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PREVALENCE OF HEPATITIS C VIRUS ANTIBODIES: A CASE STUDY OF OUTPATIENTS IN SINDH, PAKISTAN

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ABSTRACT

This study describes the status of Hepatitis C virus (HCV) prevalence in outpatients of thirteen districts of province of Sindh, Pakistan. The blood samples of outpatients (OPs) were randomly collected from >80 thousand individuals of these districts during 2018 and the sera were studied at central diagnostic Laboratory LUHMS, Hyderabad. The antibodies screening showed 14.3% HCV prevalence in the OPs. The HCV antibodies level of more than 1.0 IU /100 µL) was considered to indicate HCV. OPs were more HCV positive in females (7.96%) than males (6.36%) of OPs. Geographically the HCV prevalence was higher at Mirpurkhas (22.0%), Jamshoro (21.7), Tando Allahyar (19.8) and Sanghar (19.0%). It was around 18% in Khairpur Mirs (18%) and N. Feroze (18.3%). In other districts it ranged from 10.2 (the lowest in Tharparkar) and 15.9% in Kashmore. The districts in hand may be arranged in following order on the basis of total % HCV prevalence.

Mirpur Khas (22.0, the highest) ≈ Jamshoro (21.7) > Tando Allahyar (19.8) > Sanghar (19.0) > N. Feroze (18.3%) ≈ Khairpur Mirs (18) > Sukkur (16.0) ≈ Kashmore (15.9) ≈ Ghotki (15.6) > Dadu ((13.8) ≈ Badin (13.5) > Hyderabad (12.7) > Tharparkar (10.2, the lowest).

The order of sequence of average antibodies level in HCV+ OPs of various districts was as follows:

Tando Allahyar (60.8 ± 1.68 IU) > N. Feroze (55.5 ± 1.64 IU) > Jamshoro (48.71 ± 0.946 IU) ≈ Badin (48.14 ± 1.324 IU) ≈ Sukkur (47.3 ± 1.153 IU) > Ghotki (46.09 ± 2.915 IU) > Tharparkar (39.3 ± 3.11 IU) ≈ Khairpur Mirs (38.18 ± 1.983 IU) Hyderabad (21.10 ± 0.291 IU) > Dadu (17.60 ± 0.395 IU > Sanghar (15.9 ± 0.330 IU) > Kashmore (13.16 ± 1.146 IU) > Mirpur Khas (10.29 ± 0.296 IU).

There was difference between severity and disease prevalence in the districts. Infants and Young population appeared to have some immunity against HCV. HCV prevalence, however, increased with age positively linearly or polynomially.

Key words: Hepatitis C Virus Prevalence, Sindh, Pakistan

INTRODUCTION

Hepatitis C virus (HCV) is a pandemic viral disease, the virus is a plus-strand RNA virus, belongs to the Flaviviridae family (Lanini *et al*., 2016). It has a higher disease incidence ratio in Africa and Asia, 2.9 and 2.8% of the population (Petruzziello, 2018). The vernacular name of HCV is referred to as 'Kala Yarkan' (Bosan *et al*., 2010; Sheikh *et al*., 2019). HCV and HBV are agents of life-threatening hepatic disorders; HCV has higher prevalence with 1.3 million death incidences worldwide in a year (Ward and Hinman, 2019). About 200 million people are infected with HCV worldwide which effects c 3.3% of the World population (Wands, 2004; Bill and Porchet, 2008). It is characterized by poor appetite, vomiting, fatigue and jaundice. About 5.9 to 6% of the population is considered to be infected with HCV in Pakistan (Mahmood *et al*., 2010; Umer and Iqbal, 2016) – one in every 20 Pakistani (Al-Kanani *et al*., 2018) is a great health burden. The Punjab and Sindh are said to be the most affected provinces with HCV (Abbas *et al*., 2008). There are six genotypes of HCV of which genotype 3 is the most prevalent (80%) followed by genotype 2 (Shah *et al*., 2008). The most prevalent genotype in Pakistan is genotype 3a (Haqqi *et al*, 2019). Patoli *et al*. (2019) described type 3 genotype to be predominant in Ghotki (65.9%), Hyderabad (73.9%), Nawab Shah (87.8%), Matiari (91.6%) and Tando Allahyar (91.6%) – being impartial to gender. There are several factors to transfer the virus from person to person i.e. misuse of piercing medical instruments, barber sharp edge instruments, more importantly the lack of awareness in the general population for HCV. Blood transfusion and hypodermal piercing with instruments in multiple use or not properly sterilized may at times be the reason of transfer from person to person. The virus carrier is obviously the greatest potential transmitter of the viral infection in the population. There is no recommended vaccine available for HCV prevention (Boettler *et al*., 2019). Most studies conducted in rural and urban areas of Sindh are based on serological methods except a few

publications (Bhatti and Manzoor, 2016; Patoli *et al*. (2018) which used molecular detection of viral RNA. However, there is paucity of data from Sindh (Umer *et al*., 2010). There is a lack of data on HCV infection in patients visiting OPDs at various health facilities in Sindh. The data has generally been presented with lower sample size or minimal anthro-geographical records (Akhund *et al*., 2008; Aziz *et al*., 2010; Bosan *et al*., 2010; Ujjan *et al*., 2012). The Present study for HCV antibody was carried out with large number of individuals visiting OPDs of several health care providers in thirteen districts of Sindh (Fig. 1).

MATERIALS AND METHODS

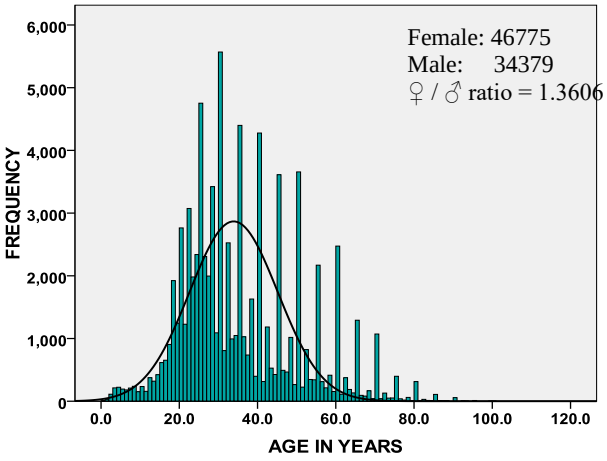
The blood samples of outpatients (OPs) were randomly collected from different private and public hospitals of the thirteen selected districts of Sindh. The study was conducted from January 2018 to December 2018 - all over the year. Each venous blood sample was taken with BD syringe with a cognizable consent of respective patient and consultants. Blood sample of 05 mL volume transferred to vacutainer serum separating tube. The tubes were inverted five times and left for 30 minutes to achieve clotting and further centrifuged at 3000 rpm for 10 minutes, the sera were separated and transported in a moist icebox to the Research and Diagnostic Laboratory of Liaquat University of Medical and Health Sciences (LUMHS), Jamshoro. The sera of samples were tested for HCV antibodies (IgM) on ELISA (AXSYM-ABBOTT, USA). The reference ranges were prescribed by company as >1.0 – 100 IU.μL^{-1} as positive. % Prevalence was calculated as %P = HCV (+) OPs / total OPs * 100). The data of each patient was recorded for their district of residence, gender and age and the antibodies status. The data was analyzed by using IBM-SPSS-21.

Number of outpatients

1. Badin - 3929
2. Dadu - 2755
3. Ghotki – 867
4. Hyderabad – 51622
5. Jamshoro – 4242
6. Kashmore – 2016
7. Khairpur Mirs – 1258
8. Mirpur Khas – 1003
9. N. Feroze – 2251
10. Sanghar – 3471
11. Sukkur – 5102
12. Tando Allahyar – 1820
13. Tharparkar – 818

Total number of outpatients
81154

Fig. 1. The districts of Sindh. Districts studied for HCV prevalence are shown by asterisk.



Age of the Outpatients
N = 81154
Mean age = 35.24 years
SE = 0.0527
Median =- 32.00
CV = 42.59%
Skewness = 0.688
SE skewness = 0.009
Kurtosis = 0.190
SE kurtosis = 0.017
Minimum = 0.5 year
Maximum = 100.0
KS-Test * = 0.116
P < 0.0001

Fig. 2. Frequency distribution of age of OPs of thirteen districts of Sindh studied for HCV. KS-test*, Kolmogorov-Smirnoff test was employed with Liliefors significance correction.

Table 1. Gender distribution of outpatients in towns studied.

District	Female OPs	Male OPs	♀ / ♂ ratio
Badin	2124	1805	1.1767
Dadu	1375	1380	0.9963
Ghotki	406	461	0.8807
Hyderabad	31638	19984	1.5832
Jamshoro	2334	1908	1.2232
Kashmore	761	1255	0.6064
Khairpur Mirs	663	595	1.1143
Mirpur Khas	500	503	0.9940
N. Feroze	1140	1111	1.026
Sanghar	1874	1597	1.1735
Sukkur	2581	2521	1.0238
Tando Allahyar	980	840	1.1667
Tharparkar	399	419	0.9523
Pooled	46775	34379	1.3606

RESULTS AND DISCUSSION

Eighty one thousand one hundred and fifty four outpatients from 13 districts of Sindh (Fig. 1) were studied for HCV by determining antibodies concentration in their blood sera. The number of OPs studied for each districts is presented in Fig. 1. A large population (51622 in number) of outpatients (63.61%) came from district Hyderabad and the lowest number of OPs (818 in number) was from district Tharparkar. In other districts, number of outpatients ranged 1003 (Mirpur Khan) and 5102 (Sukkur). District Karachi was not included in this study as several authors have investigated HCV Seroprevalence in Karachi in recent years (Janjua *et al*., 2010; Aziz et *al*., 2010; Abdullah *et al*., 2011; Khan *et al.,* 2013; Moiz *et al.,* 2014, etc.).

