

Prevalence of viral HBV and HCV among different group patients in Gujrat Pakistan

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ABSTRACT

In this study we analyzed blood samples collected from 400 high risk patients for the prevalence of an inflammatory viral disease hepatitis B virus (HBV) and hepatitis C virus (HCV) with the help of standard kit assay and Enzyme-linked immunosorbent assay (ELISA). All the samples were selected randomly from the various places of District Gujrat, Pakistan. All the selected cases were first divided into four groups according to the age and sex (Group 1, Male below age 35 years; Group 2, Male above age 35 years; Group 3, Female below age 35 years; Group 4, Female above age 35 years), each group was comprised of 100 individual patients and analyzed for different parameters for the presence of HBV and HCV in comparison with positive and negative controls. The prevalence of HBV and HCV was higher in groups 2 (22%) and 4 (39%) respectively. Assay profile revealed that the incidence of HCV was higher in female patients as compare to the male patients. The present study indicates that more than 60% of the cirrhosis and hepatocellular carcinoma in the Region is attributable to hepatitis B virus (HBV) or hepatitis C virus (HCV) infection.

Keywords: HBV; HCV; Liver Inflammation; LFTs; ELISA; ALP; Bilirubin

1. INTRODUCTION

Viral hepatitis is frequently fatal and one of commonest liver infectious (inflammatory) disease across the world [1]. Worldwide, probably 2 billion and 350 to 400 million people are chronically infected with this viral infection [2,3]. Approximately 170 million people throughout the world have chronic HCV infection and that 3 - 4 mil-

lion people are newly infected each year. Hepatitis B and C virus is present in blood, saliva, semen, vaginal secretions, menstrual blood, and to lesser extent, breast milk, and urine of infected individuals [4]. A highly resilient virus, HBV or HCV can easily transmit through contaminated blood transfusion, surgical instruments, dental surgery, sexual contacts and drug abuses, sharing of the house hold items like shaving razors, toothbrushes and also shaving from the barber [4-6].

Patients with slightly elevated ALT (1 - 2 times than the normal limit) are more likely to have transient elevation not because of the disease; however, about 30% of those with chronic HCV infection have peak ALT. In developing countries where resources and facilities may be significantly limited, the prevalence of HBV or HCV is higher as compared to the developed world [7]. In adults daily production of un-conjugated bilirubin is about 250 to 350 mg, normal values is 5 mg/kg/day, or about 400 mg/day. However in comparison to the un-conjugated the level of conjugated bilirubin is highly specific for liver disease. The serum bilirubin level is mainly associated with the extent of hepatocellular necrosis. In this regard further studies are needed to characterize the epidemiology of HBV and HCV transmission, especially in Pakistan.

The present study was conducted to investigate the incidence/prevalence of Hepatitis B and C virus in District Gujrat, Pakistan. In this study, we have analyzed a wide range of patients belonging to various health risk groups for the prevalence of HBV and HCV infection.

2. MATERIALS AND METHODS

2.1. Sample Collection

Total 400 clinically diagnosed patients were consecutively recruited for the study from various places of District Gujrat Hospitals, Pakistan. Patients were considered to have hepatitis B or hepatitis C infection if their ELIZA

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or screening test was positive and had raised alanine-aminotransferase (ALT) level.

2.2. Blood Samples

Up to 5 mL blood was drawn using sterilized disposable syringes from each patient and immediately transferred into the EDTA (anticoagulant) containing tubes. The samples were thoroughly mixed and finally centrifuged at 3000 g for 5 min. Separated serum fractions were transferred into sterilized eppendorf tubes and stored at -20°C until further use.

2.3. Determination of Hepatitis B Surface Antigen

For the purpose of quantitative determination of Hepatitis B surface antigen from a separated serum of all the selected samples, RBP4 (human) ELISA Kit was used *in vitro*. In this procedure the antigen is “trapped” between two layers of enzyme specific antibodies. The whole reaction is followed by a wash and enzyme activity of the bound material in each Microtiter well is determined by adding the substrate of the enzyme.

2.4. Liver Function Test (LFT) Profile

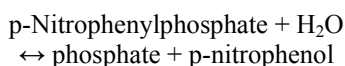
For qualitative detection of HbsAg in whole blood and serum, hepatitis B surface Antigen (HbsAg) was screened out by the use of Trinity Biotech Uni-Gold HbsAg test kit *in vitro*. Serum samples were further analyzed for bilirubin (conjugated and unconjugated), and Alkaline phosphatase (ALP).

