A CLINICAL MANIFESTATION OF HEPATITIS C AMONG THE POPULATION OF HYDERABAD, PAKISTAN

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ABSTRACT

Hepatitis C is a noteworthy public health issue around the globe, resulting in liver-related problems. Almost 80% of HCV patients suffer from chronic circumstances and are likely to have liver ailments. Approximately 6% population of Pakistan is infected with HCV. In this study of 510 samples were collected from May 2022 to September 2022 from Hyderabad and adjacent areas. 291 out of 510 samples were affected by HCV. The collected samples were screened based on ELISA using recombinant HCV antigens. Biochemical and hematological factors were performed on HCV-infected patients. RBC, Hemoglobin, WBC, MCV, MCHC, and PLT were observed normally in all age groups. But WBC and PLT are high in the 10-20 age groups. Hematocrit and MCH were decreased as compared to normal levels. Bilirubin, SGPT, and Alkaline phosphate were high in some age groups normally in all age groups. But WBC and PLT are high in the 10-20 age groups.

INTRODUCTION

Hepatitis C virus (HCV) is the utmost major contributing agent to enduring viral liver infections worldwide (Qamar, Anwar, Ahmad, Haq, Khan, Hussain, Shahzad, Ahmad, Malik, & Khan, 2021). Almost 80% of HCV patients suffer from chronic circumstances and are likely to have liver ailments (Al Kanaani, Mahmoud, Kouyoumjian, & Abu-Raddad, 2018; Alter, 2007). Approximately 5% of chronic diseases are eventually fatal due to liver failure related to hepatocellular carcinoma or cirrhosis (Rytsareva et al., 2017). The death rate of HCV patients is significantly high; about 350,000 individuals expire each year after receiving an HCV infection (Mostafa et al., 2016). It was reported by WHO that the disease of HCV infection has a great influence on public health; almost 170 million people are affected by HCV infection globally (Organization, 2017). Pakistan is the second-highest HCV-infected country in the world (Z. Khan et al., 2021), with approximately 10 million infected individuals (Mehmood et al., 2020). This occurrence is not constant throughout Pakistan but fluctuates in numerous areas of the country (Muzaffar, Hussain, & Haroon, 2008). HCV in Pakistan is highly endemic; almost 6% Pakistani population is infected actively with HCV (Umer M., 2016).

Pakistan is an underdeveloped country; around 170 million people do not have enough health and literacy facilities. According to WHO’s data report, in Pakistan, around 10 million people suffered from HCV in 2019 (Asif & College, 2019). In Pakistan, unhygienic medical treatment has been recognized as a main cause of infection (Mahmood & Raja, 2017; Moin, Fatima, & Qadir, 2018). Some other causes for the spreading of HCV are the re-usage of dental and surgical instruments, needles and syringes of infected patients, shaving from barbers and transfusions of unscreened blood (Organization, 2017; Waheed & Siddiq, 2018).

On World Hepatitis Day 2019, the Government of Pakistan publicized the Prime Minister’s Plan, aiming to reduce the incidence and chronic cases of Hepatitis C by 30% (Lim et al., 2018). To obtain this target, sufficient awareness about HCV among the people regarding indications of the disease, mode of transmission, imperative vaccination and suitable treatments.

According to the census of Pakistan in 2017(Pakistan, 2017), Hyderabad is considered the second largest city in Sindh province, having a population of 2.2 million, of which 83.35% are the urban population, which makes it the second most urbanized district of Sindh province after Karachi.

The purpose of the Present study is to investigate the evaluation prevalence of HCV through the measurement of biochemical and hematological parameters in patients of Hepatitis C among different
gender, age groups etc., in a local population of Hyderabad, Sindh, Pakistan. It was Furthermore, establishing an accurate diagnosis for Hepatitis and its variants important for planning appropriate management. Early diagnosis of Hepatitis patient will help to prevent the transmission of diseases and reduce the financial burden on the population. From our knowledge, this is the first detailed report about HCV prevalence association with biochemical and hematological reports in Hyderabad and adjacent regions of Sindh.

