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HEPATITIS B AND C VIRUS PREVALENCE IN URBAN AREA OF SOUTHERN PUNJAB MULTAN PAKISTAN, AND EPIDEMIOLOGICAL RISK FACTORS

BHUTTA AM[1*], TASAWAR Z[1], NAEEM M[1], HAYAT S[2] AND MASUD S[1]

[1]Institute of Pure and Applied Biology, Bahauddin Zakariya University Multan, Pakistan

[2]Institute of Molecular Biology and Biotechnology, The University of Lahore, Pakistan

[*]**Corresponding author E Mail: ucanfindarif@gmail.com; 00923053722626**

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ABSTRACT

Hepatitis B and C virus is invasive communicable disease of humans. That's why there is a need to find out the existence and severity of the problem along its associated risk factors in urban area of Southern Punjab Multan Pakistan. Blood samples from1087 subjects were taken who came to donate blood in pathology lab. Data was taken with the help of a comprehensive questionnaire. Blood samples were tested with latest standard techniques i.e. immune chromatographic (ICT), enzyme linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR). The data was analyzed by chi square test and Statistical Package for Social Sciences (SPSS-21).Overall prevalence of HCV and HBsAg was 24.29% and 6.62% respectively. The liable epidemiological risk factors considered in HCV positive subjects were age, gender, economic status, family history, injection therapy, visit to barber/salon, ear/nose piercing, hospitalization, surgery/dental surgery, blood transfusion, illiteracy or low education, marital status and diabetes respectively, (P> 0.05).Obtained data showed higher prevalence of HCV and reasonably high frequency of HBV, whereas the probable epidemiological risk factors considered in HCV positive subjects were statistically not significant (P> 0.05). The higher prevalence of hepatitis virus might be due to low auto-immune response of the subjects.

Keywords: Hepatitis B and C virus, Immune chromatographic, Enzyme linked immunosorbent assay, Polymerase chain reaction, auto-immune response

INTRODUCTION

Hepatitis C is a "viral time bomb" and estimated about 180 million people (3% of the world's population) are infected with hepatitis C virus (HCV), 130 million of whom are chronic carriers at risk of developing liver cirrhosis or liver cancer and an additional 3-4 million infected each year [1, 2]. Hepatitis B virus (HBV) is also considerable world health crisis especially in developing countries like Pakistan. Pakistan has an alarming situation about that silent killer [3]. The top virus-induced liver illness in many parts of the world is HCV and there is sky-scraping occurrence of hepatitis B and C in Pakistan with roughly ten million people with HCV alone [4, 5, 6].

The occurrence of hepatitis C virus varies from country to country and region to region of the same country and is less than 1% in Northern Europe, Canada and Australia, and 1% in USA and in many Europe countries, and it is more than 2% in Africa, South-East and Central Asia, and Latin America [7]. The prevalence of HCV in surrounding countries like Nepal in healthy adults is 1.0% [8], in Iran 0.87%, [9] in India 0.66% [10], in Afghanistan among women 0.31% [11] and in Pakistan among adults 6.8% [12]. The prevalence of HCV also varies within four provinces of Pakistan; the prevalence in Punjab (6.7%), in Khyber Pakhtunkhwa (1.1%), in Sindh (5%) and in Baluchistan (1.5%) respectively [13]. The previous studies have shown that HBV and HCV prevalence in Pakistani community of blood donors i.e. 2.4% (vary 1.4-11%) and 3% (vary 0.4% to 31.9%) respectively [14]. Many studies have been carried out in Pakistan to evaluate the frequency as well as liable risk factors linked with hepatitis virus andin most of studies very few risk factors were incorporated at a time in one study, however, many liable risk factors have been known globally, e.g. profession (barber industry, health care staff etc) [15], surgical procedures [16], household contact [17], blood transfusion [18], IV drug abuse [19], hemodialysis [20], dental treatment [21], skin piercing for tattoos [22], sexual contact etc. [23], but the ones responsible for the predominance of HCV in urban area of Southern Punjab Multan needs to be identified. That's why; this study was conducted for 13 variables risk factors to find out its statistically significance or association with hepatitis C virus.

MATERIALS AND METHODS

Study design

This cross-sectional study was carried out in a pathology clinical lab. Multan from 1st

January 2013 to 30th December 2015. A total of 1087 subjects of both gender were interviewed by using questionnaire arranged before conducting this study. These subjects came to Bhutta Clinical lab Multan for donating blood and some with patients as helper. Blood samples were tested for hepatitis B and C virus by standard techniques i.e. immune chromatographic (ICT), enzyme linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) by using commercials kits available in market. On the basis of clinical findings by serum analysis and comprehensive interviews, these subjects were divided into healthy 346 (without any disease) as control and unhealthy 741 (with variable diseases i.e. diabetes, obesity, hypertension) as experimental group.

