Prevalence of Hepatitis B, C and D and HIV in Sindh Province, Pakistan

CONTENTS

ACKNOWLEDGMENTS

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[1]https://apps.who.int/iris/bitstream/handle/10665/280099/WHO-CDS-HIV-19.3-eng.pdf?ua=1

EXECUTIVE SUMMARY

The first National survey on the prevalence of hepatitis B and C in Pakistan undertaken in 2008 had revealed that the prevalence of anti HCV was 5% and that of HBsAg was 2.5%. Within the Provinces, highest HCV (6.7%) was seen in Punjab followed by Sindh (5%). For HBsAg, the highest prevalence was seen in Baluchistan (4.3%) followed by Sindh (2.5%) and Punjab (2.2%).

In 2018, hepatitis B,C and D survey was undertaken in Punjab, which showed HBsAg prevalence of 2.2%, anti HCV of 8.9% and antidelta virus of 17.7% cases that were HBsAg positive . The main risk factors over the decade remain the same i.e. unsafe blood, unsafe therapeutic injections and poor infection control in the health care settings.

The 2019 survey done for hepatitis B,C and D and HIV in Sindh shows HBsAg prevalence of 1.05%, anti HCV of 6.19%, anti delta of 32.8% and HIV of 0.02%. HBV DNA was detected in 56/70 cases (80%). HCVRNA was detected in 136/349 cases (39%).

The sample size was calculated to get the provincial figures and not the district figures but district may use these figures to address the disease situation on priority. High HBsAg prevalence was seen in Kashmore (5.3%) and Sukkur (4.6%).For HCV, high prevalence was seen in Sanghar (14.5%),Mirpur Khas (10.6%), Tando Allahyar (10.4%) and Shikarpur (9.7%).

BACKGROUND

The first national hepatitis survey was done in 2007-08 and that had put Pakistan in the list of the 2nd highest HCV prevalent country in the world. Subsequently, the Prime Minister's program on hepatitis was launched in 2005 with a vision to treat hepatitis B with oral antiviral drugs and treat hepatitis C with conventional interferon. As over 85% cases in Pakistan have HCV genotype 3 therefore there were reservations about the inadequate response to interferon in our population. Interferon and ribavirin had multiple side effects and the virus clearance never crossed 60% making hepatitis C cure almost impossible. Following devolution of health in 2011-12, the PM program was devolved and all provinces developed their own chief minister's hepatitis program and took up the task of treating these cases according to their budgets.

While the treatments were being supported through the CM program, unfortunately the prevention strategies were not actively addressed and the birth dose of hepatitis B vaccine which costs 5 cents/dose and had huge advantages of preventing hepatitis B, hepatitis D and liver cancer has not been introduced in expanded program of immunization. According to WHO, all countries having a HBsAg prevalence of >2% should introduce the hepatitis B birth dose. The 3rd dose coverage of pentavalent vaccine has remained low and there is negligible guidance on the care and treatment of HBsAg positive mothers and prevention of mother to child transmission (MTCT). The risk factors for disease transmission also remain unattended and these include, improperly screened blood, unsafe injections and improper infection control in health care settings. Reuse of syringes in health care settings pose a major threat and the Ratto Dero HIV outbreak in Sindh was a clear example of such a negligence that lead to HIV in hundreds of children. Such practices are seen all over the country especially in the private care settings.

Introduction of the direct acting antivirals (DAAs) was a great break through globally for the HCV patients. These oral drugs cure HCV in few weeks with no side effects. Pakistan is fortunate to produce the generic DAAs at a very affordable cost which is lowest in the world. Following the availability of DAAs, all provincial hepatitis control programs revised their PC1s and budgets accordingly and started procuring DAAs to cure the affected population. Similarly for hepatitis B, the oral medications are also locally produced and these are affordable and efficacious.

To date slightly over 2 million HCV cases have been treated but there are 7 million more that need to be screened and treated. To calculate the real numbers that are infected and need treatment, there was desire by the provinces to undertake the survey. Punjab was the 1st province to undertake this exercise followed by Sindh. According to the figures released by the Punjab hepatitis program, the HBsAg prevalence as of 2017-18 was 2.2% and that of anti HCV is 8.9% with HCVRNA present in 59.8%. This survey shows a drop in the hepatitis B prevalence which is due to childhood immunization along with vaccination of adults. Unfortunately the hepatitis C prevalence has increased in Punjab from 6.4% to 8.9% which is very alarming and needs steps towards prevention and treatment. The Punjab survey was not representative of the districts; therefore it is difficult for them to identify which districts need more support and funding.

Sindh survey not only gives overall prevalence of hepatitis and HIV in the Province but it also gives district level figures and therefore can guide the health department to make decisions about which interventions to undertake and where to allocate more budget.

INTRODUCTION

Hepatitis B, C, D and HIV are viral infections that are transmitted through blood or body secretions. Hepatitis B is a vaccine preventable disease therefore for the prevention of hepatitis B, 3 doses of hepatitis B vaccine given at 0,1 and 6 months provides lifelong protection. WHO recommends a birth dose of monovalent hepatitis B vaccine to be given within 24 hours of birth; in countries that have an HBsAg prevalence of > 2%. In Pakistan, hepatitis B birth dose is not yet introduced in the expanded program of immunization (EPI) and children mostly receive hepatitis B as pentavalent vaccine at 6,10 and 14 weeks, leaving a 6 weeks window period for the new born to get infected. Vaccination against hepatitis B virus also protects against delta virus infection. Delta virus is most prevalent in Baluchistan and Sind province followed by Punjab. Though oral medicines are available for the treatment of hepatitis B but not everyone who has hepatitis B needs treatment and the diagnostic algorithm is complicated with lifelong treatment.

There is no vaccine for hepatitis C, therefore prevention of the risk factors is the only strategy to avoid it. The virus is called a silent killer because following exposure, the virus continues to circulate in the body for many years (over a decade) without any signs or symptoms and finally presents as chronic liver disease and cirrhosis ultimately leading to death. WHO recommends that all patients having active HCV infection should be treated using direct acting antivirals (DAAs). The development of pan genotypic DAAs and the new HCV testing and treatment algorithm developed by WHO has made its diagnosis and treatment so simple that even general physicians and the allied health care staff can treat majority of the patients while specialists may treat the complicated cases. Pakistan has also developed its National HCV guidelines with an intention to treat all cases and leave no one behind. These guidelines have been simplified further to allow general physicians to test and treat the patients at their doorstep.

HIV usually spreads in very risky behaviour men and women through sexual or intravenous drug use (IDU). Spread of the disease in general population can occur through transfusion of unscreened blood, unsafe injections and poor infection control. The outbreak of HIV in Ratto Dero district of Sindh was attributed to the reuse of syringes to give therapeutic injections for common ailments.

Methods

The study population comprised of entire population of province of Sindh including its urban and rural areas as defined in the 2017 census.

