



Serological and Epidemiological Evaluation of active HCV Infection in the Volunteer blood Donor at District Swat Khyber Pakhtunkhwa Pakistan

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ABSTRACT

Hepatitis C infection is one of the major frequent Blood born diseases caused by Hepatitis C virus. Hepatitis C virus is the main causing Agent of Liver Cirrhosis and sometime may leads to Hepatocellular carcinoma (HCC). Approximately 3% of individual of the globe is effected by hepatitis C virus (WHO). The purpose of our study to identify anti-HCV Frequency rate based on ELISA (Enzyme linked immune sorbent assay) among the blood donor of DHQ Hospital District Swat Khyber Pakhtunkhwa (KPK) Province Of Pakistan. From February 2018 to October 2018 a total of 2437 blood samples were collected in the blood bank of DHQ Hospital Swat For the Detection of anti-HCV antibodies all the samples were tasted with ELISA. Among the total blood sample only 41 (1.60%) were positive on ELISA. According to our study, The Frequency rate Of HCV Infection is even lower than that of all the other Districts of KPK and this lower prevalence is might be due to the clean and green atmosphere of District swat and might be due to improvement of the health care centers in District swat, and possibly the awareness level among the common population.

Key words: Anti HCV, ELISA, swat, Pakistan.

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INTRODUCTION

Hepatitis C infection is the main causing Agent of death and morbidity worldwide. Approximately 3% of the globe individual affected by HCV virus. Mostly Hepatitis C virus Cause liver cirrhosis and also the main Source of hepatocellular carcinoma [1]. Hepatitis C virus is enveloped, small circular, positive-sense and single stranded ribonucleic acid (RNA) virus from genus *Hepacivirus*, family *Flaviviridae* with a diameter of 50 nm [2]. Hepatitis Virus Play key role in causing Hepatitis Infection But there is also some toxic substance like (alcohol, certain drugs) and some autoimmune disease can also cause Hepatitis Infection. Hepatitis C virus is the main reason of liver cirrhosis globally since 1989 [3]. A previous study Conclude that about 130–170 million individual (2%–3%) are affected by Hepatitis C virus globally [4]. The annual death ratio of HCV infection is more than 350000 and most of the individual died due to hepatocellular carcinoma (HCC) and liver cirrhosis [5]. The morbidity ratio of Hepatitis C virus is high among all hepatitis Strain therefore its considering more danger than other hepatitis virus [6]. About 170 million individual are infected by Hepatitis C virus worldwide and their death ratio is much higher than other infectious disease [7]. The number of Hepatitis C virus Genotype is six and genotyping is very important for understanding, Diagnosis and for better treatment of HCV infection [8]. There are several way which are used for the Transmission of HCV like Sexual relation between infected and normal

individual, Blood transfusion, the lack of accurate health system and have no public awareness regarding transmission of HCV [9].

The main pathway of HCV transmission is using infected syringe, infected surgical instrument, Unhygienic food, water and organ transplantation is the main way of HCV transmission [10]. The frequency rate of HCV infection is different in different region. America, Australia and Western Europe have low frequency rate of HCV which is (< 2%) [11]. In all over the world Egypt have high prevalence rate of HCV infection which is greater than 14% [12]. Previous study show that the frequency rate of HCV infection among all blood donor of KPK province of pakistan and tribal area of pakistan is 1.65% [13].

The aim Of our study was to recognize anti-HCV frequency among volunteer blood donor who come to the blood bank of DHQ hospital District swat. While previous studies survive that have exposed HCV prevalence in different time frame [14], but as prevalence varied in respect to time, that's why to report the present HCV prevalence in Swat Area of KPK, we have conducted this study.

MATERIAL AND METHODS

The purpose of this study was to identify the frequency rate of HCV antibody among the blood donors of DHQ Hospital Swat KPK from February to October 2018. ELISA Procedure was used to recognize the prevalence of HCV antibody in the blood samples.

Blood Donors selection:

Blood samples were taken from the controlled blood donors and screened in the blood bank of DHQ Hospital Swat KPK.



Swat
Khyber Pakhtunkhwa

Fig 1: Map of District Swat

ELISA:

ELISA (Monolisa Anti-HCV, Plus version 3) technique are used to identify anti-HCV antibodies among all blood samples. According to the manufacturer's information The following method was followed for conducting ELISA.

Procedure:

For each test we used positive and negative control sera for arrange to confirm the quality of the test. Using good laboratory technique carefully established the sample distraction and screening plane. The diluted washing solution was prepared. The well from the group were taken and added the following in the particular wells of the plate in the following order.