MATERIAL AND METHODS
The present research work reported in this paper was carried out jointly at the Medical and Environmental Biotechnology Research Laboratory, Institute of Biotechnology and Genetic Engineering (IBGE), University of Sindh, Jamshoro, Pakistan and Diagnostic and Research Laboratory, ISRA University Hospital, Hyderabad, Sindh, Pakistan. Informed consent was obtained from each patient included in the study, and the study protocol conforms to the ethical guidelines of the Declaration of Helsinki 1975.

Selection criteria and history of HCV-infected patients: Both male and female patients from the age of 20-80 years were selected. The patients who refused to offer their approval were omitted. A detailed questionnaire was formulated to investigate the complete history of patients. The information obtained from the patients is based on their age and gender. The collected information about HCV patients is not reported here.

Collection of blood samples: Blood Samples of Hepatitis affected Patients were collected from ISRA University Hospital, Hyderabad. Patients belonged to Hyderabad and five adjacent areas (Tando Muhammad Khan, Matiari, Tando Jam, Jamshoro, and Hala). From each patient, 10 ml of blood was collected from a sterilized disposable syringe. The collected blood sample was immediately shifted to a tube containing an appropriate amount of anticoagulant. Each tube has given name of the patient, date and number and was transferred to the Diagnostic and Research Laboratory ISRA University Hospital, Hyderabad. 6 ml out of 10 ml collected blood of each patient was centrifuged (Kubota Model 2420, Japan) at 3500 rpm for 5 minutes. Plasma obtained from centrifugation was transferred to Eppendorf tubes and preserved in a freezer for the procedures of Complete Blood Count (CBC).

Determination of complete blood count (CBC): The stored samples of blood were used to test seven hematological parameters, including Hematocrit (Hct), Hemoglobin (Hb), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), white blood cell count (WBC) and Red blood cell count (RBC) were analyzed using a fully automatic Hematology Analyzer (Nihon-Kohden MEK-6318 K, Tokyo, Japan).

Separation of serum and determination of liver function test (LFT) and albumin test: 4 ml out of 10 ml collected blood was transferred separately into the Gel Tube (an anticoagulant-free test tube) and subsequently centrifuged (Kubota Model 2420, Japan) at 3500 rpm for 5 minutes. Blood serum was collected in cups and placed in Roche Automatic Analyzer (Cobas c 311 analyzers, Roche UK), which automatically measures the serum Alanine transaminase (ALT), Alkaline phosphatase (ALP), Albumin and Bilirubin.

Detection of hepatitis B and C virus: Hepatitis B virus (HBV) and Hepatitis C virus (HCV) tests were performed through the instrument COBAS e411, Elecsys, Roche UK. Monitor using serum samples.

RESULTS
Viral hepatitis has become main health issue in Pakistan. A cross-sectional survey of the seroprevalence of HBV and HCV antibodies (was detected through ELISA. A total of 510 patients were examined in present study, which was obtained from the Diagnostic and Research Laboratory, ISRA University Hospital, Hyderabad, Pakistan, from May 2022 to September 2022. According to results presented in Figure- 1, Out of 510, the prevalence of HBV was recorded 144 (28%) and HCV 291(57%) and Normal individuals 75(15%), respectively.
The samples of HCV patients were collected from Hyderabad and its adjacent areas. Among them, 57% of patients were from Hyderabad, 14% from Jamshoro, 12% from Tando Jam, 9% from Tando Muhammad Khan, 6% from Matiari, and 2% from Hala, as exhibited in Figure-2.

The 291 patients with HCV were categorized based on gender. Out of them, 157 (54%) patients were males, and 134 (46%) were females, as depicted in Figure-3.

Table-1: Statistical Correlation to the hepatitis patients in selected cities of Hyderabad region with their population.

<table>
<thead>
<tr>
<th>Correlations</th>
<th>Patients</th>
<th>Population</th>
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<tr>
<td>Pearson Correlation</td>
<td>1.000</td>
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Based on different ages, HCV-infected patients were divided into 7 age groups (10-20, 21-30, 31-40, 41-50, 51-60, 61-70 and 71-80 years old) as shown in Figure 4. Part of 291 patients, the prevalence of HCV-infected individuals noted different variations in age; the highest incidence was shown for the age group 41-50 years (27%), the second highest age group examined 21-30 years (23%) and the lowest prevalence was recorded 10-20 years (2 %) as compared to other under study age groups.

