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Author (s):	Ubaid Ahmad[1], Najeeb Ullah Khan[1], Maaz Iqbal[1], Muhammad Ismail[2], Adeela Mustafa[3]
Affiliation (s):	[1]The University of Agriculture Peshawar, Pakistan [2]Department of Zoology, Islamia College Peshawar [3]Khyber Medical Collage
DOI:	
History:	
Citation:	
Copyright:	
Licensing:	
Conflict of Interest:	Author(s) declared no conflict of interest

Research Article

Evaluation of Incidence, Genotypes, and Risk Factors Associated with Newly Diagnosed Hepatitis C Cases in Khyber Pakhtunkhwa (KPK), Pakistan

Ubaid Ahmad[1], Najeeb Ullah Khan[1], Maaz Iqbal[1], Muhammad Ismail[2], Adeela Mustafa[3]

[1]The University of Agriculture Peshawar, Pakistan
[2]Department of Zoology, Islamia College, Peshawar
[3]Khyber Medical Colege

Abstract

Hepatitis C is a global health concern brought on by the hepatitis C virus (HCV). The risk factors of the disease include injection drug users, contaminated medical instruments, blood transfusion, organ transplant, major surgery, dialysis, dental surgery, and acupuncture. The aim of the current study was to identify the prevalence of active HCV infection, genotypes, and their associated risk factors in newly infected patients of Khyber Pakhtunkhwa (KPK), Pakistan. A total of 230 HCV cases and 245 control subjects were enrolled. Our findings revealed that disease infection rates were higher in female patients (52.6%) than male patients (47.4%). Moreover, the disease was more prevalent in the age group 30–40 years (39.6%). The highest incidence was found in the southern region (51.30%), followed by the northern region (27.82%), and central region (18.26%). In our data set, dental surgery, major surgery, disease in family, barber community, blood received, needles, abroad travel, injection drug users, acupuncture, and organ transplantation were determined as the major associated risk factors of the disease. Odds ratio were significantly higher (10.91%) for those who used acupuncture as compared to those who did not. Similarly, odds were significantly higher (10.03%) for those who had HCV in their family, dialysis (9.377%), dental surgery (7.618%), major surgery (6.813%), barber community (5.328%), and blood received (2.252%) as compared to the control group. Also, the most frequent genotype was 3a (63%), followed by an un-typeable genotype (11.30%).

Keywords: hepatitis C virus (HCV), blood transfusion, dialysis, genotype, surgery

*Coresponding Author: najeebkhan@aup.edu.pk ; adeelaamir@gmail.com

Introduction

Chronic hepatitis C virus (HCV) infection is a worldwide health hazard [1] associated with a variety of hepatic and extrahepatic illnesses, including cancer. Hepatitis is a contagious blood viral disease caused by HCV [2]. HCV belongs to hepaciviral genus of *Flaviviridea* family and was discovered in 1989. It has a positive-sense, single-stranded RNA genome with a size of 9.5Kb that codes for a polyprotein of 3000–3030 amino acids [3]. The infective virus leads to the inflammation of the liver, manifested by the inflammatory cells in the infected liver tissue, which may progress to fibrosis, followed by cirrhosis, hepatic cellular carcinoma (HCC) (end stage of liver condition), and death [4]. HCV causes HCC by regulating various indirect mechanisms [5]. For instance, the polyprotein is processed by host and viral proteases which construct mature structural and non-structural (NS) proteins [6]. HCV core protein enters the host cell [7], where it remains present in the outside mitochondrial membrane and endoplasmic reticulum, which enhances oxidative stress. It results in the activation of key signaling pathways, such as the nuclear factor kappa B and p38 mitogen-activated protein kinase pathways [8], directing the over expression of genes involved in cytokine production and inflammation, changes in apoptotic pathways, and tumor formation. The non-structural proteins of HCV, NS3, and NS5A act as mediators to create oxidative stress and inflammation.

