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Risk factors involved in spread of HCV in patients from sub urban Rawalpindi and their association with existing genotypes

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Abstract. The current epidemiological study was designed to trace the involved risk factors in Hepatitis C Virus (HCV) spread and to identify any association between HCV genotypes and risk factors. Blood samples were taken from 400 participants and viral genotyping was performed in order to find any possible relationship between the risk factors and genotypes. Major genotypes included 3, 1, 4 and several untypeable ones with prevalence rates 65%, 22.5%, 2.75% and 9.75% respectively. Surgery and dental procedure were strongly related to the spread of genotype 3b, while genotype 1b was strongly related to blood transfusion and dental procedures as a single combination risk factor. On the other hand genotypes 1a, 3a, 4 and the untypeable genotypes, were equally affected by all reported risk factors. The probability of occurrence of genotype 3a with reference to dental procedures was 11%. Dental procedures, unsafe injection and surgical procedures are the main risk factors while the blood transfusion in combination with dental procedures has emerged as a potent risk factor in the transmission of HCV.

INTRODUCTION

A very famous member of the viral family, "flaviviridae" and genus "*Hepaciviruses*" is Hepatitis C virus (HCV), that has caused tremendous liver related morbidity and mortality in the last 30 years, since its identification, it is reported that 200 million i.e. 3% of the world population is infected (Bostan & Mahmood, 2010; Alter, 2007). HCV has 6 major genotypes and more than 50 subtypes that are mainly area specific but genotype 1 and 3 are considered universal in their distribution (Bostan *et al*., 2010) and mixed genotypes in Khyber Pakhtunkhwa (KPK) province (Afzal *et al*., 2016). Hepatitis C virus (HCV) infection is the most common blood-borne infection in the United States and is a leading cause of advanced liver disease. Over 4 million people in the U.S. and more than 170 million people worldwide have been infected by HCV infection (Mohd *et al*., 2013). Source of spread of HCV is contaminated blood and destination of virus is hepatocytes where it multiplies. Mode of disease spread in third world countries including Pakistan involve percutaneous, non-percutaneous and sporadic transmission mainly through blood transfusion, non-disposable syringes, multiple dose vials of injections, reusing of

non-disposable syringes multiple times and unnecessarily without taking into account consumer knowledge, transplantation of Organs, body piercing and tattooing, sexual exposure, parenteral transmission, inter-familial contacts and practices commonly followed in the rural and sub urban areas like sharing of razors and tooth brushes (Akhund *et al*., 2009; Alexis *et al*., 2015; Afzal *et al*., 2015; Khan *et al*., 2000).

Pakistan is hyper-endemic for HCV where according to estimates 10% of population is infected (Ahmad *et al*., 2004). Epidemiological studies and disease surveillance are important tools to get better insights of disease spread. The best means of getting useful data on HCV spread and genotypes is by performing retrospective and prospective studies. Therefore the current study from Suburban Rawalpindi will contribute in finding a relationship between existing genotypes of HCV and prominent modes of HCV transmission.

MATERIALS AND METHODS

Patients visiting the out-patient departments (OPDs) of two hospitals Fauji Foundation Hospital Rawalpindi (FFH) and Pakistan Institute of Medical Sciences Islamabad (PIMS) and reported to be anti-HCV positive were recruited for the study. Informed consent of participants was obtained through formal consent forms; and the ethical approval of the study was taken by the Institutional Review Board (IRB), Quaid-i-Azam University, Islamabad, Pakistan (Letter number No. BASR/2007-774) and ethical review board of PIMS and Fauji Foundation hospital.

Following a brief introduction about the objectives of the study, questionnaires were administered to 1200 participating patients, asking them about the potential risk factors associated with transmission of HCV such as exposure to dental procedures, barber shears, injections, child birth, surgery, blood transfusion, therapeutic injections and shared towels/tooth brushes/razors, rest were deemed sporadic. In order to verify HCV RNA positivity of the sample population, plasma was collected from the 400 randomly chosen patients out of 1200 patients taking part in the study. Extraction of HCV RNA, preparation of cDNA samples and subsequent RT-nested PCR were performed by following previously established procedures confirming the HCV RNA positivity of the patients. In order to determine the genotypes of HCV virus in the sample population, restriction fragment length polymorphism was performed by following the method described previously (Davidson *et al*., 1995).

Statistical Analysis

Binary logistic regression analysis was performed for the risk factor assessment. Pearson's chi-square analysis and statistical correlation was also applied on the data. All tests were applied using Minitab version 11 (Zgnoc, 1996) and E-Views version 3.1 software (Hill *et al*., 2000).