Defining variables

Age group: subjects were divided in to five age groups

Gender: subjects were categorized either male or female

Economic status: based on their income they were categorized into highest level of income (rich), average level of income (middle), lowest level of income (poor)

Education: no education or below primary considered illiterate, above matric considered educated

Injection therapy: on and off unsafe therapeutic injection by healthcare staff

Barbers industry: use of non-sterilized equipments or blades for hair cutting or shave or abscesses drainage or circumcision of new born baby

Ear/nose piercing: ear/nose piercing by gold smith, barbers, mother/grandmother through unsterile needles

Hospitalization: admission in ordinary hospitals either admitted or not

Dental treatment or surgical history: dental treatment for tooth cleaning/extraction or minor/major surgical history positive or not

Blood transfusion: blood or blood products multi transfusion history positive or not

Marital Status: unsafe sexual contact with life partner if positive for hepatitis virus

Family history: if someone else positive family history of hepatitis B and C other than spouse then household contact was considered positive otherwise negative

Diabetic: by analysis of blood glucose level and history considered diabetic or non diabetic

Obesity: by the person's weight in kilograms divided by the square of the height in meters considered obese or non obese

Statistical analysis

The data was analyzed by applying Statistical Package for Social Sciences (SPSS-21) and

P-value < 0.05 was considered statistically significant [24].

RESULTS

In this study overall prevalence of HCV from experimental group was 180/741 (24.29%); gender-wise analysis showed its slight preponderance in females 13.63% than males 10.66% (P> 0.005), and age-wise analysis showed its higher prevalence among (both gender) in age group of (30-45) years was 9.45% followed by (15–30) years 9.04% than (45–60) years 4.59%, (above 60) years 1.08% and (under 15) years 0.13% respectively. Chi-square test (P >0.005) indicated that there was no significant effect of age and gender on HCV prevalence, (Table 1 and Table 3).

The overall prevalence of HBV was 49/741 (6.62%); gender-wise analysis showed its high prevalence in males 4.59% than females 2.02%, and age-wise analysis showed its preponderance among (both gender) in age of (15-30) years with 4.59% followed by (30-45) years 1.75% and (45-60) years 0.0%, (above 60) years and (under 15) years 0.13% respectively. Chi-square test (P >0.005) indicated that there was no significant effect of age and gender on HBV survival status (Table 2 and Table 3).

The possible epidemiological risk factors considered in HCV positive subjects were age; (high prevalence among 15-30 and 30-45 years with 37.22% and 38.89% respectively), sex; (high frequency in females i.e. 56.11% than males 43.89%), economic status i.e. (poor 55%, middle 39.45%, rich 5.60%), family history 31.12%, injection therapy 52.23%, visit to barber/salon 58.33%, ear/nose piercing 56.67%, hospitalization 35.0%, surgery/dental surgery 20.0%, blood transfusion 6.12%, illiteracy 68.34%, marital status 88.34% and diabetes 16.20% respectively. Chi-square test (P >0.005) indicated that there was no statistically significant effect of risk factors on survival status of HCV (Table 3 and Figure 1-13).

Table 1: Age and Sex-wise Prevalence of Hepatitis C Virus

HBV Elisa			Age					Total
			under 15	15-30	30-45	45-60	Above 60	
Negative	Sex	Male	10	151	91	17	10	279
		Female	3	153	79	41	6	282
		Total	13	304	170	58	16	561
Positive	Sex	Male	1	30	24	16	8	79
		Female	0	37	46	18	0	101
		Total	1	67	70	34	8	180
Total	Sex	Male	11	181	115	33	18	358
		Female	3	190	125	59	6	383
		Total	14	371	240	92	24	741

Table 2: Age and Sex-wise Prevalence of Hepatitis B Virus

HBV Elisa			Age					Total
			under 15	15-30	30-45	45-60	Above 60	
Negative	Sex	Male	10	158	106	33	17	324
		Female	3	179	121	59	6	368
		Total	13	337	227	92	23	692
Positive	Sex	Male	1	23	9		1	34
		Female	0	11	4		0	15
		Total	1	34	13		1	49
Total	Sex	Male	11	181	115	33	18	358
		Female	3	190	125	59	6	383
		Total	14	371	240	92	24	741