- In each well we add 100 µl of sample diluents.
- In one well we add 50 µl of negative control.
- In second well we add 50 µl of positive control..
- Then we add 50 µl of sample in the next wells.

Homogenized the mixture with at least 3 aspirations or with a micro-plate shaker for 5 seconds. After the sample division the well containing sample or control changed the purple colour to blue. The plate was enclosed with adhesive film and then incubated the micro plate for 60 minutes at 37°C.

Removed the adhesive film followed by aspiration of the inside of all the wells in a liquid waste container and add a minimum of 0.370ml of washing solution into each well aspirated again and repeated the washing. Dried the strips by turning them upside down on absorbent paper. Distributed quickly 100µl of the conjugate solution into each well within the plate. Enclosed again with a new film and incubated for 30 minutes at 37°C. Washing is done five times. in dark condition we added Enzymatic solutions in each well and incubated for 30 minutes. Then added 100µl stop solution using the same sequence and same speed of division as for the development solution. the substrate colour blue for positive and pink for negative sample. After adding stopping solution the colour of positive sample become yellow and fades from the well become colourless which show negative sample. Watchfully wiped each plate bottom. Waited at least 4 minutes after the stopping solution addition and within 30 minute of stopping the reaction read the optical density at 450/620-700nm using a plate reader. Checked for agreement between the spectrophotometric and visual reading against the plate and sample distribution and recognition plan.

RESULT

A total of 2437 voluntary blood donors were initially screened for anti HCV antibody by ELISA, which show that out of the total number of volunteers 41 subjects (1.60%) were positive for anti-HCV antibodies (Table 1). Moreover, HCV prevalence was more in month of May 7 (2.52%) (Figure 1).

Table 1. Month wise HCV prevalence

Months	Donors	Anti-hcv (Elisa)+	Percentage.
February	303	6	1.98%
March	323	4	1.23%
April	314	5	1.59%
May	236	3	1.27%
June	277	7	2.52%
July	269	5	1.85%
August	253	4	1.58%
September	219	3	1.36%
October	243	4	1.64%
TOTAL	2437	41	1.60

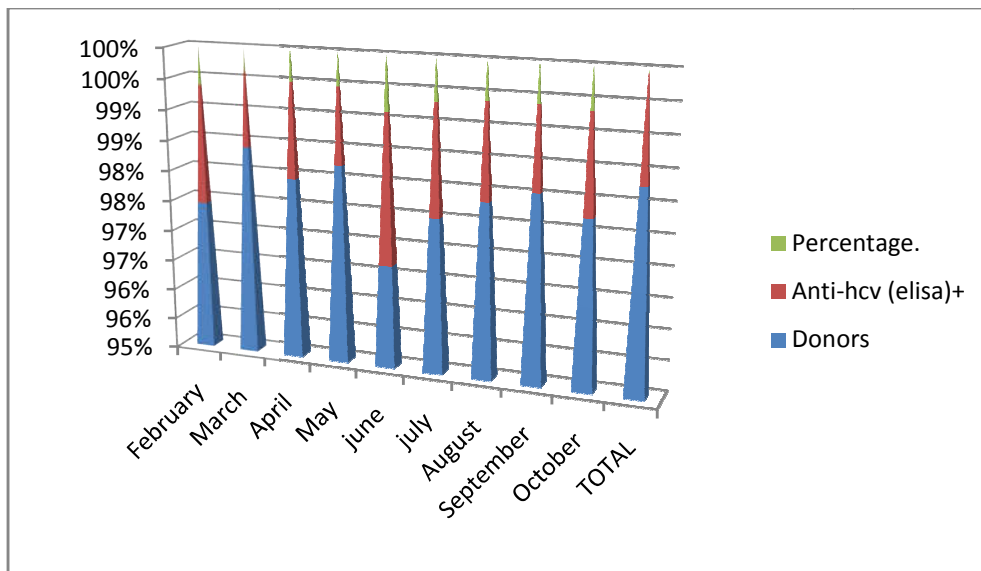
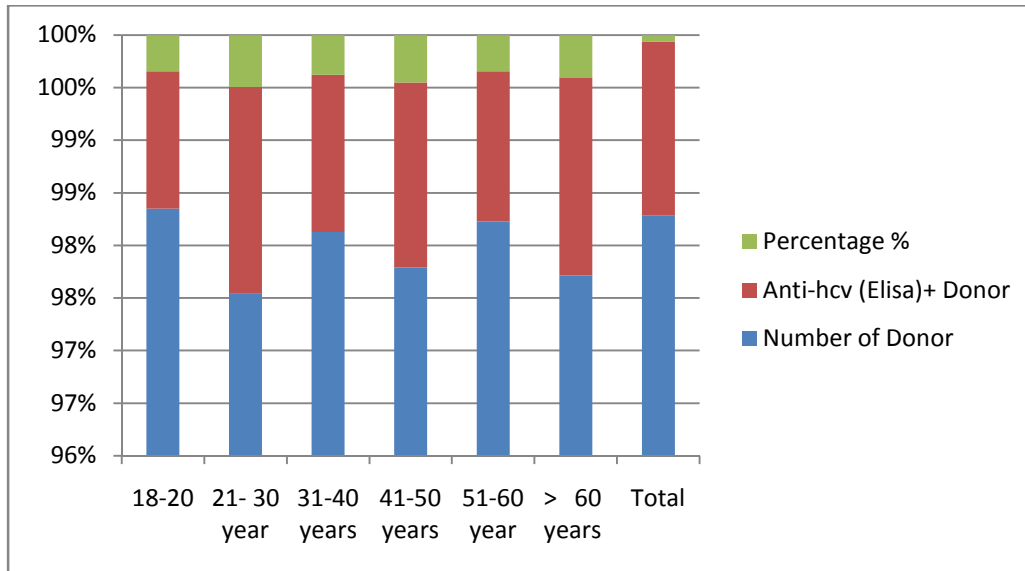