Age distribution of outpatients

The age distribution of outpatients was asymmetric – positively skewed and leptokurtic. The age of the outpatients varied from 0.5 year to 100 year (averaging to 35.24 ± 0.0327 year varying around 42.59% among the outpatients of the districts (Fig. 2). The median age was 32 years. The outpatients of lower age groups were comparatively lesser in number.

Gender composition

Amongst the total OPs (81154), 46675 OPs were female and 34379 were male (female: male ratio: 1.3606). The gender composition in terms of female: male ratio varied substantially in various districts (Table 1). This ratio varied from 0.6064 in Kashmore to 1.5832 in Hyderabad.

Table 2 presents data on gender composition for various age groups, separated by 10-year interlude, in various districts. The distribution of female and male outpatients into various age groups varied significantly in each districts indicated by the values of X^2 and the likelihood ratios. In all districts, substantial proportion of female and male outpatients belonged to the age groups, 20.1-30 and 30.1 – 40 years. In Hyderabad and Tharparkar districts three age groups (20.1-30, 30.1-40 and 40.1-50 years were more conspicuous. Generally, the lower and upper age groups had lower number of outpatients. In pooled sample of 81154 outpatients' age classes of 10.1-20, 20.1-30, 30.1-40, 40.1-50 and 50.1-60 years were important consisting of 11.56, 34.21, 21.99, 14.74 and 9.21% of the outpatients

Seroprevalence of antibodies

Percent frequency of OPs of various districts for various size classes of antibodies concentrations in terms of international units (>1 to 100 IU) is presented in Table 3. As per criterion, outpatients with ≤ 1IU of antibodies were considered non-reactive. Such Unreactive outpatients varied from districts to districts being the lowest in Mirpur Khan (78%), and Jamshoro ((78.3). The unreactivness was maximum in Tharparkar (89.9%) and Hyderabad (87.3%). It generally concentrated around 80-85 % in other districts. That is to say a larger portion of outpatients was Unreactive (HCV-). The reactive patients were 13.5% in Badin, 13.8% in Dadu, 15.6% in Ghotki, 12.7% in Hyderabad, 21.7% in Jamshoro, 15.6 % in Kashmore, 18% in Khairpur Mirs, 22.00% in Mirpur Khas, 18.3% in N. Feroze, 19.1% in Sanghar, 16.0 in Sukkur, 17.8 % in Tando Allahyar and minimally 10.1% in Tharparkar. Compositely, out of 81154 outpatients, 69529 were non-reactive and 11625 were found to be reactive (HCV +). Percent frequency of reactive OPs falling in various size classes of the antibodies concentrations (International Units) is presented in Table 4. The outpatients distributed widely. The patients of Kashmore belonged to the lowest size class of antibody concentration (> 1.0 to 10 IU) with as high percent frequency as 62.3%. Generally, the HCV (+) outpatients were associated with antibodies concentration of low magnitude (size class 10.1 – 20 IU). The outpatients of district Badin exhibited, however, more severity of disease as 65.6% of the cases were exhibited in classes of high order antibodies concentration (80.1-90 and 90.1-100 IU). OPs of Dadu and Mirpur Khas were found to be restricted in initial three classes and those of Sanghar in initial five classes of anti-bodies concentration, respectively. Majority of Dadu OPs associated with antibodies of size class 20.1-30 IU. In the pooled sample, the distribution was widespread but 62 % of the cases associated with the initial two classes of antibodies concentration (Table 4).

Table 2. Frequency distribution of gender species (female and male) of outpatients of various age groups in 13 districts of Sindh, studied for HCV. Two predominant classes are shown in bold.

Age size classes	Female	Male	Total	X^2	Likelihood ratio
District Badin (3929)					
≤ 10 years	29	45	74	306.55 P < 0.0001	324.76 P < 0.0001
10.1- 20	267	202	469		
20.1- 30	**840**	**475**	**1315**		
30.1 - 40	**486**	**436**	**922**		
40.1 - 50	302	334	636		
50.1 – 60	137	196	333		
60.1 - 70	48	84	132		
70.1 - 80	11	24	40		
80.1 - 90	4	4	8		
90.1 - 100	-	-	-		
District Dadu (2755)					
≤ 10 years	39	46	85	212.83 P < 0.0001	232.85 P < 0.0001
10.1- 20	206	97	403		
20.1- 30	**487**	**409**	**896**		
30.1 - 40	**329**	**295**	**624**		
40.1 - 50	210	212	422		
50.1 – 60	73	139	212		
60.1 - 70	26	57	83		
70.1 - 80	3	21	24		
80.1 - 90	2	4	6		
90.1 - 100	-	-	-		
District Ghotki (867)					
≤ 10 years	8	17	25	99.24 P < 0.022	5.860 P < 0.0001
10.1- 20	42	60	102		
20.1- 30	**145**	**145**	**290**		
30.1 - 40	**115**	**105**	**220**		
40.1 - 50	60	64	124		
50.1 – 60	25	43	68		
60.1 - 70	8	23	31		
70.1 - 80	1	2	3		
80.1 - 90	1	2	3		
90.1 - 100	1	-	1		
District Hyderabad (51622)					
≤ 10 years	395	603	998	3297.45 P < 0.0001	3306.96 P < 0.0001
10.1- 20	3403	2242	5645		
20.1- 30	**13484**	**5182**	**18666**		
30.1 - 40	**6731**	**4304**	**11035**		
40.1 - 50	3859	3308	7167		
50.1 – 60	2323	2437	4760		
60.1 - 70	1023	1359	2382		
70.1 - 80	349	430	779		
80.1 - 90	68	106	174		
90.1 - 100	03	13	16		
District Jamshoro (4242)					
≤ 10 years	17	40	57	212.83 P < 0.0001	232.85 P < 0.0001
10.1- 20	235	215	450		
20.1- 30	**768**	**461**	**1229**		

30.1 - 40	**537**	**377**	**914**		
40.1 - 50	411	370	781		
50.1 – 60	226	259	485		
60.1 - 70	103	133	236		
70.1 - 80	35	43	78		
80.1 - 90	2	10	12		
90.1 - 100	-	-	-		
District Kashmore (2016)					
≤ 10 years	37	103	140		
10.1- 20	132	252	384		
20.1- 30	**223**	**389**	**612**	173.19	190.63
30.1 - 40	**193**	**269**	**462**	P < 0.0001	P < 0.0001
40.1 - 50	96	130	226		
50.1 – 60	53	68	121		
60.1 - 70	20	37	57		
70.1 - 80	6	5	11		
80.1 - 90	1	2	3		
90.1 - 100	-	-	-		
District Khairpur Mirs (1258)					
≤ 10 years	16	29	45		
10.1- 20	96	99	195		
20.1- 30	**233**	**157**	**390**		
30.1 - 40	**159**	**128**	**287**	125.33	138.92
40.1 - 50	93	89	182	P < 0.0001	P < 0.0001
50.1 – 60	46	51	97		
60.1 - 70	14	32	46		
70.1 - 80	3	9	12		
80.1 - 90	2	1	3		
90.1 - 100	1	-	1		
District Mirpur Khas (1003)					
≤ 10 years	5	19	24	140.83	150.90
10.1- 20	43	40	83	P < 0.0001	P < 0.0001
20.1- 30	**152**	**126**	**278**		
30.1 - 40	**115**	**133**	**248**		
40.1 - 50	105	87	192		
50.1 – 60	53	64	117		
60.1 - 70	22	28	50		
70.1 - 80	3	5	8		
80.1 - 90	2	1	3		
90.1 - 100	-	-	-		
District N. Feroze (2251)					
≤ 10 years	28	58	86	230.71	255.64
10.1- 20	177	175	352	P < 0.0001	P < 0.0001
20.1- 30	332	299	631		
30.1 - 40	279	236	515		
40.1 - 50	194	169	363		
50.1 – 60	38	111	198		
60.1 - 70	2	39	77		
70.1 - 80	3	17	19		
80.1 - 90	-	6	9		
90.1 - 100	-	1	1		
District Sanghar (3471)					
≤ 10 years	23	43	66	304.49	324.94