2.4.1. Bilirubin

The total bilirubin in serum and plasma was determined by the method of coupling with diazotized sulfanilic acid (29 mmol/L) after the addition of caffeine (130 mmol/L), sodium benzoate (156 mmol/L) and sodium acetate (460 mmol/L). Absorbance was recorded using spectrophotometer at 578 nm. The direct and indirect bilirubin level was measured according to the method described by Saher *et al.* [8].

2.4.2. Alkaline Phosphatase

To determine the ALP level in the separated serum fractions of all patients, Human Gesellschaft für Biochemica und Diagnostica mbH (EC 3.1.3.1) kit method was used. Reaction principle is given below:



2.5. Immunochromatographic Test (ICT)

Immunochromatographic tests strips were obtained from accurate and Acon (Acon, USA). Strips were used ac-

ording to the manufacturer's instructions for the purpose of HCV screening in all of the separated serum fractions samples.

3. RESULTS AND DISCUSSION

To investigate the prevalence of HBV, all of the selected patients were first screened for the presence of HbsAg in the sera. The screening assay profile showed that total 31 cases were positive among all of the 400 analyzed samples. In comparison to the HBV the incidence of HCV was little higher among all groups and total 93 cases were HCV positive from all of the total 400 analyzed samples (Supplementary Table 1-4).

3.1. Group 1 (Male below Age 35 Years)

In this group out of the 100 selected samples only 6% were those having their HBV positive test. In the same group 14% patients were highly affected with HCV. Bilirubin assay profile showed that total 18% patients had the higher Bilirubin level (1.48 ± 1.94) as compare to the normal value (up to 1.2 mg/dl) (Figure 1). While other, 23% had the higher value of Alakaline phosphatase (345 ± 18) than that of the normal limits (80 - 306 U/L), (Figure 2).

3.2. Group 2 (Male above Age 35 Years)

This age group contained HBV and HCV positive ratio 10% and 26% respectively, out of the total 100 randomly selected samples. Bilirubin test assay revealed that 28% had the higher level of Bilirubin with maximum of 2.23 ± 16 mg/dl than the normal value while, other 37% had the higher value of Alakaline phosphatase (368 ± 92) in comparison to the normal limits (Figures 1-2).

3.3. Group 3 (Female below Age 35 Years)

In this group the prevalence of HBV was 50% lower as compare to the group 1 (Male below age 35 Years) but

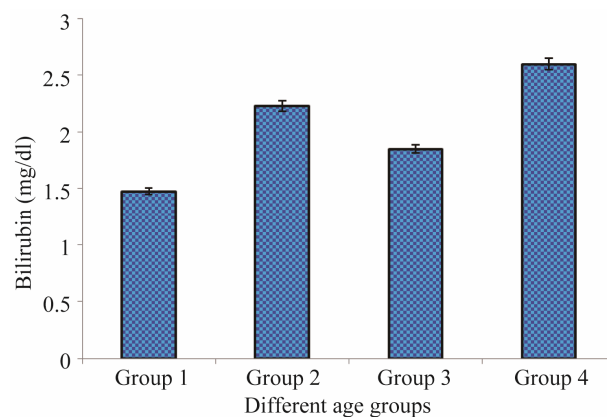


Figure 1. Bilirubin levels in patients of different age groups.

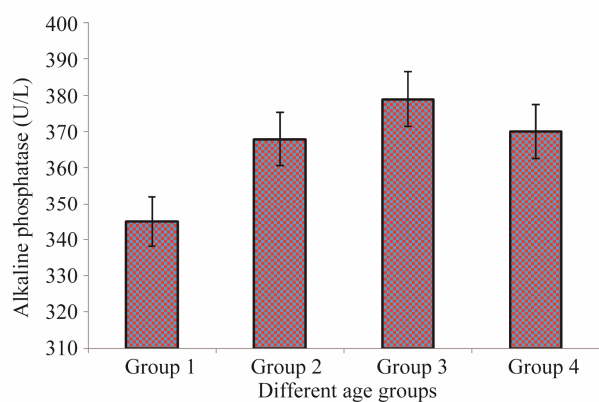


Figure 2. ALP levels in patients of different age groups.

the incidence of HCV positive cases was surprisingly high in comparison to the same age group of males. Out of 100 patients 22% had the higher level of Bilirubin (1.85 ± 24) than that of the normal limits (**Figure 1**). Total 30% were found to have higher value of Alkaline phosphatase (379 ± 19) than its normal limits (80 - 306 U/L) (**Figure 2**).

