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EPIDEMIOLOGICAL INVESTIGATIONS ON THE SEROPREVALENCE OF HEPATITIS C AND ASSOCIATED RISK FACTORS ANALYSIS IN DISTRICT GILGIT, PAKISTAN

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Abstract

Hepatitis C virus infects more than 71 million individuals worldwide. After Egypt, Pakistan is the country with high number of hepatitis C virus infections in the World. Hepatitis C infection is a major health care issue in Gilgit. Therefore, an observational cross-sectional epidemiological study was conducted to determine the sero-prevalence of HCV and analysis of associated risk factors (age, gender, vein puncture, dental surgery, blood transfusions, surgery, etc.) using enzyme-linked immunosorbent assay. In this study, a total of 364 blood samples were randomly collected from three Tehsils (Danyore, Juglot and Gilgit) in district Gilgit for anti-HCV antibody detection. Overall sero-prevalence of HCV in healthy individuals of district Gilgit was 8.2%. Sero-prevalence of HCV were found more in females (12.6%) as compared to males (3.4%). Individuals with more than 40 years of age exhibited higher sero-prevalence (14.8%) as compared to younger age groups. Gilgit had the highest sero-prevalence (11.7%) in comparison to other two Tehsils. Sero-prevalence of HCV among individuals undergone surgeries, dental procedures and blood transfusion was (17.5%), (14.5%) and (15.3%) respectively. Such a high level of HCV sero-prevalence in healthy population of district Gilgit is alarming. Immediate control measure should be taken to stop the transmission of HCV infection, by organizing different awareness sessions and workshops in different areas of district Gilgit. There is also a need to monitor all the diseased and carrier cases of HCV infection in the area for better prevention and control of the disease in near future.

Keywords
Hepatitis C, Prevalence, Genotypes, Gilgit-Baltitsan, Pakistan

1. Introduction

One of the leading causes of liver infection is the Hepatitis C virus (HCV), having the ability to infect the liver in a healthy body in multiple ways (Kish *et al.,* 2017). Initially, the virus infects the liver asymptomatically, that's why an infected person is unable to detect the disease at initial states and remains undetected for a longer duration of time (Kitiyakara *et al.,* 2017).

Due to lack of medical facilities and lack of awareness regarding HCV, still a higher prevalence of hepatitis C infection in developing countries, like Egypt, Pakistan, Iran and Afghanistan. HCV is considered a major health care dilemma (Riaz *et al.,* 2016). According to WHO the annual rate of mortality is approximately 400,000 due to blood-borne viruses such as Hepatitis C and more than 71 million populations is infected by this virus around the globe. However, recent research and study showed that the increase in the chronic cases of HCV in the world is due to the absence of a vaccine against this disease.

In developing countries, after Egypt, Pakistan is the country with a high number of hepatitis C virus cases in the World. The predominant mode of transmission of HCV is through blood transfusion followed by contaminated surgical instruments and unsafe injections (Kumar *et al.,* 2017). Several cases reported at clinical sites in Pakistan were due to the re-used of contaminated instruments and syringes during surgery in government hospitals. Especially, in rural and backward areas of Pakistan re-used of contaminated surgical instruments is common practice and this is due to the lack of medical monitoring systems in such backward regions of Pakistan (Qureshi *et al.,* 2013). Significant work and preventive measures are adopted by the government of Pakistan to limit the re-use of syringes and contaminated instrument during medical surgeries. However, due to a lack of resources government of Pakistan is unable to implement these policies at a mass scale in the whole country (Ahsan *et al.,* 2019)

According to the latest research, approximately 7.5% of people in Pakistan were infected by HCV (Ali *et al.,* 2010). Additionally, the alarming situation faced by health care facilities in Pakistan, while handling HCV is late detection of disease (when the disease enters in chronic stage). Despite the great efforts done by the government of Pakistan, HCV is still endemic in Pakistan and the occurrence of HCV is increasing annually (Rubi & Khan, 2017).