RESULTS

Females were predominant participants of the study

1200 HCV positive index cases participated in the present study but due to incomplete information provided by 75 patients they were excluded from the study and only 1125/1200 were considered for statistical analysis. There were 86% (962) females and 14% (163) males, making females the predominant studied group.

Natural history of HCV in selected group of patients

It was observed that 93% of the patients suffered from chronic HCV while only 7% were found with acute infection.

Marital status of the studied cohort

In total, 87% of the HCV positive patients were married, while 13% were unmarried and majority of the patients belonged to the 30 to 50 year age group. This was an indication that the rate of disease prevalence was higher in the married individuals.

Risk factors involved in the transmission of HCV within population

It was observed that approximately 13% males, rated injections as a main source of acquiring the infection while 91% of the females claimed surgery to be the main cause of infection. Any source of contaminated blood or blood products appeared to be capable of carrying the virus even though the source was indirect e.g. sharing tooth brushes, household commodities like body swapping jewellery, cosmetics, towels, nail cutters and trimmers, pedicure and manicure tools etc. These were exclusive to female patients, while none of male patients responded to these categories. A significant frequency difference (Table 1) was observed towards blood transfusion, dental procedures, child birth, barber shears and tattoos (p value ranges 0.05-0.001).

There was a non-significant difference for surgical procedures, therapeutic injection, needle sticks, shared towels and toothbrushes and sporadic risk factors. The foregoing figures indicate the high rate (45.1%) for the use of therapeutic injections. Unsafe injection practices are common in suburban areas where inexperienced or untrained quacks use either non-disposable syringes or unsterilized injecting equipments. Injections were reported by 12.8% males and 87.2% females to be the potential cause of catching the infection (Table 1). The risk factors were also explored according to different age groups (Table 2).

Binary logistic regression analysis of HCV genotypes and risk factors

Binary logistic regression analysis of specific risk factors for genotypes was applied to find statistical significance in terms of odd ratios (OR) and confidence intervals (CI), where genotype 1a showed statistically no significance between risk factors and the genotypes (Table 3). Genotype 1b, when analyzed using binary logistic regression analysis for a few prominent and specific risk factors, showed statistically no significant association except for blood transfusion and dental procedures (OR=3.46, $p< 0.054$). Furthermore, binary logistic regression analysis of specific risk factors for genotype 3a showed no statistically significant association between risk factors and genotypes (Table 3).

The statistical analysis of genotype 3b, by binary logistic regression analysis for prominent and specific risk factors, showed a statistically significant association between risk factors and genotypes (Table 3) where surgery and dental procedure had a significant association with the spread of genotype 3b (OR=4.99, 95% CI= 1.62-15.34, $p< 0.005$). Binary logistic regression analysis of specific risk factors for genotype 4 and untypable genotypes displayed statistically

Table 1. Most common potential risk factors observed in the studied cohort of patients. Pearson's chi-square analysis and statistical correlation was also applied on the data.

Risk factors	Overall percentage (numbers)	Percentage (Numbers)		χ^2
		Females	Males	
Therapeutic injections	45.1(507)	87.2 (442)	12.8 (65)	1.89
Surgical procedures	43.6(490)	91 (446)	9 (44)	2.27
Dental procedure	43.4 (488)	88.7 (433)	11.3 (55)	7.26**
Blood transfusion	25.9(291)	93.5 (272)	6.5 (19)	20.07***
Sporadic	17.3(195)	83.1 (162)	16.9 (33)	1.13
Child birth	5.2 (59)	100 (59)	0 (0)	10.55**
Barbers shears	3 (34)	2.9 (1)	97.1 (33)	192.93***
Shared towels/Tooth Brushes/Razors	1.2 (13)	100 (13)	0 (0)	2.23
Needle stick injuries	1.0 (12)	83.33 (10)	16.66 (2)	1.73
Tattoos	0.4 (5)	60 (3)	40 (2)	8.39**

d.f =1; **p<0.01; ***P<0.001

Table 2. Age groups wise distribution of different risk factors. Pearson's chi-square analysis and statistical correlation was also applied on the data