3.4. Group 4 (Female above Age 35 Years)

In comparison to the all other three groups the patients belong to this group were highly affected with HBV and HCV. Total occurrence of HBV and HCV positive percentage was 12 and 30% respectively. In this age group 38% patients have higher level of Bilirubin (2.6 ± 11) while, 45% have higher level of Alkaline phosphatase (370 ± 56) as compare to the normal limits (**Figures 1-2**).

Many of the earlier studies from various regions of Pakistan have reported high prevalence of HCV (20.5% - 60%), [9,10]. The poor dental procedures are the major source of HBV or HCV exposure (39.7%) that is followed by injections (16.6%) and finally the surgical unsterilized items (16.6%) [11]. In an earlier study, Herve [12] reported that the transmitting risk of these viruses has drastically been reduced by screening for their antibodies before blood transfusion. In the present study we observed higher level of Bilirubin that indicates the biliary inflammation, which may be intrahepatic or extra hepatic and this increased value of Bilirubin, is related to liver hepatitis [13]. In the present study serum ALP values were little higher than many of the previously conducted studies.

Our data suggest that the prevalence of HBV and HCV infection was higher in person with age higher than 35. Similar findings have also been reported previously by Leung [14]. It has been reported in literature that blood transfusion continues to cause hepatitis B or C in those countries, where donor blood is not screened for HBV [15]. The present study indicates that blood transfusion is

taking part in transmission of viral infection. Previously, HBV transmission by blood transfusion has also been reported in Pakistan by [16].

4. CONCLUSION

Lack of proper blood screening facilities and unawareness about the possible transmission routes of HBV and HCV are contributing a great deal towards the spread of the infection among the population. In this regard further studies are needed to characterize the epidemiology of HBV and HCV transmission, especially in Pakistan.

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Supplementary Data Only for Review**Table 1.** Data of incidence of HBV and HCV in Group 1 (Male below age 35 years).

Sr. No.	HBsAg (+ve)	HbsAg (-ve)	Cut off value of HBsAg	HCV (+ve)	HCV (-ve)	Cut off value of HCV
1.		0.007	0.105		0.016	0.150
2.		0.065	0.105	2.521		0.150
3.		0.009	0.105		0.080	0.150
4.		0.021	0.105		0.005	0.150
5.		0.007	0.105		0.001	0.150
6.		0.012	0.105		0.004	0.150
7.		0.010	0.105		0.017	0.150
8.		0.016	0.105		0.010	0.150
9.		0.018	0.105		0.011	0.150
10.		0.020	0.105		0.033	0.150
11.		0.011	0.105		0.023	0.150
12.		0.010	0.105		0.009	0.150
13.		0.049	0.105		0.021	0.150
14.		0.091	0.105		0.037	0.320
15.		0.014	0.105	2.569		0.320
16.		0.017	0.105		0.021	0.320
17.		0.086	0.105		0.021	0.320
18.		0.021	0.105		0.052	0.320
19.		0.040	0.105		0.078	0.320
20.		0.065	0.105		0.028	0.320
21.		0.041	0.105	3.222		0.320
22.		0.015	0.105	2.218		0.320
23.		0.005	0.105		0.014	0.233
24.		0.019	0.105	2.451		0.150
25.		0.035	0.105		0.010	0.150
26.		0.007	0.105		0.012	0.150
27.		0.014	0.105		0.005	0.150
28.		0.010	0.105	0.867		0.150
29.		0.005	0.105		0.008	0.150
30.		0.012	0.105		0.005	0.150
31.		0.010	0.105		0.007	0.150
32.		0.009	0.105		0.008	0.150
33.		0.010	0.105		0.010	0.150
34.		0.013	0.105	3.446		0.150
35.		0.022	0.105		0.014	0.150
36.		0.016	0.105		0.009	0.150
37.		0.018	0.105		0.014	0.150
38.		0.012	0.105		0.010	0.150
39.		0.013	0.105	3.786		0.150
40.		0.015	0.105	1.809		0.150
41.		0.021	0.105		0.011	0.150