Statistical analysis revealed that the incidence of HCV is increasing in rural areas by 25% as compared to urban areas. This indicates that unhygienic living conditions, lack of facilities and awareness in the healthcare system, which ultimately lead to an increase in the prevalence of HCV in rural areas (Umer & Iqbal, 2016). Based on this information it is important to study the prevalence of HCV from both socio-economic and medical perspectives.

According to Arshad & Ashfaq (2017), the HCV prevalence in Pakistan differs in each province of Pakistan. There, a study reported that the

prevalence of HCV is more in Balochistan as compared to Federally Administrated Tribal Areas. However, it is important to know that the population of these two provinces is low as compared to other provinces of the state. Statistical analysis shows that the rate of prevalence of HCV is 25.77% in Balochistan, 6.07% in Khyber Pakhtunkhwa, 5.46% in Punjab, 3.37% in Federally Administrated Area and 2.55% in Sindh (Arshad & Ashfaq, 2017). According to Al Kanaani *et al.* (2018), the rate of prevalence of HCV is 6.2% in the general population of Pakistan as compared to clinical individuals more prone to disease which is 34.5%. This study also shows that the risk of acquiring HCV in medical staff is higher around 12.8% as compared to the general population. The most important factor revealed by this study is that the highest incidence 55.9% was found in populations suffering from liver abnormalities. This shows that HCV is directly related to liver diseases. And according to this study 1 out of 20 Pakistanis is a carrier of HCV, making HCV a threatening health care dilemma in the country (Al Kanaani *et al.,* 2018).

The number of studies conducted on the HCV prevalence in the country has determined that the pre-dominate genotype of HCV is 3a, and this genotype is responsible to infect 63% of overall cases of HCV reported in Pakistan. Statistical analysis also shows that the individuals of age group greater than 40 are more likely to infect by the 3a genotype of HCV (Riaz *et al.,* 2016).

Unfortunately, no previous study on the seroprevalence of hepatitis C has been conducted in District Gilgit, the capital of Gilgit Baltistan Northern area of Pakistan. Keeping in view the importance of HCV infection and its growing prevalence in the country, the proposed study plan aimed to find out the seroprevalence of anti-HCV antibodies in healthy populations of district Gilgit and to explore the associated risk factor.

2. Materials and Methods

An observational cross-sectional epidemiological study was conducted in District Gilgit to detect the prevalence of anti-HCV antibodies and to assess the role of associated risk factors with this disease. This current study was conducted from 2017 to 2018 in three tehsils of district Gilgit (Gilgit, Danyore and Juglot). For sampling purposes, we selected five main hospitals i.e. CMH Gilgit, Aga Khan Medical Centre Gilgit (AKMC), Government Civil Hospital, Safe-ul-Rehman Government Hospital and DHQ Hospital.

2.1 Data Collection

A questionnaire was designed and presented to each individual with their permission to get basic information like age, gender, area, surgical interventions, dental procedures body piercing and tattooing. The prior consent for sampling was taken from the Ethical Review Committee of Government College University Faisalabad.

2.2 Samples Size

Blood samples were collected from healthy individuals of different age groups ranging from

(10-25, 25- 40, 40 and above) years from all the three Tehsils (Gilgit, Danyore and Juglot) of District Gilgit by non-probability convenience sampling method. Samples were collected from all of the tehsils. The sample size was designed by considering the expected prevalence of 15%, 95% confidence level and 5% of the ideal absolute accuracy (Nafees *et al.,* 2007).

The following formula was used for sample size calculations. Where:

n = required sample size Pexp = expected prevalence d = desired absolute precision

2.3 Sample Collection

n = 1 .962Pexp (1 - Pexp)d2

A whole 3 ml blood was collected in sterile syringes, transferred into plain red top test tubes and centrifuged for 10 minutes at 1,500 rpm to separate the serum. Serum was collected in Eppendorf tubes and an identification code was given to each sample. These Eppendorf tubes were stored at -20°C and then shift to the Post Graduate Laboratory of the Department of Microbiology Government College University, Faisalabad.