A part from 291 HCV Male and female patients of different age groups affected individuals were also further distinguished according to gender. In males, the maximum occurrence was found 33% in the group of age 41-50 years and lowest 4% in the age 10-20 years as compared to other male age groups. In females, the maximum occurrence was observed 29% at the age of 21-30 years and lowest percentage 2% at the age of 61-70 years, correspondingly as summarized in Figure-5 in comparison to other age groups of females. In contrast, no female was found infected in the age group 10-20 years.
Usually, patients with Hepatitis C have normal hemoglobin levels. In present study, as depicted in Figure- 6, 20 to 70 age groups showed normal hemoglobin concentrations of 11.29 gm/dl, 12.15 gm/dl, 12.26 gm/dl, 11.85 gm/dl, 12.42 gm/dl, but in 71-80, age group showed slightly reduce hemoglobin 9.45 gm/dl and second reduce hemoglobin 10.15 gm/dl in 10-20 years age group.

According to Figure-7, all patients of HCV infected showed low hematocrit (Hct) levels. However, 71-80 age group showed significantly lowest Hct 29.1% and second lowest Hct 30.4% in 10-20 years age group as compared to other age groups Hct of Hepatitis C affected patients.
In patients of HCV infection, MCV of the following age groups: 10-20, 21-30, 41-50 and 51-60 years were found with normal range (76-96 FL). Whereas the 31-40 age group illustrated an MCV value (73.1 FL) which was below the normal range; however, last two age groups, 61-70 and 71-80 are adjacent to the normal MCV range as shown in Figure-9. Mean MCH was approximately slightly reduced in patients of HCV in current study except for age group 10-20. The lowest MCH was detected in 31to 40 (24.75 PG) and 61 to 70 (24.77 PG) age groups. In comparison, only 10 to 20 age group showed normal MCH (27.95 PG) in their blood (Figure-10). MCHC in all age groups of under-study HCV patients showed in normal ranges, i-e 30-35 gm/dl (Figure-11).
Figure-9: Mean Corpuscular Volume (MCV) levels in HCV patients (Normal ranges= 76-96 FL)

Figure-10: Mean Corpuscular Hemoglobin (MCH) levels in HCV patients (Normal ranges= 27-32 PG)

Figure-11: Mean Corpuscular Hemoglobin Concentration (MCHC) in HCV patients (Normal range= 30-35 gm/dl)
White Blood Cell (WBC) was found normal (4-11×10⁹/L) in the patients of HCV in 21 to 80 years age groups. Whilst only 10 to 20 age group, Hepatitis C patients’ WBC examined higher (14.97×10⁹/L) as compared to other age groups as reported in Figure-12.

Platelet count was found to be approximately normal (150-400×10⁹/L) in all patients of HCV in 21 to 80 years age groups. While in 10 to 20 age group showed highest (490×10⁹/L). Platelet as compared to normal (Figure-13)

According to Figure-14, the highest Bilirubin level (19.2 mg/dl) was found in 10-20 age group, followed by 21-30 age group (1.91 mg/dl). On the other hand, patients of HCV of age groups 10-20, 21-30 and 51-60 also possessed high levels of Bilirubin as compared to normal levels.
The ALT values in patients of HCV were found to be high in 10-60 age groups. The highest ALT values (190 IU/L) were found in age group 10-20 followed by age group 21-30 (141 IU/L). While only age group 71-80 showed below-normal (33.6 IU/L) ALT values as depicted in Figure-15.

According to Figure-16, HCV-infected patients of age group 10-20 possessed abnormally high ALP value (308 IU/L) followed by age group 41-50 (192 IU/L). In contrast, other age groups showed normal ALP range.
Alkaline Phosphatase (ALP) values in HCV patients (Normal ranges: 40-129 U/L)

Albumin levels in the patients of HCV infected in all age groups was found normal except for age group 10-20 which showed slightly lower than normal albumin levels (2.9 gm/dl) as depicted in Figure-17.

DISCUSSION

In Pakistan, Hepatitis C virus (HCV) infections are considered the primary public health apprehensions. This viral infection is spreading promptly in the general public of Pakistan owing to several economic and social factors. Although several potent antiviral treatments are available in the country, the disease liability in general public has not dropped. This may be due to the asymptomatic nature of this infection which consequences in early detection of the virus. For an efficacious control of the infection in the population of Pakistan, scientific analysis is required.

