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Prevalence of Hepatitis C Virus in Urban Ghettos of Twin Cities

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Abstract.- The present study was undertaken to identify the factors that are involved in the spread of Hepatitis C Virus (HCV) in the low socioeconomic regions of the "twin cities" (Rawalpindi and Islamabad) in Pakistan. For this purpose the presence of HCV RNA was analyzed in 503 blood samples collected at random along with the demographic data. The study revealed an unusually high prevalence of HCV RNA in Rawalpindi (17%) vs. Islamabad (4%). Factors considered to be higher risk for HCV spread did not appear as significant to explain this difference, including blood transfusion, therapeutic injections, dental, hospital, barber, and beauty salon visits, skin piercing, tattooing, and needle stick injuries. These data highlight the urgent need to understand other unknown risk factors operating in poor urban ghettos associated with HCV spread to slow down and/or prevent its epidemic spread worldwide.

Key words: Hepatitis C Virus, HCV RNA, risk factors, HCV seroprevalence, HCV transmission.

INTRODUCTION

Hepatitis C virus (HCV) is spreading at alarming rates in developing countries like Pakistan, where the prevalence in the general population varies from 0.5-31.9%, depending upon the cohort being studied (Ali *et al*., 2010; Delarocque *et al*., 2007). The primary routes of HCV transmission are parenteral and nosocomial, involving exposure to infected blood; however, increasingly, HCV is spreading in a non-parenteral manner (Abe and Inchauspe, 1991); Karmochkine *et al*., 2006; Mastromatteo *et al*., 2001). A study of household contacts of HCV-infected thalassemia patients revealed that over 20% of the household contacts had anti-HCV antibodies and were infected with the same virus, suggesting that HCV spread was not from an exogenous source but from the index patient (Akhtar *et al*., 2002). Interestingly, these and other studies that have analyzed the presence of HCV RNA in saliva/sputum/gingival washes have revealed that HCV RNA can be isolated from infected patients, suggesting other ways of transmitting the virus such as sharing of toothbrushes or being bitten by infected individuals (Abe and Inchauspe, 1991; Mastromatteo *et al*., 2001; Dusheiko *et al*., 1990).

Since low socioeconomic status is among the potential risk factors for HCV spread (Akhtar *et al*., 2002; Dusheiko *et al*., 1990), the present study analyzed the low socioeconomic population of the twin cities to determine the behavior patterns involved in the transmission of HCV in this group. Towards this end, low socioeconomic colonies (ghettos) within the twin cities of Rawalpindi and Islamabad were chosen. Rawalpindi is an ancient city that has developed haphazardly into a moderately large congested metropolitan with a mixture of affluent and poor areas. In contrast, Islamabad is a modern city with an affluent population that lives in well-planned sectors interspersed with unauthorized sprawling ghettos of low income colonies that developed over time to provide the residential and commercial needs of the city.

MATERIALS AND METHODS

Ethics committee approval

Prior to initiation, the study design was approved by the Shifa College of Medicine/Shifa

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International Hospital Ethics Committee/ Institutional Review Board.

Study population

Random, healthy individuals living in seven ghetto colonies in Rawalpindi and five such colonies in Islamabad representing nearly every other household in these areas were administered a questionnaire regarding life style choices, including, visits to the barber, beauty salon, dentist, tattoo parlor, body piercing, blood transfusions, therapeutic injections, surgical procedures, use of illicit drugs, number of multiple sexual partners, etc. From these households a total of 503 blood samples were also collected, ensuring that only one sample was collected per household.

Sample collection

Blood samples were collected in vacutainers without additives (Becton Dickinson). These samples were stored at 4^{o}C during transport and serum was separated within 5 hours of collection and stored at -70^{o}C till further use.

RNA isolation and HCV amplification

RNA was isolated from the serum with the help of Roche AMPLICOR® HCV Specimen Preparation Kit, version 2.0, as per the manufacturers recommendations and amplified using the Roche AMPLICOR® HCV Amplification Kit, version 2.0, as described by the manufacturer. The 255 bp thus amplified was resolved on 1.5% agarose gels, visualized under UV transillumination and documented using a digital camera.

To reconfirm the results of the positive samples, viral RNA was re-extracted from stored frozen sera and used for RT-PCR using an Enzyme linked Immunosorbant Assay (ELISA) based detection method (Roche AMPLICOR® Hepatitis C Virus (HCV) Test, version 2.0). The validation of both the techniques showed that any one of them could be used for HCV RNA analysis.