Sampling framework:

The sampling framework of Federal Bureau of statistics was used which was implemented by the Sindh Bureau of Statistics. The provinces was stratified into Urban and rural areas. Each city or town is divided into many enumeration blocks. Each enumeration block has 200-250 households with well-defined boundaries and maps. The list of enumeration blocks and villages/mouzas/dehs as prepared by population census organization were used as sampling framework. Enumeration blocks and villages were called as primary sampling units from urban and rural areas or domains.

Stratification plan:
Urban domains

Large cities: Large cities like Karachi, Hyderabad, Sukkur are considered large cities and are dealt with as a separate stratum. These are further sub stratified into low, middle and high income areas depending on the information collected from these enumeration blocks at the time of updating the urban areas sampling frame or household lists.

Remaining Urban Areas: All cities and towns that are left after excluding the large population cities are grouped under another stratum called other urban areas. Each division in remaining urban areas constitutes a stratum.

Rural domains

In rural domain each district is considered as an independent stratum.

Sample size

To address the primary objectives of the survey, the sample size was estimated using the sample size formula (equation 1) using an expected prevalence for HBV, HCV and HIV, inflated by the anticipated non-response rate (20%) and multiplied by the number of strata (n=29). For the calculation of the sample size, the following parameters were considered:[2]

	Expected prevalence	Desired absolute precision	Anticipated non-response	Sample Size (district)	Number of strata	Total sample size (Province)
HBV	3.5%	±3.5%	20%	199	29	5771
HCV	7%	±5%	20%	189	29	5481
HIV	0.5%	±0.25	20%	-	-	5706

Confidence interval: 95% and DEFF: 1.5

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[2] See more details in the 2015 WHO reference manual for vaccination coverage cluster surveys, annex B –1 for cluster surveys[15].

Equation 1: sample size calculation formula

$$n = deff \times \frac{N\hat{p}\hat{q}}{\frac{d^2}{1.96^2}(N-1) + \hat{p}\hat{q}}$$

where:

n=sample size

deff=design effect

N=population size

p= estimated prevalence

q= 1-p

d=desired absolute precision or absolute level of precision

Based on the assumptions above, the total sample size for HBV was calculated to be 5771 individuals, for HCV 5481 individuals and for HIV 5706. Since the estimated sample size for HBV is greater than the rest, the total sample size for the survey was adjusted to be 5771 (199 individuals per district). Based on the expected household average size (5 members per HH), 40 households in each district were selected. Eight clusters of 5 households each was allocated using PPES in each district to reach the sample size, which represents a total of 1160 households, meaning 40 households in each district (Appendix I).

Sample Design:
A stratified two stage sample design was used for this survey

Contract

WHO country office made a contract agreement with the Sindh health department. The budget was transferred to health department. All the procurements were done by the hepatitis program manager following government rules and conditions.

Dr Huma Qureshi who had undertaken the national hepatitis B and C survey in 2007-08 was contracted by WHO to undertake the Sindh hepatitis B,C,D and HIV survey.

RDTs, ELISA and NAT testing
WHO prequalified rapid device tests (SD Bioline) were procured by the program manager for HBsAg and anti HCV. For HIV three rapid tests were procured with first being Alere kit (HIV combo). Those who were positive on this test were run another rapid test using Unigold device. Those who were positive on Unigold were run a 3rd rapid test using SD Bioline kit.

NAT testing was done using the already available geneXpert machines of the TB program installed in the District Head Quarter Hospitals (DHQs). These machines were programed by the local distributor of Cepheid for the qualitative and quantitative viral load testing of HBV and HCV. The HBV and HCV GeneXpert cartridges were procured by the hepatitis program.

For delta virus, ELISA testing was done in batches on the already stored sera.

For HIV, only rapid test were run by the field teams in the field following the already agreed protocol. No venous blood was taken for further testing of HIV.

Survey teams:

Each survey team comprised of an enumerator from the Sindh bureau of statistics for selection of enumeration block and the selection of the households. There was a male interviewer who also worked as a supervisor of the team, a female interviewer and a phlebotomist. Following the identification of the households by the statistics enumerator, the male interviewer took the permission from the head of the household and interviewed the males while female interviewer interviewed the females. The phlebotomist performed finger prick for rapid test of HBV, HCV and HIV. He was also responsible to do a venepuncture and collected blood for cases that were positive for hepatitis B or C on rapid test.

A total of 4 teams were trained with each given 7 districts to cover, except for team D which covered 8 districts. The teams and their assigned districts are at appendix II.

Training of teams:

Two days training of the teams was conducted on how to select the household, their approach to the head of the household to get consent to collect household information, how to motivate the individuals to participate in the survey and blood testing, how to pose the question and fill the questionnaire, how to label the devices, how to counsel the persons before the blood test and after a positive blood test and how to undertake a finger prick for rapid test and how to interpret the results. Hands on training on rapid devices was done to give them confidence on using these devices and interpret their results.

Land marks and routes used to reach the primary sampling unit.

Team supervisor was entrusted with the job of marking the route on a paper to reach the primary sampling unit. Important sites like hospital, mosque, school or shop were used as a reference. The door of each household selected was also marked with unique identifier of HEP/PSU number/household number.

The community notables or people with good reputation in the community were approached prior to the survey to take them into confidence about the survey and their participation. The medical superintendent of the district hospital was also taken on board to keep him in the loop and ensure the availability of lab and its technicians for running the NAT test on geneXpert.

Survey Questionnaire:

Each survey questionnaire was translated from English to Urdu and later back translated. Once ready they were run as pilot on few individuals who were not part of the survey. After further corrections the questionnaires were finalised.

For each household, a household information questionnaire was filled. This included the basic information about all individuals who were presently living in that selected house, like their names, age, gender, relation with the head of the household etc.

Information about each individual present in the house at the time of survey and who consented to be part of the survey was collected on the individual form. This form included information about education, marital status, and exposure to risk actors of disease. For each individual including children, a separate form was filled. Information from individuals 18 years or older was collected directly from them while for those who were less than 18 years, the information was collected from their parents or caregivers. A consent form was filled for every individual who was aged 18 years or older while for children, the assent was taken from parents or caregivers.

Report form was filled for every individual that gave blood for rapid testing. All negative persons were informed of their healthy status while for those with a positive HBsAg or anti HCV test, venous blood was collected for further tests and they were informed to collect their report from the DHQ later. Those found to be positive on NAT testing for HCV were linked to the hepatitis program for the provision of the medicines.

Data collection procedure

Trained field workers visited the selected household and provided information about the survey and relevant documents to the head of the household or the respondent and requested for his/her participation in the survey. After collecting information about the household on the household information, they obtained informed consent from each individual who was present in the house. Once agreed, individual information was collected through direct interviews followed by pre-test counselling and blood testing.

Rapid blood test:

Following aseptic procedures the middle finger of the left hand was pricked using a lancet. A drop of blood was placed on Hepatitis B,C and HIV kit after proper labelling of the devices. Diluent was added as per recommendations of the manufacturer and results were read after 5-10 minutes. Appearance of 2 pink/red lines in the window of the device was taken as positive test while one line was taken as negative. If no line appeared the test was taken as invalid and repeated. For HIV, the 1st rapid test was Alere (HIV combo). Those cases that came positive on 1st test were run on 2nd rapid test using Unigold device. Those who were positive on 2nd test were run on the third rapid test using SD Bioline kit.

