 seconds at 95 C and stage 3 had two temperature cycle first 20 seconds at 95 C and second 40 seconds at 60 C, stage 3 was repeated 42 times.

SGPT and SGOT

SGPT and SGOT enzymesactivity was measured on Hitachi 902 Automated Chemistry analyzer using the Roche IFCCkits at 340 nm filter based on the principle of a coupled equilibrium enzymatic kinetic reaction.
For SGPTtest in the first reaction SGPT catalyzed -ketoglutarate to L-glutamate and pyruvate in the presence of L-alanine which was followed by the conversion of pyruvate

and NADH to L-lactate and NAD^+ by the action of lactate dehydrogenase enzyme (LDH). The rate of decrease of NADH was directly proportional to the rate of formation of pyruvate, and thus the SGPT activity.
Similarly the SGOT test principle was in the first reaction SGOT catalyzed -ketoglutarate to L-glutamate and oxaloacetate in the presence of L-aspartate which was followed by the conversion of oxaloacetate and NADH to L-malate and NAD^+ by the action of malate dehydrogenase enzyme (MDH). The rate of decrease of NADH was directly proportional to the rate of formation of oxaloacetate, and thus the SGOT activity.

Results

The HCV PCR results proved 23 (25%) subjects out of 92 were infected with active HCV infection which is quite high if we compare it with general population, in Pakistan, which is 4.7%, described by [12]. Remaining 69 (75%) were not having HCV infection as shown in the figure1. Major factor responsible for this high prevalence rate is not only because of the poor hygienic life style but also that the drug addicts are the most deprived and isolated part of the community.
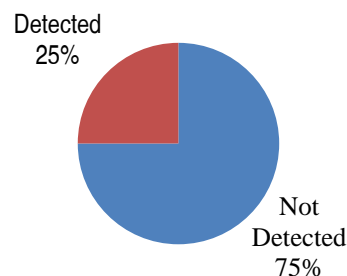
HCV Prevalence in Drug Addicts

Figure 1: HCV Prevalence in Drug addicts

Out of the 23 HCV PCR positive subjects only 05 (22%) were taking drugs orally but the remaining 18 (78%) were injection drug users. This proves the use of injection or needle for taking drugs in drug addicts is one of the most largest cause of spreading HCV infection rapidly and the data also shows the most of the HCV infected IDU`s were in their first year of drug addiction some even more less as shown in figure2.
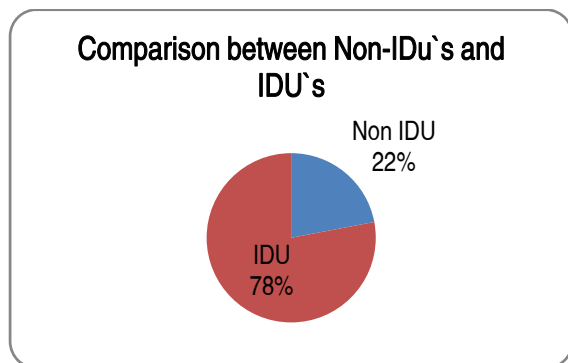
Figure2: Comparison between Non-IDU and IDU

HCV prevalence for the each rehabilitation centerturned out 10%, 38%, 08% and 40% in Silverlining Lahore, Silverlining Sheikhupura, Roshan Mustaqbil Lahore and Panah Lahore respectively.

SGPT and SGOT

The results presented a wide variation among all subjects but to sum up in the HCV negative group the lowest and highest SGPT and SGOT reported was 08 U/L, 21 U/L and 119 U/L, 95 U/L with a average of 27 U/L and 34 U/L respectively where the same for the HCV positive group proved to be 23 U/L, 27 U/L, 320 U/L, 187 U/L and mean 92 U/L and 72 U/L respectively. HCV positive drug addicts proved to have elevated level of both SGPT and SGOT which indicates the HCV active infection.

Discussion

HCV is blood-borne virus causing the Hepatitis C, which affects, pandemicaly, over the whole globe as shown by the WHO statistics that about 123 million people worldwide have hepatitis due to HCV having, 2% of the whole world population, the Egypt most affected, up to 19% [13]. The major risk factors include, intravenous drug use (IDU), blood transfusion, tattoos, history of hospitalization, history of sexually transmitted diseases (STD) and hemodialysis [14]. The major routes of transmission include per cutaneous, per mucosa and perinatal but there is also no recognizable route of transmission in many cases of HCV infection [15]. The very high prevalence rate of HCV infection, in some part of the world, suggests that there might be some unknown vectors or route of transmission for HCV infection [16].

Injecting drug users (IDU) are at highest risk for developing HCV infection worldwide [17]. In United States the IDU`s are the largest category having HCV infection and still having the increasing number of new infections every year. The figures prove that among the IDU`s 80% to 95% are infected with HCV and most of them, 80%, develop infection even in first year of needle injecting practice. Moreover the active IDU`s are on the high risk of developing re-infections by other HCV genotypes [18]. The main route of viral transmission is parenteral by sharing syringes, needle equipment and also sexual behavior [19]. HCV outbreaks, in Indonesia, China, Vietnam, Eastern Europe and central Asia, are linked predominantly to the injecting drug use and same is true for Pakistan. Geographically Pakistan is neighbor of the foremost opiate producer country, the Afghanistan, and is involved in most of the trafficking of the same. According to the recent United Nation figures about 0.8% of opium is used country wide in Pakistan. Among the 200 IDUs analyzed for HCV in Khyber Pakhtunkhwa 48 (24%) were found positive which is alarmingly high [20].