The ideal transmission mode of HCV is either through blood or blood-related products. It was noticed that the supply of blood was contaminated with an unidentified agent causing post-transfusion non-A and non-B hepatitis [9]. In developed countries, post-transfusion hepatitis C has become relatively rare. The incidence of transfusion associated hepatitis, traced from 1970 to 1998, decreased from 33% to the nearly eliminated HCV transmission caused by the effectiveness of a series of donor screening interventions [10]. In developing countries, where HCV testing of donated blood is not feasible, receiving blood items remains a dominant source of HCV infection. The prevalence of anti-HCV among intravenous drug users ranged from 31% to 98% [11]. It has been reported that injection drug use accounts for 60% and 80% of HCV infections in USA [12] and Australia, respectively [13]. In developing countries, HCV transmission is mainly via unsafe therapeutic injections [14] resulting in 2.34 million HCV infections every year, approximately [15]. The

transmission of HCV through unhygienic injection equipment has been an issue in most developing countries [16]. Other sources of HCV transmission [17] include activities involving the potential for percutaneous exposure to blood or blood derived body fluids, such as tattooing [18], acupuncture [19], sharing cottons [20], and other biologically plausible modes of transmission including body piercing, cosmetic procedures, and commercial barbering [21].

HCV has six major genotypes and eighty subtypes with their specific sequences [22], [23], [24]. HCV genotypes 1, 2, and 3 are found throughout the world, while their relative prevalence differs from place to place. HCV genotype 1 is the most common genotype, accounting for 83.4 million cases (46.2% of all HCV cases), worldwide. HCV genotype 3 is the next most prevalent genotype with 54.3 million cases (30.1%), worldwide. In Nepal [25], India [26], and Pakistan [27], HCV-3 (predominantly 3a) is the most common genotype [28]. The high incidence of HCV genotype 3 is a serious concern because compared to other genotypes, genotype 3 is associated with a higher risk of liver consequences and overall mortality in all HCV patients [29]. In the current study, we aimed to find the incidence of active HCV infection and associated risk factors in newly infected individuals among the population of Khyber Pakhtunkhwa (KPK), Pakistan.

Materials And Methods

Study Design and Sampling

In this cohort study, a total of 230 HCV cases and 245 healthy control subjects were enrolled. All the patients and healthy blood donors were recruited from Khyber Teaching Hospital (KTH), Peshawar. Ethical approval from the competent authority of KTH was taken along with the written consent of the patients. The objectives of the study were explained to the patients before taking their data. Demographic data, such as age, home town, locality, economic status, and time period of the disease were collected via a standard questionnaire along with 2–3 CC blood.

Hepatitis C Virus Screening

All the samples were screened for anti-HCV using ICT (Immuno-Chromatographic test), ELISA (Enzyme Linked Immune Sorbent Assay) and RT (Real Time PCR).

HCV Genotyping

The anti-HCV PCR-positive patients were further subjected to HCV genotyping. /Complete follow up was conducted of all the pateints. The results were obtained in the form of a document to find out their respective genotypes. HCV genotyping was peformed using real-time hybridization fluorescence detection method. For subtyping, HCV RNA was reverse transcribed into cDNA by using MMLV reverse transcriptase enzyme. The cDNA was subjected to two rounds of PCR amplification. PCR products were electrophoresed on 2% agarose gel, stained with ethidium bromide, and visualized on UV transilluminator.

Statistical Analysis

The odds ratio and significance level of all the possible risk factors were assessed by using binary logistic regression view via the Statistical Package for Social Sciences (IBM SPSS STATISTICS V20). One-way ANOVA was carried out to determine the differences between the different conditions (age, gender, and other parameters) of patients and control.

Results

Demographic Characteristics of HCV Positive Cases

A total of 300 newly infected patients and 245 healthy controls were questioned and conveniently sampled for the current study. Out of 300 HCV infected cases, 230 were found to be positive for HCV using ICT, ELISA, and PCR, of which 121 were female and 109 were male patients (Table 1). Based on age difference, patients were categorized into five different age groups, namely below 30 years, 31–40 years, 41–50 years, 51–60 years, and above 60 years. Our results indicated that HCV infection was high in age group 31–40 (35.2%), followed by age group 41–50 (23.5%) (Table 2).