Age groups	Risk factors									
	Therapeutic injections	Surgical procedures	Dental procedures	Blood transfusion	Sporadic	Child birth	Barber shears	Shared towels/tooth brushes/Razors	Needle stick injuries	Tattoos
19 <	4.2 (21)	1.0 (5)	1.0 (5)	2.1(6)	7.3 (14)	0 (0)	14.7 (5)	7.7 (1)	16.66 (2)	20 (1)
20-29	12.2 (61)	7.6 (37)	6.5 (31)	5.2 (15)	13.0 (25)	10.2 (6)	14.7 (5)	7.7 (1)	8.33 (1)	40 (2)
30-39	21.7 (108)	26.5 (129)	23 (110)	26.4 (76)	29.0 (56)	39.0 (23)	29.4 (10)	38.5 (5)	8.33 (1)	20 (1)
40-49	49.2 (245)	51.5 (251)	52.7 (252)	52.8 (152)	36.3 (70)	42.4 (25)	29.4 (10)	30.8 (4)	66.66 (8)	0 (0)
50-59	12.0 (60)	12.5 (61)	15.3 (73)	12.5 (36)	14.5 (28)	8.5 (5)	11.8 (4)	15.4 (2)	0 (0)	20 (0)
60>	0.6 (3)	0.8 (4)	1.5 (7)	1.0 (3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
χ^2	15.24	38.68	53.73	21.57	13.40	8.37	12.62	2.02	7.02	9.59

Table 3. Binary logistic regression analysis of the specific risk factors for HCV infection from existing genotypes. Pearson's chi-square analysis and statistical correlation was also applied on the data

Genotype	Risk factor	Odd ratios	95% CI	P values
1a	Unknown	0.85	0.28-2.59	0.774
	Dental procedure	1.01	0.43-2.35	0.988
	Barber shears	0.90	0.34-2.33	0.820
	Injections	0.64	0.28-1.48	0.298
	Child birth	0.84	0.14-5.08	0.851
	Surgery and dental procedure	0.77	0.20-2.90	0.695
	Blood transfusion and dental procedure	0.49	0.15-1.55	0.225
1b	Unknown	1.76	0.28-11.11	0.548
	Dental procedure	0.90	0.26-3.15	0.875
	Barber shears	1.40	0.39-4.98	0.606
	Injections	3.12	0.82-11.86	0.695
	Child birth	0.00	0.00	0.999
	Surgery and dental procedure	0.00	0.00	0.998
	Blood transfusion and dental procedure	3.46	12.20	0.054*
3a	Unknown	0.86	0.38-1.93	0.715
	Dental procedure	0.90	0.49-1.65	0.741
	Barber shears	0.75	0.38-1.49	0.412
	Injections	0.85	0.47-1.54	0.594
	Child birth	0.37	0.08-1.63	0.190
	Surgery and dental procedure	0.55	0.21-1.45	0.226
	Blood transfusion and dental procedure	1.63	0.79-3.37	0.186
3b	Unknown	1.63	0.49-5.40	0.423
	Dental procedure	1.62	0.69-3.80	0.270
	Barber shears	1.73	0.69-4.33	0.241
	Injections	1.73	0.75-4.00	0.199
	Child birth	4.21	0.81-21.82	0.086
	Surgery and dental procedure	4.99	1.62-15.34	0.005**
	Blood transfusion and dental procedure	0.24	0.05-1.09	0.064
4	Unknown	0.65	0.06-7.13	0.727
	Dental procedure	0.40	0.06-2.86	0.361
	Barber shears	0.78	0.07-8.61	0.839
	Injections	0.41	0.06-2.90	0.372
	Child birth	0.00	0.00	0.999
	Surgery and dental procedure	1.50	0.14-16.09	0.739
	Blood transfusion and dental procedure	1.01	0.09-11.57	0.995
untypable	Unknown	0.76	0.24-2.46	0.650
	Dental procedure	0.85	0.36-2.00	0.703
	Barber shears	0.86	0.32-2.34	0.772
	Injections	0.87	0.36-2.08	0.754
	Child birth	1.75	0.34-8.98	0.505
	Surgery and dental procedure	0.40	0.08-2.09	0.280
	Blood transfusion and dental procedure	0.79	0.27-2.33	0.664

*p< 0.05 **p<.005

insignificant association between risk factors and genotypes (Table 3).

Relationship between genotypes and risk factors

Probabilities were calculated in terms of percentages, for the 400 patients from whom the genotypes were determined. The probabilities of occurrence of genotype 1a, 3a and 3b caused by some unknown risk factors in the current study were found to be 4%, 10% and 2%, respectively. However, the untypability (U) caused by the unknown risk factors was also reported in 3% of total

cases analysed. Surgery was reported to be a potential risk factor having 4% association with transmission of 3a genotype, while its association was found negligibly small (1%) with acquisition of genotype 1a and untypable cases. Meanwhile, blood transfusion also had a negligible effect on the transmission of genotype 3b, U and 1a and only 1% probability of occurrence of genotype 3a with reference to blood transfusion exists. There is no association between blood transfusion on the transmission of genotypes 1b and 4.