10.1- 20	194	173	357	P < 0.0001	P < 0.0001
20.1- 30	709	403	1112		
30.1 - 40	473	380	853		
40.1 - 50	270	313	583		
50.1 – 60	145	176	321		
60.1 - 70	61	79	140		
70.1 - 80	7	21	28		
80.1 - 90	1	6	7		
90.1 - 100	-	-	-		
District Sukkur (5102)					
≤ 10 years	35	108	143	420.117	446.348
10.1- 20	330	361	691	P < 0.0001	P < 0.0001
20.1- 30	**801**	**661**	**1462**		
30.1 - 40	**604**	**519**	**1123**		
40.1 - 50	455	408	863		
50.1 – 60	216	291	507		
60.1 - 70	92	114	206		
70.1 - 80	22	49	71		
80.1 - 90	3	9	12		
90.1 - 100	-	1	1		
District Tando Allahyar (1820)					
≤ 10 years	13	20	33	146.31	165.31
10.1- 20	104	72	176	P < 0.0001	P < 0.0001
20.1- 30	335	239	574		
30.1 - 40	261	200	461		
40.1 - 50	142	157	299		
50.1 – 60	77	99	176		
60.1 - 70	37	42	79		
70.1 - 80	9	11	20		
80.1 - 90	2	0	2		
90.1 - 100	-	-	-		
District Tharparkar (818)					
≤ 10 years	6	7	13	105.94	129.07
10.1- 20	39	39	78	P < 0.004	P < 0.0001
20.1- 30	158	129	287		
30.1 - 40	91	89	180		
40.1 - 50	48	72	120		
50.1 – 60	34	49	83		
60.1 - 70	13	24	37		
70.1 - 80	6	8	14		
80.1 - 90	4	1	5		
90.1 - 100	-	1	1		
Pooled Data set (81154)					
≤ 10 years	651	1138	1789	4622.7	3890.9
10.1- 20	5258	4127	9385	P < 0.0001	P < 0.0001
20.1- 30	18686	9074	27760		
30.1 - 40	10373	7472	17845		
40.1 - 50	6250	5713	11963		
50.1 – 60	3494	3983	7477		
60.1 - 70	1505	2051	3556		
70.1 - 80	457	653	1110		
80.1 - 90	96	152	248		
90.1 - 100	5	16	21		

Table 3. Percent frequency of outpatients in various size classes of antibodies concentration as I.U. units detected in outpatients of 13 districts of Sindh. Outpatients detected with antibodies ≤ 1.0 I.U. were considered non-reactive (HCV -) and those with higher values were rated as reactive (HCV +).

Districts Of Sindh	Size classes of antibodies international units												% Total HCV (+)
	≤ 1	1.1-10	10.1-20	20.1-30	30.1-40	40.1-50	50.1-60	60.1-70	70.1-80	80.1-90	90.1-100	>100	
A	**86.5**	1.0	2.0	1.3	2.0	2.6	1.2	0.7	0.8	0.5	2.1	0.10	13.5
B	**86.2**	2.8	4.5	6.2	0.3	-	-	-	-	-	-	-	13.8
C	**84.4**	1.7	5.2	-	0.2	2.9	1.6	1.2	0.6	0.9	2.7	-	15.6
D	**87.3**	3.7	6.2	0.1	0.5	0.6	0.4	0.3	0.3	0.2	0.4	0.009	12.7
E	**78.3**	1.2	3.0	1.8	3.5	3.0	2.3	1.8	1.3	0.8	2.9	0.10	21.7
F	**84.4**	9.6	3.3	0.1	0.4	0.1	0.2	-	0.2	0.2	0.2	0.20	15.6
G	**82.0**	2.3	6.4	-	0.7	2.6	1.7	0.9	1.2	0.8	1.4	-	18.0
H	**78.0**	7.9	14.0	0.2	-	-	-	-	-	-	-	-	22.0
I	**81.7**	1.4	2.0	1.7	2.0	2.0	2.5	1.1	0.8	0.5	5.10	-	18.3
J	**80.9**	4.3	9.6	3.8	1.4	0.03	-	-	-	-	-	-	19.1
K	**84.0**	1.7	4.3	0.02	0.5	1.8	1.9	1.5	1.0	0.7	2.6	-	16.0
L	**82.2**	0.8	2.9	0.10	1.5	2.0	2.4	2.3	1.0	1.3	5.69	0.10	17.8
M	**89.9**	0.9	2.5	1.0	1.2	1.3	0.8	0.9	0.4	0.2	0.9	-	10.1
Pooled	**85.7**	**3.3**	**5.6**	**0.6**	**0.9**	**0.9**	**0.7**	**0.6**	**0.4**	**0.3**	**1.0**	**0.03**	**14.3**

A = Badin; B, Dadu,; C, Ghotki; D, Hyderabad; E, Jamshoro; F, Kashmore; G, Khairpur Mirs; H, Mirpur Khas; I, N. Feroze; J, Sanghar; K, Sukkur; L, Tando Allahyar; and M, Tharparkar

Table 4. Percent frequency of reactive (HCV +) outpatients falling in various size classes of antibodies concentration (international units) in 13 districts of Sindh. Outpatients detected with antibodies concentration ≤ 1.0 I.U. were considered non-reactive (HCV -) and those with higher values (> 1 to 100 IU were rated as reactive (HCV +). Only reactive outpatients' data was included in this analysis.

Districts of Sindh (Number of HCV + cases)	Size Classes of Antibodies Concentration in Terms of International Units										
	> 1.0 – 10.0 units	10.1-20 units	20.1-30.0 units	30.1-40.0 units	40.1 – 50.0 units	50.1-60.0 units	60.1-70.0 units	70.1-80.0 units	80.1-90.0 units	90.1-100.0 units	≥ 100 units
Badin (530)	7.0	14.9	10.0	15.1	12.8	9.1	5.1	6.0	40	**15.6**	0.4
Dadu (381)	20.2	32.8	**45.2**	1.8	-	-	-	-	-	-	-
Ghotki (135)	8.1	**33.4**	-	1.58	11.1	11.1	8.1	3.7	6.0	17.0	-
Hyderabad (6556)	29.1	**48.5**	0.9	4.9	4.4	3.6	2.4	1.8	1.5	3.4	0.1
Jamshoro (920)	5.7	**13.4**	8.7	16.0	13.9	10.5	8.1	6.0	4.1	**13.4**	0.4
Kashmore (321)	**62.3**	**26.8**	0.9	2.2	1.1	1.0	2.6	-	0.9	2.5	0.3
Khairpur Mirs (226)	12.8	**35.5**	-	3.1	15.1	9.7	4.9	6.6	4.9	7.5	-
Mirpur Khas (221)	36.2	**63.3**	0.5	-	-	-	-	-	-	-	-
N. Feroze (411)	7.5	10.7	9.3	11.4	10.7	9.5	5.9	4.1	3.2	**27.2**	0.5
Sanghar (662)	22.4	**50.4**	19.9	7.1	0.2	-	-	-	-	-	-
Sukkur (819)	10.9	**26.6**	0.1	2.9	11.8	11.3	9.5	6.6	4.3	15.8	0.2
TandoAllahyar (360)	4.2	14.4	-	8.3	10.0	12.3	11.4	5.2	5.3	**28.2**	0.6
Tharparkar (83)	9.6	**24.1**	9.7	12.0	13.3	7.2	9.6	3.7	2.4	8.4	-
Pooled (11625)	23.10	**38.9**	4.7	6.0	6.2	5.2	3.4	2.8	2.1	7.1	0.2

A = Badin; B, Dadu,; C, Ghotki; D, Hyderabad; E, Jamshoro; F, Kashmore; G, Khairpur Mirs; H, Mirpur Khas; I, N. Feroze; J, Sanghar; K, Sukkur; L, Tando Allahyar; and M, Tharparkar

District-wise location and dispersion of antibodies concentration in HCV (+) outpatients

Table 5 describes the location and dispersion parameters of antibodies concentration detected in HCV (+) OPs of various districts. Distribution of antibodies concentration in all districts was asymmetrical as indicated by

Kolmogorov- Smirnoff test with Lilliefors correction. Coefficient of variability associated with the data for various districts was moderate to very high i.e. 42.7 % in Mirpur Khas and 155% in Kashmore.

Table 5. Location and dispersion parameters of antibodies concentration (expressed in international units) observed in HCV (+) outpatients of 13 districts of Sindh. Only reactive outpatients' data was included in this analysis.

Statistics	Districts of Sindh												
	A	B	C	D	E	F	G	H	I	J	K	L	M
N	530	381	135	6556	920	321	226	221	411	662	819	360	83
Mean	**48.14**	**17.60**	**46.09**	**21.10**	**48.71**	**13.16**	**38.18**	**10.29**	**55.5**	**15.9**	**47.3**	**60.8**	**39.3**
SE	1.324	0.395	2.915	0.291	0.946	1.146	1.983	0.296	1.64	0.330	1.153	1.68	3.11
CV (%)	63.33	43.77	73.49	111.7	58.9	155.9	78.09	42.7	60.1	53.6	69.8	52.4	72.2
G1	0.400	-0.453	0.329	1.949	0.347	3.058	0.635	-0.66	0.11	0.299	0.194	-0.219	0.661
Sg1	0.106	0.125	0.209	0.030	0.081	0.136	0.162	0.164	0.12	0.095	0.085	0.129	0.264
G2	-1.00	-0.775	-1.329	2.962	-0.83	8.850	-0.83	-0.46	-1.38	-0.03	-1.30	-1.17	-0.481
Sg2	0.212	0.249	0.414	0.060	0.16	0.271	0.322	0.326	0.240	0.19	0.171	0.256	0.523
Min	1.1	1.09	1.40	1.10	1.06	1.03	1.02	1.01	1.29	1.03	1.01	1.01	1.06
Max	100.7	32.80	100.0	100.5	100.7	100.2	100.0	20.91	100.1	42.03	100.3	100.7	100.0
K-ST	0.085	0.109	0.219	0.343	0.066	0.337	0.238	0.132	0.158	0.054	0.191	0.140	0.133
p	***	***	***	***	***	***	***	***	***	***	***	***	***
Curve	AS	AS	AS	AS	AS	AS	AS	AS	AS	AS	AS	AS	AS

A = Badin; B, Dadu,; C, Ghotki; D, Hyderabad; E, Jamshoro; F, Kashmore; G, Khairpur Mirs; H, Mirpur Khas; I, N. Feroze; J, Sanghar; K, Sukkur; L, Tando Allahyar; and M, Tharparkar

CV (%), Coefficient of variability; G1, Skewness; Sg1, SE of skewness; G2, Kurtosis; Sg2, SE of Kurtosis; K-ST, Kolmogorov-Smirnoff test with Lilliefors significance correction. ***, $p < 0.0001$; AS, Asymmetrical.