2.4 Serological Serological Examination

Enzyme link immunosorbent Assay (ELISA) was performed to detect Anti-HCV antibodies in all serum samples collected from healthy individuals of all three tehsils of district Gilgit.

2.5 Enzyme-Linked Immunosorbent Assay (ELISA).

By using RecombiLISA HCV IgG by CTK (Biotech, USA) kit for quality analysis of IgG of Hepatitis C Virus (HCV) in serum samples. The principle of the ELISA kit is based on an indirect ELISA technique for the detection of IgG of HCV in serum, having 100% sensitivity and 100% specificity. All serum samples were processed through ELISA according to guidelines provided by the manufacturer.

2.6 Statistical Analysis

All the data obtained were tabulated in the excel spreadsheet. Chi-square test and Odds Ratio analysis was done to check the association of risk factors with HCV infection using SPSS® software.

3. Results

The current study was conducted in three different Tehsils of District Gilgit (Gilgit, Danyore and Juglot) to detect the seroprevalence of hepatitis C Virus and associated risk factors in healthy individuals of district Gilgit. A total of 364 serum samples were collected from healthy individuals of different age groups to detect the prevalence of HCV. Serum samples were analysed by using ELISA for detection of seroprevalence of HCV in a healthy population of district Gilgit. Out of 364 serum samples collected from three tehsils of district Gilgit, 30 serum samples were positive for IgG HCV and the overall seroprevalence of HCV detected in district Gilgit was 8.2%.

It was observed that people of tehsil Gilgit had high seroprevalence of HCV infection (11.7%), followed by tehsil Juglot and Danyore, 9.3% and 4.41% respectively. Based on age groups, a higher seroprevalence of HCV 14.8% was detected in the age group above 40 years.

Results showed that Blood transfusion, surgical and dental procedures were found to be statistically significant (P<0.05). Whereas, age, gender, geographical distribution, barber visits and body piercing/tattooing were found to be statistically non-significant (P>0.05). Predominant risk factors associated with Hepatitis C infection were Blood transfusion, surgical and dental procedures. The seroprevalence of HCV associated with surgical procedures was observed as (17.5%), followed by (3.4%) in non-operated individuals. A total of 124 individuals undergo dental procedures and out of 124 individuals, 18 (14.5%) were positive for IgG HCV, as compared to 240 individuals who did not undergo any dental procedure 12 (5%) were positive for HCV. The results prove the seroprevalence of HCV associated with dental procedures is higher. Total 78 individuals with blood transfusion were analyzed for IgG HCV and the results showed that

out of 78, 12 (15.3%) individuals were positive. Whereas, 286 individuals with non-blood transfusion, 18 (6.3%) were positive for IgG HCV. The results showed that the seroprevalence of HCV in blood transfused individuals was higher. Barber visitors showed (6.9%) seroprevalence as compared to non-visitors (9.5%).

Similarly, the association of HCV with Body Piercing and Tattooing was (8.4%). Whereas, the seroprevalence of HCV in individuals, who did not undergo this procedure was (8.2%) (Table 1).

Table 1: Risk Factors (Age, Gender, Area, Dental Surgery, Blood Transfusions, Surgery, Body Piercing/ Tattooing Etc.) Associated With HCV Infection.

