

## Healthcare Related Factors Associated with Mono-Infection and Co-Infection of Bloodborne Viruses in Lasbela and Quetta, Baluchistan

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### Abstract

**Objective:** To determine the frequency of human immunodeficiency virus, hepatitis B virus and hepatitis C virus infection, and to compare the prevalence and risk factors of mono-infection versus co-infection.

**Method:** The comparative, cross-sectional study was conducted in selected hospitals in Lasbela and Quetta districts of Balochistan, Pakistan, from November 2022 to April 2023, and comprised patients of either gender aged  $\geq 18$  years visiting the participating hospitals. The patients were screened using an immunochromatographic test kit, and were divided into mono-infected with human immunodeficiency virus group 1, mono-infected with hepatitis C virus group 2, mono-infected with hepatitis B virus group 3, co-infected with two or three viruses group 4, and healthy controls group 5. Factors associated with co-infection versus mono-infection of blood-borne viruses were identified. Data was analysed using SPSS 21.

**Results:** Of the 1992 subjects, 1043(52.4%) were females, 949(47.60%) were males, and 864(43.4%) were aged 30-44 years. Of the total, 75(3.8%) subjects were in group 1, 175(8.8%) in group 2, 88(4.4%) in group 3, 79(4%) in group 4, and 1,575(79.1%) in control group 5. In group 4, 27(34.1%) subjects had human immunodeficiency virus and hepatitis B virus, followed by 26(32.9%) having hepatitis B virus and hepatitis C virus, 17(21.5%) having human immunodeficiency virus and hepatitis C virus, and 9(11.3%) having triple infection. Among other findings, history of surgical procedures, male gender and rural residence were significantly associated with co-infection ( $p < 0.05$ ).

**Conclusion:** Strengthening infection control practices in district hospitals, expanding vaccination coverage, incorporating supplementary testing methods, and promoting community awareness campaigns about safe injection and transfusion practices are to control the risk of life-threatening virus in the community.

**Keywords:** Hepatitis C, Hepatitis B, Mono-infections, Co-infection, Prevalence, Balochistan.

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### Introduction

Blood-borne viruses are a serious public health concern due to their high prevalence, morbidity and mortality rates.<sup>1</sup> A range of diseases is associated with mono-infection and co-infection of blood-borne viruses, including chronic hepatitis, acquired immunodeficiency syndrome (AIDS), liver cancer and liver cirrhosis.<sup>2,3</sup> Hepatitis B virus (HBV), human immunodeficiency virus (HIV) and hepatitis C virus (HCV) are the three most common blood-borne viruses.<sup>4,5</sup>

HBV, HIV and HCV contribute substantially to the global burden of disease.<sup>6</sup> Although these viruses share several transmission routes, important differences exist.<sup>7</sup> For example, HBV is about 100 times more infectious than HIV and 10 times more infectious than HCV, while, unlike HIV, it is not transmitted via breastfeeding or vertical

transmission in the same way.<sup>8,9</sup>

Research in Baluchistan has primarily focused on prevalence. For example, Wali et al. in 2019 reported 20.8% HCV prevalence among male blood donors in Quetta, while Lodhi et al. in 2019 reported HBV prevalence 16.1%, HCV prevalence 43.2%, and 1.6% co-infection among dialysis patients.<sup>10-12</sup> However, to our knowledge, not many studies have systematically examined healthcare-related risk factors for mono-infection versus co-infection in Balochistan.

In Pakistan, HIV and viral hepatitis remain serious public health issues affecting millions. Given the paucity of epidemiological data in Balochistan, the current study was planned to determine the frequency of HIV, HBV and HCV infection, and to compare the prevalence and risk factors of mono-infection versus co-infection.

### Patients and Methods

The multi-centre, hospital-based, comparative, cross-sectional study was conducted to at four hospitals in Lasbela and Quetta districts of Balochistan, Pakistan, from November 2022 to April 2023. The hospitals included Jam Ghulam Qadir Government Hospital, Hub, Lasbela, rural health centre (RHC) Winder, Lasbela, Gaddani Jail Hospital,

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and Bolan Medical Complex, Quetta.

After approval from the institutional ethics review board of Shaheed Zulfiqar Ali Bhutto Institute of Science and Technology (SZABIST), Karachi, the sample size was estimated using the OpenEpi calculator<sup>13</sup> based on data reported by a 2010 study<sup>14</sup> with 80% power and 95% confidence interval (CI). The sample was raised using systematic random sampling from among those visiting the participating hospitals. The first patient was chosen randomly, and then every 5th patient from outpatient department (OPD) registers who met inclusion criteria of patients of either gender aged  $\geq 18$  years. was approached. Those with other acute infections (dengue, malaria, typhoid), mental disorders, cancer or history of immunosuppressive treatment were excluded.