Data analysis

Statistical significance was determined using the Pearson's Chi Square (χ^2) and Chi Square Test for Linear Trends (CSLT) along with determination of the Odds Ratio (OR) and confidence intervals (CI). Both the uni- and multivariate analyses were carried out using SPSS for Windows version 16.0 (Chicago, IL).

RESULTS AND DISCUSSION

The low socioeconomic regions of Rawalpindi have a higher HCV seroprevalence rate than those of Islamabad

A total of 503 random, healthy individuals living in seven poor ghettos in Rawalpindi (n=303) and five such colonies in Islamabad (n=200) were administered a questionnaire regarding lifestyle choices detailed in Materials and Methods above and tested for the presence of HCV RNA in blood to determine recent exposures to HCV. Table I shows the number, location, and sex-wise breakdown of the test population and their HCV status. The Rawalpindi sample was proportional between the two sexes; however, there was a preponderance of females in three of the five Islamabad colonies tested due to the timing of the sampling (morning) when most of the males were away at work.

Test of the HCV status of the combined cohort revealed an overall 11.9% (n=60) HCV prevalence rate for the total (n=503) sampled population, which is higher than the reported worldwide prevalence rates of 2.2%, but within the range reported for the general population in Pakistan (0.5-31.9%) (Ali *et al*., 2010). However, when the sample group was divided according to their respective cities, there was a drastic difference in the prevalence rate of the two regions, with Rawalpindi exhibiting an unusually high rate 17% (n=52) as compared to Islamabad's rate of only 4% (n=8) (Table I). This difference was statistically significant (OR = 4.97; χ^2 =19.87; *p* value = 8.3 x 10^{-6}) and was unexpected since the two sampling groups came from similar low socioeconomic ladders of the society.

Age-wise increase in HCV prevalence rate in the infected population

To determine the reasons for the higher rates of infection in Rawalpindi, the general demographics of the test population were analyzed (Table II). Age-wise analysis of the total sampled population in the two cities revealed that there was a

Table I.- Demographic analysis and Hepatitis C virus (HCV) prevalence in the low socioeconomic regions of Rawalpindi and Islamabad.

Sampling Colony	Total n (%)	HCV RNA(+) n (%)	Male n (%)	HCV RNA(+) n (%)	Female n (%)	HCV RNA(+) n (%)
Rawalpindi						
Ratta Pul	44 (14)	3 (7)	20 (45)	0 (0)	24 (55)	3 (7)
Shamsabad	41 (13)	8 (19)	20 (49)	3 (7)	21 (51)	5 (12)
Gawal Mandi	35 (12)	6 (17)	18 (51)	2 (6)	17 (49)	4 (11)
Iqbal Town	47 (16)	10 (21)	26 (55)	6 (13)	21 (45)	4 (9)
Pirwadhai Proper	44 (14)	15 (34)	22 (50)	11 (25)	22 (50)	4 (9)
Pirwadhai Surroundings	47 (16)	8 (17)	22 (47)	4 (9)	25 (53)	4 (9)
Gulistan Colony	45 (15)	2 (4)	19 (42)	0 (0)	26 (58)	2 (4)
Total	**303 (100)**	**52* (17)**	**147 (49)**	**26 (9)**	**156 (51)**	**26 (9)**
Islamabad						
66th Colony	50 (25)	2 (4)	13 (26)	0 (0)	37 (74)	2 (4)
Faisal Colony	32 (16)	3 (9)	7 (22)	1 (3)	25 (78)	2 (6)
Musharaf Colony	18 (9)	0 (0)	9 (50)	0 (0)	9 (50)	0 (0)
Hansa Colony	51 (26)	1 (2)	19 (37)	1 (2)	32 (63)	0 (0)
French Colony	49 (24)	2 (4)	26 (53)	2 (4)	23 (47)	0 (0)
Total	**200 (100)**	**8 (4)**	**75* (37)**	**4 (2)**	**125 (63)**	**4 (2)**

*Statistically significant difference: OR 4.97; Chi square = 19.87, p value = 8.3 x 10^6.

statistically significant trend in the number of infected individuals with increasing age. This was observed by the increase in prevalence from 3% in the <20 age group to nearly 20% in the >51 year age-group (Table II; CSLT = 8.49; p value = 0.003). This observation remained significant when the data were analyzed to study trends between the two cities (Table II). Within Rawalpindi, the prevalence increased from 5% progressively to 23% (CSLT = 7.87; p value =0.005), while in Islamabad, the prevalence increased from 0% to 16% (CSLT = 8.13; p value =0.004). However, this trend was significant only among the males when the data were split between the sexes despite the males and females having comparable n numbers (data not shown). Furthermore, the Rawalpindi population (either male or female) exhibited an earlier exposure to the virus than Islamabad, which cannot be attributed to a lower number of individuals tested in the older category since in both cities, the n number was higher in the <20 year age group (n=60 and 25, respectively) than the >51 age group (n=22 and 19, respectively) (Table II).