On the other hand, dental procedures had an 11% association with the spread of genotype 3a, while the genotypes: 3b, untypable and 1a had a 3% association with dental procedures (Table 4). However, all other genotypes were statistically non significantly associated with surgery, blood transfusion, dental procedures and child birth. Barber shears and child birth as independent risk factors had no specific association with the spread of any particular genotype. However, the combined effect of 2 risk factors i.e., blood transfusion and dental procedures had negligible association with some of the reported genotypes (Table 5).

Genotype 3a had only 2% association in each, with a multiple risk factor combination surgery, dental procedure and child birth and risk factor combination surgery and child birth. While 3% probability of occurrence for genotype 3a is due to multiple risk factors combination of surgery and dental procedures. A risk factor with multiple options such as surgery, blood transfusion and dental procedure had only 1% probability for 3a and untypable genotypes while all other genotypes in relation to this particular risk factor had no association whatsoever. Surgery in combination with dental procedure, as reported by many of the index cases had only 1% association with genotype 1a, 3% with genotype 3a and 1% with untypable genotypes. Similarly, surgery in combination with blood transfusion was also found to be a potential risk factor having 1% association with transmission of genotypes 3a and 3b (Table 5).

DISCUSSION

Hepatitis C Virus (HCV) being one of the devastating viruses has been investigated by many researchers. Its epidemiology including involved risk factors and its circulating genotypes has been extensively reported. Many studies reported unequal numbers of females to males (Fakeeh & Zaki, 1999; Lwin *et al*., 2007; Armstrong *et al*., 2006; Plamondon *et al*., 2007). Previously reported by (Al-Moslih & Al-Huraibi, 2001) and (NIH, 2002) that majority of the reported cases were chronic and the number of acute cases was very low, as is the case in present study. Because HCV remains asymptomatic in most of cases, only 20-25% of the patients reported to the hospitals with some presenting complaints (Herrine, 2002).

The HCV infection was pronounced in married individuals falling in the 30-49 age groups. A similar trend was observed and reported by several other studies for married people falling in the age range of 30-50, which agreed with results of the current study (Irfan & Afreen, 2004; Perez *et al*., 2005). In our study, the majority of HCV positive cases were non-addicts which is in agreement with the research studies (Wang *et al*., 2002) showing the spread of disease among non-addicts. Majority of the index cases belonged to low socioeconomic levels; and these results were in agreement with the studies reporting the prevalence of HCV among individuals belonging to low income levels. Similarly it was found that the prevalence of HCV disease had a strong association with formal education, as majority of the reported cases were illiterate. This trend was similar to those reported in previous studies (Gifford *et al*., 2005; Khan *et al*., 2008). This might be due to the fact that lack of formal education in Pakistan results in a dearth of knowledge in population at large with regards to modes of transmission of HCV and appropriate precautionary measures.

In the present study regarding the professions of the index cases, it was observed that health care providers were not

Table 4. Probability table for specific risk factors with reference to reported genotypes. Pearson's chi-square analysis and statistical correlation was also applied on the data

Genotypes	Sporadic	Surgery	Blood transfusion	Dental procedure	Barber shears	Therapeutic injections	Child birth
1a	0.044 (4%)	0.015 (1%)	0.009 (0%)	0.031 (3%)	0.006 (0%)	0.009 (0%)	0 (0%)
1b	0.009 (0%)	0.003 (0%)	0 (0%)	0.015 (1%)	0 (0%)	0 (0%)	0 (0%)
3a	0.103 (10%)	0.044 (4%)	0.012 (1%)	0.113 (11%)	0.009 (0%)	0.025 (2%)	0.009 (0%)
3b	0.028 (2%)	0.009 (0%)	0.003 (0%)	0.037 (3%)	0.009 (0%)	0.015 (1%)	0 (0%)
4	0.009 (0%)	0 (0%)	0 (0%)	0.009 (0%)	0 (0%)	0.006 (0%)	0 (0%)
U	0.031(3%)	0.015 (1%)	0.003 (0%)	0.031 (3%)	0.009 (0%)	0.003 (0%)	0.009 (0%)

Table 5. Probability table for multiple risk factors with reference to reported genotypes. Pearson's chi-square analysis and statistical correlation was also applied on the data