Devices were pasted on the individual form to ease their tracking

Nucleic Acid Test (NAT):

In cases of positive rapid B or C test, 8 ml of whole blood was taken from the vein using aseptic procedure. This blood was poured in the gel tube, labelled and kept in a cool box after clotting. It was transported to the DHQ laboratory for NAT testing to see the presence of the virus. At the DHQ, the gel tube was centrifuged to separate the serum. The serum was extracted and stored in another tube after proper marking of the name of the individual and the PSU. This sample was frozen at minus 40 degrees for nucleic acid testing for RNA. After NAT testing all samples were again frozen at minus 40 degrees for further use.

Enzyme linked Immunoassay (ELISA) for anti-Delta:

All HBV positive sera were frozen and sent to Karachi for antidelta testing using ELISA.

Post-test counselling information was shared only with the individuals who tested positive for either HBV or HCV or HIV or both. They were further encouraged to give venous samples for further confirmation of disease using NAT testing and treatment if required.

Field workers revisited the households two times within two consecutive days to include members who were not present at the time of the survey or for households that were locked. If the individual was unable to join the survey despite repeated attempts of the team, he/she were counted as absent (non-response).

Field teams used a cluster log sheet to track the number of households visited and completed interviews, number of tests performed and blood specimens collected, number of households abandoned, absent and refusals.

After completion of each primary sampling unit, the forms along with devices were checked for their completeness of information and then placed in polythene bags and sealed. Each bag was marked with the name of the PSU and the number of households that were covered in the survey.

Data entry:

All questionnaires were brought to Karachi and data entry was done in EPI INFO. Data was checked for consistency by another data entry person.

Statistical Analysis:

The data feeding and analysis was done on computer package SPSS (Statistical Packages of Social Sciences) version 19.0. Clinical characteristics were summarized in terms of frequencies, percentages and 95% of Confidence Interval for qualitative categorical variables, mean±S.D for quantitative/continuous variables i.e. age and years of education, etc. Statistical comparison in between groups, was performed using student t-test and ANOVA for quantitative variables and Chi-square/fisher test (for values<5) for qualitative variables. In all statistical analysis only p-value <0.05 was considered significant.

Referral and linkage to care:

All HBsAg positive cases along with their HBVDNA and anti delta reports were referred to the hepatitis program for further evaluation by the experts. All HCVRNA positive cases were referred to the hepatitis program for initiation of treatment using DAAs.

Results:

The survey was started on 1st November 2019 and finished in mid-June 2020. Due to tight time lines, teams had to undertake interview and testing till late in the evenings especially for people who were not present at the time of the visit of the field teams. As the survey was done in winters, with extremely low temperatures, therefore RDTs were very slow to show the positive bands. In many cases it took over 20 minutes to become positive. Errors were also noted in geneXpert reporting in 64 cases. A team from Cepheid and local distributor and the TB control program technicians worked for almost 3-4 weeks to sort the issue; which was found to be low room temperature causing improper homogenization of the serum and thus leading to error while cartridges were being run. To complete the numbers, the blood samples were collected again from the same 50 individuals in June 2020 while 14 cases refused testing. For these 50 blood samples, geneXpert was not used and HCV RNA was done at the hepatitis control program's laboratory.

A total of 1153 households from 29 districts of Sindh province were visited. There were 7133 household members and 6769 (94.9%) individuals were present at the time of survey. A total of 6672 (98.6%) underwent screening for HBV, HCV and HIV. There was an average of 5.8 persons per house. Refusal was seen in 97 people (1.4%).

Out of 6769 household members present, there were 3214 (47.5%) males, 3547 (52.4%) females and 08 transgenders (0.1%).The age breakup and district wise numbers is shown in Figure 1 (appendix I).

Information on education was available in 3181 individuals out of 3684 (86.3%). Majority {1818 (57.2%)} were illiterate. Five years of education were completed by 413 (13%) persons, 6-10 years of education was completed by 508 (16%), 263 (8.3%) had completed 11-12 years of education and 179 had education of over 12 years.

Family history of liver disease was present in 411 cases (11.2%). Past history of treatment for HCV was present in 61 cases (15%).
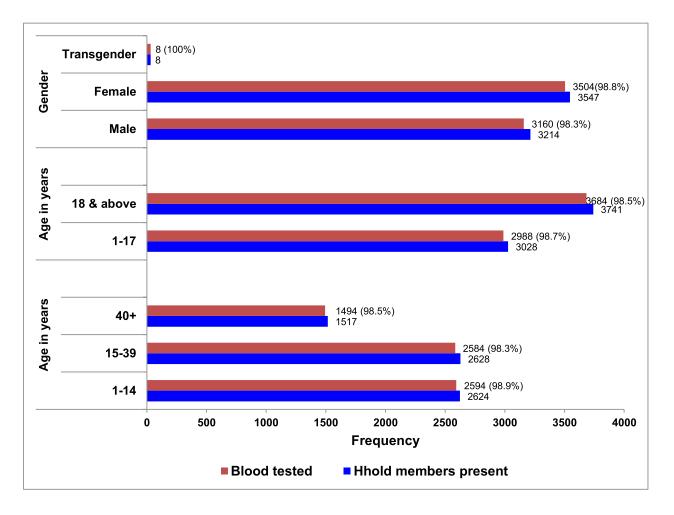
Figure 1: Age and Gender distribution of the population

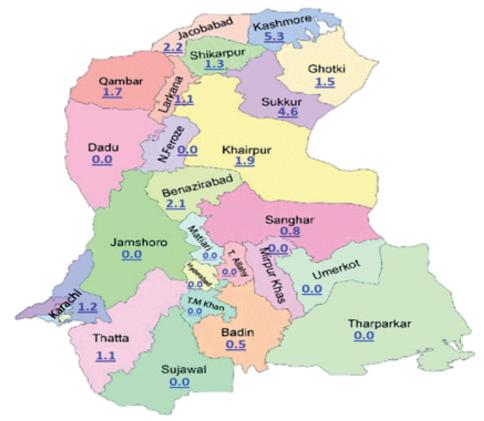## Overall HBsAg, HBV DNA and anti-Delta in Sindh

Out of 7133 individuals, 6672 (93.5%) were screened for HBsAg using rapid test. Overall HBsAg was detected in 70 (1.05%) persons with 42 males (1.33%) and 28 (0.80%) females. There were 8 transgender children and all were HBsAg negative. Family history of liver disease was present in 14 (20.0%) cases. The prevalence of HBsAg with 95% confidence interval and gender wise distribution is shown in table 1. The scatter of HBsAg prevalence in Sindh Province is shown in figure 2.