After taking written informed consent, the participants were screened for HIV, HBV and HCV using immunochromatographic test (ICT) kits (Manufacturer: Accu-Tell, USA) with finger-prick blood samples. The subject were divided into mono-infected with HIV group 1, mono-infected with HCV group 2, mono-infected with HBV group 3, co-infected with two or three viruses group 4, and healthy controls group 5. Positive cases were reported to physicians as an ethical requirement to ensure linkage to care and counselling. Non-HBV subjects were facilitated for HBV vaccination.

Data was collected a structured closed-ended questionnaire that was developed in English, and was translated into Urdu and Sindhi. It was administered by two male and two female interviewers fluent in Balochi, Sindhi and Pashto languages. The tool was pilot-tested on 10% of participants.

Data was analysed using SPSS 21. Descriptive statistics were presented as mean $\pm$ standard deviation for continuous variables. Frequencies and percentages were used for categorical variables. Chi-square and independent t-tests were used with  $p \leq 0.05$  being the marker of significance. Bivariate logistic regression was first performed, and variables having  $p < 0.2$  were entered into a multivariate model using backward elimination. Crude odds ratios (cOR) and adjusted odds ratios (aOR) with 95% CIs were calculated. Model diagnostics included the Hosmer–Lemeshow goodness-of-fit test, Nagelkerke  $R^2$ , and receiver operating characteristic (ROC) curve analysis. Multicollinearity was assessed (Variance Inflation Factor  $< 2.0$  for all predictors). Stratified analysis comprised

subgroup comparisons across age, gender and residence categories to check effect modification.

## Results

Of the 1992 subjects, 1043(52.4%) were females, 949(47.60%) were males, and 864(43.4%) were aged 30–44 years. Of the total, 75(3.8%) subjects were in group 1, 175(8.8%) in group 2, 88(4.4%) in group 3, 79(4%) in group 4, and 1,575(79.1%) in control group 5. Within group 4, 27 (34.1%) subjects had HIV and HBV, followed by 26 (32.9%) having HBV and HCV, 17 (21.5%) having HIV and HCV, and 9 (11.3%) having triple infection. Demographic characteristics of subjects in each group were noted (Table 1).

**Table-1:** Socio-demographic and healthcare-related characteristics of the participants.

Factors	Total n (%)	HIV n (%)	HCV n (%)	HBV n (%)	Co-infected n (%)	Healthy n (%)
Overall	1992 (100)	75 (3.8)	175 (8.8)	88 (4.4)	79 (4.0)	1575 (79.1)
<b>Age group (years)</b>						
18–29	852	20 (2.3)	52 (6.1)	45 (5.3)	31 (3.6)	704 (82.6)
30–44	864	55 (6.4)	90 (10.4)	27 (3.1)	46 (5.3)	646 (74.8)
45–59	185	0 (0)	29 (15.7)	14 (7.6)	2 (1.1)	140 (75.7)
60+	91	0 (0)	4 (4.4)	2 (2.2)	0 (0)	85 (93.4)
<b>Gender</b>						
Male	949	65 (6.8)	88 (9.3)	47 (5.0)	58 (6.1)	691 (72.8)
Female	1043	10 (1.0)	87 (8.3)	41 (3.9)	21 (2.0)	884 (84.8)
<b>Residence</b>						
Rural	750	14 (1.9)	59 (7.9)	31 (4.1)	36 (4.8)	610 (81.3)
Urban	1242	61 (4.9)	116 (9.3)	57 (4.6)	43 (3.5)	965 (77.7)

**Table-2:** Comparison of co-infection and mono-infection cases with respect to healthcare-related factors.

Factor	Co-infection n (%)	Mono-infection n (%)	cOR (95% CI)	p-value
Surgical procedure history	33 (44.0)	60/225 (26.7)	2.16 (1.26–3.72)	0.005
Abortion history (N=124)	14/17 (82.3)	58/107 (54.2)	3.94 (1.07–14.52)	0.035
Blood transfusion history	17 (22.7)	59/225 (26.2)	0.83 (0.45–1.53)	0.540
Dental treatment history	17 (22.7)	51/225 (22.7)	1.00 (0.54–1.87)	1.000
Unsafe injection use	27 (36.0)	62/225 (27.6)	1.48 (0.85–2.58)	0.166
Home delivery (n=124)	10/17 (58.8)	57/107 (53.3)	1.25 (0.44–3.54)	0.670

cOR: Crude odds ratio, CI: Confidence interval; Variables with  $p < 0.2$  in bivariate analysis were subjected to multivariate analysis.