Continued

42.		0.018	0.105		0.012	0.150
43.		0.011	0.105	2.294		0.150
44.		0.018	0.105		0.016	0.150
45.		0.007	0.105		0.009	0.150
46.		0.025	0.105		0.011	0.150
47.		0.016	0.105		0.074	0.150
48.		0.029	0.105		0.014	0.150
49.		0.010	0.105		0.012	0.150
50.		0.038	0.105		0.009	0.150
51.		0.018	0.105		0.009	0.150
52.		0.009	0.105		0.009	0.150
53.	3.885		0.105		0.013	0.150
54.		0.013	0.105		0.010	0.150
55.		0.013	0.105		0.011	0.150
56.		0.008	0.105		0.008	0.150
57.		0.015	0.105		0.009	0.150
58.		0.016	0.105		0.008	0.150
59.		0.042	0.105		0.015	0.150
60.		0.015	0.105		0.014	0.150
61.		0.008	0.105		0.012	0.150
62.		0.023	0.105		0.017	0.150
63.		0.008	0.105		0.016	0.150
64.		0.021	0.105		0.009	0.150
65.		0.008	0.105		0.010	0.150
66.		0.008	0.105		0.007	0.150
67.		0.008	0.105		0.063	0.150
68.		0.009	0.105		0.006	0.150
69.		0.009	0.105		0.007	0.150
70.		0.007	0.105		0.011	0.150
71.		0.019	0.105		0.012	0.150
72.		0.018	0.105		0.010	0.150
73.		0.024	0.105		0.010	0.150
74.		0.005	0.105		0.006	0.150
75.		0.025	0.105		0.009	0.150
76.	3.463		0.105		0.006	0.150
77.		0.009	0.105		0.007	0.150
78.		0.014	0.105	1.883		0.150
79.		0.013	0.105		0.004	0.231
80.	3.744		0.105		0.039	0.231
81.		0.005	0.105		0.003	0.231
82.		0.009	0.105		0.008	0.231
83.	3.941		0.105		0.006	0.231
84.		0.004	0.105		0.004	0.380

Continued

85.		0.015	0.106	3.162		0.380
86.		0.046	0.106		0.074	0.380
87.		0.078	0.106		0.086	0.380
88.		0.027	0.106		0.071	0.380
89.		0.031	0.106		0.004	0.186
90.		0.006	0.078		0.003	0.186
91.		0.008	0.078		0.005	0.186
92.		0.009	0.078	3.077		0.186
93.		0.006	0.078		0.003	0.186
94.		0.005	0.078	2.425		0.186
95.		0.055	0.233		0.016	0.250
96.		0.043	0.137		0.011	0.250
97.	3.629		0.137		0.003	0.210
98.		0.020	0.088		0.013	0.210
99.		0.007	0.110		0.008	0.210
100.	3.763		0.110		0.006	0.210

Table 2. Data of incidence of HBV and HCV in Group 2 (Male above age 35 years).

Sr. No.	HBsAg (+ve)	HbsAg (-ve)	Cut off value of HBsAg	HCV (+ve)	HCV (-ve)	Cut off value of HCV
1.		0.008	0.105		0.032	0.150
2.		0.037	0.105	2.998		0.150
3.		0.008	0.105		0.008	0.150
4.		0.008	0.105		0.008	0.150
5.		0.034	0.105		0.034	0.150
6.	3.643		0.105		0.019	0.150
7.		0.033	0.105		0.010	0.150
8.	3.933		0.105		0.021	0.150
9.		0.022	0.105		0.016	0.150
10.		0.011	0.105		0.022	0.150
11.	3.483		0.105		0.014	0.150
12.		0.005	0.105		0.009	0.150
13.		0.031	0.105		0.009	0.150
14.		0.052	0.105		0.009	0.150
15.		0.015	0.105		0.020	0.150
16.	3.394		0.105		0.011	0.150
17.		0.013	0.105	2.840		0.150
18.		0.005	0.105		0.007	0.150
19.	3.741		0.105	3.551		0.150
20.	1.127		0.105		0.009	0.150
21.		0.005	0.105		0.007	0.150
22.		0.001	0.105		0.004	0.231
23.		0.005	0.105		0.011	0.231
24.		0.029	0.106		0.008	0.231