Table 1: Prevalence of HBsAg according to gender

Gender	Subject tested	HBsAg +ve	
		No. (%)	95% C.I.
Male	3160	42 (1.33)	0.93 – 1.73
Female	3504	28 (0.80)	0.50 – 1.09
Transgender	8	0	-
Total	6672	70 (1.05)	0.80 – 1.29

Figure 2: Scatter of HBsAg in Sindh

Prevalence of HBsAg in Sindh (District Wise)

*All values are in percentage

Age wise distribution is shown in table 2. Overall HBsAg was detected in 70 cases. Prevalence was 0.3% in children less than 5 years of age and it peaked to 2.1% in those aged between 25-34 years. Gender wise, in those aged age <5 years, there was no male child infected with HBsAg but there were two female children infected with HBsAg. The HBsAg prevalence increased with age. In those aged between 25-34 years, there were 3.5% males and 1.1% females showing strong male preference for the disease. There were 8 transgender children out of whom 4 were under 5 years of age and 4 were between 5-11 years of age and all were HBsAg negative.

Table 2: Prevalence of HBsAg according to Age and Gender

Age in years	Overall				Male				Female			
	Subject Tested	HBs Ag Positive			Subject tested	HBs Ag Positive			Subject tested	HBs Ag Positive		
		No.	%	95% C.I.		No.	%	95% C.I.		No.	%	95% C.I.
< 5	633	2	0.32	0.12 - 0.75	318	0	0.00	-	311	2	0.64	0.25 - 1.53
5-11	1492	9	0.60	0.21 - 1.00	745	6	0.81	0.16 - 1.45	743	3	0.40	0.05 - 0.86
12-17	863	7	0.81	0.14 - 1.25	410	5	1.22	0.02 - 1.93	453	2	0.44	0.17 -1.05
18-24	808	16	1.98	1.02 - 2.94	356	9	2.53	0.90 - 4.16	452	7	1.55	0.41 - 2.69
25-34	954	20	2.10	1.27 - 3.13	405	14	3.46	1.86 - 5.54	549	6	1.09	0.22 - 1.96
35-44	761	8	1.05	0.33 - 1.78	356	4	1.12	0.03 - 2.22	405	4	0.99	0.02 - 1.95
45-54	540	4	0.74	0.02 - 1.46	259	3	1.16	0.14 - 2.46	281	1	0.36	0.34 - 1.05
55-64	361	2	0.55	0.21 -2.32	159	0	0.00	-	202	2	0.99	0.38 - 2.36
65-74	203	2	0.99	0.37 - 2.34	121	1	0.83	0.79 - 2.44	82	1	1.22	1.16 - 3.60
≥ 75	57	0	0.00	-	31	0	0.00	-	26	0	0.00	-
Total	6672	70	1.05	0.80 - 1.29	3160	42	1.33	0.93 - 1.73	3504	28	080	0.50 - 1.09

Transgender 8:   4 (under 5 years) and 4 (5-11 years of age),all HBsAg -ve

Risk Factors for Disease:

The risk factors for the disease are summarised in table 3. Family history of chronic liver diseases and Shaving at barbers appeared as the major risk factors with odds ratio (OR) of 2.5 and 2.2 respectively while other risk factors were not significant because the OR was 1 or slightly over 1.

Table 3. Factors Associated with Chronic HBV along with Odds Ratio

Variables		No. of Subject	Prevalence of HBV	OR (95% C.I.)
Live or have ever lived with someone **Chronic Liver disease** member	Yes	444	13 (2.92%)	** 2.53 (1.27 – 4.95)
	No	3222	39 (1.18%)	Reference
Shaved by a traditional barber (Males)	Yes	952	23 (2.42%)	2.20 (0.93 – 5.38)
	No	719	8 (1.11%)	Reference
Hospitalized in the last 12 months	Yes	793	13 (1.64%)	1.21 (0.61 – 2.36)
	No	2872	39 (1.36%)	Reference
Tattooed, pierced or received acupuncture	Yes	488	8 (1.64%)	1.19 (0.51 – 2.64)

	No	3184	44 (1.38%)	Reference
Received any therapeutic injection in the last 12 months	Yes	2623	38 (1.45%)	1.08 (0.57 – 2.11)
	No	1046	14 (1.34%)	Reference
Received an invasive dental treatment	Yes	455	5 (1.10%)	0.75 (0.26 – 1.98)
	No	3217	47 (1.46%)	Reference
Received a blood transfusion	Yes	290	3 (1.03%)	0.71 (0.21 – 2.37)
	No	3361	49 (1.46%)	Reference

** Statistically significant ($p < 0.01$)

District wise HBsAg prevalence is shown in table 4. HBsAg prevalence was highest in Kashmore (4.8%) followed by Sukkur (4.6%), Jacobabad (2.2%) and Shaheed Benazirabad (2.1%).

Table 4: Prevalence of HBsAg in various districts (n=6672)

	No. of Subject	Blood tested	HBsAg positive		*1SD/2SD
			No.	%	
Kashmore / Kandkot	235	208	10	4.80	1 SD
Sukkur	188	173	8	4.62	1 SD
Jacobabad	254	226	5	2.21	
Shaheed Benazirabad	237	236	5	2.12	
Karachi Central	233	199	4	2.01	
Karachi West	272	249	5	2.01	
Ghotki	288	260	5	1.92	
Khairpur	286	265	5	1.89	
QamberS hahdadKot	276	241	4	1.66	
Shikarpur	267	227	3	1.32	
Malir	249	235	3	1.28	
Thatta	291	274	3	1.09	
Larkana	308	277	3	1.08	
Korangi	219	210	2	0.95	
Karachi East	259	235	2	0.85	
Sanghar	249	242	2	0.83	
Badin	223	219	1	0.46	
Dadu	257	250	0	0.00	
Hyderabad	180	179	0	0.00	
Jamshoro	254	243	0	0.00	
Karachi South	229	203	0	0.00	
Matiari	228	219	0	0.00	
MirpurKhas	249	235	0	0.00	
NaushahroFiroze	266	255	0	0.00	
Sajawal	204	196	0	0.00	

TandoAllahyar	228	220	0	0.00	
Tando M Khan	225	224	0	0.00	
Tharparkar	237	237	0	0.00	
UmerKot	242	235	0	0.00	
	7133	6672	70	1.05	

*Districts having >1 standard deviation prevalence when compared with provincial data

Nucleic Acid Test:

HBVDNA levels were run on geneXpert in 70 samples that were found HBsAg positive on rapid test. Overall 56 out of 70 (80%) samples showed the presence of HBVDNA and only 14 showed no HBVDNA (Table 5).

Table 5: Nucleic Acid Test (HBVDNA) Results in HBsAg positive cases

		NAT Results (HBV DNA)		
	Positive	Detected	Not detected	Error
HBV	70	56 (80.0%)	14	-

The HBVDNA viral load in the individuals along with age and gender is shown in table 8. Levels over 20,000 were found in 16 persons and age wise all were under 15 years and majority (9) were females(table 6).