Fig. 2. Comparative HCV prevalence in different months:

Table 2 show. Age wise Prevalence of HCV Donor:

Age Group	Number of Donor	Anti-hcv (Elisa)+ Donor	Percentage %
18-20 years	377	5	1.32%
21- 30 year	397	8	2.01%
31-40 years	394	6	1.52%
41-50 years	389	7	1.79%
51-60 year	413	6	1.45%
> 60 years	467	9	1.92%
Total	2437	41	1.60%

**Fig3. Age wise Prevalence of HCV Donor:****DISCUSSION**

Hepatitis C infection is major problem around the world. The frequency rate of hcv infection is higher than Egypt which is considered is the second highest prevalence globally. The increasing ratio of mortality and morbidity associated with HCV infection is due to unhygienic food, water, poor health care facility and lack of social awareness regarding HCV infection in different part of the country [15]. Previous study use different technique to identify the seroprevalence of HCV infection in Pakistan which show 3.3% to 5.3% [16]. Donation records and serum of all donors reporting to the DHQ Hospital Swat, Pakistan from February 2018 to October 2018 were analyzed.

Mean of the current study was to identify the prevalence of HCV in the volunteer blood donors, through Elisa technique in District Swat KPK. In this study, screening of blood by Elisa technique revealed that 1.60% of the blood donors were positive for anti-HCV which is well in the range of previously reported anti-HCV prevalence like studies conducted by [17-20]. According to these the prevalence rates of HCV in blood donors was 1.05%, 1.89%, 2.23%, and 4.5%, respectively. Studies conducted in other parts of the country including Kurram Agency [21] Interior Sindh [22] and Hyderabad [23] has shown prevalence rates of 1.1%, 5.7%, 3.45% respectively. The prevalence ratio of HCV infection in 2018 is 1.2% was recorded at district Mardan [24]. The another study was conducted in district Charsadda to identify the frequency rate of HCV infection which show 0.94% HCV and HBV 1.47, 0.6 prevalence of syphilis respectively in 2018 [25-27]. But in the present study we analyzed all the donor blood samples by Elisa which indicated that 1.60% of the blood donors had antibodies against HCV [Table. 1]. Looking to HCV prevalence in different regions of KPK province, it is obvious that prevalence of HCV is variable and mostly is more than our study prevalence.

This may be due to variation in the subjects' involvement, procedure used for identification, area of involvement and possibly seasons of study. In comparison to all of the above studies conducted either in KPK or in Pakistan, the affinity of HCV prevalence is in downward order that is it is almost lower than the previous.

This might be due to development in the health care facilities adopted by the patients or adopted for the patients in different hospitals, or might be due to enhanced awareness regarding extent of infection and possibly due to movement of refugees that had come from Afghanistan.

Moderately the percentages of HCV positive cases were more in June which was 2.52% [Table.1]. This might be due an effect of the season load as in summer the chance of getting HCV infection is more, so patients after getting infection have to screen themselves.

CONCLUSION

HCV prevalence in the volunteers blood donors was lower (1.60%) than the previous studies conducted in Khyber Pkhtunkhwa.

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