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Prevalence of Hepatitis 'C' in Apparently Suspected Individuals of Southern Punjab by Highly Sensitive Chemiluminescence Immunoassay

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ABSTRACT

Background: The prevalence of hepatitis C virus (HCV) infection is increasing worldwide .Hepatitis C virus (HCV) infections accounts for substantial proportions of the world wide liver disease. This study was aimed to assess the prevalence of Anti-HCV among suspected patients for hepatitis by community doctors attending collection centers of Aga Khan University Hospital's clinical laboratory Karachi in Southern Punjab.
Place and Duration: A retrospective study was conducted from January 2011 to June 2011 at Multan Stat Laboratory of Aga Khan University Hospital.
Methods: A total of 3341 suspected patients were received at collection centers and included in this study. The screening was performed by using the Elecsys Anti-HCV II assay kit on Cobas e 411 immunoassay analyzer. Data were entered and analyzed using SPSS version 16 statistical package.
Results: From the total of 3341 suspected hepatitis patients, 2045 (61.20%) were males and 1296 (38.80%) were females. Anti-HCV was found reactive in 1002 (29.99%) borderline 20 (0.59%) and non-reactive in 2319 (69.42%). Out of the total individuals, 589 (17.63%) males and 413 (12.36%) females were Anti-HCV reactive. . . The overall seroprevalence was found to be 29.99%. LODHRAN (70%), VEHARI (49.55%), KHANEWAL (44%) and BAHAWALNAGER (37.7%) are most affected districts for HCV infection.
Conclusion: The overall prevalence of HCV is alarming high. Males were more affected. It is required to prevent the disease by giving education to the health personal as well as to general population.

Keywords: Hepatitis C virus, Prevalence, Southern Punjab

INTRODUCTION

Hepatitis C virus (HCV) belongs to the Flaviviridae family and is a plus-stranded RNA virus[1]. HCV isolates are classified into 6 genotypes that differ in their nucleotide sequence by 30%–35% and into multiple subtypes that differ in their nucleotide sequence by 20%–25%[2]. Approximately 3% of the world population (or about 170 million people) may be infected with HCV[3,4]. Infection with hepatitis C virus (HCV) may lead to disabling symptoms, cirrhosis and hepatocellular carcinoma[5,6], and reportedly account for a significant proportion of endstage liver disease[7]. World Health Statistics 2008 lists cirrhosis of the liver as the 18th commonest cause of mortality in the world, and it is estimated that by 2030, liver cancer will become the 13th commonest cause[8]. In Pakistan 10 million people are presumed to be infected with HCV[9]. It has been demonstrated that nearly 50 % patients with hepatocellular carcinoma ((HCC) in Pakistan are anti- HCV[10]. The prevalence of hepatitis C virus (HCV) infection is increasing worldwide. HCV is predominantly transmitted by means of percutaneous exposure to infected blood.[11-12] in developed countries, most new HCV infections are related to intravenous drug abuse. HCV may also be transmitted by means of acupuncture, tattooing, and sharing razors. Needle stick- injuries in the health care setting result in a 3% risk of HCV transmission[13-14]. Vertical transmission also remains the only route of acquisition of the virus by children in most developed countries[15]. Some health care procedures, i.e., surgical and dental treatments, have recently been indicated as risk factors for acute HCV[16]. In most individuals anti-HCV antibodies appear 2 to 8 weeks after exposure, although it can last for as long as nine months[17,18]

A recently concluded survey[19] found HCV prevalence within provinces as 5% in Sindh, 6.7% in Punjab, 1.1% in NWFP and 1.5% in Baluchistan. In a community based study in Punjab, Pakistan,

6.5% HCV seroprevalence was recorded[20]. Hospital-based studies have revealed prevalence rates of anti-HCV antibodies that are 9% in Mardan and 17.77% in Faisalabad[21,22].