Characteristics	Total Examined	Positive	Negative	Percentage	P-value
Gender					
Male	174	6	168	3.44%	0.185
Female	190	24	166	12.63%	
Ages Groups					
10-25	152	6	146	3.94%	
25-40	90	6	84	6.66%	0.105
40 Above	122	18	334	8.24%	
Tehsils					
Gilgit	120	14	106	11.66%	
Danyore	136	6	130	4.41%	0.878
Juglot	108	10	334	9.25%	
Surgery					
Operated	126	22	104	17.46%	0.003
Non-Operated	238	8	230	3.36	
Dental Procedures					
Operated	124	18	106	14.51%	0.044
Non-Operated	240	12	228	5.00%	
Blood Transfusion	78	12	66	15.34%	0.001

YES	286	18	168	6.29%	
NO					
Barber visits	174	12	162	6.89%	0.561
Visitors	190	18	172	9.47%	
Non-visitors					
Body Piercing/Tattooing	190	16	174	8.42%	0.933
YES	174	14	160	8.04%	
NO					

4. Discussion

In Pakistan Hepatitis C virus (HCV) infection is a crucial health care problem, which leads to acute and chronic infections that causes liver damage, cirrhosis and hepatocellular carcinoma. Hepatitis C virus is a blood-borne virus; which is transmitted from one person to another through exposure to small amounts of blood. HCV is transmitted through the usage of unsafe injections, usage of contaminated instruments during surgeries in the healthcare system, and it may also transmit through transfusion of unscreened blood and blood products. About 185 million people are infected by Hepatitis C worldwide. After Egypt, Pakistan is the country with the highest seroprevalence of hepatitis C virus (HCV) infections on the earth. Almost 10 million people in Pakistan suffer from hepatitis C (Ashraf *et al.*, 2018). According to World Health Organization (WHO), a significant number of people with hepatitis C infection may enter into chronic stages such as cirrhosis or liver cancer.

About 399,000 people die from hepatitis C each year mainly due to cirrhosis and hepatocellular carcinoma worldwide. The current study was conducted in District Gilgit. Randomly blood samples were collected from healthy individuals of three Tehsils of District Gilgit i.e. Gilgit, Danyore and Juglot. Unfortunately, no such type of study was conducted previously in this area to represent the seroprevalence of HCV. This study aims to detect seroprevalence of Hepatitis C Virus in healthy people of District Gilgit and to judge the role of certain factors (age, gender needle pricks, dental procedures, blood transfusion, surgery etc.) associated with HCV.

A total of 364 serum samples were randomly collected from healthy individuals of different age groups i.e. (10-25, 25-40, 40 and above) years from all the three Tehsils of District Gilgit. The results of the current study showed that out of 364 serum samples 30 (8.2 %.) were positive for anti-HCV antibodies. Such a high prevalence of HCV in this area may be due to: (1) Reuse of contaminated blood products and instruments during a blood transfusion. (2) Reuse of contaminated surgical instruments in hospitals during surgery and in dental procedures. (3) Reuse of contaminated razors by barbers.

The results of the current study prove seroprevalence of HCV is more in females (12.6%) as compared to males (3.4%). The gender-based association of the prevalence of HCV infection was statistically non-significant ($P > 0.05$). Our results are also favoured by the

finding of Anwar *et al.* (2011) who also set up a statistically non-significant difference in the frequency of HCV in males and females. Similarly, e Ammara *et al.* (2015) also reported the non-significant difference in the prevalence of HCV between males and females. However, our finding was found contradictory to Umumararungu *et al.* (2017), who said a higher HCV prevalence in males as compared to females in Rwanda. The possible reason for such a high prevalence of HCV in females is due to the use of contaminated and improper sterilised equipment in Caesarean section operations in different government hospitals of district Gilgit.

The results showed that HCV prevalence was highest in those individuals having age more than 40 years (14.8 %), followed by age 25-40 years (6.7%), and 10-25 years (3.9%). The prevalence of HCV infection associated with age was statistically non-significant ($P > 0.05$). The finding of Arshad *et al.* (2012) favoured our findings those 41-50 years' individuals are more prone to HCV infection. Our findings are also favoured by Ahmad *et al.* (2006), who reported a high prevalence of HCV in the 55-64 years age group as compared to the 25-43 years age group. (Akhtar *et al.* (2016)) also reported a high prevalence in the age group of 41-50 years. The possible reason for this may be the weak immune system, dietary imbalance and secondary infections.