Table 3: Epidemiological Variables Considered in HCV Positive Subjects

S.NO	PARAMETERS	HCV^+ N= (180)		Chi Square	P-Value
	Total subjects	(n)	(%)		
1	Age				
	under 15	1	0.56%	0.0002	1.0000
	15-30	67	37.22%		
	30-45	70	38.89%		
	45-60	34	18.89%		
	Above 60	8	4.44%		
2	Gender				
	Male	79	43.89%	0.1722	0.6782
	Female	101	56.11%		
3	Economic Status				
	(Poor)	99	55.00%	0.0054	0.9973
	(Middle)	71	39.45%		
	(Rich)	10	5.60%		
4	Injection Therapy	94	52.23%	0.0000	1.0000
5	Visit to Barber/Saloon	105	58.33%	0.4444	0.5050
6	Ear/Nose Piercing	102	56.67%	0.0000	1.0000
7	Hospitalization	63	35.00%	0.0000	1.0000
8	Surgery/Dental surgery	36	20.00%	0.6905	0.4060
9	Blood Transfusion	11	6.12%	0.0000	0.9990
10	Illiteracy or low education	123	68.34%	0.0685	0.7935
11	Marital Status	159	88.34%	0.0000	0.9984
12	Diabetic	29	16.20%	0.0000	1.0000
13	Family history of hepatitis virus	56	31.12%	0.0001	0.9905