Overall, gender analysis revealed that there was a preponderance of females in the tested population from Islamabad colonies- 63% females verses 51% in the Rawalpindi cohort (Table I). Gender analysis of the HCV positive population within the two cities revealed that there was no sexual bias in the proportions of males or females infected (Table II). However, interestingly, almost all of the infected individuals were married (OR = 4.6; χ^2 = 7.5; p = 0.006) irrespective of sex (Table II) with only 3 out of the 60 infected individuals being unmarried (one female and two males of 18-24 years of age; data not shown).

Multivariate analysis of the HCV(-) and (+) individuals revealed a significant association with age and marital status, as indicated by Wilk's Lambda value of 0.897 (p = 0.000) (data not shown). While univariate analysis indicated that age was the only factor that could be significantly associated with HCV(+) individuals (F= 19.683; p = 0.000), suggesting biases in data collection were probably responsible for the association of marriage with HCV positivity.

Usual high risk factors not associated with increased HCV transmission in Rawalpindi

Next, a number of risk factors were evaluated based on the administered questionnaire including, blood transfusions, therapeutic injections,

Table II.- Analysis of demographic feature association with the transmission of hepatitis C virus (HCV) in the twin urban ghettos*.

Demographic factors		Rawalpindi + Islamabad (n=503)				Rawalpindi (n=303)				Islamabad (n=200)			
		Total Tested (n)	Nos (n) HCV+ (%)	OR (95% CI)	Chi Square (p value)	Total Tested (n)	Nos (n) HCV+ (%)	OR (95% CI)	Chi Square (p value)	Total Tested (n)	Nos (n) HCV+ (%)	OR (95% CI)	Chi Square (p value)
Age group (years)	<20	86	3 (3)	-	**CSLT =**	60	3 (5)	**1.0**	**CSLT =**	25	0 (0)	NA	**CSLT =**
	21-50	376	49 (13)	-	**8.49**	221	44 (20)	**4.8**	**7.869**	156	5 (3)	NA	**8.13**
	>51	41	8 (20)	-	**(0.003)**	22	5 (23)	**5.6**	**(0.005)**	19	3 (16)	NA	**(0.004)**
Gender	Male	221	30 (14)	1.32 (0.772-2.254)	1.0	147	26 (18)	1.07 (0.594-1.943)	0.06	74	4 (5)	1.74 (0.462-6.573)	0.60 (.43)
	Female	282	30 (11)		(.31)	156	26 17)		(0.813)	126	4 (3)		
Married	Yes	414	57 (14)	**4.6 (1.482-14.098)**	**7.5 (0.006)**	248	49 (20)	**4.27 (1.354-13.386)**	**6.48 (0.010)**	166	8 (4)	∞ (0.436-∞)	1.71 (0.19)
	No	89	3 (3)			55	3 (5)			34	0 (0)		

*Numbers in bold reflect statistically significant relationships

hospitalizations, visits to dentists, barbers, and beauty salons, skin piercing, tattooing and needle stick injuries (Table III). Multivariate analysis of HCV negative (-) and positive (+) individuals within Rawalpindi and Islamabad with the mentioned risk factors revealed that none of these factors were significantly associated with an increased transmission of HCV infection in these populations (data not shown). This is despite the fact that there was an unusually high use of therapeutic injections among the Islamabad cohort compared to the Rawalpindi one (Table III). Conversely, tattooing was observed to be a more common practice in the Rawalpindi ghettos (Table III), but still was not found to be significantly associated with HCV spread (data not shown). These data suggest that risk factors in addition to the ones studied must be operational in Rawalpindi, resulting in much higher rates of HCV positivity compared to Islamabad.