Hyderabad is considered the second largest and most populated city in Sindh province of Pakistan. The present study investigated the evaluation prevalence of HCV through the measurement of biochemical and hematological parameters in patients of Hepatitis C virus in a local population of Hyderabad, Sindh and its adjacent areas.

We applied statistical technique correlation to check the hepatitis patients in selected cities of Hyderabad region with their population hence we conclude that the relationship between population and no. of patients is the positive correlation that is 0.90, which shows that the hepatitis patients increase as increases of population (Table-1).

According to existing results, out of 510 participants prevalence of HBV antibodies was found 144 (28%) and HCV 291 (57%) in all age groups. In reported literature of different cities of Sindh (Larkana, Thatta, Nousheroferoz, and Karachi), Pakistan, it was
revealed by (Samo, Laghari, Baig, Khoso, & Hygiene, 2021) that HCV prevalence ranges from 3.2% to 25.1%, while, in Nawabshah city HCV 14.3% and HBV 6.7%. However, in current result regarding HCV prevalence is compatible with reports from other cities province of Khyber Pakhtunkhwa, Pakistan such as Mardan, HCV prevalence was 11.7%(Desikan & Khan, 2017), Peshawar where the HCV prevalence was 12.9% and in Tehsil Daggar District Buner 34.1% individuals were recorded in all age groups(Qamar, Anwar, Ahmad, Haq, Khan, Hussain, Shahzad, Ahmad, Malik, Khan, et al., 2021). Furthermore, in Farash town, located in Islamabad, the capital of Pakistan prevalence of Hepatitis B virus was 9%, and that of Hepatitis C infection was 33%(M. Asad, F. Ahmed, H. Zafar, & S. J. P. j. o. m. s. Farman, 2015). The results of our findings are quite higher (HBV 28% and HCV 57%) as compared to all above-reported studies as well as also in another study conducted in 2009 at Liaquat University Eye Hospital, Hyderabad(S. A. Junejo, N. A. Khan, & A. A. J. P. J. M. S. Lodhi, 2009) which showed 4.6% patients were infected from HBV and 13.3% from HCV. In this connection, our results are quite alarming and proved that the prevalence of Hepatitis B and Hepatitis C has spiked up in Hyderabad since last decade. The increased rate of HBV and HCV prevalence may be caused by transmission due to the lack of public consciousness(ABBASI, F, ARTMI, K, KADIR, S, SATHIAKUMAR, & health, 2014) and might be an increase in population. Several reports(Ambreen, Younas, Rasool, & Ali, 2016; Muhammad Naveed, Muhammad, & Umar Farooq, 2016) have been published that highlight the growing occurrence rate of these viral diseases for years. However, variation in reported results available in literature exhibited very unpredictable verdicts, possibly because most of the reports were revealed by consideration of small terrestrial constituency or containing only a precise population and small size of sample(KHALID et al., 2015).Based on a detail study, it was revealed that HCV infection rates in males was higher (54%) than in females (46%), giving a preponderance of males over to the female. Similar findings reported by different workers that higher prevalence in males than females were proved with results obtained by (I. Ullah et al., 2021) that hepatitis C infection was more common in males 53.58% than females 46.42%. (Jan, Awan, & Bioll, 2020) in Bannu KPK(male 64.44% and female 35.56%), (N. Ullah et al., 2021) in Mardan KPK(61.38% vs 38.62%), (M. Khan, Malik, Din, Ali, & Ahmad, 2018) from Takht Bhai, Mardan, Pakistan (76.47% vs. 23.53%), (M. Khan, et al., 2018) from Batkhela, Malakand District, Pakistan (56.52% vs. 43.48%), (S. A. Junejo, N. A. Khan, & A. A. Lodhi, 2009) also reported high prevalence in male 53.4% and female 46.6%, (M. Asad, F. Ahmed, H. Zafar, & S. Farman, 2015) have revealed that 52% males and 48% females were diagnosed Hepatitis C in Pakistan. Dissimilar results have also reported about male and female prevalence of HCV from other cities of Pakistan such as in Nawabshah the prevalence of HCV in male participants was 15.5% whereas in female participants was 12.9% (Samo et al., 2021), in Swat District (Z. Khan et al., 2021) have established that infection rate in males was higher 11.8% with HCV than females 10.9%, however, these observations proved that the percentage of the male is higher than female but results of both are low than present findings. It is suggested that the reasons of higher prevalence of HCV in males than females may be due to more social contacts of males than females in our culture, also cause the response of unsafe blood transfusion, unhygienic usage of razors and syringes (Raja, Janjua, & Infection, 2008). The lowest frequency of HCV in females may be associated to less exposure to HCV risk circumstances due to males influencing society of the area and also the estrogen hormone in females is deliberated to perform a key role in the spontaneous elimination of HCV infection(Arlig et al., 2000).