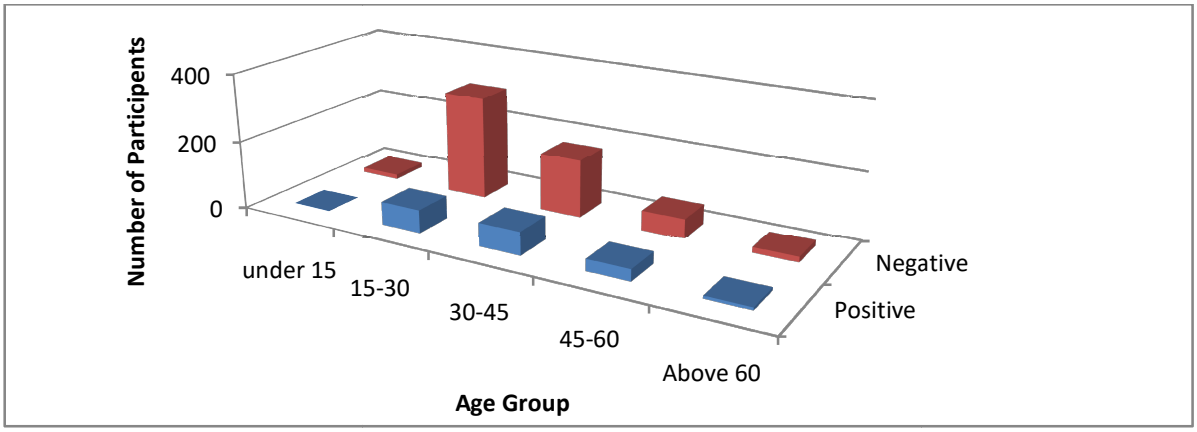

Figure 1: Age group versus hepatitis C virus

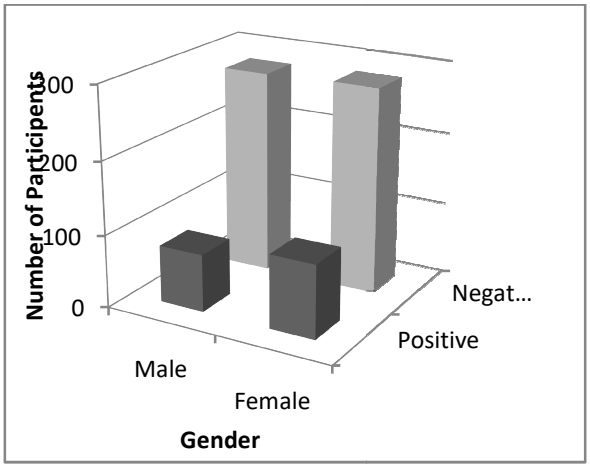

Figure 2: Gender versus HCV

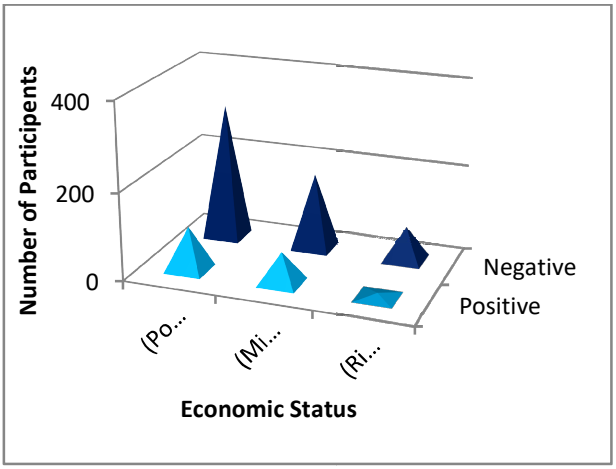

Figure 3: Economic status versus HCV

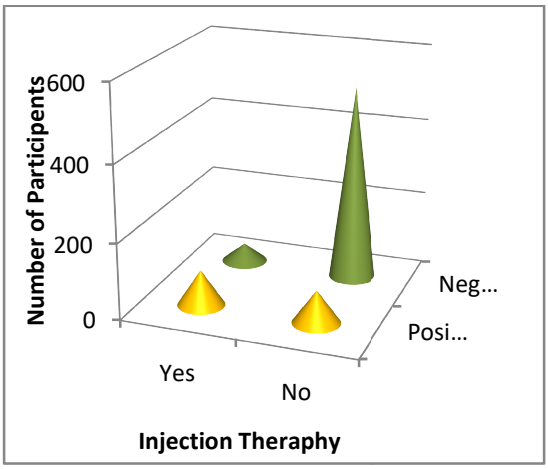

Figure 4: Injection Therapy versus HCV

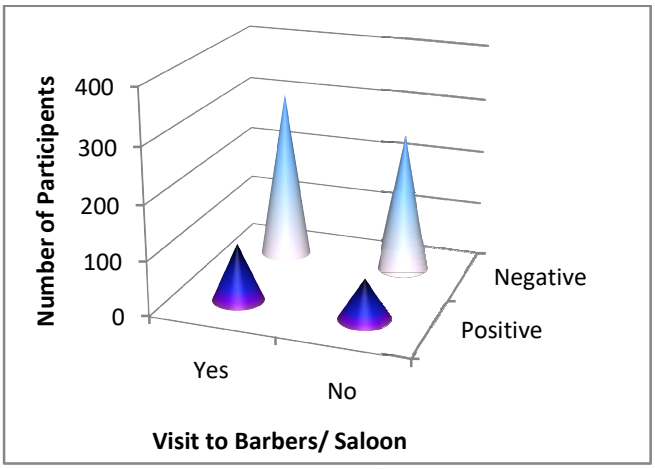

Figure 5: Visit to Barbers/saloon versus HCV

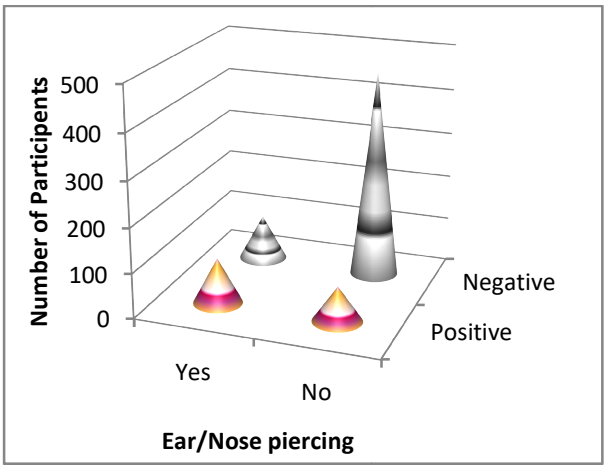

Figure 6: Ear/nose piercing versus HCV

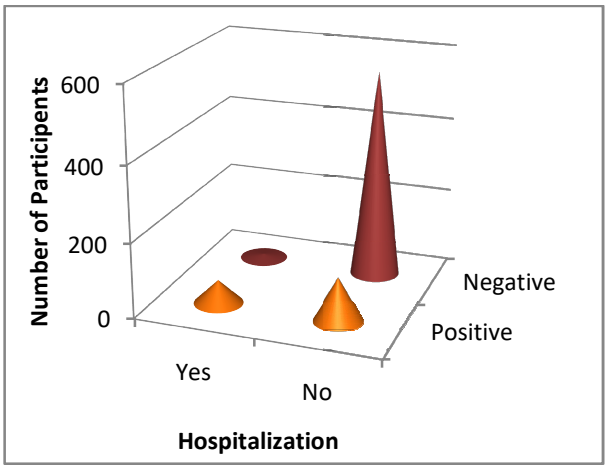

Figure 7: Hospitalization versus HCV

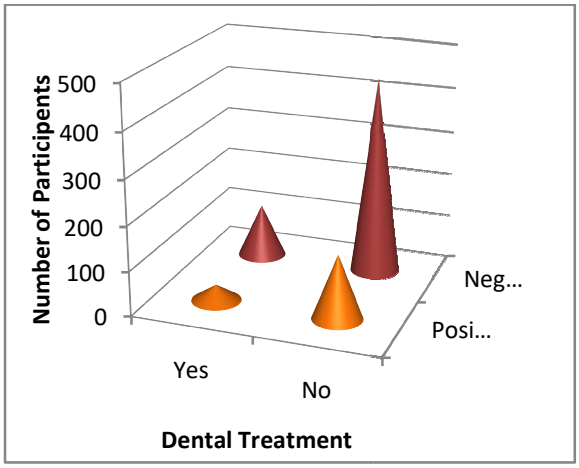

Figure 8: Dental treatment versus HCV

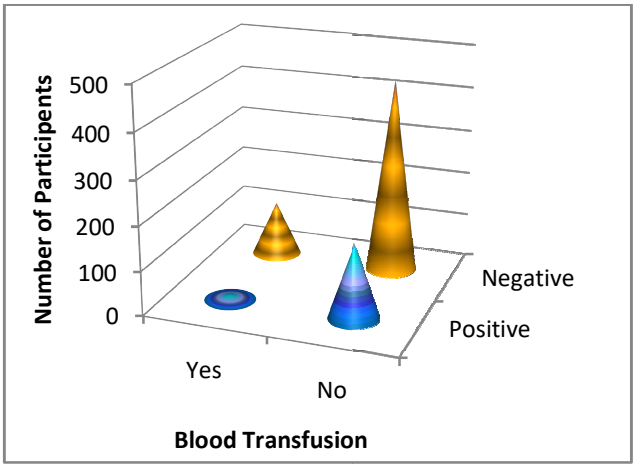