Table 1: Gender-wise Distribution of HCV Positive Cases

Count		Location				Total
		Afghanistan	Southern KP	Northern KP	Central KP	
Gender	Female	2	14	35	70	121
	Male	4	28	29	48	109
Total		6	42	64	118	230

Table 2: Age-wise Distribution of HCV Positive Cases

Age-group	No. of samples	Male	Female
Below 30	45 (19.6%)	20 (18.3)	25 (20.6)
31-40	81 (35.2%)	33 (30.2)	48 (39.6)
41-50	54 (23.5%)	25 (22.9)	29 (23.9)
51-60	38 (16.5%)	21 (19.2)	17 (14.04)
Above 60	12 (15.3%)	10 (9.17)	2 (1.6)
Total	230	109	121

Odds Ratio (OR) in Relation to HCV Risk Factors

The odds ratio and significance level of all possible risk factors were assessed via simple and forward selection using binary logistic regression view through Statistical Package for Social Sciences (IBM SPSS STATISTICS V20). The package was used to analyze and find out the percept distribution of qualitative records the various infected patients and control group. The results revealed that the following categorical variables (Table 3a, 3b, 3c) as significant risk factors in comparison to control. The odds showed that the disease causing factors were statistically associated with HCV transmission. The odds were significantly higher (10.91%) for those patients who used acupuncture and were HCV positive as compared to control, followed by patients with HCV in the family (10.03%), dialysis (9.377%), dental surgery (7.628%), major surgery (6.813%), barber community (5.328%), and blood received (2.25%).

Table 3a. Dependent Variables in Coding

Original Value	Inner Value
Yes	1
No	0

Table 3b. Coding of All Definite Variables

		Frequency	Parameter coding (1)
Travel Abroad	Yes	42	1.000
	No	432	.000
Blood Received	Yes	69	1.000
	No	405	.000
I-D-Users	Yes	38	1.000
	No	436	.000
Smoking	Yes	65	1.000
	No	409	.000
Needles	Yes	43	1.000
	No	431	.000
Barber	Yes	61	1.000
	No	413	.000
O. Transplantation	Yes	20	1.000
	No	454	.000
Major Surgery	Yes	104	1.000
	No	370	.000
Disease in family	Yes	88	1.000
	No	386	.000
Piercing	Yes	20	1.000
	No	454	.000
Acupuncture	Yes	27	1.000
	No	447	.000
Dialysis	Yes	52	1.000
	No	422	.000
Dental Surgery	Yes	108	1.000
	No	366	.000

Table 3c. Definite Variables in Calculation

Variables	B	S.E.	Wald	Df	Sig.	Exp (B)
Blood Received	.812	.377	4.624	1	.032	2.25
Barber	1.673	.490	11.658	1	.001	5.328
Major Surgery	1.919	.330	33.728	1	.000	6.813
Dental Surgery	2.030	.330	37.865	1	.000	7.618
Dialysis	2.238	.619	13.082	1	.000	9.377
Acupuncture	2.389	.883	7.329	1	.007	10.907
Disease in family	2.306	.378	37.113	1	.000	10.031
Constant	-2.151	.215	100.408	1	.000	.116

Possible Risk Factors for HCV Transmission

A basic objective of our study was to find out the possible risk factors of HCV transmission in the newly infected individuals. All the HCV infected patients and also the control group were questioned as per the questionnaire. The results revealed by applying descriptive statistics and frequency was shown in the case study contating the control group also.the risk factors were significantly associated with HCV transmission (Figure 1). Among all risk factors, dental surgery (37.39%) was found to be the most possible route risk factor for HCV infection, followed by major surgery (34.34%), HCV history in family (20.34%), barber community (23.47%), blood received (23.04%), dialysis (20.86%), needles reuse (16.08%), travel abroad (13.47%), ID-users (13.04), acupuncture (10.86%), and organ transplantation (6.95%).
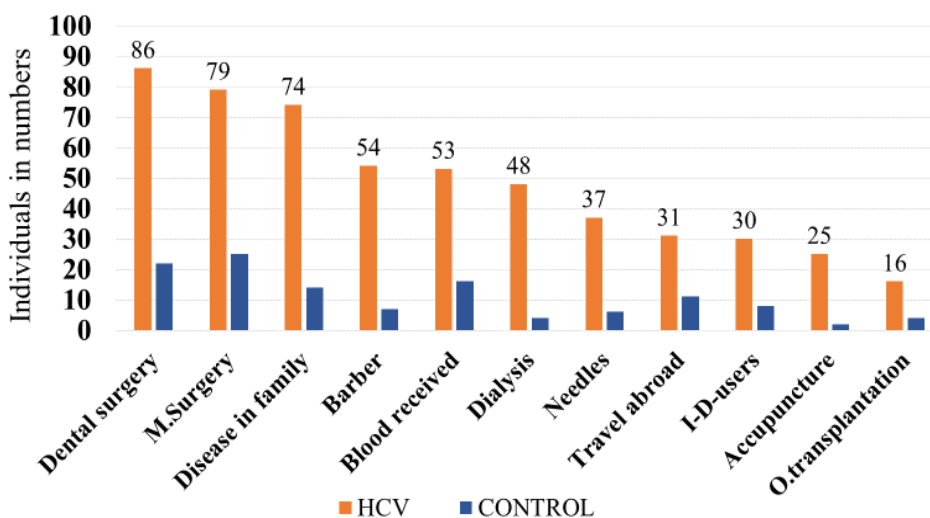