The present study was conducted to evaluate the sero-positivity of HCV in both sexes in the population, reporting to different collection points of Aga Khan laboratory Hospital and to determine its prevalence in various districts in Southern Punjab.

MATERIAL & METHODS
This retrospective study was carried out in the Aga Khan Stat laboratory Multan, with samples collected from all collection points in different areas in Southern Punjab between the months of January and June 2011.

The study included blood specimens for routine testing for Anti-HCV received from individuals suspected for hepatitis by doctors at eighteen collection centers of Aga Khan Lab in different districts Dera Gazi Khan(1),Rahim Yar Khan (3), Rajanpur (1),Layyah (1), Vehari (2), Muzaffarghar (1), Bahawalpur (1), Bahawalnagar (1), Multan (5), Khanewal (1), Lodhran (1) of Southern Punjab during study period. District Pakpattan is not included because there is no Aga Khan Collection center in Pakpattan .

A total of 3341 patients were received at collection centers and included in this study. All ages and either sex were included. Samples were collected by using tubes containing separating gel and centrifuged prior to testing. The screening was performed in Aga Khan Multan Stat Lab by using the Elecsys Anti-HCV II assay kit on Cobas e 411 immunoassay analyzer.

The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and Cobas e immunoassay analyzer. The reactive and non-reactive controls of Anti-HCV were run along with the samples.

PRINCIPLE OF THE TEST
The Elecsys Anti-HCV II assay kit is a third-generation test. The Elecsys Anti-HCV II assay uses peptides and recombinant antigens representing core, NS3 and NS4 proteins for the determination of anti-HCV antibodies (23,24).Sandwich principle is used.

STATISTICAL ANALYSIS
Data were collected from computerized record of Aga Khan Laboratory University Karachi . Permission was obtained from the Head of Stat Laboratories, Aga Khan University hospital before the data collection. Data was analyzed using SPSS version 16.0. Percentages were calculated directly for Anti-HCV in different gender and for district-wise individuals having Anti-HCV positivity . The level of significance was set at $p < 0.05$.

RESULTS
Out of the total 3341 individuals , 2045 (61.20%) were males and 1296 (38.80%) were females: Anti-HCV was found reactive in 1002 (29.99%) borderline 20 (0.59%) and non-reactive in 2319 **(69.42%).**
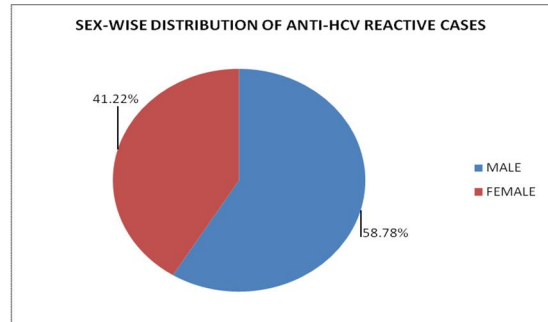
Table 1

Total No. of patients screened for anti-HCV	3341
Total No. of male patients screened for anti-HCV	2045(61.20%)
Total No. of female patients screened for anti-HCV	1296(38.80%)
Total No. and percentage of anti-HCV non reactive patients	2319(69.42%)
Total No. and percentage of anti- HCV borderline patients	20(0.59%)
Total No. and percentage of anti-HCV reactive patients	1002(29.99%)
Total No. of anti-HCV reactive male patients	589
Percentage of anti-HCV reactive male patients from total patients	17.63%
Total No. of anti-HCV reactive female patients	413
Percentage of anti-HCV reactive female patients from total patients	12.36%
Percentage of anti-HCV reactive male patients from anti-HCV screened male patients	28.80%
Percentage of HCV reactive female patients from anti-HCV screened female patients	31.86%