In the distribution of seroprevalence of HCV according to the geographical locations, out of 120 serum samples collected from healthy individuals in tehsil Gilgit, 14 (11.7%) were positive and had the highest seroprevalence of HCV infection, followed by tehsil Juglot were out of 108 serum samples 10 samples were positive with 9.3% of seroprevalence of HCV. Whereas, it was observed that in tehsil Danyore, where 6 serum samples were positive from the total of 136 serum samples and had the lowest seroprevalence of HCV 4.41%. The prevalence of HCV infection associated with geographical locations was statistically non-significant ($P> 0.05$). The possible reasons for the high occurrence of HCV in Gilgit may be due to the reuse of contaminated blood products and instruments during a blood transfusion. Reuse of contaminated surgical instruments in government hospitals during surgery and dental procedures. Sero-prevalence of HCV associated with surgical procedures it was observed that out of a total of 126 individuals having surgery, 22 were found positive (17.5%) as compared to 238 non-operated individuals, 8 were positive (3.4%). The results showed that higher seroprevalence was found in operated individuals than non-operated personals included from all three tehsils of district Gilgit and this difference was statistically significant ($P \leq 0.05$). Our findings are favoured by M. Ali *et al.* (2011) who reported that the rate of prevalence of HCV was elevated in surgically operated people as compared to non-operated individuals. Similarly, Ijaz-ul-Haque Taseer *et al.* (2010) also reported a high prevalence of HCV in surgically operated persons. The most

important and possible reason for high prevalence in individuals having surgery is a reuse of improperly sterilized and contaminated surgical instruments in government hospitals during surgery.

A total of 124 individuals underwent the dental procedure and out of 124 individuals, 18 were positive (14.5%) as compared to 240 individuals who did not undergo dental procedures 12 were positive (5%). The results showed that the seroprevalence of HCV associated with dental procedures is higher. This difference was statistically significant ($P \leq 0.05$). Our results are similar to Jafri *et al.* (2006) who concluded that dental procedures were a contributing risk factor in getting HCV infection. Javed *et al.* (2008) also found that people having dental procedures were more prone to HCV. The possible reason for this may be the reuse of improperly sterilized and contaminated surgical instruments in dental clinics and hospitals during dental procedures.

Hepatitis C Virus is a blood born pathogen and seroprevalence of HCV is associated with blood transfusion. Total 78 individuals with blood transfusion were tested and the results showed that out of 78, 12 individuals were positive (15.3 %) whereas in 286 non-transfused individuals 18 were positive (6.3%). The results showed that the seroprevalence of HCV in blood transfused individuals was higher. This difference was statistically significant ($P \leq 0.05$). Naheed (2012) reported similar findings that support the results of the present study. Khan *et al.* (2018) also reported that people having blood transfusion was more prone to HCV infection. This is due to the reuse of contaminated instruments during blood transfusion in developing countries like Pakistan. Pakistan has the National Institute of Health Services Regulations & Coordination Government of Pakistan. For the implementation of International Health Regulations (IHR) (2005) in Pakistan. According to this before blood transfusion, the blood donors must be screened before transfusion for Transfusion Transmissible Infections (TTI). In this practice, the donor blood is a screen for the presence of one or more of the TTI infections. The false-negative results during rapid identification methods result in the transmission of infection.

A total of 174 individuals who visited barbershops 12 individuals were positive. The prevalence of HCV associated with barber visits was (6.9%) as compared to 190 individuals of which 18 were positive who did not visit barbershops and the prevalence of HCV was (9.5%). The prevalence of HCV infection associated with barber visits was statistically non-significant ($P> 0.05$). Our present study partially contradicts the study of Waheed *et al.* (2009) who reported that barber visiting was a major contributing factor in HCV prevalence. The possible reason may be reused of contaminated razors by barbers.