Table 6: HBsAg and HBV DNA positivity according to gender and age

	Tested	HBsAg Positive	HBV DNA				
			Detected	Viral load (IU/ml) - Values			
				< 2000	2000 to 10,000	10,000 to 20,000	> 20,000
Overall	6672	70	**56 (80.0%)**	29 (51.8%)	10 (17.8%)	1 (1.8%)	16 (28.6%)
Gender							
Male	3160	42	**33 (78.6%)**	20 (66.6%)	5 (15.2%)	1 (3.0%)	7 (21.2%)
Female	3504	28	**23 (82.1%)**	9 (39.1%)	5 (21.7%)	-	9 (39.1%)
Transgender	8	-	-	-	-	-	-
Age in years							
< 5	633	2	2 (100%)	-	-	-	2 (100%)
5-11	1492	9	8 (88.9%)	2 (25.0%)	-	-	6 (75.0%)
12-17	863	7	7 (100%)	5 (83.3%)	-	-	1 (16.7%)
18-24	808	16	11 (68.7%)	4 (36.4%)	2 (18.2%)	-	5 (45.4%)
25-34	954	20	16 (80.0%)	12 (75.0%)	3 (18.7%)	-	1 (6.3%)
35-44	761	8	7 (87.5%)	4 (57.1%)	2 (28.6%)	1 (14.3%)	-
45-54	540	4	2 (50.0%)	1 (50.0%)	-	-	1 (50.0%)
55-64	361	2	1 (50.0%)	-	1 (100%)	-	-
65-74	203	2	2 (100%)	1 (50.0%)	1 (50.0%)	-	-
≥ 75	57	-	-	-	-	-	-
Total	6672	70	56 (80.0%)	29 (51.8%)	10 (17.8%)	1 (1.8%)	16 (28.6%)

Anti Delta Prevalence in Sindh:

Out of 70 HBsAg positive cases, frozen samples were available for 64 cases. These frozen samples were brought to Karachi and run as a batch using ELISA. Anti delta was detected in 21 cases (32.8%). There were 39 (61%) males and 25 females (39%) out of which 16 (41%) males and 5 (20%) females were delta positive. Age and gender distribution is shown in table 7. Three children/adolescents between 5-17 years were anti delta positive and over all disease was mostly noted between 18-34 years of age.

Table 7: Age, gender distribution of antidelta virus

Gender	Subject tested	Delta + ve	
		No.	%
Overall	**64**	**21**	**32.8**
Male	39	16	41.0
Female	25	5	20.0
Age in years			
< 5	2	-	

5-11	9	3	33.3
12-17	7	1	14.3
18-24	14	7	50.0
25-34	19	7	36.8
35-44	8	2	25.0
45-54	2	1	50.0
55-64	1	-	-
65-74	2	-	-

District wise delta virus distribution is shown in table 8. Delta virus was mostly found in Sanghar, Khairpur and Sukkur.

Table 8: Delta virus in various districts

Districts	Tested	Delta Positive	
		No.	%
Sanghar	2	2	100.0
Khairpur	5	3	60.0
Sukkur	7	4	57.1
Thatta	2	1	50.0
Jacobabad	5	2	40.0
Shaheed Benazir	5	2	40.0
QamberShahdadKot	3	1	33.3
Larkana	3	1	33.3
Shikarpur	3	1	33.3
Kashmore / Kandkot	10	3	30.0
Ghotki	4	1	25.0
Badin	1	0	-
Karachi Central	3	0	-
Karachi East	2	0	-
Karachi West	5	0	-
Korangi	2	0	-
Malir	2	0	-
Total	64	21	32.8

Overall Anti HCV and HCV RNA in Sindh

Rapid test for anti HCV was run on 6672 persons out of a population of 7133. Overall 413 (6.2%) cases were anti HCV positive; indicating exposure to hepatitis C virus. According to gender, anti HCV was detected in 183 (5.8%) males and 230 (6.6%) females. Family history of HCV was present in 65 (18.6%) cases. Past history of treatment for HCV was present in 61 cases (15%).
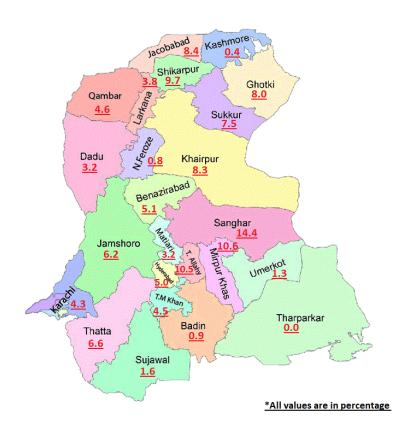
The prevalence of anti HCV with 95% confidence interval and the gender is shown in table 9. The scatter of HCV prevalence in Sindh is shown in Figure 3

Table 9: Prevalence with 95% Confidence Interval of Anti HCV according to gender

		Anti HCV +ve	
Gender	Subject tested	No. (%)	95% C.I.
Male	3160	183 (5.79)	4.98 – 6.61
Female	3504	230 (6.56)	5.74 – 7.38
Transgender	8	0	–
Total	6672	413 (6.19)	5.61 – 6.77

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Prevalence of HCV in Sindh
(District Wise)

All values are in percentage

The age wise distribution of anti HCV is shown in table 10. Overall anti HCV prevalence increased with the age. Its prevalence was 1.3% in those aged 12-17 years and doubled to 2.6% in 18-24 years age group and from there on increased manifolds. Highest prevalence of 14% was seen from 45 years onward. Within the gender, anti HCV exposure in women was higher than men in ages 25-44 years, after it equalled. All transgender children were anti HCV negative.

Table 10: Prevalence of Anti HCV according to Age and Gender (with 95% Confidence Interval)

Age in Years	Overall				Male				Female			
	Subject tested	Anti HCV Positive			Subject tested	Anti HCV Positive			Subject tested	Anti HCV Positive		
		No.	%	95% C.I.		No.	%	95% C.I.		No.	%	95% C.I.
< 5	633	1	0.16	0.00 – 0.47	318	0	0.00	-	311	1	0.32	0.00 – 0.95
5-11	1492	4	0.27	0.01 – 0.53	745	1	0.13	0.00 - 0.40	743	3	0.40	0.00 – 0.86
12-17	863	11	1.27	0.53 – 2.02	410	6	1.46	0.30 - 2.63	453	5	1.10	0.14 – 2.07
18-24	808	22	2.72	1.60 – 3.84	356	12	3.37	1.50 – 5.25	452	10	2.21	0.86 – 3.57
25-34	954	82	8.60	6.82 – 10.37	405	26	6.42	4.03 – 8.81	549	56	10.20	7.67 – 12.73
35-44	761	89	11.70	9.41 – 13.98	356	34	9.55	6.50 – 12.60	405	55	13.58	10.24 – 16.92
45-54	540	88	16.30	13.18 – 19.41	259	46	17.76	13.11 – 22.42	281	42	14.95	10.78 – 19.12
55-64	361	68	18.84	14.80 – 22.87	159	31	19.50	13.34 – 25.65	202	37	18.32	12.98 – 23.65
65-74	203	40	19.70	14.23 – 25.18	121	22	18.18	11.31 – 25.05	82	18	21.95	12.99 – 30.91
≥ 75	57	8	14.04	5.02 – 23.05	31	5	16.13	3.18 – 29.08	26	3	11.54	0.00 – 23.82
Total	6672	413	6.19	5.61 – 6.77	3160	183	5.79	4.98 – 6.61	3504	230	6.56	5.74 – 7.38

Transgender 8: 4 (under 5 years) and 4 (5 11 years of age),all were Anti HCV negative

Risk Factors for HCV:

Risk factors for HCV are summarised in table 11. Family history of liver disease, therapeutic injections, blood transfusion and hospitalization showed a strong association with HCV prevalence as the Odds ratio was more than 1.3 in all.