Figure 1. Possible Risk Factors for HCV Transmission in Khyber Pakhtunkhwa

Frequency Distribution of HCV Genotypes

All HCV positive patients were assessed for HCV genotypes (Figure 2). Out of 230 HCV samples, genotype 3a (63.04%) was found to be the most abundant, followed by an un-typeable genotype (11.3%), 1a (9.1%), 2a

(6.5%), 3b (4.8%), 2b (1.3%), 1b+3a (1.30%), 1b (0.86%), 1a+3a (0.86%), and 3a+3b (0.43%).
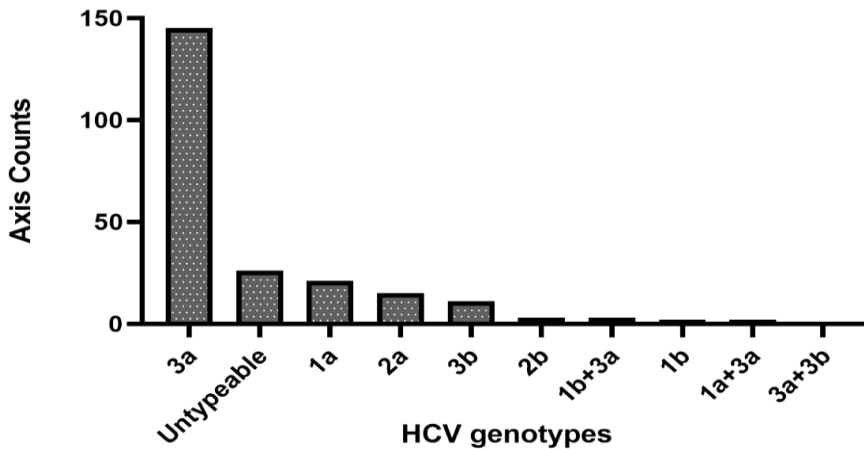

Figure 2. Frequency Distribution of HCV Genotypes

HCV RNA was reverse transcribed into cDNA by using MMLV reverse transcriptase enzyme. The cDNA was subjected to two rounds of PCR amplification, which utilized genotype specific primers.

Associated Risk Factors of HCV Genotype 3a and 3b

Demographic data showed a higher percentage of association between risk factors and HCV genotype 3a. The high percentage of barber community (72.2%) was found to be the main associated risk factor for genotype 3a transmission, followed by dental surgery (65.1%), blood received from infected individuals (62.3%), major surgery (62%), HCV in the family (59.5%), dialysis (58.3%), and acupuncture (48%) (Figure 3a). A higher percentage association was also observed between risk factors and HCV genotype 3b. The percenatge of infected individuals who used acupuncture (16%) was found to be the main association factor for HCV transmission, followed by major surgery (7.6%), dental surgery (7%), dialysis (6.2%), HCV in the family (4.1%), and barber community (1.9%) (Figure 3b).
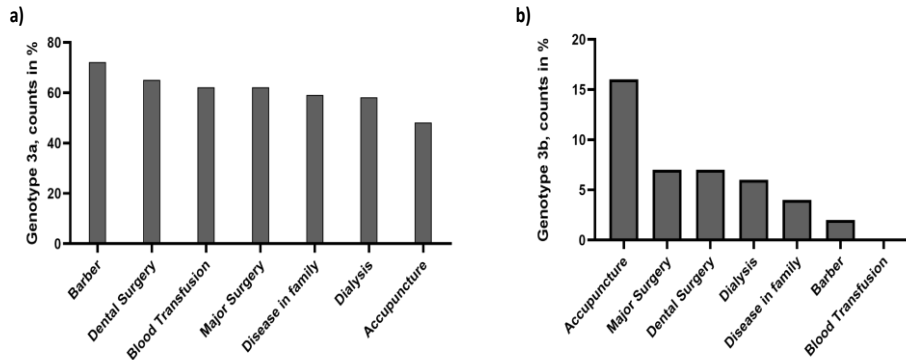
Figure 3. Associated Risk Factors of HCV Genotypes 3a and 3b. a) Association between various risk factors and 3a genotype. b) Association between various risk factors and 3b genotype.