Figure 9: Blood transfusion versus HCV

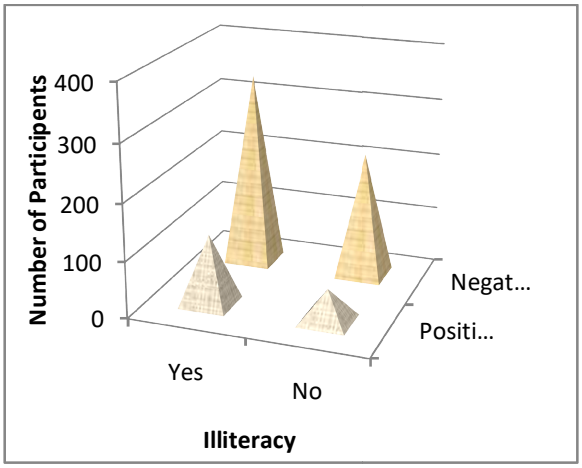

Figure 10: Illiteracy versus HCV

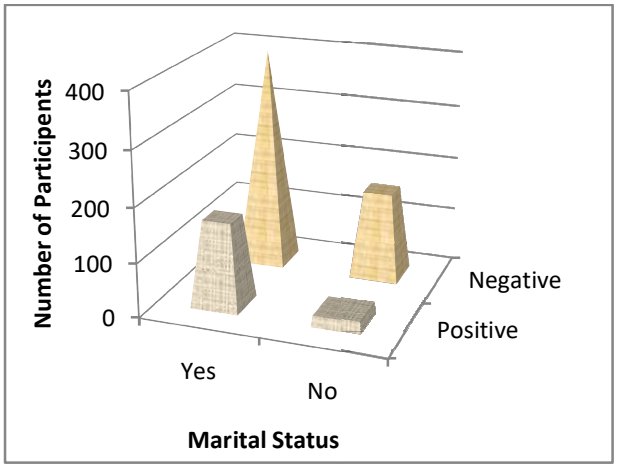

Figure 11: Marital status versus HCV

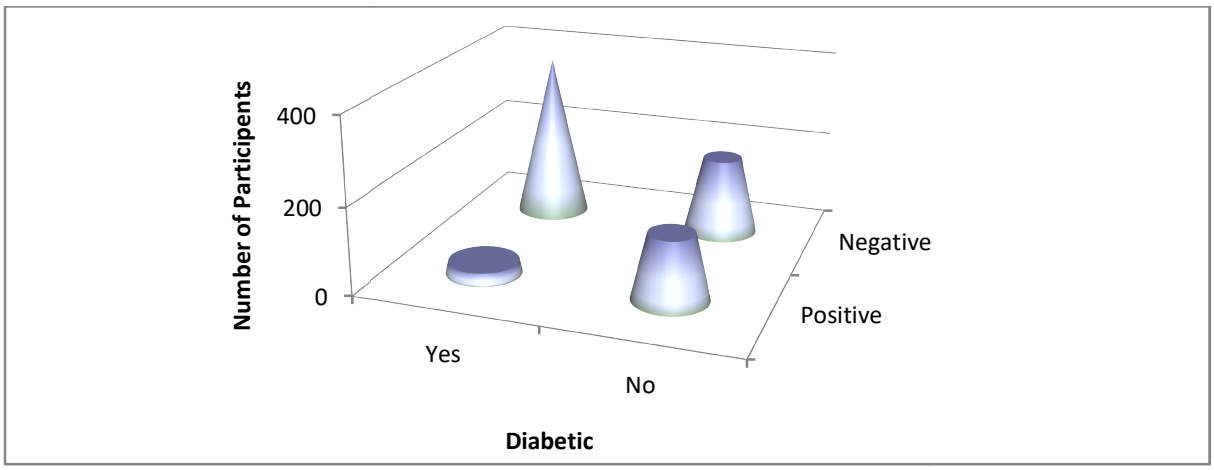

Figure 12: Diabetis versus HCV

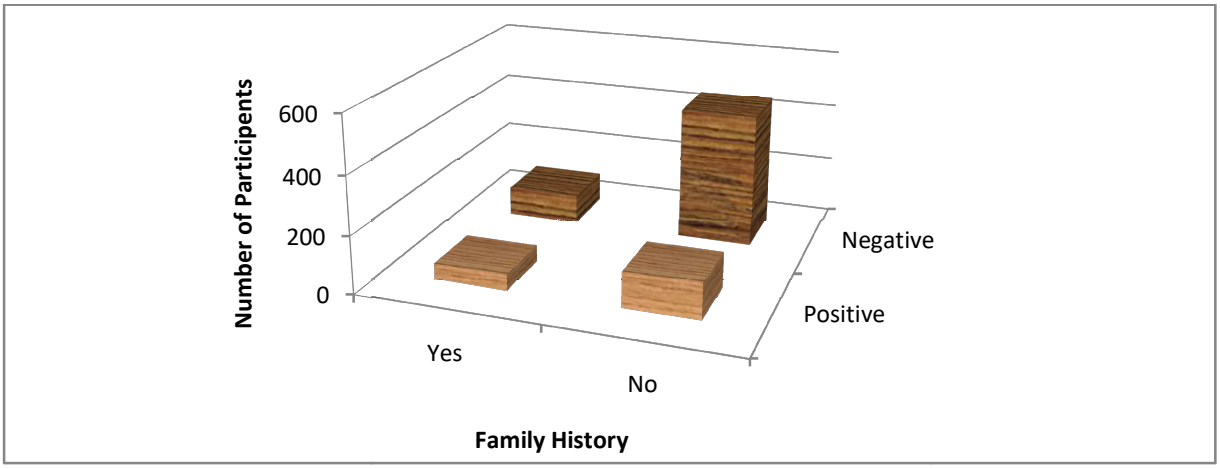

Figure 13: Family history or household contact of hepatitis virus versus HCV

DISCUSSION

Blood borne diseases like hepatitis virus are transmitted intermittently and may account for wide difference in prevalence within a nations, provinces or communities. In the current study observed prevalence of HCV was higher and frequency of HBV was also reasonably high. In spite of that HBV is totally preventable as observed in certain Far Eastern countries, which have achieved much in this regard [25]. Though, overall frequency of HBV is getting down than HCV due to utilization of HBV immunization since the early 1980s [26], similar outcomes were by Pakistan Medical Research Council that overall frequency of HBV is getting down than HCV in general population of Pakistan [27], and the current study results regarding to high frequency of HCV than HBV are appraised by a study conducted in Southern Punjab showed HCV prevalence 7.8% than 2.3% HBV [28]. However, still suggested as community base needs to be more attentive as the disease load of HBV was not decreasing in our community instead of that vaccination is available.