This was rather unexpected but not surprising given the observation that the source of HCV transmission in 10-70% of the cases cannot be attributed to known risk factors (Kerzman *et al*., 2007; Kumar *et al*., 2007). In support of this, two French studies have shown that HCV transmission could not be correlated with either shaving at barber/beauty shops, tattooing, piercing or visits to the dentist (Delarocque *et al*., 2007; Karmochkine *et al*., 2006). Similarly, a study from Egypt was unable to find any defined risk factors for HCV transmission among children (El-Raziky *et al*., 2007), while in India over 62% of HCV(+) pregnant women could not be ascribed a risk factor (Kumar *et al*., 2007). Given that HCV can be isolated from saliva/sputum/gingival washes, the role of oral transmission in these or other cases cannot be ignored (Akhtar *et al*., 2002; Dusheiko *et al*., 1990), especially the observation that inoculations with saliva from chronically infected HCV carrier chimpanzees can transfer HCV infection to the test animals (Abe and Inchauspe, 1991).

As injecting drug users (IDUs) are the primary source of HCV spread worldwide (Delarocque *et al*., 2007), we also considered this factor among our test population, as well as having multiple sex partners, a high risk activity for HCV transmission. Unfortunately, due to taboos associated with these subjects, none of the

Table III.- Analysis of high risk behavior with the transmission of Hepatitis C Virus (HCV) in the twin urban cities.

		Rawalpindi + Islamabad (n=503)				Rawalpindi (n=303)				Islamabad (n=200)			
		Total Tested (n)	Nos (n) HCV+ (%)	OR (95% CI)	Chi Square (p value)	Total Tested (n)	Nos (n) HCV+ (%)	OR (95% CI)	Chi Square (p value)	Total Tested (n)	Nos (n) HCV+ (%)	OR (95% CI)	Chi Square (p value)
Therapeutic injections	Yes	171	7 (4)	0.225 (0.102-0.497)	15.1 (<0.001)	9	1 (11)	0.6 (0.095-3.782)	0.24 (>0.05)	162	6 (4)	0.69 (0.152-3.110)	0.19 (>0.05)
	No	332	53 (16)			294	51 (17)			38	2 (5)		
Blood transfusions	Yes	46	6 (13)	1.12 (0.466-2.701)	0.06 (>0.05)	25	5 (20)	1.23 (0.456-3.331)	0.15 (>0.05)	21	1 (5)	1.21 (0.188-8.115)	0.03 (>0.05)
	No	457	54 (12)			278	47 17)			177	7 (4)		
Blood donations	Yes	76	7 (9)	0.72 (0.30) 19-1.61	0.63 (>0.05)	54	6 (11)	0.55 (0.229-1.336)	1.69 (>0.05)	22	1 (5)	1.16 (0.180-7.752)	0.02 (>0.05)
	No	427	53 (12)			249	46 (18)			178	7 (4)		
Hospitaliza-tions	Yes	189	26 (14)	1.314 (0.765-2.258)	0.96 (>0.05)	90	20 (22)	1.62 (0.872-2.998)	2.31 (>0.05)	99	6 (6)	3.19 (0.715-14.147)	2.17 (>0.05)
	No	314	34 (11)			213	32 (15)			101	2 (2)		
Dentist visits	Yes	180	23 (13)	1.13 (0.653-1.965)	0.19 (>0.05)	113	20 (18)	1.06 (0.578-1.953)	0.04 (>0.05)	67	3 (5)	1.2 (0.307-4.709)	0.06 (>0.05)
	No	323	37 (11)			190	32 (17)			133	5 (4)		
Barber visits	Yes	133	21(16)	1.46 (0.662-3.225)	0.86 (>0.05)	89	18(20)	1.38 (0.582-3.264)	0.52 (>0.05)	44	3 (7)	2.12 (0.284-15.417)	0.42 (>0.05)
	No	88	10 (11)			58	9 (16)			30	1 (3)		
Beauty parlor visits	Yes	19	0 (0)	0 (0.000-0.954)	2.34 (>0.05)	11	0 (0)	0 (0.00-1.732)	2.26 (>0.05)	8	0 (0)	0 (0.000-15.363)	0.28 (>0.05)
	No	263	29 (11)			145	25 (17)			118	4 (3)		
Skin piercing	Yes	335	41 (12)	1.09 (0.617-1.939)	0.09 (>0.05)	205	36(18)	1.09 (0.576-2.066)	0.07 (>0.05)	130	5 (4)	0.595 (0.095-3.782)	0.02 (>0.05)
	No	168	19 (11)			98	16 (16)			70	3 (5)		
Tattooing	Yes	119	14 (12)	0.98 (0.523-1.839)	0.00 (>0.05)	95	13(14)	0.69 (0.351-1.346)	1.18 (>0.05)	24	1 (4)	1.05 (0.163-6.961)	0.00 (>0.05)
	No	384	46 (12)			208	39 (19)			176	7 (4)		
Needle stick injuries	Yes	15	2 (13)	1.14 (0.282-4.659)	0.03 (>0.05)	9	1 (11)	0.6 (0.095-3.782)	0.24 (>0.05)	6	1 (17)	5.34 (0.751-41.022)	2.58 (>0.05)
	No	488	58 (12)			294	51 (17)			194	7 (4)		