Associated Risk Factors of HCV Genotypes 2a and 2b

Demographic data showed a higher percentage association between risk factors and HCV genotype 2a (Figure 4a). The percentage of infected individuals with HCV in the family (9.5%) was found to be the main associated factor for HCV transmission, followed by blood received (9.4%), dental surgery (5.8%), dialysis (4.2%), acupuncture (4%), major surgery (3.8%), and barber community (1.9%). Demographic data also showed a higher percentage association between risk factors and HCV genotype 2b (Figure 4b). The percentage of infected individuals who used acupuncture (8%) was found to be the main associated factor for HCV transmission, followed by dialysis (2.1%), blood received from infected individuals (1.9%), and dental surgery (1.2%).
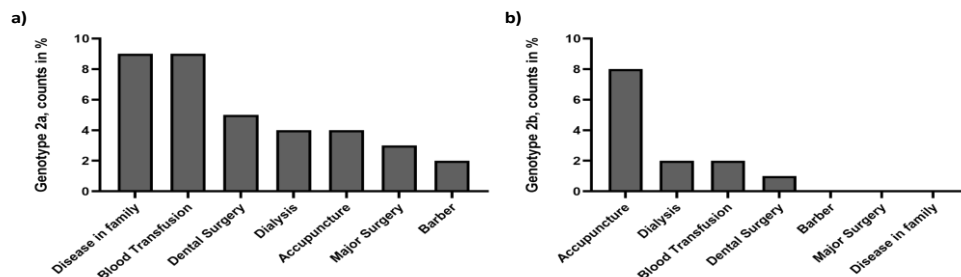

Figure 4: Associated Risk Factors of HCV Genotypes 2a and 2b. a) Association between various risk factors and 2a genotype. b) Association between various risk factors and 2b genotype.

Associated Risk Factors of HCV Genotypes 1a and 1b

Demographic data showed a higher percentage association between risk factors and HCV genotype 1a (Figure 5a). The percentage of patients using dialysis (12.5%) was found to be the main associated risk factor for HCV genotype 1a transmission, followed by blood received from infected individuals (11.3%), individuals with HCV in the family (10.8%), major surgery (7.6%), barber community (7.4%), dental surgery (5.8%), and acupuncture (4%). The percentage of infected individuals who /belonged to the same barber community (3.7%) was found to be the main associated factor in genotype 1b transmission, followed by those who belonged to the same dental surgery community (2.3%) (Figure 5b).
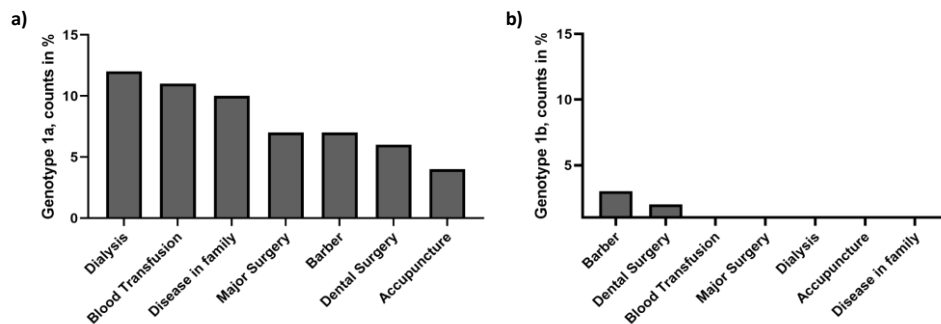

Figure 5. Associated Risk Factors of HCV Genotypes 1a and 1b. a) Association between various risk factors and genotype 1a. b) Association between various risk factors and genotype 1b.