Table 11. Factors Associated with Chronic HCV along with Odds Ratio

Variables		No. of Subject	Prevalence of HCV	OR (95% C.I.)
Live or have ever lived with someone **Chronic Liver Disease** member	Yes	444	96 (21.62%)	** 2.69 (2.08 – 3.47)
	No	322	299 (9.27%)	Reference
Received any therapeutic injection in the last 12 months	Yes	2623	315 (12.01%)	** 1.63 (1.26 – 2.09)
	No	1046	81 (7.74%)	Reference
Received a blood transfusion	Yes	290	43 (14.83%)	* 1.50 (1.05 – 2.09)
	No	3361	352 (10.47%)	Reference
Hospitalized in the last 12 months	Yes	793	110 (13.87%)	* 1.47 (1.16 – 1.86)
	No	2872	284 (9.89%)	Reference
Tattooed, pierced or received acupuncture	Yes	488	71 (14.55%)	* 1.49 (1.14 – 1.97)
	No	3184	325 (10.21%)	Reference

Received an invasive dental treatment	Yes	455	59 (12.97%)	* 1.33 (0.98 – 1.77)
	No	3217	335 (10.41%)	Reference
Shaved by a traditional barber (Males)	Yes	952	105 (11.03%)	1.15 (0.83 – 1.58)
	No	719	70 (9.74%)	Reference

Statistically significant * P<0.05, ** p<0.01

District wise anti HCV prevalence is shown in table 12. The prevalence was highest in Sanghar (14.5%) followed by Ghotki (11.5%), Mirpur Khas (10.6%) and Tando Allahyar (10.4%). Using 1 or 2 standard deviation from the mean prevalence in the province, the high prevalence districts are shown as 1 or 2 SD.

Table 12: Prevalence of Anti HCV with district (n=6672)

Districts	No. of Subject	Blood tested	Anti HCV positive		
			No.	%	Standard Dev.
Sanghar	249	242	35	14.46	2 SD
Ghotki	288	260	30	11.54	1 SD
Mirpur Khas	249	235	25	10.64	1 SD
Tando Allahyar	228	220	23	10.45	1 SD
Shikarpur	267	227	22	9.69	1 SD
Jacobabad	254	226	19	8.41	1 SD
Khairpur	286	265	22	8.30	1 SD
Dadu	257	250	19	7.60	
Sukkur	188	173	13	7.51	
Thatta	291	274	18	6.57	
Malir	249	235	15	6.38	
Jamshoro	254	243	15	6.17	
Hyderabad	180	179	11	6.15	
Karachi Central	233	199	11	5.53	
Korangi	219	210	11	5.24	
Karachi West	272	249	13	5.22	
Shaheed Benazirabad	237	236	12	5.08	
Badin	223	219	11	5.02	
Qamber Shahdadkot	276	241	11	4.56	
Tando M Khan	225	224	10	4.46	
Karachi South	229	203	9	4.43	
Larkana	308	277	11	3.97	
Naushahro Firoz	266	255	9	3.53	
Karachi East	259	235	8	3.40	
UmerKot	242	235	8	3.40	
Tharparkar	237	237	8	3.38	
Matiari	228	219	7	3.20	
Kashmore / Kandkot	235	208	4	1.92	
Sajawal	204	196	3	1.53	
Total	7133	6672	413	6.19	

Nucleic Acid Test (NAT):

HCVRNA was run on geneXpert on 332 samples and RT PCR on 17 samples making a total of 349 (84.5%) out of 413 anti HCV samples. Others (64) refused to give the blood sample. HCVRNA was detected in 210 samples (60.2%) and no HCVRNA was detected in 125 samples (35.8%). HCV RNA could not be run on 14 samples due to an error detected in geneXpert cartridge.

Age and gender wise HCVRNA distribution is shown in table 13. HCVRNA was detected in 64.2% females and 55.1% males. Viral detection was maximally seen from 25 years onwards.

Table13: Age and gender wise HCVRNA distribution in HCV cases

	Tested	Anti HCV Positive	HCVRNA Tested	HCV RNA		
				Positive	Negative	Error
Overall	6672	413 (6.19%)	349 (84.5%)	210 (60.2%)	125 (35.8%)	14 (4.0%)
Gender						
Male	3160	183 (5.79%)	156 (85.2%)	86 (55.1%)	65 (41.7%)	5 (3.2%)
Female	3504	230 (6.56%)	193 (83.9%)	124 (64.2%)	60 (31.1%)	9 (4.7%)
Transgender	8	0		–	–	–
Age in years						
< 5	633	1 (0.16%)	1 (100%)	–	1 (100%)	–
5-11	1492	4 (0.27%)	4 (100%)	1 (25.0%)	2 (50.0%)	1 (25.0%)
12-17	863	11 (1.27%)	11 (100%)	5 (45.4%)	5 (45.4%)	1 (9.1%)
18-24	808	22 (2.72%)	22 (100%)	12 (54.5%)	10 (45.5%)	–
25-34	954	82 (8.60%)	75 (91.5%)	52 (69.3%)	22 (29.3%)	1 (1.3%)
35-44	761	89 (11.70%)	71 (79.8%)	48 (67.6%)	20 (28.2%)	3 (4.2%)
45-54	540	88 (16.30%)	78 (88.6%)	53 (68.0%)	22 (28.2%)	3 (3.8%)
55-64	361	68 (18.84%)	49 (22.1%)	21 (42.9%)	24 (49.0%)	4 (8.2%)
65-74	203	40 (19.70%)	30 (75.0%)	10 (33.3%)	19 (63.3%)	1 (3.3%)
≥ 75	57	8 (14.04%)	8 (100%)	8 (100%)	–	–
Total	6672	413 (6.19%)	349 (84.5%)	210 (60.2%)	125 (35.8%)	14 (4.0%)

Risk Factors for HCV:

Amongst the various risk factors questioned for HCV infection, having lived with someone suffering from hepatitis appeared as the major risk factor seen in 21.6% cases with an odds ratio of 2.69 indicating a strong association of disease spread within the house members due to exposure to infected body fluids. Other risk factors were all coming from the health care settings like receiving therapeutic injections (12%), receiving blood transfusion (14.8%), getting hospitalized (13.8%) or undergoing dental treatment (12.9%). Community spread through tradition rituals like ear and nose piercing, tattooing and head shave etc were also contributory factors in 14.5% cases. (table 14)