Genotypes	Surgery and blood transfusion	Surgery and dental procedure	Blood transfusion and dental procedure	Dental procedure and therapeutic injections	Surgery and child birth	Surgery blood transfusion and dental procedure	Surgery, dental procedure and child birth	Surgery, blood transfusion, dental procedure and child birth
1a	0.006 (0%)	0.018 (1%)	0.003 (0%)	0.003 (0%)	0.009 (0%)	0 (0%)	0 (0%)	0.003 (0%)
1b	0.003 (0%)	0.006 (0%)	0.003 (0%)	0 (0%)	0.003 (0%)	0 (0%)	0.006 (0%)	0.006 (0%)
3a	0.015 (1%)	0.031 (3%)	0.009 (0%)	0.003 (0%)	0.028 (2%)	0.012 (1%)	0.025 (2%)	0.012 (1%)
3b	0.015 (1%)	0.009 (0%)	0 (0%)	0.009 (0%)	0 (0%)	0.009 (0%)	0.006 (0%)	0 (0%)
4	0.003 (0%)	0 (0%)	0 (0%)	0 (0%)	0.003 (0%)	0 (0%)	0 (0%)	0 (0%)
U	0 (0%)	0.012 (1%)	0.003 (0%)	0.012 (1%)	0.006 (0%)	0.012 (1%)	0 (0%)	0 (0%)

at an increased risk of disease acquisition; and similar observations were also reported by others. The major risk factors reported by HCV positive patients for transmission of HCV included unsafe therapeutic injections, dental procedures and surgeries. Unsterilized injections were also reported to be the highest risk factor in transmission of HCV by studies undertaken in some other parts of Pakistan (Kermode, 2004; Raja & Janjua, 2008). In the present study, a strong relationship between dental procedures and HCV positivity was found, which also is in agreement with another report (Latorre *et al*., 2002). This might be a result of lack of proper training for dentists, frequent use of unsterilized dental equipments and lack of infrastructure at local hospitals (Karaca *et al*., 2006).

Unsafe surgical procedures were found to be the second important risk factor in the transmission of HCV (Table 2) in the current study. Some of studies reported it to be low risk factor (4%) (Alter *et al*., 1992; Kelen *et al*., 1992). In contrast, the surgical procedures were among the risk factors in the current study (Table 2). Surgical procedures require the presence of patients at hospitals for a long term. The rate of prevalence of HCV was reported to be higher in the hospitalized patients than in the general public (Wesler & Alter, 2002). The hospitalized HCV positive patients pose a high risk for the spread of HCV. Furthermore, incautiously performed phlebotomies and injudicious application of multiple injections serve as a major reservoir for the acquisition of HCV (Esteban *et al*., 1996). Some other reasons behind unsafe surgical procedures as a major risk factor might be the poorly equipped resources, weak infrastructure, untrained staff, increasing power breakdowns and ineffective screening of blood donors (Akhtar & Moatter, 2004; Aslam & Syed, 2005). The present epidemiological study found a significant association between commercial barbering and HCV positivity in the male index cases, which might be caused by unawareness of barbers belonging to third world countries about the basic concepts of personal hygiene and transmission of blood borne pathogens such as viral entry in the body via skin microtrauma.

Besides, the three main risk factors observed during the present study, there are some other factors involved in the transmission of HCV in the population such as contaminated needles, surgical equipment, surgical disposables, blood transfusions, and self pricks during the procedure (Janjua & Nizamy, 2004). Results obtained from the present study were only based on the reported risk factors by patients behind acquisition of HCV, but they do not lead us to identify possible relationships between route of transmission and genotypes due to implication of more than one risk factors in the transmission of HCV (Idrees, 2008). For instance, genotype 3 is generally correlated with intravenous drug use and genotype 4 with patients undergoing haemodialysis or frequentation of commercial barbers. Similarly, the transmission of genotype 1b was significantly associated with blood transfusion and dental procedures ($p<0.05$), while the significant association ($p<0.005$) was found between the spread of genotype 3b surgeries and dental procedures, indicating the involvement of more than one risk factor in the transmission of HCV genotypes (1b, 3b). The results obtained from the present study showed genotype 3 as the predominant genotype, which were in disagreement with those reported by previous studies, which prevalence of genotype 2 (Idrees *et al*., 2008; Sarwat *et al*., 2008; Davis, 1999) Moreover, in Faisalabad (Pakistan), genotype 3 was observed to be a predominant genotype along with many untypable genotypes (Davis, 1999; Nasir, 2004).

CONCLUSION

Dental procedures, unsafe injection and surgical procedures are the main risk factors while the blood transfusion in combination with dental procedures has emerged as a potent risk factor in the transmission of HCV.

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Conflict of interest:
We declare no conflict of interest for this study

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