Continued

25.		0.029	0.106	3.169		0.380
26.		0.043	0.137		0.040	0.380
27.	3.629		0.137		0.110	0.380
28.		0.040	0.137	3.464		0.250
29.		0.038	0.137		0.016	0.250
30.		0.038	0.080		0.011	0.250
31.		0.088	0.210		0.003	0.250
32.		0.042	0.210		0.004	0.250
33.		0.039	0.210		0.004	0.185
34.		0.025	0.210		0.030	0.210
35.		0.008	0.210		0.019	0.210
36.		0.015	0.210	3.646		0.210
37.		0.021	0.210		0.004	0.210
38.		0.021	0.210		0.022	0.210
39.		0.023	0.190	3.469		0.210
40.		0.012	0.190		0.007	0.210
41.		0.018	0.190	3.068		0.210
42.		0.017	0.105	3.060		0.210
43.		0.014	0.105		0.052	0.210
44.		0.018	0.105		0.002	0.190
45.		0.023	0.105		0.002	0.190
46.		0.022	0.105	2.353		0.190
47.		0.032	0.105	3.164		0.190
48.		0.022	0.105		0.001	0.190
49.		0.173	0.260	3.140		0.190
50.		0.060	0.260	3.287		0.190
51.		0.063	0.260		0.004	0.190
52.		0.074	0.260		0.004	0.190
53.	0.249		0.260	3.097		0.190
54.		0.220	0.260		0.017	0.190
55.		0.078	0.260		0.003	0.190
56.		0.020	0.110		0.007	0.230
57.		0.013	0.110			0.230
58.		0.016	0.110	3.964		0.230
59.		0.011	0.110	3.962		0.230
60.		0.019	0.110	3.672		0.230
61.		0.009	0.110		0.066	0.230
62.		0.034	0.115		0.024	0.230
63.		0.025	0.115	3.957		0.230

Continued

64.	0.030	0.115	3.963		0.230
65.	0.042	0.115		0.021	0.230
66.	0.040	0.115		0.017	0.180
67.	0.008	0.150	3.344		0.180
68.	0.010	0.150		0.004	0.180
69.	0.018	0.150	3.208		0.180
70.	0.103	0.150		0.004	0.180
71.	0.078	0.150		0.008	0.180
72.	0.022	0.107		0.002	0.180
73.	0.012	0.107		0.172	0.411
74.	0.007	0.107		0.139	0.411
75.	0.051	0.107		0.117	0.411
76.	0.016	0.150	3.270		0.411
77.	0.135	0.150		0.003	0.411
78.	0.017	0.150		0.203	0.411
79.	0.014	0.090	1.609		0.300
80.	0.049	0.111		0.003	0.300
81.	0.057	0.111	2.974		0.300
82.	0.037	0.107		0.226	0.300
83.	0.028	0.107		0.009	0.300
84.	0.025	0.099		0.003	0.300
85.	0.022	0.099			0.300
86.	0.025	0.099			0.300
87.	0.024	0.099			0.300
88.	0.043	0.099		0.003	0.190
89.	0.027	0.184		0.004	0.190
90.	0.020	0.082		0.003	0.190
91.	0.017	0.090		0.003	0.190
92.	0.018	0.090		0.003	0.250
93.	0.019	0.090		0.011	0.250
94.	0.015	0.090	3.220		0.250
95.	0.011	0.090		0.004	0.190
96.	0.022	0.106		0.004	0.190
97.	0.022	0.106		0.007	0.190
98.	0.019	0.102	2.832		0.193
99.	0.008	0.102		0.036	0.193
100.	0.015	0.072		0.037	0.193

Table 3. Data of incidence of HBV and HCV in Group 3 (Female below age 35 years).