Table 14: Risk factors for HCV with Odds ratio

Variables		No. of Subject	Prevalence of HCV	OR (95% C.I.)
Live or have ever lived with someone having **Chronic Liver Disease**	Yes	444	96 (21.62%)	** 2.69 (2.08 − 3.47)
	No	322	299(9.27%)	Reference
Received any therapeutic injection in the last 12 months	Yes	2623	315(12.01%)	** 1.63 (1.26 − 2.09)
	No	1046	81(7.74%)	Reference
Received a blood transfusion	Yes	290	43 (14.83%)	* 1.50 (1.05 − 2.09)
	No	3361	352 (10.47%)	Reference
Hospitalized in the last 12 months	Yes	793	110 (13.87%)	* 1.47 (1.16 − 1.86)
	No	2872	284 (9.89%)	Reference
Tattooed, pierced or received acupuncture	Yes	488	71 (14.55%)	* 1.49 (1.14 − 1.97)
	No	3184	325 (10.21%)	Reference
Received an invasive dental treatment	Yes	455	59 (12.97%)	* 1.33 (0.98 − 1.77)
	No	3217	335 (10.41%)	Reference
Shaved by a traditional barber (Males)	Yes	952	105 (11.03%)	1.15 (0.83 − 1.58)
	No	719	70 (9.74%)	Reference

Overall Coinfection with HBV+HCV in Sindh

Out of 7133 individuals 6672 (93.5%) were screened for HBsAg and anti HCV using rapid test. Three cases (0.04%) were positive for both HBsAg and anti HCV.Gender wise coinfection was detected in 2 males and 1 female (Table 15). Coinfection was seen in 1 case each in age group 18-24, 45-54 and55-64 years.

Table 15: Prevalence of HBV and HCV Coinfection (with 95% C.I.)

Gender	Subject tested	Both HBsAg &Anti HCV Positive	
		No. (%)	95% C.I.
Male	3160	2 (0.06)	0.02 – 0.15
Female	3504	1 (0.03)	0.03 – 0.08
Transgender	8	0	–
Total	6672	3 (0.04)	0.01 – 0.10

HBsAg and Anti HCV in Children and Adolescents in Sindh

A total of 633 children less than 5 years of age were screened for HBsAg using rapid test. Overall HBsAg was detected in two (0.32%) children who were both females. There were a total of 2355 children and adolescents between 5-17 years and out of these 15 (0.6%) were HBsAg positive. In this age group, males were twice more affected with the virus than females. There were 8 transgender children and all were HBsAg negative. The 95% confidence interval and gender distribution is shown in table 16.

Table 16: Prevalence of HBsAg according to Age and Gender in Children and Adolescents

Age in years	Overall				Male				Female			
	Subject Tested	HBs Ag Positive			Subject tested	HBs Ag Positive			Subject tested	HBs Ag Positive		
		No.	%	95% C.I.		No.	%	95% C.I.		No.	%	95% C.I.
< 5	633	2	0.32	0.12 – 0.75	318	0	0.00	-	311	2	0.64	0.25 – 1.53
5-11	1492	9	0.60	0.21 – 1.00	745	6	0.81	0.16 – 1.45	743	3	0.40	0.05 – 0.86
12-17	863	7	0.81	0.21 – 1.41	410	5	1.22	0.02 – 1.93	453	2	0.44	0.17 –1.05
Total	2988	18	0.60	0.32 – 0.88	1473	11	0.75	0.31 – 1.19	1507	7	0.46	0.12 – 0.81

Transgender 8:   4 (under 5 years) and 4 (5-11 years of age), all were HBsAg negative

Out of 18 HBsAg positive children, delta virus was not detected in any child aged less than 5 years, while it was detected in 4 cases (22.2%) cases (table 17).

Table 17: Delta virus in Children and Adolescents

Gender	Subject tested	Delta + ve	
		No.	%
Overall	18	4	22.2
Male	11	3	27.3
Female	7	1	14.3
Age in years			
< 5	2	-	-
5-11	9	3	33.3
12-17	7	1	14.3

The anti HCV was tested in 633 children who were less than 5 years of age and only one (0.16%) female child had detected anti HCV. Anti HCV was checked in 2355 children/ adolescents between 5-17 years of age and it was detected in 15 (0.6%). The prevalence of anti HCV increased to 0.27% in children between 5-11 years of age and 1.27% in those between 12-17 years. Gender wise there was equal distribution between two genders (table 18). There were 8 transgender children and all were anti HCV negative.

Table 18: Prevalence of Anti HCV according to Age and Gender in Children and Adolescents

Age in Years	Overall				Male				Female			
	Subject Tested	Anti HCV Positive			Subject tested	Anti HCV Positive			Subject tested	Anti HCV Positive		
		No.	%	95% C.I.		No.	%	95% C.I.		No.	%	95% C.I.
< 5	633	1	0.16	0.00 – 0.47	318	0	0.00	-	311	1	0.32	0.00 – 0.95
5-11	1492	4	0.27	0.01 – 0.53	745	1	0.13	0.00 - 0.40	743	3	0.40	0.00 – 0.86
12-17	863	11	1.27	0.53 – 2.02	410	6	1.46	0.30 - 2.63	453	5	1.10	0.14 – 2.07
Total	2988	16	0.54	0.27 - 0.80	1473	7	0.48	0.12 - 0.83	1507	9	0.60	0.21 - 0.99

Transgender 8: 4 (under 5 years) and 4 (5-11 years of age), all were Anti HCV negative

Overall HIV in Sindh

The 1st rapid HIV test was run on all cases using Alere kit (HIV combo). Those who were positive on this test were run another rapid test using Unigold device. Those who were positive on Unigold were run a 3rd rapid test using SD Bioline kit. Only 2 cases were positive on all 3 tests, giving a prevalence of 0.03%

Both these subjects were husband and wife aged over 40 years belonging to Karachi. Both were negative for HBsAg and anti HCV.

Comparison of 2008 and 2019 survey data

The HBV prevalence data of previous survey (2008) was compared with the present data (2019) to see the trends over the decade (table 19). Over all the HBsAg prevalence has gown down from 2.5% to 1.1%. Except for Sukkur where the HBV prevalence has gone up from 1.5% to 4.6% all other districts showed a down ward trend, which is very encouraging and is due to the high HB vaccination in children through EPI and CSOs and partners and adult vaccination through public and private sector.