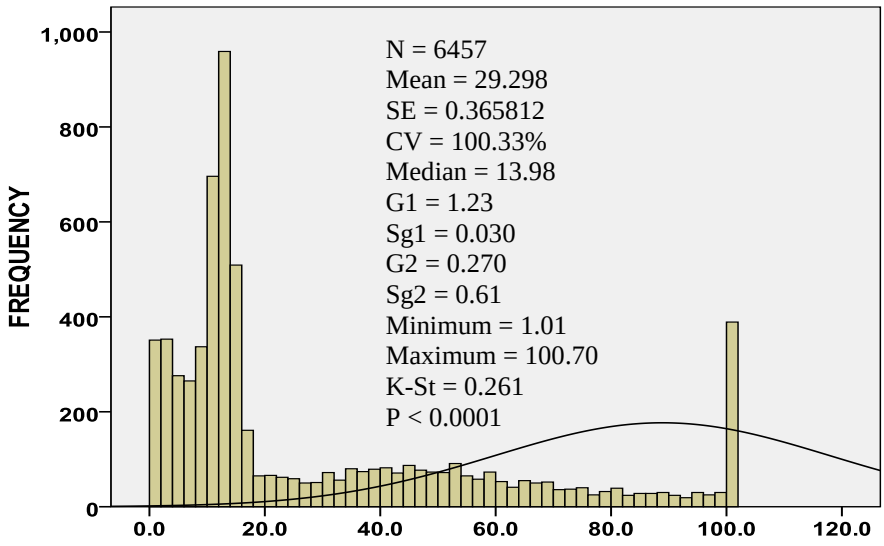


Fig. 3. Distribution of HCV (+) female outpatients (N = 6457) from the pooled data into various size classes of antibiotic concentration (I.U).

The order of sequence of average antibodies level in HCV+ OPs of various districts may be described as follows:

Tando Allahyar (60.8 ± 1.68 IU) > N. Feroze (55.5 ± 1.64 IU) > Jamshoro (48.71 ± 0.946 IU) ≈ Badin (48.14 ± 1.324 IU) ≈ Sukkur (47.3 ± 1.153 IU) > Ghotki (46.09 ± 2.915 IU) > Tharparkar (39.3 ± 3.11 IU) ≈ Khairpur Mirs (38.18 ± 1.983 IU) Hyderabad (21.10 ± 0.291 IU) > Dadu (17.60 ± 0.395 IU > Sanghar (15.9 ± 0.330) > Kashmore (13.16 ± 1.146 IU) > Mirpur Khas (10.29 ± 0.296 IU).

It follows from the results that on average concentration of antibodies in sera of the OPs, the disease severity was most intense in district Tando Allahyar and relatively least severe in Mirpur Khas.

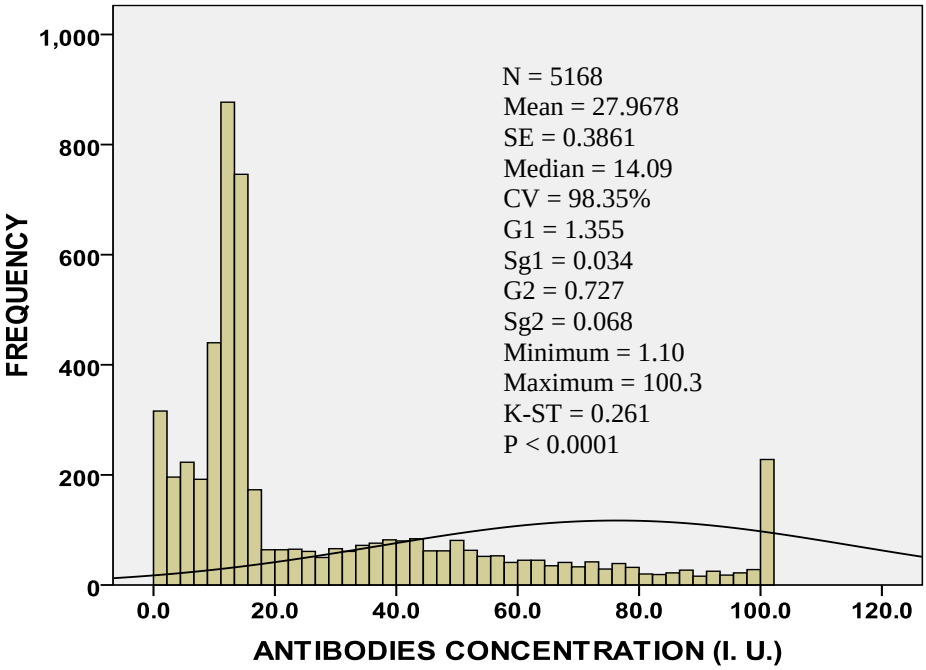


Fig. 4. Distribution of HCV (+) male outpatients (N = 5168) from the pooled data into various size classes of antibodies concentration (I.U).

Table 6. Location and dispersion parameters of antibodies concentration (expressed in international units) observed in HCV (+) OPs of all districts investigated. Only reactive outpatients' data was included in this analysis. CV (%), Coefficient of variability; G1, Skewness; Sg1, SE of skewness; G2, Kurtosis; Sg2, SE of Kurtosis; K-ST, Kolmogorov-Smirnoff test with Lilliefors correction for significance. ***, $p < 0.0001$; AS, Asymmetrical.

Statistics	All Districts Pooled		
	♀	♂	♀ + ♂
N	6457	5168	11625
Mean	29.298	27.967	28.710
SE	0.3658	0.3861	0.2651
CV (%)	100.33	98.35	98.51
G1	1.230	1.355	1.285
Sg1	0.030	0.034	0.023
G2	0.270	0.727	0.463
Sg2	0.061	0.060	0.045
Min	1.01	1.10	1.01
Max	100.7	100.3	100.7
K-ST	0.261	0.241	0.260
p	***	***	***
Curve	AS	AS	AS

Gender relations of antibodies concentration in HCV (+) OPs

Antibodies concentration varied widely in HCV OPs in a range of > 1.0 to 100 IU, both in female and male outpatients but generally concentrated in lower size classes of > 1.0 to 20 IU (Fig. 3 and 4 and Table 6). The antibodies concentration in pooled data of female (N = 6457) and male (N = 5168) outpatients averaged to 29.298 ± 0.3658 (CV: 100.33%) and 27.9678 ± 0.3861 IU (CV: 98.35%) respectively and tended to distribute asymmetrically. Antibodies level, however tended to be significantly higher in female outpatients than male ($t = 2.499$, $p < 0.020$). Antibodies level in pooled female and males HCV (+) OPs averaged to 28.71 ± 0.2651 IU, varied around 98.51% and also distributed asymmetrically. The average of composite sample didn't differ from mean of female sample ($t = 1.2845$, NS) and male sample ($t = 1.580$, NS) significantly.

Gender relations of HCV

Table 7 presents the gender relationship with HCV in various districts of Sindh in form of contingency tables

Table 7. Gender relationship of HCV in thirteen districts of Sindh. N = Number of total outpatients

Gender	Number (Percentage) HCV (-)	Number (Percentage) HCV (+)	Total	X^2	Likelihood ratio
District Badin (N = 3929)					
Female	1804 (84.93%)	260 (12.24 %)	2124	6.170, p < 0.013	6.150, p < 0.013
Male	1535 (85.04%)	270 (14.95%)	1805		
District Dadu (N = 2755)					
Female	1189 (86.47%)	186 (13.52%)	1375	0.210, p < 0.647	0.210, p < 0.647
Male	1185 (85.86%)	195 (14.13%)	1380		
District Ghotki (N = 867)					
Female	336 (82.76%)	70 (17.24%)	406	1.621, p < 0.203	1.617, p < 0.203
Male	396 (85.90%)	65 (14.09%)	461		
District Hyderabad (N = 51622)					
Female	27791 (87.84%)	3847 (12.16%)	31638	21.54 , p , 0.0001	21.399, p , 0.0001
Male	17275 (86.44%)	2709 (13.56%)	19984		
District Jamshoro (N = 4242)					
Female	1794 (76.86%)	540 (23.14%)	2334	6.409, p < 0.011	6.437, p , 0.012
Male	1528 (80.08%)	380 (19.91%)	1908		
District Kashmore (N = 2016)					
Female	648 (85.15%)	113 (14.85%)	761	1.053, p < 0.305	1.061, p < 0.335
Male	1047 (83.43%)	208 (16.57%)	1255		
District Khairpur Mirs (N = 1258)					
Female	566 (85.37%)	97 (14.60%)	663	10.576, p < 0 .001	10.534, p < 0.001
Male	466 (78.32%)	129 (21.68%)	595		
Mirpur Khas (N = 1003)					
Female	376 (75.20 %)	124 (24.8%)	500	4.441, p < 0.035	4.449, p < 0.035
Male	406 (80.72%)	97 (19.28%)	503		
District N. Feroze (N = 2251)					
Female	935 (82.02%)	205 (17.98%)	1140	0.118, p < 0.731	0.118, p < 0.731
Male	905 (81.46%)	206 (18.54%)	1111		
District Sanghar (N = 3471)					
Female	1505 (80.31%)	369 (19.69%)	1874	1.008, p , 0.315	1.010, p < 0.315
Male	1304 (81.65%)	293 (18.35%)	1597		
District Sukkur (N = 5102)					
Female	2168 (83.99%)	413 (16.00%)	2581	0.010, p < 0.920	0.010, p < 0.950)
Male	2115 (83.19%)	406 (16.10%)	2521		
District Tando Allahyar (N = 1820)					
Female	785 (80.10%)	195 (19.89%)	980	0.019, p < 0.892	0.019, p < 0.892
Male	675 (80.36%)	165 (19.64%)	840		
District Tharparkar (818)					
Female	361(90.48%)	38 (9.52%)	399	0.331, p < 0.565	0.332, p < 0.565
Male	374 (89.26%)	45 (10.74%)	419		
Pooled data set (N = 81154)					
Female	40318 (86.20%)	6457 (13.80%)	46775	24.350, p < 0.0001)	24.268, p < 0.0001)
Male	29211 (84.96%)	5168 (15.03%)	34379		