Mostly Pakistani people living with low health and low educational standard and are ranked to 146th out of 187 countries reported by Human Development Index of the United States. In the present study the possible epidemiological risk factors considered in HCV positive subjects were age; (high prevalence among 15-30 and 30-45 years), sex; (predominance in females than males), economic status i.e. (high prevalence among poor and middle than rich), family history, injection therapy, visit to barber/salon, ear/nose piercing, hospitalization, surgery/dental surgery, blood transfusion, illiteracy, marital status and diabetes respectively, (P >0.005). The results

of the present study are in agreements with the previous study by Razi-ul-Hussnain *et al* [29] and Shafiq *et al* [30] who found similar risk factors in their studies.

The present results regarding to age-wise showed high prevalence of HCV in age group of (30-45) years i.e. 9.45% followed by (15–30) years with 9.04% respectively and less success rate in other age groups (P> 0.05).The predominance of HCV in middle age group might be due to frequent exposure to risk factors that causes this virus infection or due to low immunity of the subjects and similar results were assessed by different studies [30-35].Whereas, the present study results are statistically not in accordance to the previous study in district Multan in which age was statistically significant versus HCV prevalence by Amin *et al*[36].

Gender-wise analysis showed its slight preponderance in females 13.63% than males 10.66%,(P> 0.05).The higher prevalence of HCV among females than males in the present study might be due to more exposure to anti-HCV, which was quite evident by their life style or due to low auto-immune response of the subjects. These results are comparable with the previous studies [33, 34]. While, the current study results are not in accordance to the studies in which males had higher frequency of HCV than females [28, 36-38], it might be due to low socioeconomic sampling area or high-risk profession or geographical differences or low auto-immune response of the host. Whereas the current results are statistically in accordance to the study conducted in district Multan by Amin *et al* [36].

The present study results regarding to economic status and illiteracy are consistent with the previous studies. As reported by Haider [39] poverty of Pakistan is a supporting factor for the prevalence of hepatitis virus, Henderson [40] also reported that HCV infection was inversely associated with socioeconomic stress, and similar results by Yaseen *et al* [41] and Guerra *et al* [42] that as total income of household's is raised then the possibility of hepatitis virus infection decreases. Unfortunately, in Pakistan literacy rate is 43% only[43] and reported as a supporting risk factor in prevalence of HCV and HBV among 56.5% illiterate prisonersby Niamatullah *et al*[44]. In the present study out of 180 HCV positive subjects 68.34% were illiterate and belonged to low income level. Similar outcomes were also by other studies [42, 45]. Whereas, the present study results are statistically not significant and in accordance to a study conducted in district Peshawar by Shafiq *et al* [30].

In the present study unsafe injection therapy and hospitalization was another possible risk factor. As estimated by WHO people in South East Asia receives average of four injections annually[46], according to Pasha *et al*[47] persons who had more than four injections per year were 11.9 times more expected to acquire HCV. Globally by healthcares approximately 2 million infections acquired from germ-infested injections during hospitalization and may account approximately 40% of the entire HCV infections [48]. Similar outcomes in other studies have established a relationship between hepatitis virus and injection therapy or sharing of contaminated syringes/needles during hospitalization with poor medical measures by health care staff [14, 31, 46, 49-51], Catherine *et al* [52] also reported similar risk factors for HCV, HIV and HBV infection in the Kabul Afghanistan.

The findings of the present study regarding dental treatment/surgery are in agreement with the previous studies that HCV infection extends to the rest of the society might be through unsafe dental treatment/surgery for tooth cleaning/extraction or injections therapy in hospitals and rude clearance of hospitals wastes in Pakistan [53-55]. Ali *et al* [56] reported 14.2% to 16.2% HCV infection among group undergoing dental surgery by quacks in Khyber Pakhtunkhwa Pakistan. Similar conclusion was also evaluated by other studies [21, 32]. Whereas, in the present study the association between dental treatment/surgery and HCV was statistically not significant ($P>0.05$) and not in accordance to a study conducted in district Peshawar ($P<0.05$), by Shafiq *et al* [30].

Barbers/saloon industry for hair or nail cutting, shaving, ear/nose piercing, minor abscess drainage or circumcision of new born baby might be a causative agent for HCV in the current study. The present study results are in agreements by different studies in community of Rawalpindi and Islamabad [57-59]. Similar outcomes about the high-risk activity of barber industry were also assessed by previous studies [31, 32, 50, 51]. The present study results are also in accordance to previous study conducted in Peshawar and Multan, in which barbers/saloon was statistically not significant with HCV prevalence [30,36]and according to Hamid *et al* [60] females were likely to be higher victims of HCV than males because of higher contact to surgery or ear/nose piercing particularly in Balochistan.