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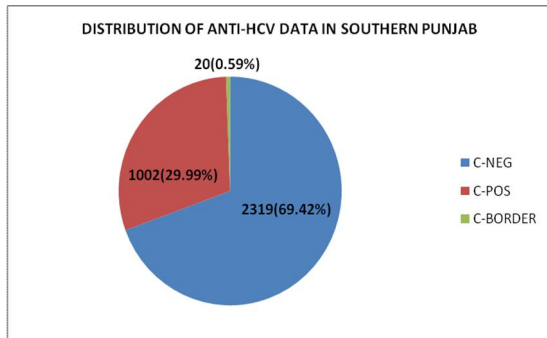
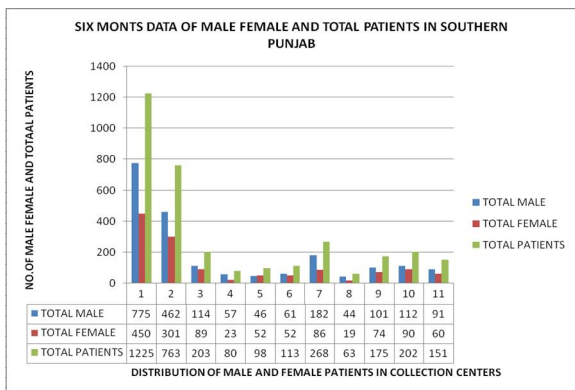
Graph 1

Graph 2

Table 2: District-Wise Male and Female Distribution of Total Patients

	Districts	Total Male	Total Female	Total Patients
1	MULTAN*	775(63.26%)	450(36.74%)	1225
2	RAHIM YAR KHAN**	462(60.55%)	301(39.45%)	763
3	BAHAWALPUR	114(56.15%)	89(43.84%)	203
4	LODHRAN	57(71.25%)	23(28.75%)	80
5	BAHAWALNAGER	46(46.93%)	52(53.07%)	98
6	VEHARI***	61(53.98%)	52(46.01%)	113
7	DERA GHAZI KHAN	182(67.91%)	86(32.08%)	268
8	RAJAN PUR	44(69.84%)	19(30.15%)	63
9	KHANEWAL	101(57.71%)	74(42.29%)	175
10	LAYYAH	112(55.44%)	90(44.55%)	202
11	MUZAFAR GARH	91(60.26%)	60(39.74%)	151
	GRAND TOTAL	2045(61.20%)	1296(38.80%)	3341

MULTAN* (MN-MP-MT-MU-SJ-05 POINTS)		
VEHARI*** (VEHARI-BUREWALA-02 POINTS)		
RAHIM YAR KHAN **(RAHIM YAR KHAN-SADIQABAD-KHANPUR-03 POINTS)		

Table 3: District-Wise Distribution of Female Patients for Anti-HCV

District	NEG Female	POS Female	% POS Female	Borderline Female	Total Female
Multan	319	130	28.88%	1	450
Rahim Yar Khan	210	90	29.9%	1	301
Bahawalpur	60	29	32.58%	0	89
Lodhran	13	10	43.47%	0	23
Bahawalnager	27	25	48.07%	0	52
Vehari	29	22	42.3%	1	52
Dera Ghazi Khan	67	19	22.09%	0	86
Rajan Pur (JP)	14	4	21.05%	1	19
Khanewal	40	34	45.94%	0	74
Layyah	55	34	37.77%	1	90
Muzafar Garh	43	16	26.66%	1	60
	877	413	31.86%	6	1296

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Graph 2

Table 2 , Graph 2 show total number of suspected patients and % ages of male and female subjects in different districts of Southern Punjab. Maximum samples(1225) were collected during study period in Multan (5 collection points) followed by (763) in Rahim Yar Khan(3 collection points) and Dera Ghazi Khan(268).