with values of Chi square (X^2) and likelihood ratio for each district, which indicated significant difference in the reactivity (- /+) between female and male outpatients. Gender wise strength of HCV (-) and HCV (+) varied from district to district. On the basis of X^2 and the likelihood ratio, some districts such as Badin, Hyderabad, Jamshoro, Khairpur Mirs and Mirpur Khas exhibited significant difference in HCV due to gender. In these districts - male OPs tended to have somewhat higher HCV+ cases. Such a difference was not significant in districts Dadu, Ghotki, Kashmore, N. Feroze, Sanghar, Sukkur, Tando Allahyar and Tharparkar. The pooled sample for all districts, however, indicated significant difference for HCV reactivity in female and male outpatients. Male OPs had higher number of HCV + cases (Table 7).

Table 8. Frequency distribution of HCV (-) and HCV (+) OPs of various age groups in 13 districts of Sindh, studied for HCV. (% Prevalence = HCV (+) outpatients / total outpatients X 100).

Age size classes	HCV(-)	HCV (+)	% Prevalence (%P)	X^2	Likelihood ratio
District Badin					
≤ 10 years	71	3	4.05	191.08	200.19
10.1- 20	444	25	5.33	P < 0.0001	P < 0.0001
20.1- 30	1181	134	10.19		
30.1 - 40	781	141	15.29		
40.1 - 50	519	117	18.40		
50.1 – 60	267	66	**19.82**		
60.1 - 70	98	34	**25.76**		
70.1 - 80	31	9	**22.50**		
80.1 - 90	7	1	12.50		
District Dadu					
≤ 10 years	84	1	1.18	165.39	176.01
10.1- 20	375	28	6.95	P < 0.0001	P < 0.0001
20.1- 30	**795**	**101**	11.27		
30.1 - 40	**528**	**96**	15.38		
40.1 - 50	345	77	18.25		
50.1 – 60	166	46	9.75		
60.1 - 70	61	22	**26.51**		
70.1 - 80	17	7	**29.17**		
80.1 - 90	3	3	**50.00**		
District Ghotki					
≤ 10 years	25	-	Zero	137.47	135.28
10.1- 20	99	3	2.94	P < 0.0001	P < 0.0001
20.1- 30	**261**	**29**	**10.0**		
30.1 - 40	**186**	**34**	**15.45**		
40.1 - 50	92	32	25.80		
50.1 – 60	42	26	38.23		
60.1 - 70	24	7	22.58		
70.1 - 80	1	2	66.67		
80.1 - 90	1	2	66.67		
District Hyderabad					
≤ 10 years	981	17	1.70	2447.10	2496.83
10.1- 20	5393	252	4.56	P < 0.0001	P < 0.0001
20.1- 30	**17137**	**1529**	8.19		
30.1 - 40	**9413**	**1622**	14.70		
40.1 - 50	5637	1530	**21.35**		
50.1 – 60	3739	1021	**21.45**		
60.1 - 70	1936	446	**18.72**		
70.1 - 80	665	114	14.63		
80.1 - 90	154	20	11.50		
90.1 - 100	11	5	**31.25**		
District Jamshoro					
≤ 10 years	55	2	3.50	315.33	340.18
10.1- 20	417	33	7.33	P < 0.0001	P < 0.0001
20.1- 30	**1048**	**181**	14.73		
30.1 - 40	**686**	**228**	24.97		
40.1 - 50	**554**	**227**	29.07		
50.1 – 60	335	150	30.93		
60.1 - 70	158	78	33.06		
70.1 - 80	61	17	21.79		
80.1 - 90	8	4	33.33		

% P = - 3.137 + 0.788 (ageCMP) – 0.006 (ageCMP) ^{2} ± 3.573
t = 4.160 ($p < 0.006$), t = -3.196 ($p < 0.019$)
$R = 0.911$, $R^2 = 0.830$, $F = 14.611$ ($p < 0.005$)
Spearman rho = 0.733 ($p < 0.025$), N = 9

% P = -2.857 + 0.478 (ageCMP) ± 7.249, N = 9
t = 5.114 ($p < 0.001$)
$r = 0.888$, $r^2 = 0.789$, $F = 26.452$ ($p < 0.001$)
Spearman rho = 0.900 ($p < 0.001$)

% P = -10.432 + 0.843 (ageCMP) ± 10.373, N = 9
t = 6.298 ($p < 0.0001$)
$r = 0.922$, $r^2 = 0.850$, $F = 39.666$ ($p < 0.0001$)
Spearman rho = 0.946 (0.0001)

% P = 3.930 + 0.217 (ageCMP) ± 6.28, N = 10
t = 3.140 ($p < 0.014$)
$r = 0.743$, $r^2 = 0.552$, $F = 9.86$ ($p < 0.014$)
Spearman rho =0.661 ($p < 0.038$)

% P = -3.475 + 1.008 (ageCMP) – 0.007 (ageCMP) ^{2} ± 4.663
t = 4.074 ($p < 0.007$), t = -2.776 ($p < 0.032$)
$R = 0.932$, $R^2 = 0.869$, $F = 19.98$ ($p < 0.002$), N = 9
Spearman rho = 0.833 ($p < 0.005$)

District Kashmore					
≤ 10 years	137	3	2.14	140.95	161.36
10.1- 20	357	27	7.03	P < 0.0001	P < 0.0001
20.1- 30	**516**	**96**	**15.69**		
30.1 - 40	**372**	**90**	**41.59**		
40.1 - 50	171	55	24.34		
50.1 – 60	90	31	25.62		
60.1 - 70	43	14	24.56		
70.1 - 80	6	5	45.45		
80.1 - 90	3	-	Zero		
District Khairpur Mirs					
≤ 10 years	43	2	4.44	166.51	177.44
10.1- 20	183	12	6.15	P < 0.0001	P < 0.0001
20.1- 30	**339**	**51**	**13.08**		
30.1 - 40	**232**	**55**	**19.16**		
40.1 - 50	136	45	24.73		
50.1 – 60	63	34	35.05		
60.1 - 70	29	17	36.96		
70.1 - 80	6	6	50.00		
80.1 - 90	1	2	66.66		
90.1 - 100	-	1	100.0		
District Mirpur Khas					
≤ 10 years	23	1	4.17	107.06	120.26
10.1- 20	74	9	10.84	P < 0.0001	P < 0.0001
20.1- 30	**231**	**47**	**16.91**		
30.1 - 40	**192**	**56**	**22.58**		
40.1 - 50	144	**48**	25.0		
50.1 – 60	81	36	30.77		
60.1 - 70	32	18	36.0		
70.1 - 80	4	4	50.0		
80.1 - 90	1	2	66.6		
District N. Feroze					
≤ 10 years	83	3	3.49	137.04	158.20
10.1- 20	323	29	8.24	P < 0.0001	P < 0.0001
20.1- 30	526	**105**	16.64		
30.1 - 40	407	**108**	20.97		
40.1 - 50	197	83	22.86		
50.1 – 60	141	57	28.79		
60.1 - 70	57	20	25.97		
70.1 - 80	14	5	26.32		
80.1 - 90	8	1	11.11		
90.1 - 100	1	-	Zero		
District Sanghar					
≤ 10 years	62	4	6.06	319.94	341.74
10.1- 20	336	21	5.88	P < 0.0001	P < 0.0001
20.1- 30	**998**	**114**	10.25		
30.1 - 40	**650**	**213**	24.97		
40.1 - 50	**417**	**166**	28.47		
50.1 – 60	**220**	**101**	31.46		
60.1 - 70	96	44	31.43		
70.1 - 80	25	6	21.43		
80.1 - 90	5	3	42.86		
90.1 - 100	-	-	-		
District Sukkur					
≤ 10 years	140	3	2.09	316.68	340.87
10.1- 20	640	51	7.38	P < 0.0001	P < 0.0001
20.1- 30	**1326**	**155**	**10.60**		
30.1 - 40	**904**	**219**	**19.50**		
40.1 - 50	675	193	22.36		

% P = 4.024 + 0.481 (ageCMP) ± 10.287
t = 3.027 ($p < 0.023$
$r = 0.777$, $r^2 = 0.600$, $F = 9.132$ ($p < 0.023$)
Spearman rho = 0.833 (0.010), N = 8

%P = -10.898 + 0.929 (ageCMP) ± 10.844
t = 7.78 ($p < 0.0001$)
$r = 0.940$, $r^2 = 0.883$, $F = 60.51$ ($p < 0.0001$)
Spearman rho = 0.999 ($p < 0.0001$), N =10