The present study results regarding to blood transfusion and HCV association are consistent with the previous might be a threat

due to improper screening of blood in our community. In Pakistan each year approximately 1.5 million blood products is transfused, and that transfusion is roughly planned and contributes to serious communicable diseases i.e. HBV and HCV [61]. Similar outcomes have been reported by other studies that unsafe blood transfusion by health care staff in Pakistan are a common risk factor [30, 62, 63]and similar results were appraised in Lebanon and Korea by Naman *et al* [64] and Kwon and Bae [65], whereas the association between blood transfusion and HCV in the present study results are statistically not significant (P>0.05),and statistically not in accordance to the study conducted in Peshawar (P<0.05), by Shafiq *et al* [30].

The present study regarding to marital status as a risk factor are consistent with the previous studies [51, 66-68]. Similar outcomes have been reported by Niamatullah *et al* [44] who reported higher prevalence of HBV and HCV in married group 7.6% and 7.8% respectively. Basit *et al* [37] also reported more prevalence among married (23.1%) than unmarried (20.7%). Hepatitis virus transmission in spouse might be due to lack of knowledge that has pre exposure before marriage which was then transmitted to life partner or it might be due to household sharing i.e. tooth brushes, common raiser, toilet, under garments and towels etc. Whereas, inter spousal transmission somewhat conflicting in different studies [45, 69] incomplete or no proof of HCV transmission between life partners was found in other studies [23,70]. However, the present study results marital status with HCV are statistically not significant (P>0.05), and not in accordance to the study conducted in Peshawar (P<0.05) by Shafiq *et al* [30].

The present results regarding to prevalence of HCV with diabetes showed its high prevalence among non diabetic than diabetic with 16.20% prevalence (P> 0.05). The present study results are in agreements with the previous studies [71, 72]. While not in accordance to other studies in which higher frequency of HCV was in diabeticthan non-diabetic [73, 74].In another study it was claimed that diabetes was linked with more susceptibility to acquiring HCV infection [75], although diabetes frequently occurs globally and among those subjects who were old age, obese, genetic susceptibility, low socioeconomic status and chronic HCV infection; might be a contributing factor to the growth of diabetes through progressive liver damage [76]. Whereas, the association between diabetes and HCV in the present study was statistically not significant and in

accordance to a study conducted in district Peshawar by Shafiq *et al* [30].

Family history or household contacts of positive family history in the present study as a risk factors are in agreement with a study conducted by Shazi and Abbas [55] that the high-risk activities in the transmission of HCV was household contacts of positive family history by the use of common toilet, tooth brushes or sexual contact etcwas2.5 times more than those of the common population. Similar outcomes were evaluated by other studies [77-79]. Whereas the results of the present study are statistically not significant with positive family history and in accordance to a study conducted in District Multan by Amin *et al* [36].

As it is known that hepatitis virus is present in blood, body fluids, urine, semen, saliva secretions and sweat as well, which may easily transfer to healthy individuals. The higher prevalence of hepatitis C in urban area of Southern Punjab District Multan Pakistan could be on the following basis that it might be due to low economic status, quackery, barber activity (for shaving, ear/nose piercing or abscess drainage), family history, marital status, hospitalization, injection therapy, unsafe blood transfusion, illiteracy and lack of awareness. But, the present study results are statistically not significant (P>0.05). So, it might be due to low auto-immune response of the host or this rising trend might be due to coverage of high risk groups or better strategies or better analytical facilities or due to positive cases of HCV and HBV who got infection in the past years and had been identified now indicate a boost of new cases. The actual burden of HCV and HBV might be more than current or previous results, in spite of that HBV is totally preventable as observed in certain Far Eastern countries, which have achieved much in this regard [25]and as suggested by National Institute for Health and Care Excellence that high-risk community should be immunized against HBsAg infection [80].

CONCLUSION

Obtained data showed shocking prevalence of HCV and reasonably high frequency of HBV and the liable epidemiological risk factors considered in HCV positive subjects might be contributing factors to acquiring a new infection of HCV because statistically not significant (P> 0.05)and their relative importance is not yet clear. Because mostly subjects from control and experimental groups were also observed with these risk factors but still negative for HCV. Therefore, this higher prevalence of HCV in urban area might be due to low auto-immune response of the host. The results of the present study

are supported by Takaki *et al* [81] that in most of the cases HCV is cleared automatically by powerful immune system i.e. natural or cell mediated immunity, whereas in some cases with weak immune system, antibodies are not produced against HCV due to low auto-immuneresponse of the host and ultimately acquired a infection of HCV.

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