Table 3 and graph 3 show that the maximum %age (48.07%) of Anti-HCV reactive female subjects was detected in district BAHAWALNAGER ,followed by KHANEWAL

(45.94%), LODHRAN (43.47%), VEHARI (42.3%), LAYYAH (37.77%), BAHAWALPUR (32.58%), RAHIM YAR KHAN (29.9%), MULTAN(28.8%), MUZAFARGARH (26.66%), DERA GHAZI KHAN(22.09%) while the lowest frequency was observed in district RAJAN PUR(21.05%).
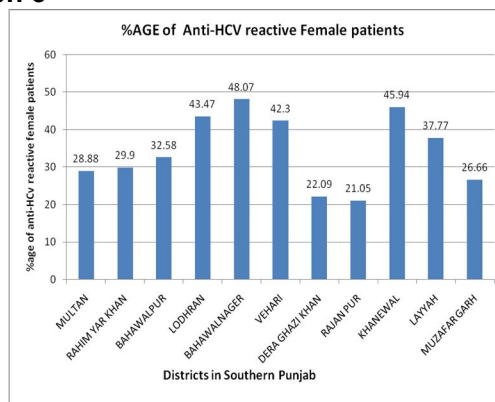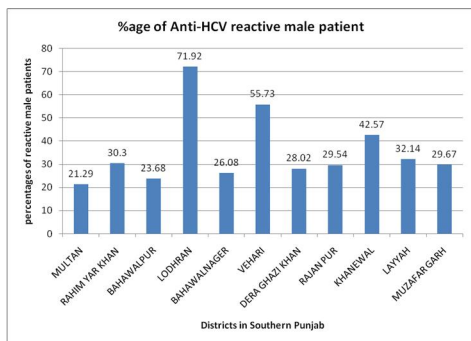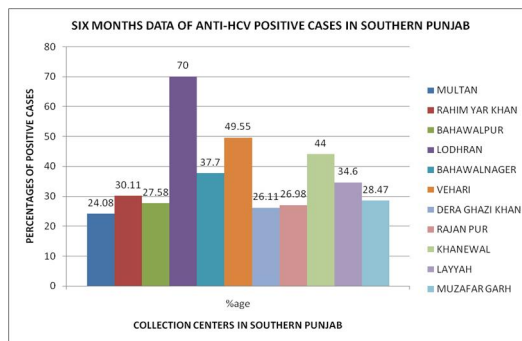
Graph 3

Table 4: District-Wise Distribution of Anti-HCV Male Patients

District	NEG Male	POS Male	% Age POS	Borderline Male	Total Male
Multan	607	165	21.29%	3	775
Rahim Yar Khan	319	140	30.3%	3	462
Bahawalpur	87	27	23.68%	0	114
Lodhran	16	41	71.92%	0	57
Bahawalnager	34	12	26.08%	0	46
Vehari	27	34	55.73%	0	61
Dera Ghazi Khan	129	51	28.02%	2	182
Rajan Pur (JP)	30	13	29.54%	1	44
Khanewal	57	43	42.57%	1	101
Layyah	72	36	32.14%	4	112
Muzafar Garh	64	27	29.67%	0	91
	1442	589	28.80%	14	2045

Graph 4

Graph 5

Table 4 and graph 4 show that the maximum %age of Anti-HCV reactive male subjects was detected in district LODHRAN (71.92%), followed by VEHARI (55.73%), KHANEWAL (42.57%). LAYYAH (32.14%), RAHIM YAR KHAN (30.3%), MUZAFARGARH (29.67%) , RAJAN PUR(JP) (29.54%), DERA GHAZI KHAN(28.02%), BAHAWALNAGAR (26.08%), BAHAWALPUR (23.68%), while the lowest frequency was observed in district MULTAN (21.29%),

Graph 5 shows that LODHRAN (70%), VEHARI (49.55%), KHANEWAL (44%) and BAHAWALNAGAR (37.7%) are most affected districts for HCV infection.

DISCUSSION

Hepatitis C virus infection is endemic not only in Pakistan but also all over the world (25).Many studies have been published about the prevalence of hepatitis C in different regions of Pakistan. Many studies are available about the prevalence of HCV in patients and in healthy persons but not in patients suspected as having hepatitis. In our study the suspected patients for hepatitis , referred by consultants for investigation were screened for HCV.