Table 19. Comparison of High Prevalence HBsAg Districts of Sindh in the two surveys

District	2008	2019
Sukkur	1.5	4.6
Jacobabad	3.3	2.2
Khairpur	6.3	1.9
Ghotki	5.9	1.5
Shikarpur	3.3	1.3
Karachi	1.4	1.2
Larkana	4.3	1.1
Thatta	3.1	1.0
Sanghar	2.8	0.8
Nosheroferoz	4.0	0.0
MirpurKhas	3.0	0.0
Hyderabad	2.6	0.0
Total	2.5	1.1

Similarly for HCV, the comparison between the two surveys is shown in table 20. Here the situation is totally different when compared to the hepatitis B. Almost all districts showed a rise in the HCV prevalence when compared from the past. In Sanghar and many other districts, the prevalence of HCV increased two folds in a decade.

Table 20.   High HCV Prevalence Districts of Sindh in the two surveys

District	Previous HCV	Current HCV
Sanghar	7.8	14.5
Ghotki	12.7	11.5
Mirpur Khas	4.9	10.6
Shikarpur	5.3	9.7
Jacobabad	5.3	8.4
Khairpur	3.3	8.3
Sukkur	3.7	7.5
Thatta	5.4	6.6
Hyderabad	5.7	6.2
Dadu	7.2	7.6
Total	5.0	6.2

Comparison of HBsAg and Anti HCV in children

In children aged less than 5 years of age, the HBsAg prevalence in 2008 was 1.3% which has dropped to 0.32% in the present survey. The HBsAg prevalence in 5–19 years in the last survey was 1.7% which has now dropped to 0.6% in children aged 5–17 years. Overall 1% drop in the prevalence of HBsAg is noted over the last decade.

The anti HCV prevalence in children less than 5 years of age in the 2008 survey was 1.9% which has now dropped to 0.16% in the current survey. The anti HCV in 5–19 years in the last survey was 2.1% which is now 0.54% in children aged 5–17 years. In children, overall 1.6% decrease in the anti HCV prevalence is seen over the last decade.

Appendix-III: Age and district wise distribution of the population

Characteristics	Household members	HHold members present		Blood test done		Blood test refused	
		No.	%	No.	%	No.	%
Overall	**7133**	**6769**	**94.9**	**6672**	**98.6**	**97**	**1.4**
Age in years							
1-14	2687	2624	97.7	2594	98.9	30	1.2
15-39	2827	2628	93.0	2584	98.3	44	1.7
40+	1619	1517	93.7	1494	98.5	23	1.5
1-17	3112	3028	97.3	2988	98.7	40	1.3
18 & above	4021	3741	93.0	3684	98.5	57	1.5
Gender							
Male	3488	3214	92.1	3160	98.3	54	1.7
Female	3637	3547	97.5	3504	98.8	43	1.2
Transgender	8	8	100.0	8	100.0	0	0.0
Districts							
Badin	223	220	98.7	219	99.5	1	0.5
Dadu	257	250	97.3	250	100.0	0	0.0
Ghotki	288	264	91.7	260	98.5	4	1.5
Hyderabad	180	179	99.4	179	100.0	0	0.0
Jacobabad	254	231	90.9	226	97.8	5	2.2
Jamshoro	254	243	95.7	243	100.0	0	0.0
Karachi Central	233	203	87.1	199	98.0	4	2.0
Karachi East	259	250	96.5	235	94.0	15	6.0
Karachi South	229	210	91.7	203	96.7	7	3.3
Karachi West	272	254	93.4	249	98.0	5	2.0
Kashmore / Kandkot	235	211	89.8	208	98.6	3	1.4
Khairpur	286	269	94.1	265	98.5	4	1.5
Korangi	219	217	99.1	210	96.8	7	3.2
Larkana	308	281	91.2	277	98.6	4	1.4
Malir	249	239	96.0	235	98.3	4	1.7
Matiari	228	223	97.8	219	98.2	4	1.8
MirpurKhas	249	235	94.4	235	100.0	0	0.0
NaushahroFiroze	266	255	95.9	255	100.0	0	0.0
QamberShahdadKot	276	247	89.5	241	97.6	6	2.4
Sajawal	204	196	96.1	196	100.0	0	0.0
Sanghar	249	242	97.2	242	100.0	0	0.0
ShaheedBenazirabad	237	236	99.6	236	100.0	0	0.0
Shikarpur	267	239	89.5	227	95.0	12	5.0
Sukkur	188	176	93.6	173	98.3	3	1.7
TandoAllahyar	228	220	96.5	220	100.0	0	0.0
Tando M Khan	225	224	99.6	224	100.0	0	0.0
Tharparkar	237	237	100.0	237	100.0	0	0.0
Thatta	291	283	97.3	274	96.8	9	3.2
UmerKot	242	235	97.1	235	100.0	0	0.0
Total	7133	6769	94.9	6672	98.6	97	1.4

Appendix-II: Teams and their districts

Team A	Team B	Team C	Team D
1.Karachi central	8. Badin	15. MirpurKhas	22. Ghotki
2.Karachi east	9. Dadu	16. ShaheedBenazirabad	23. Khairpur
3.Korangi Karachi	10. Hyderabad	17. NowsheroFeroz	24. Sukkur
4.Malir Karachi	11. Jamshoro	18. Sanghar	25. Jaccobabad
5.Karachi South	12. Mitiari	19. Tharparkar	26. Kashmore
6.Karachi west	13. Sajawal	20. Tando Muhammad Khan	27. Larkana
7.Thatta	14. Tando Allah Yar	21. Umerkot	28. Qambar
			29. Shikarpur
Team A	Team B	Team C	Team D
1.Karachi central	8. Badin	15. MirpurKhas	22. Ghotki
2.Karachi east	9. Dadu	16. ShaheedBenazirabad	23. Khairpur
3.Korangi Karachi	10. Hyderabad	17. NowsheroFeroz	24. Sukkur
4.Malir Karachi	11. Jamshoro	18. Sanghar	25. Jaccobabad
5.Karachi South	12. Mitiari	19. Tharparkar	26. Kashmore
6.Karachi west	13. Sajawal	20. Tando Muhammad Khan	27. Larkana
7.Thatta	14. Tando Allah Yar	21. Umerkot	28. Qambar
			29. Shikarpur

Appendix-I: Number of clusters, households and sample size by district, Sindh province

District	Number of clusters	Number of HHs	Sample size
Badin	8	40	199
Dadu	8	40	199
Ghotki	8	40	199
Hyderabad	8	40	199
Jacobabad	8	40	199
Jamshoro	8	40	199
Karachi Central	8	40	199
Karachi East	8	40	199
Karachi South	8	40	199
Karachi West	8	40	199
Kashmore	8	40	199
Khairpur	8	40	199
Korangi	8	40	199
Larkana	8	40	199
Malir	8	40	199
Matiari	8	40	199
MirpurKhas	8	40	199
NaushahroFiroze	8	40	199
QambarShahdadkot	8	40	199
Sanghar	8	40	199
ShaheedBenazirabad	8	40	199
Shikarpur	8	40	199
Sujawal	8	40	199
Sukkur	8	40	199
TandoAllahyar	8	40	199
Tando Muhammad Khan	8	40	199
Tharparkar	8	40	199
Thatta	8	40	199
Umerkot	8	40	199
TOTAL	**232**	**1160**	**5771**