% P = -1.890 + 0.690 (ageCMP) ± 5.364
t = 9.961 ($p < 0.0001$)
$r = 0.966$, $r^2 = 0.934$, $F = 99.212$ (p , 0.0001)
Spearman rho = 0.999 ($p < 0.0001$), N = 9

% P = - 4.793 + 1.159 (ageCMP) – 0.011(ageCMP) ^{2} ± 3.442
t = 3.350 ($p < 0.0001$), t = -5.509 ($p < 0.0001$)
R = 0.942, R2 = 0.888, F = 23.734 ($p < 0.001$)
Spearman = 0.0600 ($p < 0.088$), N = 9

% P = 4.291 + 0.405 (ageCMP) ± 6.881, N = 9
t = 4.555 ($p < 0.003$)
$R = 0.748$, $r^2 = 0.748$, $F = 20.478$ ($p < 0.003$)
Spearman rho = 0.800 ($p < 0.010$)

50.1 – 60	375	131	25.84		
60.1 - 70	154	52	25.25		
70.1 - 80	58	13	18.31		
80.1 - 90	10	2	16.66		
90.1 - 100	1	-	Zero		
District Tando Allahyar					
≤ 10 years	32	1	3.03	192.39	205.41
10.1- 20	166	10	5.68	P < 0.0001	P < 0.0001
20.1- 30	503	71	12.37		
30.1 - 40	358	**103**	22.34		
40.1 - 50	212	**87**	29.9		
50.1 – 60	122	54	30.68		
60.1 - 70	51	28	35.44		
70.1 - 80	15	5	25.0		
80.1 - 90	1	1	50.0		
District Tharparkar					
≤ 10 years	12	1	7.69	76.89	78.90
10.1- 20	75	3	3.85	P < 0.267	P < 0.218
20.1- 30	269	**18**	6.27		
30.1 - 40	155	**25**	13.89		
40.1 - 50	101	**19**	15.83		
50.1 – 60	78	5	6.02		
60.1 - 70	27	10	27.02		
70.1 - 80	12	2	14.29		
80.1 - 90	5	-	Zero		
90.1 - 100	1	-	Zero		
Pooled Data set					
≤ 10 years	1748	41	2.29	3767.87	3890.91
10.1- 20	8882	503	5.36	P < 0.0001	P < 0.0001
20.1- 30	25130	**2631**	9.48		
30.1 - 40	14864	**2980**	16.70		
40.1 - 50	9283	**2680**	22.40		
50.1 – 60	5719	**1758**	23.51		
60.1 - 70	2766	790	22.22		
70.1 - 80	915	195	17.57		
80.1 - 90	207	41	16.53		
90.1 - 100	15	6	28.57		

$\%P = -5.010 + 0.996\ (ageCMP) - 0.009\ (ageCMP)^2 \pm 2.476$
$t = 7.582\ (p < 0.0001),\ t = -6.164\ (p < 0.001)$
$R = 0.965,\ R2 = 0.931,\ F = 40.701\ (p < 0.0001)$
Spearman rho = 0.567 ($p < 0.112$), N = 9

$\%\ P = 1.250 + 0.501\ (ageCMP) \pm 6.501$
$t = 5.967\ (p < 0.0001)$
$r = 0.914,\ r^2 = 0.836,\ F = 35.604\ (p < 0.001)$
Spearman = 0.900 ($p < 0.001$), N = 9

$\%\ P = 4.052 + 0.195\ (ageCMP) \pm 3.373$
$t = 1.977\ (p < 0.095)$
$r = 0.628,\ r^2 = 0.395,\ F = 3.910\ (p < 0.095)$
Spearman = 0.570 ($p < 0.139$), N 8

$\%\ P = 5.161 + 0.226\ (ageCMP) \pm 5.23,\ N = 10$
$t = 3.918\ (p < 0.004)$
$r = 0.811,\ r^2 = 0.657,\ F = 15.35\ (p < 0.004)$
Spearman rho = 0.709 ($p < 0.022$)

Table 9. Distribution of number of HCV (+) outpatients in initial age-group up to 10 years of age in various districts of Sindh.

Districts														
Age	A	B	C	D	E	F	G	H	I	J	K	L	M	N
	3*	1	0	17	2	3	2	1	3	4	3	1	1	41
0.5	-	-	-	0	-	-	-	-	-	-	-	-	-	0
1	0	0	0	1	0	0	0	0	0	0	0	0	0	1
2	0	0	0	1	0	0	0	0	0	0	0	0	0	1
3	0	0	0	1	0	1	1	0	0	1	2	0	0	4
4	0	0	0	1	0	1	0	0	0	0	0	0	0	4
5	1	0	0	2	0	0	0	0	0	1	1	0	0	4
6	0	0	0	1	0	0	0	0	0	0	0	0	1	3
7	1	0	0	2	0	0	0	0	0	0	0	0	0	3
8	1	0	0	3	2	0	1	0	0	1	0	0	0	8
9	0	1	0	0	0	0	0	1	2	0	0	0	0	4
10	0	0	0	5	0	1	0	0	1	1	0	1	0	9

*, Total number of HCV (+) cases in the initial age group up to 10 years of age.

Acronyms for Districts of Sindh

A = Badin; B, Dadu; C, Ghotki; D, Hyderabad; E, Jamshoro; F, Kashmore; G, Khairpur Mirs; H, Mirpur Khas; I, N. Feroze; J, Sanghar; K, Sukkur; L, Tando Allahyar; M, Tharparkar and N, pooled data for 13 districts.

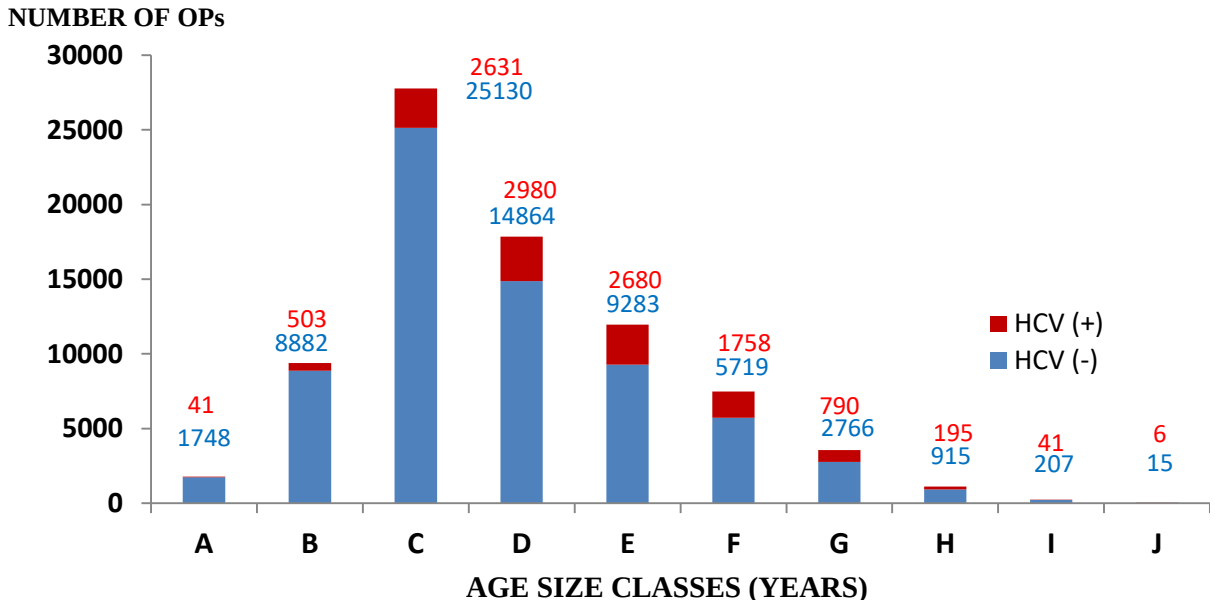


Fig. 4. Number of HCV (-) and HCV (+) outpatients in the pooled sample of outpatients from 13 districts of Sindh. Age size classes: A, ≤ 10.0 years; b, 10.1-20; C, 20.1 – 30 …… and J, 90.1-100 years.

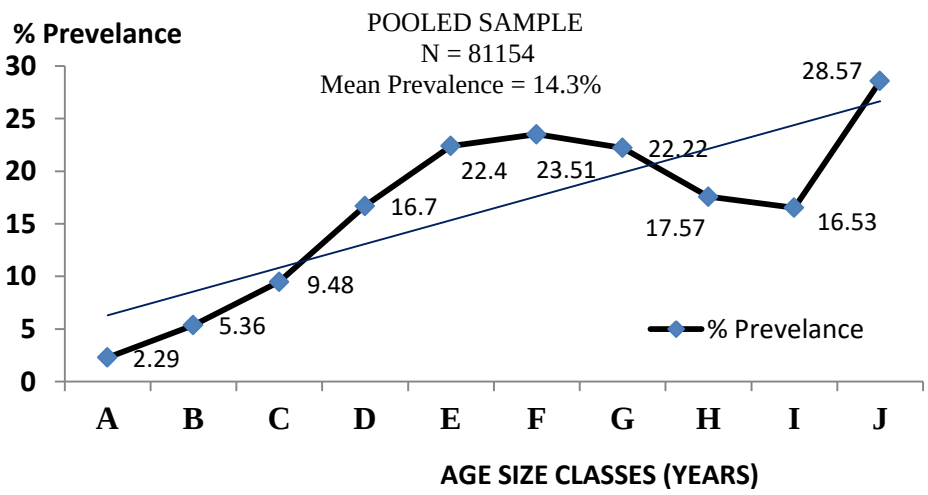


Fig. 5. Percent Prevalence of HCV in various age groups of OPs pooled, N = 81154) studied from 13 districts of Sindh, Age Classes: A, ≤ 10.0 years; B, 10.1-20.0; C, 20.1-30; D, 30.1-40; E, 40.1-50; F, 50.1-60; G, 60.1-70; H, 70.1-80; I, 80.1-90 and J, 90.1-100 years.