The dominance of HCV in Males over females may be caused by their more therapeutic consultation and due to more self-motivation and the vigorous role of male in society. In addition, it is difficult to predict confidentially that the greater number of HCV occurrences in males is due to the bias of the gender for the infection process or otherwise, if a primary hematological survey should be accomplished. Based on age group, significant differences were observed in the prevalence of HCV in both genders. The frequency of HCV also assorted concerning age, but the utmost prevalence 27% was detected in age groups 41-50 years. In age groups above 50 years (51 to 80 years), a decreasing trend of active HCV prevalence was observed. The decrease in percentage to 13% in the age group of 51-60 years, 7% in 61-70 and 10% in > 70 years. It is suggested that decreasing the prevalence of HCV in the age group 70 or above in present study may cause due to collection of small size of patients of HCV or a death ratio higher in the age of 70 or above by different diseases or naturally in the Pakistani population, unlike the developed countries. It was not surprising that our findings were different as compared to the findings of others because several researchers have reported different percentages of prevalence of HCV in different age groups as (Ahsan et al., 2019) have reported that in different cities of Punjab, a considerably extraordinary frequency was detected in 41–60 years old as over 33%. (M. Khan, et al., 2018) from Batkhela, Malakand District reported that infection of HCV was maximum, 31.71% in 41-60 years age group. (Gilani et al., 2021) reported utmost prevalence of Hepatitis C was observed in 61–70 years age group, (Jan et al., 2020) found that in District Bannu, Khyber Pakhtunkhwa, infection of HCV infection was maximum, 33.33% in 16-30 years age group. (N. Ullah et al., 2021) found that in district Mardan, Khyber Pakhtunkhwa, the age group 21-40 years was
found to be most active towards hepatitis C infection (47.09%). (Ahsan et al., 2019) found that in patients of Khyber College of Dentistry Peshawar, the age group 21-40 years was revealed to be most seropositive towards HCV infection (44.55%). Our findings preclude that patients in age group 41-50 were highly infected with HCV. Our results are in agreement with (Niu, Zhang, & Tong, 2016), who described that as age increased above 40 years, the rate of HCV infection also increased. It was unveiled from our results that least HCV infected patients were found in age group 10 to 20 years, this is may be due to strong immune system in younger than older. Alternations in hematological and biochemical variables have been examined and described in the Hepatitis C infection. For early diagnosis the complication related to severe Hepatitis C infection, biochemical and hematological examinations have a vital role. This helps to extensively care for the patients and prevent death that may result from such complications. The result of this study shows that the Hepatitis C infection resulted in the alternations of a few parameters.