participants responded in the affirmative to these queries. To corroborate the claim about illicit drug use, arms of the participants were checked during sampling to determine presence of tell-tale signs of needle pricks. Based upon the current data, the involvement of the above two risk factors still cannot be ruled out which is a major weakness of this study.

Pirwadhai and Faisal Colony reveal unusually high prevalence rates compared to their surroundings

To get better insights into the dynamics of HCV spread in the two metropolitan ghettos, the colony-wise distribution of the tested populations was further analyzed. Such an analysis revealed that each city had its own "hot spot" of infection, where HCV prevalence rates were much higher than the neighboring areas. Within Rawalpindi, the area of Pirwadhai proper revealed a prevalence rate of 34%, exactly twice that of the overall prevalence rate of 17% for Rawalpindi (Table II), a difference that was statistically significant (OR: 3.1; $\chi^2 = 10.4$; p value = 0.001). Similarly, within Islamabad, Faisal Colony had a prevalence rate of 9%, more than double that for the overall rate of 4% for Islamabad (Table II). At the other end of the spectrum, Gulistan Colony in Rawalpindi revealed a low prevalence rate of 4%, more characteristic of Islamabad, while Musharaf Colony in Islamabad revealed no cases of HCV at all, probably due to the lower number of individuals tested (n=18). Similarly, Hansa Colony in Islamabad showed a low prevalence rate of only 2% (Table II).

Pirwadhai proper (34% HCV RNA(+)) is a culminating point for many of the small water channels that come together primarily at this point before the sewage-contaminated dirty water flows into the Soan River in the southern part of Islamabad. It is a highly congested area of the very poor living in extremely dilapidated conditions. To ascertain the environmental impacts in this area, the adjoining relatively better socioeconomic population was screened for HCV prevalence (Pirwadhai surroundings, Table II). The result for this cohort matched the results obtained from the rest of Rawalpindi, showing a prevalence rate of 17% and the difference between the two regions, Pirwadhai proper and surroundings, was statistically significant (OR: 2.59; $\chi^2 = 3.72$; p value = 0.05, data not shown). Pirwadhai proper is known to be a hot spot for HIV infection as well and it has been postulated that the co-existence of the two viruses might help their general spread in the population, as has been well documented (Ali *et al*., 2010; Wolff *et al*., 2007). Thus, this could be partly responsible for the higher HCV infection rate in the Pirwadhai proper area.

Pirwadhai proper also maintains a huge bus terminal used by migrant population of the two cities where long distance truck drivers rest before moving onwards. To determine whether the source of virus was coming from these drivers, they were also screened (n=39; numbers not included in overall Rawalpindi data). Surprisingly, results revealed only a 7.6% prevalence rate (3/39), a rate at *par* with that observed overall for Rawalpindi, however, not high enough to explain the high rates observed in Pirwadhai proper. This strongly suggests that in addition to high risk sexual behavior and drug routes, HCV is spreading in unknown, but very effective manner, within Pirwadhai proper.

Finally, better awareness and/or better access to healthcare facilities may have additionally contributed to the differences in the spread of HCV in the two cities as observed by a much higher use of therapeutic injections by the Islamabad cohort compared to Rawalpindi (Table III).

CONCLUSIONS

Overall, our results reveal that most of the important risk factors associated with HCV infection were not significant in explaining the substantial differences observed between the economically disadvantaged ghettos of the two twin metropolis, except perhaps some effects of better education/awareness and better access to healthcare of the Islamabad cohort. Thus, this study highlights the need for characterizing the "other" significant unknown risk factors operational in urban settings to slow down the spread of HCV nationally and globally.