Age relations of HCV prevalence

The number of HCV (-) and HCV (+) OPs in various age groups separated with 10-year interlude is presented in Fig. 4 and the HCV prevalence in Fig. 5. Percent prevalence of HCV in various age groups of outpatients of 13 districts is given in Table 8. Percent prevalence varied significantly amongst various age groups in all districts and tended to the direct function of age. It tended to be low in lower age groups and high in higher age groups. In each district HCV prevalence was low in outpatients of the initial age group of ≤ 10.0years varying from zero (district Ghotki) to 7.6 9% (district Tharparkar) averaging to 3.349 ± 0.568% with variability around 61.18%.

HCV+ OPs were distributed in all age of all districts. Four age groups (A, B, C, and D (20.1-30, 30.1-40, 40.1-50 and 50.1-60, respectively) were important. The age group A and B were prominent in districts Badin, Dadu, Ghotki, Kashmore, Khairpur Mirs and N. Feroze. In three districts (Mirpur Khan, Jamshoro and Tharparkar, three age groups (A, B and C) were found to be prominent as regards to the occurrence of HCV+ cases. Tando Allahyar exhibited age groups B and C to contain relatively larger number of HCV+ cases. However, four age groups (A, B, C and D) had substantially larger number of HCV cases in district Hyderabad, Sukkur and Sanghar. Likely, in pooled sample, there were four age groups (A, B.C and D) which had large number of HCV+ cases (2631, 2980, 2680 and 1758, respectively). As described by several workers (Abbas *et al*., 2008; Qureshi *et al*., 2010; Ghanghro and Ghanghro, 2016), Patoli *et al*., 2018) also observed that mono or dual hepatitis increases with age. Although small sample size was limitation in their study, Patoli *et al*. (2018) found maximum frequency of HCV cases (37.5%) in age group of 51-60 years. Patoli *et al*. (2019) have described that middle aged people (31-50 years) were

more susceptible to HCV infection. In southern Pakistan, Kakepoto *et al*. (1996) reported high seropositive population (30-47%) in 21-41 years of age during study of blood donors' population.

Percent prevalence appeared to increase with age of the OPs in all districts as indicated by Pearson correlation and regression analysis between age class mid points (ageCMP) and % prevalence of HCV and nonparametric correlation of Spearman (rho). These parameters related positively and linearly in nine districts – Dadu, Ghotki, Hyderabad, Tando Allahyar, Kashmore, Khairpur Mirs, Mirpur Khas, Sanghar and Tharparkar where as they were exhibited by quadratic or second degree polynomial relationship in four districts - Badin, Jamshoro, N. Feroze and Sukkur. The best fit regressive equations are posted for each district in Table 8. In pooled sample for all districts the % HCV prevalence also increased with increasing magnitude of ageCMP significantly in linear fashion (Pearson r = 0.811; Spearman rho: 0.709) (Table 8, Fig. 5) varying from 2.29% in initial ageCMP to 28.57% in last age group of 90.1 to 100 years of age.

Table 10. Summary of HCV (+) outpatients in various districts of Sindh and the % prevalence of virus as per present study.

Districts	Total outpatients	HCV (+) ♀	HCV (+) ♂	Total HCV (+)	% Prevalence In town		
					♀	♂	Total
Badin	3929	260	270	530	6.6	6.9	13.5
Dadu	2755	186	195	381	6.6	7.1	13.8
Ghotki	867	70	65	135	8.0	7.5	15.6
Hyderabad	51622	3847	2709	6556	7.5	5.3	12.7
Jamshoro	4242	540	380	920	12.7	9.0	21.7
Kashmore	2016	113	208	321	5.6	10.3	15.9
Khairpur Mirs	1258	97	129	226	7.7	10.3	18.0
Mirpur Khas	1003	124	97	221	12.4	9.7	22.0
N. Feroze	2251	205	206	411	9.1	9.2	18.3
Sanghar	3471	369	293	662	10.6	8.4	19.0
Sukkur	5102	413	406	819	8.09	8.0	16.0
Tando Allahyar	1820	195	165	360	10.7	9.0	19.8
Tharparkar	818	38	45	83	4.6	5.5	10.2
Pooled sample	81154	6457	5168	11625	7.96	6.36	14.3

It may be deduced from Table 8 that prevalence associated with age rather ageCMP, in two fashions – linearly and quadratic manner. The districts showing linear relationships were found to exhibit following order of sequence on the basis of regression coefficient, "b" i.e., the slope value of increase of prevalence with age.

Khairpur Mirs (0.929) > Ghotki (0.843) > Mirpur Khas (0.690) > Tando Allahyar (0.501) > Kashmore (0.481) ≈ Dadu (0.478) > Sanghar (0.405) > Hyderabad (0.217) > Tharparkar (0.195) - i.e., highest age related rate of increase of HCV prevalence was in Khairpur Mirs and the lowest in Tharparkar.

It is apparent from Table 9, presenting frequency distribution of HCV (+) OPs in the initial age class of ≤ 1-10 years of age, and the data supplemented in Appendix I that there were only 41 HCV (+) cases in this class for all districts studied. There was no HCV (+) case amongst the infants of 0.5 year of age. There was only one HCV (+) case in 1- year old OPs and one HCV (+) case in 2-year old OPs of district Hyderabad. Here large number of OPs (51622) was studied and there were only 17 HCV (+) cases in all in the initial age class (merely 0.033%). All districts exhibited HCV (+) patients infrequently even for OPs of 3 -10 year of age. It follows from the results that infants and younger babies somehow possess immunity for this disease. Our data based on very large sample size, appear to strongly validate the contention of young age immunity against HCV in local population of Sindh. Patoli *et al*. (2018), from 108 patients of district Hyderabad, found 79 male and 29 female seropositive HCV mono-infection cases in eight age groups differentiated with equal interlude of 10 years. None of these seropositives associated with age group of 1-10 years of age and only two female seropositive cases were reported in age group II of 11-20 years of age. The remaining HCV (+) cases distributed differentially in higher age groups. Although they couldn't discuss this fact, their data indicated to the younger age immunity to the virus in Hyderabad population.

Table11. HCV prevalence for some areas of Sindh reported in literature.

Area in Sindh	Seroprevalence (%)			
	Adult healthy population			
	Male	Female	Total	Reference
Larkana (2009)	7.37	4.12	6.66	Shaikh *et al* (2009)
Larkana (2006)*	2.88	-	2.88	Malik *et al*. (2008)
Dadu (2006)*	2.23	-	2.23	Malik *et al*. (2008)
Ghotki (2006)	6.64	-	6.64	Malik *et al*. (2008)
Jamshoro (2006)*	Zero	-		Malik *et al*. (2008)
Jacobabad (2006) *	4.9	-	2.35	Malik *et al*. (2008)
Kambar Shahdad Kot (2006)*	4.9	-	4.9	Malik *et al*. (2008)
Karachi periurban (2010)	19	26	23.83	Janjua *et al*. (2010)
Karachi (general population)	-	-	5.2	Hakim *et al*. (2008)
Karachi	-	-	4.8	Qureshi *et al*. (2010)
Karachi	-	-	4.7	Umer and Iqbal (2010)
Karachi	-	-	6.0	Umer and Iqbal (2016)
Karachi (2010)	2.8	5.23	3.17	Abbas *et al*. (2010).
Karachi (2011)	-	-	9.75	Abdullah *et al*. (2011)
Karachi (2018)	-	-	6.2	Al-Kanani *et al*. (2018)
Khairpur	6.71	-	6.71	Malik *et al*. (2008)
Kashmore (2006)*	1.96	-	1.96	Malik *et al*. (2008)
Nawabshah (2006) *	9.32	-	9.32	Malik *et al*. (2008)
N. Feroze (2006) *	2.66	-	2.66	Malik *et al*. (2008)
Sanghar (2006) *	28.37	-	28.37	Malik *et al*. (2008)
Shikarpur (2006)*	-	-	3.33	Malik *et al*. (2008)
Sukkur (2006)*	5.37	-	5.37	Malik *et al*. (2008)
Thatta / N. Feroze (2010)	-	-	25.1	Aziz *et al*. (2010)
	Blood donors			
Mirpur Khas (2009)	-	-	15.05	Abbas *et al*. (2009).
Karachi (2011)	-	-	1.9	Borhany *et al*. (2011)
Karachi (2013)	-	-	2.0	Ansari *et al*. (2012)
Karachi (2013)	-	-	2.61	Khan *et al*. (2013)
Karachi (2014)	-	-	1.65	Moiz *et al*. (2014)
Interior Sindh (2004 and 2007	-	-	7.2, 8.9	Mujeeb and Pearce (2008)
	Hepatitis C patients with liver disease			
Karachi (2010)	-	-	50	Ahmad *et al*. (2010)
Hyderabad (2014)	-	-	66	Munaf *et al*. (2014)
Karachi (2010)	-	-	59.4	Ahmad *et al*. (2010)
	High Risk population (pregnant women)			
Karachi (2009)	-	-	1.8	Aziz *et al*. (2011)
Karachi (2011)	-	-	5.79	Sami *et al*. (2009)
Hyderabad	-	-	4.7	Bibi *et al*. (2013)

*, all male subjects.