Sr. No.	HBsAg (+ve)	HbsAg (-ve)	Cut off value of HBsAg	HCV (+ve)	HCV (-ve)	Cut off value of HCV
1.		0.016	0.105		0.037	0.150
2.		0.009	0.105		0.007	0.150
3.		0.041	0.105		0.008	0.150
4.		0.011	0.105		0.009	0.150
5.		0.016	0.105	2.949		0.150
6.		0.027	0.105		0.005	0.150
7.		0.006	0.105		0.019	0.150
8.		0.014	0.105		0.006	0.150
9.		0.009	0.105		0.011	0.150
10.		0.017	0.105		0.023	0.150
11.		0.019	0.105	1.383		0.150
12.		0.012	0.105		0.032	0.150
13.		0.010	0.105		0.011	0.150
14.		0.012	0.105		0.020	0.150
15.	0.147		0.105		0.013	0.150
16.		0.041	0.105		0.015	0.150
17.		0.011	0.105	2.964		0.150
18.		0.018	0.105	2.675		0.150
19.		0.013	0.105		0.013	0.150
20.		0.004	0.105		0.010	0.150
21.		0.004	0.105		0.029	0.150
22.		0.012	0.105		0.039	0.320
23.		0.019	0.105		0.025	0.320
24.		0.040	0.105		0.021	0.320
25.		0.021	0.105		0.037	0.320
26.		0.014	0.105		0.047	0.320
27.		0.030	0.105		0.078	0.320
28.		0.079	0.105		0.014	0.320
29.		0.020	0.105	3.055		0.320
30.		0.011	0.105	2.900		0.320
31.		0.014	0.105		0.063	0.320
32.		0.074	0.105		0.032	0.320
33.		0.047	0.105		0.024	0.320
34.		0.023	0.105		0.020	0.320
35.		0.018	0.105		0.103	0.320
36.		0.016	0.105		0.013	0.320
37.	3.669		0.105		0.069	0.320
38.		0.013	0.105		0.065	0.320
39.		0.031	0.105		0.112	0.320
40.		0.016	0.105	1.372		0.320
41.		0.009	0.105		0.006	0.233
42.		0.069	0.105		0.011	0.233

Continued

43.		0.008	0.105		0.014	0.233
44.		0.008	0.105	2.307		0.233
45.		0.015	0.105		0.006	0.150
46.		0.012	0.105		0.021	0.150
47.		0.012	0.105		0.009	0.150
48.		0.014	0.105		0.070	0.150
49.		0.007	0.105		0.051	0.150
50.		0.012	0.105	3.759		0.150
51.		0.010	0.105	3.417		0.150
52.		0.011	0.105	1.190		0.150
53.		0.006	0.105	2.488		0.150
54.	3.957		0.105		0.010	0.150
55.		0.011	0.105		0.006	0.150
56.		0.014	0.105		0.017	0.150
57.		0.016	0.105	3.007		0.150
58.		0.013	0.105	1.800		0.150
59.		0.011	0.105		0.009	0.150
60.		0.009	0.105	2.567		0.150
61.		0.009	0.105		0.005	0.150
62.		0.006	0.105	2.273		0.150
63.		0.013	0.105		0.007	0.150
64.		0.013	0.105		0.004	0.150
65.		0.009	0.105	2.904		0.150
66.		0.016	0.105		0.007	0.150
67.		0.012	0.105	1.070		0.150
68.		0.006	0.105	3.671		0.150
69.		0.013	0.105		0.008	0.150
70.		0.011	0.105		0.023	0.150
71.		0.013	0.105	3.929		0.150
72.		0.024	0.105	1.176		0.150
73.		0.007	0.105		0.009	0.150
74.		0.009	0.105		0.010	0.150
75.		0.039	0.105		0.010	0.150
76.		0.021	0.105		0.010	0.150
77.		0.009	0.105		0.007	0.150
78.		0.018	0.105	2.608		0.150
79.		0.018	0.105		0.009	0.150
80.		0.013	0.105		0.008	0.150
81.		0.015	0.105		0.009	0.150
82.		0.008	0.105		0.009	0.150
83.		0.010	0.105		0.008	0.150
84.		0.008	0.105	3.565		0.150
85.		0.014	0.105		0.013	0.150

Continued

86.	0.008	0.105	0.032	0.150
87.	0.013	0.105	0.007	0.150
88.	0.010	0.105	0.007	0.150
89.	0.010	0.105	0.005	0.150
90.	0.007	0.105	0.012	0.150
91.	0.012	0.105	0.008	0.150
92.	0.011	0.105	0.011	0.150
93.	0.011	0.105	0.007	0.150
94.	0.011	0.105	0.017	0.150
95.	0.059	0.105	0.007	0.150
96.	0.005	0.105	0.008	0.150
97.	0.005	0.105	0.005	0.150
98.	0.006	0.105	0.006	0.231
99.	0.006	0.105	0.016	0.231
100.	0.023	0.106	0.191	0.231

Table 4. Data of incidence of HBV and HCV in Group 4 (Female above age 35 years).