The association of HCV with Body Piercing and Tattooing was observed and found that the prevalence of HCV in Piercing and Tattooing

individuals was (8.4%) whereas, the prevalence of HCV in individuals who do not undergo this procedure was (8.2%). The prevalence of HCV infection associated with Body Piercing and Tattooing was statistically non-significant (P> 0.05). Results of a study conducted by Ahmad *et al*. (2009) favoured our study who concluded that body piercing and Tattooing individuals had more prevalence of HCV as compared to non-pierced. However, our results contradict Jafri *et al.* (2006), who reported that tattooing and piercing were not associated with HCV infection. The possible reason may be the use of contaminated not sterilized equipment at home and tattooing shops for body piercing and tattooing. It was concluded that out of 364 serum samples collected from three tehsils of district Gilgit, 30 serum samples were positive for IgG HCV and the overall seroprevalence of HCV detected in district Gilgit was 8.2%.

5. Conclusion

Seroprevalence of Hepatitis C virus in district Gilgit was 8.2%. The following factors significantly contribute to the occurrence and transmission of Hepatitis C infection among healthy population: (1) reuse of contaminated blood products and instruments during Blood transfusion (2) Reuse of contaminated surgical instruments in hospitals during surgery and dental procedures (3) Reuse of contaminated razors by barbers. It is therefore recommended that more research work is needed in this area to find out the genotyping of HCV. Moreover, a comprehensive awareness campaign highlighting information about disease transmission, prevention and immunization is required for the common people of these beautiful areas of Northern Pakistan. Future work may also include awareness level of HCV or any blood-borne infectious diseases in the study areas.

6. References

Ahmad, A., Ahmad, B., Ali, A., & Ahmad, Y. (2009). Seroprevalence of HBsAg and anti-HCV in general healthy population of Swat district with frequency of different HCV Genotypes. *Pakistan Journal of Medical Sciences*, 25(5), 744-748.

Ahmad, I., Khan, S. B., Rehman, H., Khan, M. H., & Anwar, S. (2006). Frequency of Hepatitis B and Hepatitis C among cataract patients. *Gomal Journal of Medical Sciences*, 4(2).

Ahsan, A., Khan, A. Z., Javed, H., Mirza, S., Chaudhary, S. U., & Shahzad-ul-Hussan, S. (2019). Estimation of hepatitis C prevalence in the Punjab province of Pakistan: a retrospective study on general population. *PloS one*, 14(4), e0214435.

Akhtar, N., Ilyas, M., Muhammad, K., Shams, S., Saeed, K., & Asadullah, A. (2016). Prevalence of Hepatitis C virus infections among the general population of Buner, Khyber Pakhtunkhwa, Pakistan. *Biomedical Research and Therapy*, 3(12), 1003-1017.

Al Kanaani, Z., Mahmud, S., Kouyoumjian, S. P., & Abu-Raddad, L. J. (2018). The epidemiology of hepatitis C virus in Pakistan: systematic review and meta-analyses. *Royal Society Open science*, 5(4), 180257.

Ali, A., Ahmad, H., Ali, I., Khan, S., Zaidi, G., & Idrees, M. (2010). Prevalence of active hepatitis c virus infection in district Mansehra Pakistan. *Virology Journal*, 7(1), 1-5.

Anwar, M. S., Nafees, M., & Nabi, U. (2011). Sero-prevalence of HCV and associated infections with HIV and HBV among prisoners in Lahore. *Biomedica*, 27(2), 119-1122.

Arshad, A., & Ashfaq, U. A. (2017). Epidemiology of hepatitis C infection in Pakistan: current estimate and major risk factors. Critical Reviews™ in *Eukaryotic Gene Expression*, 27(1).

Ashraf, I., Mehmood, Y., Yousaf, H., Riaz, H., & Sarwar, F. (2018). Detection of hepatitis c virus in blood donors; Correlation of hepatitis c with intravenous drug use/abuse/addict, type of blood donor and multiple-pricks. *Journal of Medical Sciences*, 26(2), 164-168.