Associated Risk Factors of HCV Un-typeable and Mixed Genotypes

Demographic data showed a higher percentage association between risk factors and un-typeable genotypes. The percentage of people using acupuncture (20%) was found to be the main associated factor for un-typeable genotype transmission, followed by major surgery community (15.2%), infected individuals with HCV in the family (14.9%), dialysis (14.6%), blood received from infected individuals (11.3%), barber community (11.1%), and dental surgery (9.3%) (Figure 6a). A higher percentage association was found between risk factors and HCV genotypes 1b+3a. The percentage of infected individuals who underwent dialysis (2.1%) was found to be main associated factor with genotypes 1b+3a transmission, followed by blood received (1.9%), barber community (1.9%), major surgery (1.3%), and dental surgery (1.2%).

Demographic data also showed a higher percentage association between risk factors and HCV genotypes 3a+3b. The frequency of infected individuals who underwent major surgery (1.3%) was found to be main associated with genotypes 3a+3b transmission, followed by dental surgery (1.2%), and blood received from infected individuals (1%).
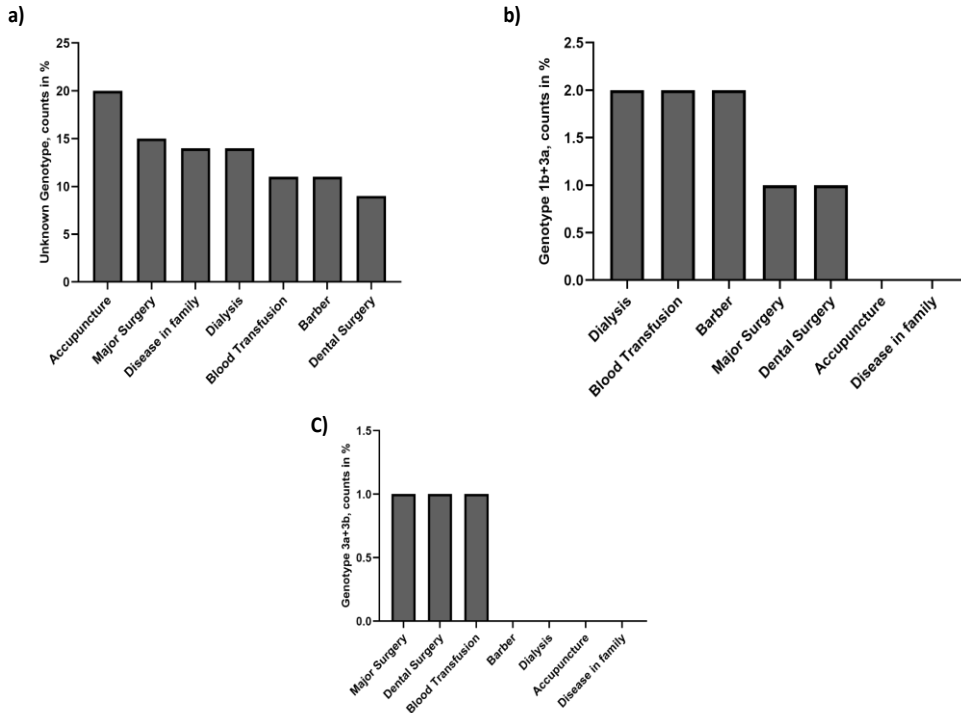

Figure 6: Associated Risk Factors of HCV Un-typeable and Mixed Genotypes. a) Association between various risk factors and un-typeable genotypes. **b)** Association between various risk factors and 1b+3a genotypes. **c)** Association between various risk factors and 3a+3b genotypes.

Discussion

Hepatitis is a transmissible blood viral ailment caused by the hepatitis C virus (HCV). HCV is a worldwide health hazard related to numerous hepatics and hepatic disorders, such as malignancies. The most essential transmission modes of HCV are blood or blood associated products. The known global risk factors of the disease include injection drug users, contaminated medical instruments, blood transfusion, organ transplant, major surgery, dialysis, household items, dental surgery, and acupuncture.