In present study as depicted in Fig-6, 21-30 and up to 70 years age groups showed normal Hb concentration whereas in 10-20 and 71-80 years age groups showed a slightly reduced than normal Hb. It was noted that the age group 41-50, which in our study showed the highest prevalence of HCV in present report, exhibit normal Hb. These results were harmony by (Bukhari & Zafar, 2013) and (Asghar, Anjum Zia, Jafri, Ahmed, & Amjad, 2017), who revealed a normal range of hemoglobin in HCV patients. However, current findings were different from the studies described by (Chen et al., 2008) that the hemoglobin is higher in HCV patients. Moreover, another study carried out (Abdullah, 2018) in Saudi Arabia showed a lower level of hemoglobin. Hemoglobin is decreased in chronic cases when there is lower liver efficiency, which increases the breakdown of red blood cells. The Hct values showed significantly below normal in all age groups of Hepatitis C. The Hct and Hb are closely linked to each other hence low values may be indicated the presence of anemia in under-study patients. Hepatitis C has an impact on the red cells, the main components of the CBC. In under-studied Hepatitis C patients, RBC were found to be normal in all age groups, only 10-20 and 70-80 years age groups showed slightly reduced RBC than other age groups but in normal range. In Hepatitis C patients according to present data, MCV was found normal in all age groups except age group 31-40, which showed below normal range. Overall there were no significant abnormalities were observed in MCV values. On the other hand, values of MCH were noted to be slightly reduced than normal in patients of HCV in current study except age group 10-20. Furthermore, MCHC values were estimated and were found normal in all age groups. In the present report, age group 21-80 years represented a normal count of WBC except 10-20 years age group, which showed a high WBC count (Asghar et al., 2017) mentioned normal value of the Hb; however, (Tsai et al., 2015) have revealed that the HCV-infected patients also displayed considerably higher Hb, WBC and RBC count and level of Hct. Platelet count was found normal in under-study of Hepatitis C patients in 21 to 80 years age group, while only 10 to 20 years old group contained greater than normal platelet count. Our results were different from those (Bukhari & Zafar, 2013) and many published studies found lower than normal range of platelets and reported that, thrombocytopenia is an initial diagnostic indicator for HCV infection (de Almeida et al., 2004; Wang, Yao, Wang, Chang, & Chou, 2004). On the other hand, Wang et al., (Wang et al., 2004) mentioned that an HCV-infected group, which also had chronic hepatitis and cirrhosis, had a mean platelet count greater than normal range.

Our studies also include liver function tests (LFT) of patients, which is an important biochemical marker used to detect liver diseases. The liver function test includes serum analysis of serum ALP, Albumin and Bilirubin. The level of bilirubin was estimated in patients of HCV and found to be significantly high in age groups 10-30 and 41-60 years as compared to other age groups. Our results are consistent with the results of (Abdullah, 2018) and (Ashraf et al., 2016), which shows high level of bilirubin. Several literatures documented that the increased level of bilirubin reflects bile duct damage or liver cell damage. High levels of bilirubin can cause jaundice (Tasneem, 2016). (Giannini, Testa, & Savarino, 2005) have reported that Jaundice occurs in about 20%–33% of cases of acute hepatitis C infection.

Higher values of ALT in HCV-positive patients were found in this present study. The highest elevated values of ALT were observed in age group of 10-20 years. Similarly, high level of ALT was also found in HCV patients, as reported by (Abdullah, 2018), (Hassanpour & Karami, 2017) and (Asghar et al., 2017). It has been reported by (Woreta & Alqahtani, 2014) that variable ALT levels (ranging from normal to high) are related to chronic HCV infection. Continual, normal levels of ALT have been related to a lower advancement and incidence of cirrhosis in Hepatitis patients.

In HCV-infected patients, the value of ALP was found to be high in all age groups. Similar results were also documented by (Abdullah, 2018),(Asghar et al., 2017) and (Ashraf et al., 2016). In liver diseases, increased ALP amount is release into plasma instead of impaired biliary secretion. It has been reported (Hsu et al., 1996) that level of ALP increased with increasing age, body mass index, C-reactive protein, monocyte count, serum uric acid, lead, diabetes, and smoking, lesion of the liver and cardiovascular disease.

Albumin levels in the patients HCV infected in all age groups were found to be normal except for age group 10-20, which showed slightly lower than
normal albumin levels. These findings are different from those (Abdullah, 2018), who reported a significant reduction in albumin levels and (Hassanpour & Karami, 2017) mentioned a slightly low albumin level.

CONCLUSION
Based on reported data in the present study, the HCV prevalence was documented in different age and gender groups, exhibiting its occurrence has been higher with the increase in age. The percentage of HCV infection in male individuals was almost higher as compared to females. WBC and PLT are high in the 10-20 age groups. Hematocrit and MCH were decreased as compared to normal. Bilirubin, SGPT, and Alkaline phosphate were high in some age groups but also normal in other age groups. The higher Hepatitis C prevalence shows that the overall population in Hyderabad is constant exposure to the risk of HCV infection. There is an immediate requirement to introduce active population-based health awareness programs among the people about paths of dispersal and hindrance from Hepatitis virus.

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Disclosure statement: The authors have no conflicts of interest to declare.

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