Agglomerative clustering of various districts of Sindh on the basis of disease severity in HCV cases

To elucidate similarity amongst the districts studied, agglomerative clustering of districts on Euclidean distances amongst them was accomplished by employing Ward's (1963) method on the basis of frequency distribution of antibodies concentration in the sera of HCV (+) outpatients of various districts. At group extraction level of 7.5% dissimilarity amongst various districts, three clusters were recognized.

Cluster A was composed of seven districts - Badin, Jamshoro, Tharparkar, N. Feroze, Tando Allahyar, Ghotki and Sukkur; **Cluster B** stood alone for district Khairpur Mirs and **Cluster C** was composed of Hyderabad, Mirpur Khas, Sanghar, Kashmore and Dadu. The mean level of antibodies in these clusters was highest in cluster A (49.85 ± 2.619 IU), medially located in cluster B (38.14 ± 1.98 IU) and the lowest in cluster C (15.62 ± 1.802 IU). That is to say that disease severity was of relatively low order in Hyderabad, Mirpur Khas, Sanghar, Kashmore and Dadu, moderately severe in Khairpur Mirs and highly severe in districts such as Badin, Jamshoro, Tharparkar, N. Feroze, Tando Allahyar, Ghotki and Sukkur.

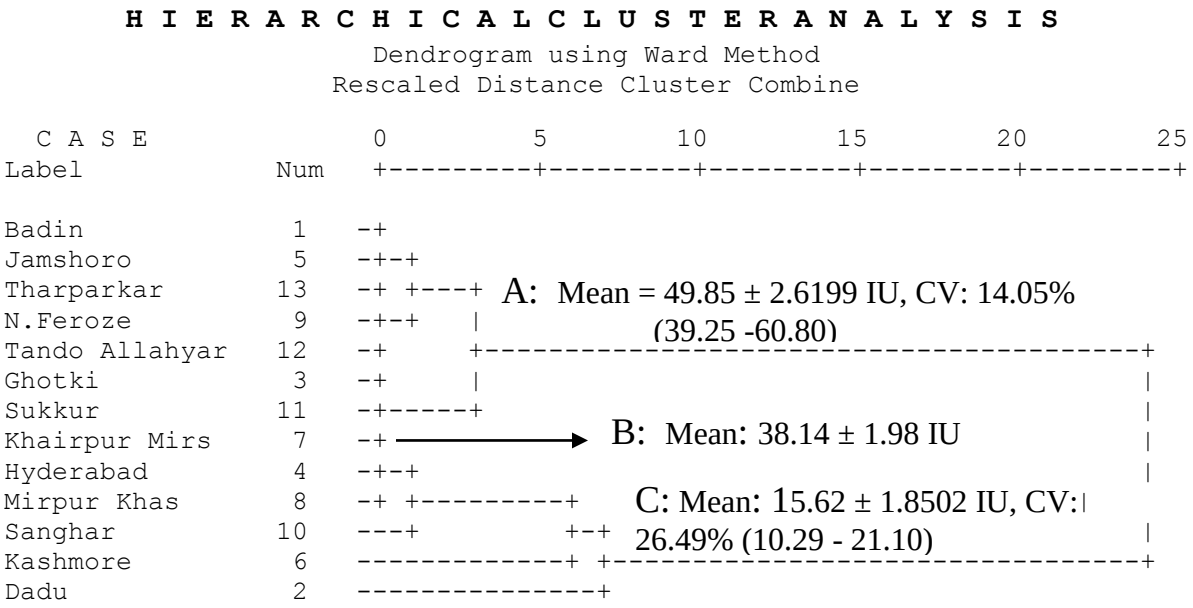


Fig. 6. Dendrogram showing clustering of various districts on the basis of % frequency distribution of antibodies (International units) detected in blood sera of the HCV (+) outpatients.

Appendix I. Status of HCV in young (1-2 years of age) outpatients of various districts of Sindh.

District	HCV status in Youngs
Badin (N: 3929)	Youngest one-year old, 1 in number, Male, **HCV (-)**
Dadu (N: 2755)	Youngest one-year old, I in number , Male, **HCV (-)**
Ghotki (N: 867) *	Youngest two- year old, 2 in number, Male, **HCV (-)**
Hyderabad (N: 51622)	Youngest Half-year old, 7 in number, two male, five female, All **HCV (-)** One-Year old, 18 in number, One **HCV (+)** Two-year old, 52 in number, Two **HCV (+)**
Jamshoro (N: 4242)	One-year old, 2 in number, One male, One Female, Both **HCV (-)**
Kashmore (N =2016)	One-year old, 1 I number, **HCV (-)** Two- year old, 16 in number, All **HCV (-)**
Khairpur Mirs (N: 1258)	Youngest two-year old, 4 in number, All **HCV (-)**
Mirpur Khas (N: 1003)	Youngest three-year old, 4 in number, All **HCV (-)**
N. Feroze (N: 2251)	Youngest two-year old, 4 in number (3 male; 1 Female), All **HCV (-)**
Sanghar (N: 3471)	Youngest one-year old, 1 in number, Female, (HCV (-) Two –year old, 5 in number (3 female, two male), All **HCV (-)**
Sukkur (N: 5102) *	Youngest two-year old, 12 in number, (2 female, 10 male), All **HCV (-)**
Tando Allahyar (N: 1820)	Youngest one-year old, I in number, Male, **HCV (-)** Two year old, 1 in number, male, **HCV (-)**
Tharparkar (N: 818) *	Youngest two-year old, 2 in number (1 female, 1 male), Both HCV (-)

*, Even three-year old outpatients were HCV (-).

HCV prevalence in various districts of Sindh

The HCV prevalence as determined in the present studies of thirteen districts of Sindh is presented in Table 10. % prevalence of HCV varied significantly amongst the districts studied. It ranged from 4.6 (Tharparkar) to 12.7 % (Jamshoro) in females and 5.3 (Hyderabad) to 10.3 (Kashmore and Khairpur Mirs) in male outpatients. The prevalence was comparatively lower in females than in males in five districts (Badin, Dadu, Kashmore, Khairpur Mirs and Tharparkar). HCV was almost equally prevalent in female and males in district N. Feroze and Sukkur. In six districts such as Ghotki, Hyderabad, Jamshoro, Mirpur Khas, Sanghar and Tando Allahyar, the prevalence was comparatively lower in male outpatients than female one. The total prevalence was maximum in Mirpur Khas (22.0%) followed by Jamshoro (21.7%). It was minim in Tharparkar (10.2%). The districts in hand may be arranged in following orders on the basis of prevalence in female and male OPs and total (♀ + ♂) HCV prevalence.

% prevalence (Female): Jamshoro (12.7) ≈ Mirpur Khas (12.4) > Tando Allahyar (10.7) ≈ Sanghar (10.6) ≈ N. Feroze (9.1) > Sukkur (8.09) ≈ Ghotki (8.0) > Khairpur Mirs (7.7) ≈ Hyderabad (7.5) > Badin (6.6) = Dadu (6.6) > Kashmore (5.6) > Tharparkar (4.6)

% prevalence (Male): Kashmore (10.3) + Khairpur Mirs (10.3) > Mirpur Khas (9.7) > N. Feroze (9.2) ≈ Jamshoro (9.0) = Tando Allahyar (9.0) > Sanghar (8.4) > Sukkur (8.0) > Ghotki (7.5) ≈ Dadu (7.1) ≈ Badin (6.6) Tharparkar (5.5) ≈ Hyderabad (5.3)

Total prevalence (Female + Male): Mirpur Khas (22.0, the highest) ≈ Jamshoro (21.7) > Tando Allahyar (19.8) > Sanghar (19.0) > N. Feroze (18.3%) ≈ Khairpur Mirs (18) > Sukkur (16.0) ≈ Kashmore (15.9) ≈ Ghotki (15.6) > Dadu ((13.8) ≈ Badin (13.5) > Hyderabad (12.7) > Tharparkar (10.2, the lowest).

The compositional similarity between females and males OPs on the basis of % prevalence was calculated with Czekanowski's (1913) index to be 97.1%. However, general prevalence in pooled outpatients' data appeared to be larger in females (7.96%) than males (6.36%) i.e. the total prevalence amounted to 14.3%. This is higher than several estimated values reported for general population earlier (Table 11) but lower than that for Karachi periurban estimate of 2010 reported by Janjua *et al*. (2010) Sanghar in 2006 (Malik *et al*., 2008) and Thatta / N. Feroze 2010 (Aziz *et al*., 2010). Estimate on the basis of present studies based on larger sample size should be more reliable regarding Sindh. Haqqi *et al* (2019) reported nearly 6.1 % of the population of Pakistan to be infected with HCV and Ahsan *et al* (2009) gave slightly higher statistic of 6.34% in general population of Punjab. The frequency of HCV infection is reported to vary in different provinces of Pakistan and even in different groups of the same community (Al-Kanani *et al*., 2018). The prevalence of hepatitis C, as reported in earlier published reports may be viewed to vary not only in different geographical areas of Sindh but the categories of the people (Table 11). WHO (2021) have recognized Ghotki, Sanghar and Dadu as high HCV prevalence areas in Sindh. According to the present studies prevalence in all districts of Sindh is high but more in Mirpur Khas, Jamshoro, Tando Allahyar, N. Feroze, Khairpur Mirs, Sukkur and Kashmore districts.

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