HCV arising as a mega health problem especially for developing countries the situation becomes worse if we focus the drug users including both IDU and non IDU. The present study aims to discover the HCV prevalence in the drug addicts the most deprived and most of the time poor part of our community. HCV RT-PCR is the most advanced analytical method for HCV diagnosis available in Pakistan which we selected also the SGPT and SGOT enzyme test activity was measured by using the Roche kits one of the most up to date biochemistry kits manufacturer and the automation used was Hitachi 902.

As the results proved 25% of 92 subjects from four different centers showed active HCV infection. There is a wide variety of HCV prevalence rate among drug addicts all around the world reported in indexed articles. A research done in Khyber Pakhtunkhwa conducted by [21] reports 24% IDU`s having active HCV infection, confirmed by HCV PCR, which is almost consistent with my results and importantly in this study the PCR testing was adopted, as I have done, inspite of anti HCV antibody testing, [22] also reported 28% HCV (PCR) prevalence. [23,24] showed 61.8% and 74.2% HCV seroprevalence by testing the anti HCV antibodies. [25] proved 63% HCV seroprevalence in drug addicts. There is also a low rate of 10.3% HCV seroprevalence in high risk group proved by [26] in a review study covering whole Pakistan.

Among the 23 HCV detected subjects 78% were IDU and only 22% were non IDU. High prevalence percentages in Silverlining Sheikhupura (38%) and Panah Lahore (40%) was because of all the drug addicts in these centers were [27] estimated about 80% to 90% IDU`s in USA have HCV infection which almost resembles my study results which is 78%, the only thing creating the small fraction of difference may be the sample volume which obviously with limited resources I studied only 92 subjects. [28] proved 89% of IDU`s infected with HCV in the islands Puerto Rico and Puerto Rican, USA. Another study done in England and Wales by [29] determined more than 60% of HCV infection among IDU`s which is slightly less than my findings. [30] described

about 86% of IDU`s were HCV seropositive in United States of America. [31], screening the whole Pakistan,reported 57% HCV prevalence among IDU`s which is noticeably less as compared to my findings the reason may be that I covered Lahore and its peripheries only.

HCV develops acute liver hepatitis and chronic live complication including cirrhosis, chronic liver hepatitis and liver carcinoma and SGPT is the main marker indicating the liver function. The normal SGPT level in our community ranges from 5 to 55 U/L. The elevated level indicates the liver malfunction as proved by my study. [32] studying HCV prevalence in Faisalabad reported the SGPT mean 79.4 U/L among the HCV positive cases which supports my finding of 92 U/L SGPT mean among HCV positive subjects.

Major factor responsible for this high prevalence rate is not only because of theunhygienic life style but also that the drug addicts are the most neglected part of the community.

Conclusion

The study was done on a closed group of drug addicts selected through the 04 different rehabilitation centers in Lahore. The, HCV RAN PCR, results show 25% prevalence of HCV among the selected 92 drug addicts which proves that the drug addicts to be the higher risk group for developing the blood borne infections specially HCV. Furthermore among the 23 HCV positive subjects 18 (78%) were injection drug users and only 05 (22%) were taking drugs orally which proves the IDU`s to be the highest risk group according to my research conclusion. Furthermore the mean results of SGPT and SGOT 92 U/L and 72 U/L respectively, the liver function markers, were also much elevated of the HCV positive subjects as compared to the same of HCV negative group which were 27 U/L and 34 U/L respectively. The following figure 3 shows all the results and their comparison.
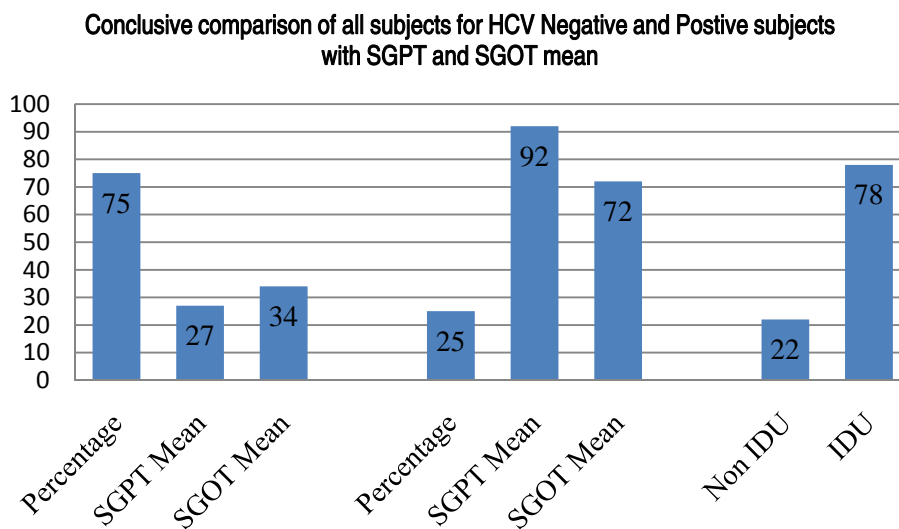
Conclusive comparison of all subjects for HCV Negative and Postive subjects with SGPT and SGOT mean

Figure 3: Conclusive graph showing all the results and comparison

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