Numerus procedures have been adopted to control the disease, including proper screening and awareness campaigns, both in health professionals and general public. However, theworldwide situation of the disease is still alarming, particularly in developing countries, such as Pakistan. In this study, we aimed to find out the exact situation of risk factors in the newly diagnosed cases of HCV. A total of 300 HCV cases and 245 healthy individuals were included in the current study. Out of the 300 HCV cases, 230 samples were found to be positive for active HCV using ICT, ELISA, and RT-PCR. Our data set showed that HCV infection occurred more in female patients as compared to male patinets. Moreover, the disease was more prevalent in age group 31–40 years, followed by age group 41–50 years (Table 2). Recently, a similar conclusion was reached by an author from Pakistan [30]. Our results further showed that the most probable risk factors of HCV cases were dental surgery, followed by major surgery, and family history. These risk factors of HCV transmission have been very well known for the last 2–3 decades [28], [31]. It indicates that in Pakistan, there is a lack of proper guidelines for health professionals who consequently indulge in malpractices, even though these risk factors have been well known to everyone including the majority of the general public. Government officials need to ensure compliance with proper and strict guidelines for reusing the dental and surgical equipment.

The most prevalent genotype in our data set was 3a (63.04%), followed by the un-typeable genotype (11.3%), 1a (9.1%), 2a (6.5%), 3b (4.8 %), 2b (1.3%), 1b+3a (1.30%), 1b (0.86%), 1a+3a (0.86%), and 3a+3b (0.43%). Similarly, a recent study conducted by Pakistani authors from 2015 to 2018 on HCV cirrhotic patients in Peshawar hospitals [32] concluded that the prevalence of HCV genotype 3a was 47.9%, 2a and 3b was 11% each, 1a was 6%, and 1b was 1%; while, 4.1% had mixed genotypes and 18.7% had un-typable genotypes. Additionally, HCV genotype 3 was identified as the most common genotype in Bangladesh, Nepal, and India [25, 33, 34]. Our sample included a substantial proportion of un-typable genotypes in contrast to the earlier findings..

Our study identified the higher percentage association between risk factors and HCV genotype 3b. The percentage of infected individuals who used acupuncture (16%) was found to be the main associated factor for HCV transmission, followed by major surgery (7.6%), dental surgery (7%), dialysis (6.2%), HCV in the family (4.1%), and barber community

(1.9%). Our study also showed a higher percentage association between risk factors and HCV genotype 3a+3b. The percentage of infected individuals who underwent major surgery (1.3%) was found to be main associated factor with genotype 3a+3b transmission, followed by dental surgery (1.2%), and blood received from infected individuals (1%). Blood transfusion, dangerous intravenous injection, and surgery are all instances of unsafe medical procedures. However, many people acquire HCV while visiting barber shops, beauty salons, and hair salons. A large percentage of people denied ever having been exposed to HCV. Moreover, we found no evidence of vertical transmission. A considerable number of HCV infections occur outside of dangerous medical procedures incosmetics, as well as hair and barber businesses. This is presumably a result of the use of razors, clippers, cuticle pushers, nail files, nail buffers, brushes, or any other piercing equipment.

However, additional nationwide investigations are still required to provide a complete picture of the distribution of HCV genotypes in association with gender, age, risk factors, and pretreatment viral load. The results of this study clearly showed some gaps in the epidemiology of HCV and its associated risk factors in the Khyber Pakhtunkhwa population.

Conclusion

In contrast to the outcomes of our previous research conducted a decade ago, the results showed that 3a is the most common HCV genotype. Female patients were found to have a somewhat higher prevalence of HCV. Moreover, the majority of those infected were middle-aged (30-40 years). The findings implicate that all high and extremely high viral loads are frequently found in all genotypes, although the most prevalent subtype 3a is associated with low and intermediate viral loads. The majority of people in Khyber Pakhtunkhwa contract HCV because international standards are not being followed, especially when it comes to blood transfusion and surgery. Prior knowledge of pretreatment viral load and HCV genotype must be considered in national planning strategies, as it would minimize the side effects of antiviral medication, reduce the economic bioburden, and improve response rates.

Conflict of Interests

The authors have no conflict of interests.

Funding

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