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	No	332	53 (16)			294	51 (17)			38	1 (3)		
Blood transfusions	Yes	46	6 (13)	1.12 (0.466-2.701)	0.06 (>0.05)	25	5 (20)	1.23 (0.456-3.331)	0.15 (>0.05)	21	1 (5)	1.21 (0.138-8.115)	0.05 (>0.05)
	No	457	54 (12)			278	47 17)			177	7 (4)		
Blood donations	Yes	76	7 (9)	0.72 (0.30) 19-1.61	0.63 (>0.05)	54	6 (11)	0.55 (0.229-1.336)	1.69 (>0.05)	22	1 (5)	1.16 (0.180-7.752)	0.02 (>0.05)
	No	427	53 (12)			249	46 (18)			178	7 (4)		
Hospitaliza-tions	Yes	189	26 (14)	1.314 (0.765-2.258)	0.96 (>0.05)	90	20 (22)	1.62 (0.872-2.998)	2.31 (>0.05)	99	6 (6)	3.19 (0.715-14.147)	2.17 (>0.05)
	No	314	34 (11)			213	32 (15)			101	2 (2)		
Dentist visits	Yes	180	23 (13)	1.13 (0.653-1.965)	0.19 (>0.05)	113	20 (18)	1.06 (0.578-1.953)	0.04 (>0.05)	67	3 (5)	1.2 (0.307-4.709)	0.05 (>0.05)
	No	323	37 (11)			190	32 (17)			133	5 (4)		
Barber visits	Yes	133	21(16)	1.46 (0.662-3.225)	0.86 (>0.05)	89	18(20)	1.38 (0.582-3.264)	0.52 (>0.05)	44	3 (7)	2.12 (0.284-15.417)	0.42 (>0.05)
	No	88	10 (11)			58	9 (16)			30	1 (3)		
Beauty parlor visits	Yes	19	0 (0)	0 (0.000-0.954)	2.34 (>0.05)	11	0 (0)	0 (0.00-1.732)	2.26 (>0.05)	8	0 (0)	0 (0.000-15.363)	0.28 (>0.05)
	No	263	29 (11)			145	25 (17)			118	4 (3)		
Skin piercing	Yes	335	41 (12)	1.09 (0.617-1.939)	0.09 (>0.05)	205	36(18)	1.09 (0.576-2.066)	0.07 (>0.05)	130	5 (4)	0.595 (0.095-3.782)	0.02 (>0.05)
	No	168	19 (11)			98	16 (16)			70	3 (5)		
Tattooing	Yes	119	14 (12)	0.98 (0.523-1.839)	0.00 (>0.05)	95	13(14)	0.69 (0.351-1.346)	1.18 (>0.05)	24	1 (4)	1.05 (0.163-6.961)	0.00 (>0.05)
	No	384	46 (12)			208	39 (19)			176	7 (4)		
Needle stick injuries	Yes	15	2 (13)	1.14 (0.282-4.659)	0.03 (>0.05)	9	1 (11)	0.6 (0.095-3.782)	0.24 (>0.05)	6	1 (17)		
	No	488	58 (12)			294	51 (17)			194	7 (4)		

Table II.- Analysis of demographic feature association with the transmission of hepatitis C virus (HCV) in the twin urban ghettos*.

Demographic factors		Rawalpindi + Islamabad (n=503)				Rawalpindi (n=303)				Islamab	
		Total Tested (n)	Nos (n) HCV+ (%)	OR (95% CI)	Chi Square (p value)	Total Tested (n)	Nos (n) HCV+ (%)	OR (95% CI)	Chi Square (p value)	Total Tested (n)	Nos (n) HCV+ (%)
Age group (years)	<20	86	3 (3)	-	**CSLT = 8.49 (0.003)**	60	3 (5)	**1.0**	**CSLT = 7.869 (0.005)**	25	0 (0)
	21-50	376	49 (13)	-		221	44 (20)	**4.8 5.6**		156	5 (3)
	>51	41	8 (20)	3.19 (0.715-14.147)		22	5 (23)			19	3 (16)
Gender	Male	221	30 (14)	1.32 (0.772-2.254)	1.0 (.31)	147	26 (18)	1.07 (0.594-1.943)	0..06 (0.813)	74	4 (5)
	Female	282	30 (11)	1.2 (0.307-4.709)		156	26 17)			126	4 (3)
Married	Yes	414	57 (14)	**4.6 (1.482-14.098)**	**7.5 (0.006)**	248	49 (20)	**4.27**	**6.48 (0.010)**	166	8 (4)
	No	89	3 (3)			55	3 (5)	**(1.354-13.386)**		34	0 (0)

*Numbers in bold reflect statistically significant relationships