Sr. No.	HBsAg (+ve)	HbsAg (-ve)	Cut off value of HBsAg	HCV (+ve)	HCV (-ve)	Cut off value of HCV
1.		0.004	0.105		0.014	0.150
2.		0.005	0.105	2.509		0.150
3.		0.032	0.105	2.995		0.150
4.		0.013	0.105		0.024	0.150
5.		0.008	0.105		0.028	0.150
6.		0.039	0.105		0.011	0.150
7.		0.013	0.105		0.040	0.150
8.		0.012	0.104	3.075		0.190
9.		0.059	0.104		0.011	0.190
10.		0.033	0.104		0.005	0.230
11.		0.086	0.260		0.026	0.230
12.		0.069	0.260	2.823		0.180
13.		0.011	0.110	2.571		0.180
14.		0.014	0.110		0.117	0.180
15.		0.011	0.110		0.003	0.180
16.		0.033	0.115	2.436		0.180
17.		0.054	0.115		0.007	0.180
18.		0.050	0.115		0.359	0.411
19.		0.011	0.150	2.783		0.300
20.		0.043	0.150		0.066	0.300
21.		0.031	0.150		0.122	0.300
22.		0.126	0.150		0.005	0.300
23.		0.017	0.150	2.576		0.300
24.		0.020	0.090	2.400		0.300
25.		0.015	0.090	2.258		0.300

Continued

26.		0.021	0.090	2.167		0.190
27.		0.042	0.111	1.621		0.190
28.		0.044	0.111		0.031	0.250
29.		0.045	0.111	2.945		0.190
30.	3.496		0.099	2.962		0.190
31.		0.019	0.099	3.070		0.190
32.	1.511		0.099	3.090		0.190
33.		0.022	0.099		0.004	0.190
34.		0.017	0.184		0.001	0.190
35.		0.030	0.184		0.016	0.190
36.		0.020	0.184	2.138		0.190
37.		0.022	0.184		0.036	0.182
38.		0.023	0.082	2.700		0.182
39.		0.019	0.090	2.659		0.182
40.		0.023	0.090	3.221		0.182
41.	3.635		0.090	2.064		0.182
42.		0.021	0.090		0.002	0.184
43.		0.026	0.090		0.004	0.184
44.		0.022	0.090		0.006	0.184
45.		0.018	0.090		0.006	0.184
46.		0.011	0.090	1.662		0.184
47.		0.013	0.090		0.007	0.185
48.		0.019	0.090		0.004	0.185
49.		0.025	0.106		0.017	0.185
50.		0.026	0.106		0.012	0.185
51.		0.020	0.106		0.002	0.185
52.		0.002	0.063		0.027	0.185
53.		0.027	0.071	3.150		0.184
54.		0.025	0.071		0.108	0.184
55.		0.008	0.071	0.436		0.184
56.		0.022	0.071		0.132	0.184
57.		0.025	0.071		0.009	0.184
58.		0.024	0.071	2.850		0.193
59.		0.021	0.071	2.687		0.193
60.		0.014	0.071	2.768		0.193
61.		0.005	0.071	2.780		0.193
62.		0.019	0.071	2.621		0.195
63.		0.063	0.143		0.070	0.195
64.		0.076	0.143	2.465		0.195
65.		0.085	0.143		0.005	0.184
66.		0.065	0.143	2.627		0.184
67.		0.055	0.143		0.002	0.184
68.		0.060	0.143		0.010	0.184

Continued

69.		0.048	0.143	2.951		0.184
70.		0.069	0.143		0.012	0.184
71.		0.064	0.143		0.003	0.184
72.		0.064	0.143		0.003	0.184
73.		0.054	0.143		0.003	0.184
74.		0.052	0.143	2.855		0.184
75.		0.014	0.092	2.532		0.184
76.		0.035	0.093	2.728		0.184
77.		0.016	0.093		0.009	0.184
78.	3.530		0.100		0.006	0.184
79.		0.005	0.100		0.005	0.184
80.		0.016	0.100	2.516		0.184
81.		0.007	0.100	2.709		0.184
82.		0.012	0.100		0.005	0.184
83.		0.017	0.106	2.981		0.184
84.		0.010	0.083		0.006	0.184
85.		0.010	0.103		0.004	0.184
86.			0.102		0.004	0.184
87.	2.938	0.043	0.072	3.178		0.184
88.					0.007	0.184
89.					0.003	0.184
90.	3.741			2.082		0.189
91.	1.127			2.109		0.189
92.	3.741			2.104		0.189
93.					0.010	0.189
94.	1.127			0.534		0.184
95.	2.222			2.702		0.184
96.				2.854		0.184
97.	3.741			2.774		0.184
98.					0.007	0.192
99.					0.005	0.192
100.	1.127			3.115		0.192