Sero-prevalence of Hepatitis C virus in different parts of Pakistan, reported in the last 5 years, is from 2.2%-13.5% (26). The highest seroprevalence of hepatitis C has been reported from Lahore (13.5%) (27). Our study results are comparable with the studies reported with in country .The prevalence of HCV among the total subjects was reported 21.7% in another study(28) in the general population of Lahore. Aslam & Aslam (2001) have reported that in Gujranwala, the occurrence of anti-HCV-positive serology was 23.8%.(29). From Islamabad, Nasir Khokhar, et al found 24% HCV prevalence in patients on long term hemodialysis(30). In present study the prevalence of anti-HCV antibodies has been observed higher in male population than female. Prevalence of anti-HCV in males (17.30%) as compared to females(12.68%) was reported by (31) which is similar with our study. Similarly, the other studies have reported higher percentage among males as compared to females(32). The high prevalence of anti-HCV antibodies in males could be attributed to their exposure status to HCV risk factors.

Wide variations was observed in district-wise distribution of results within Southern Punjab in our study .The higher %ages of reactive Anti-HCV were noted in districts LODHRAN (70%), VEHARI (49.55%), KHANEWAL (44%) and BAHAWALNAGER (37.7%). In another study frequency of anti-HCV in young adults from different districts of Southern Punjab was reported high in Bahawalpur and Lodhran (33) .In a small demographics based survey for HCV prevalence,the worst affected region is that of Southern Punjab, especially Khanewal and Hafizabad where the incidence has been reported to be as high as 35&37%(34);.Prevalence of Hepatitis C infection in rural population of Rahim Yar Khan was reported 23.8%in the study (35).

Figure 1: illustrates the differences in prevalence among the provinces and districts. (36)
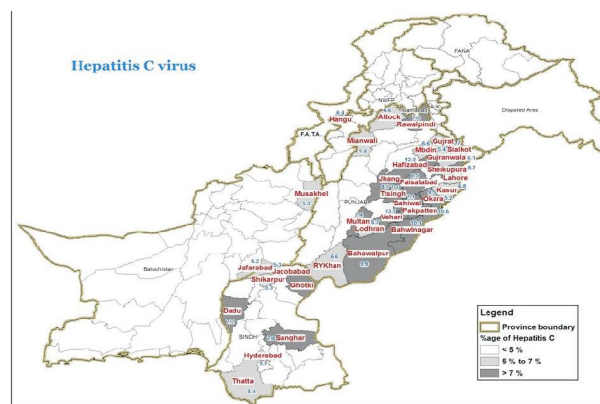
The high prevalence in our study is alarming. The differences in the prevalence rates between our study and those reported elsewhere are due to several factors . .These differences are the populations selected, the use of different methodologies with different sensitivities and the enrollment of both sexes of all ages in the sample population. In this study we used a third generation ECLIA with high sensitivity and specificity and we analysed the laboratory based data on suspected subjects for hepatitis referred by doctors to collection centers of Aga Khan Lab for screening of Anti-HCV.

CONCLUSION

With the increasing rate of Hepatitis C infection in Pakistan, the use of screening has become a vital tool in preventive measure for the disease. In order to prevent HCV infection in our country government should take aggressive steps towards awareness programs involving both the media and public sectors organizations. . It is required to prevent the disease by giving education to the

health personal as well as to general population. Awareness programs regarding Hepatitis C at School level should be started to save our younger generation.

LIMITATIONS OF STUDY

This study took place at collection points and Agha Khan Multan stat lab in Southern Punjab, and the results may not apply to other regions of Pakistan.

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AUTHORS' CONTRIBUTIONS

JA designed the study and advised about the protocols. JA and HF carried out sampling, experimental procedures and manuscript preparation.JA critically reviewed and approved the manuscript. All authors read and approved the final manuscript.

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