Ammara, U., Mansoor, S., Saeed, M., Amin, I., Briddon, R. W., & Al-Sadi, A. M. (2015). RNA interference-based resistance in transgenic tomato plants against Tomato yellow leaf curl virus-Oman (TYLCV-OM) and its associated betasatellite. *Virology Journal*, 12(1), 1-12.

Ijaz-ul-Haque Taseer, F. I., Hussain, L., Safdar, S., Mirbahar, A. M., & Faiz, S. A. (2010). Frequency of anti-HCV, HBsAg and related risk factors in pregnant women at Nishtar Hospital, Multan. *Journal of Ayub Medical College* Abbottabad, 22(1), 13-16.

Jafri, W., Jafri, N., Yakoob, J., Islam, M., Tirmizi, S. F. A., Jafar, T., *et al*. (2006). Hepatitis B and C: prevalence and risk factors associated with seropositivity among children in Karachi, Pakistan. *BMC Infectious Diseases*, 6(1), 1-10.

Javed, S., Nasreen, G., Muhammad, I., Rehman, A. U., Jamila, F., & Muhammad Yasir, A. (2008). Seroprevalence of hepatitis B and hepatitis C in health care workers in Abbottabad.

Khan, J., Khan, B. T., Ayaz, A., Salman, M., & Khan, K. (2018). Hepatitis-C virus prevalence in district Shangla and evaluation of screening tests for anti-HCV. *Pakistan Journal of Zoology*, 50(4).

Kish, T., Aziz, A., & Sorio, M. (2017). Hepatitis C in a new era: a review of current therapies. *Pharmacy and Therapeutics*, 42(5), 316.

Kitiyakara, T., Redmond, S., Unwanatham, N., Rattanasiri, S., Thakkinstian, A., Tangtawee, P., (2017). The detection of

hepatocellular carcinoma (HCC) from patients' breath using canine scent detection: a proof-of-concept study. *Journal of Breath Research*, 11(4), 046002.

Kumar, T., Ahmad, N., Hayat, M. K., Gao, B.-X., Faisal, S., Ilahi, N.(2017). Prevalence and genotypic distribution of hepatitis C Virus in Peshawar KPK, Pakistan. *Hayati Journal of Biosciences*, 24(1), 22-25.

Nafees, M., Bhatti, M., & Haq, I. (2007). Sero-prevalence of HCV Antibodies in population attending Madina Teaching hospital, Faisalabad. *Annals of King Edward Medical University*, 13(4), 260-260.

Naheed, F. (2012). Frequency and determinants of hepatitis C virus infection among females admitted for gynaecological surgeries. *Journal of Surgery Pakistan International*, 17(1), 24-26.

Qureshi, H., Mohamud, B. K., Alam, S. E., Arif, A., & Ahmed, W. (2013). Treatment of hepatitis B and C through national programme—an audit. *The Journal of Pakistan Medical Association*, 63(2), 220-224.

Riaz, H., Latif, M. Z., & Qureshi, M. A. (2016). HCV Prevalence and its Predominant Genotypes in Sargodha Region of Pakistan. Cell, 333, 4428870.

Rubi, G., & Khan, M. A. (2017). Gene Polymorphism of Responder, Relapser and Non-Responder of Chronic Hcv Infection among Ethnic Tribes of Punjab Pakistan. *Journal of Clinical Immunology Research*, 1(1), 1-5.

Umer, M., & Iqbal, M. (2016). Hepatitis C virus prevalence and genotype distribution in Pakistan: Comprehensive review of recent data. *World Journal of Gastroenterology*, 22(4), 1684.

Umumararungu, E., Ntaganda, F., Kagira, J., & Maina, N. (2017). Prevalence of hepatitis C virus infection and its risk factors among patients attending Rwanda military hospital, Rwanda. *BioMed Research International*, 2017.

Waheed, Y., Shafi, T., Safi, S. Z., & Qadri, I. (2009). Hepatitis C virus in Pakistan: a systematic review of prevalence, genotypes and risk factors. *World Journal of Gastroenterology*, 15(45), 5647.