**Table-3:** Multivariate analysis of factors between co-infection and mono-infection cases.

Factor	cOR (95% CI)	p-value	aOR (95% CI)	p-value
Gender (Male vs Female)	3.09 (1.70–5.64)	0.000	2.58 (1.29–5.14)	0.007
Residence (Rural vs Urban)	1.96 (1.14–3.37)	0.015	2.20 (1.03–4.71)	0.042
Ethnicity (Sindhi ref.)				
Balochi	4.08 (1.73–9.60)	0.001	2.54 (0.84–7.69)	0.100
Brohi	2.23 (0.84–5.94)	0.108	3.50 (1.00–12.27)	0.050
Others	2.91 (1.19–7.11)	0.019	3.02 (0.93–9.81)	0.066
Education (None ref.)				
Matric or above	2.64 (1.38–5.06)	0.003	2.91 (1.12–7.58)	0.029
Surgical procedure (Yes vs No)	2.16 (1.26–3.72)	0.005	2.22 (1.04–4.75)	0.039

cOR: Crude odds ratio, CI: Confidence interval.

Among other findings, history of surgical procedures, male gender, and rural residence were significantly associated with co-infection (Table 2).

In terms of healthcare-related exposure, 23% (458) of the participants had a history of blood transfusion, among whom 6.4% (29) had HCV, 5% (23) had HIV, 3.5% (16) had HBV, and 3.7% (17) were co-infected. The majority (81.4% (373)) of transfused patients had no viral infection. Model diagnostics indicated adequate fit, while multicollinearity was not observed (Table 3).

## Discussion

In the present study, serological tests indicated anti-HCV status in 8.8% of the subjects, HBV in 4.4%, HIV in 3.8%, HBV+HCV co-infection in 1.3%, HIV+HBV co-infection in 1.4%, HIV+HCV in 0.9%, and triple infection in 0.9%. The results were consistent with national surveys,<sup>15</sup> while HIV prevalence appeared higher, possibly because some study sites were sentinel sites for HIV. The co-infection rate in the current study (4%) was lower than that reported in Tunisia (4.8%) and Egypt (34%).<sup>16,17</sup>

We further observed that males were at three times higher risk of co-infection when compared with mono-infection, which suggests a gender disparity in blood-borne viruses' co-infection. This could be due to differences in biological factors, exposure risks or behaviours. This finding was consistent with other studies in Asia.<sup>18,19</sup> HBV was more prevalent in males, while HCV was more prevalent among females, which were consistent with findings from China.<sup>20</sup>

Living in rural areas was also associated with co-infection, highlighting differences in healthcare access, hygiene and social networks.<sup>21</sup> Although ethnicity did not always reach statistical significance, higher odds were noted among Balochi and Brohi groups, suggesting that cultural and healthcare-seeking behaviour differences may play a role.

Interestingly, participants with formal education at matric level or above had higher odds of co-infection in the current study. This may reflect differing exposure behaviours rather than education itself, and warrants further exploration.

The significant association between surgical procedures and co-infection suggests healthcare exposures may contribute to transmission. This underscores the urgent need for stricter infection-control measures, sterilisation practices, and training of healthcare staff in the studied areas.<sup>22</sup>

Factors that did not reach statistical significance may reflect the small sample size and sparse data bias. With only limited co-infected cases, the power to detect true

associations was limited. Future studies should include larger, population-based samples to confirm these associations.

Furthermore, while behavioural risk factors, such as sexual practices, injection drug use, and history of blood transfusion, play a crucial role in the transmission dynamics of blood-borne infections, these aspects were not explored by the study.

The current study has several important limitations. limited time and budget; thus, only ICT kits (Manufacturer: Accu-Tell, USA) were used. This raises the possibility of outcome misclassification. Besides, the sample size was relatively small and based only on two districts, which restricts external validity. Further, as a cross-sectional study, causal relationships could not be established. Finally, sparse data bias was available for multivariate models, since logistic regression ideally requires  $\geq 10$  events per predictor variable.

Despite the limitations, however, the current study is one of the first done in Lasbela and Quetta to explore healthcare-related risk factors for co-infection, providing locally relevant insights. Large-scale, population-based studies are recommended to validate the current findings.

## Conclusion

Strengthening infection control practices in district hospitals, expanding vaccination coverage, incorporating supplementary testing methods, and promoting community awareness campaigns about safe injection and transfusion practices are key to control the risk of life-threatening virus in the community.

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**Author Contribution:**

**NR:** Concept, methodology, data collection, analysis, drafting and critical revision.

**MK:** Supervision, study design, statistical analysis guidance and final review.

**STA:** Literature review, data interpretation, writing and formatting.