# Factors associated with HIV infection among children in Larkana District, Pakistan: a matched case-control study

Fatima Mir[1] (MsCR), Apsara Ali Nathwani[1] (MSc),Victoria Simms[2] (PhD), Syed Hani Abidi[3] (PhD), Amna Rehana Siddiqui[4] (PhD), Aneeta Hotwani[5] (MSc), Sikander Ali Memon[6] (MBBS), Saqib Ali Shaikh[6] (MSc), Jamila Soomro[7] (MSc), Sharaf Ali Shah[8] (MSc), Baseer Achakzai[9] (MSc), Sofia Furqan[9] (MSc), Quaid Saeed[9] (MSc), Palwasha Khan[2] (PhD), Helen A Weiss[2] (PhD), Syed Faisal Mahmood[10] (MBBS), Rashida Abbas Ferrand[1,11] (PhD)

1. Department of Pediatrics and Child Health, Aga Khan University, Karachi, Pakistan
2. MRC Tropical Epidemiology Group, Department of Infectious Disease Epidemiology, London School of Hygiene & Tropical Medicine, London, United Kingdom
3. Department of Biological and Biomedical Sciences, Aga Khan University, Karachi, Pakistan
4. Department of Community Health Sciences, the Aga Khan University, Karachi, Pakistan
5. Infectious Disease Research Laboratory, Department of Pediatrics and Child Health, Aga Khan University, Karachi Pakistan
6. Sindh AIDS Control Programme, Ministry of Health, Sindh, Pakistan
7. Public Health Wing, Ministry of Health, Sindh, Pakistan
8. Bridge Consultants Foundation
9. National AIDS Control Program, Pakistan
10. Section of Infectious Disease, Department of Internal Medicine, Aga Khan University, Karachi, Pakistan
11. Department of Clinical Research, London School of Hygiene & Tropical Medicine, London, United Kingdom

Corresponding author:
Dr Fatima Mir
Associate Professor, Department of Paediatrics and Child Health
Aga Khan University
National Stadium Rd
Karachi,
Sindh 74800
Pakistan
Tel: +92 3005844610
Email: fatima.mir@aku.edu

## Abstract

Background: In April 2019, an HIV outbreak predominantly affecting children occurred in Larkana District, Sindh, Pakistan. By December 2019, 881 of 21962 children screened had tested positive for HIV. We conducted an individually-matched case-control study to assess factors associated with HIV infection.

Methods: Between July and December 2019, 406 cases (defined as individuals aged <16 years registered for pediatric HIV care in Larkana city) and controls (individuals without HIV matched 1:1 by age, sex and neighbourhood) were sampled concurrently. An interviewer-administered questionnaire was used to collect data on possible risk factors for HIV acquisition and a blood sample was collected from all participants for hepatitis B and C serology. Mothers of all participants underwent HIV testing. Odds ratios were estimated using conditional logistic regression to assess factors associated with HIV infection.

Findings: Of 406 case-control pairs recruited, 401 were analyzed. The prevalence of hepatitis B surface antigen and hepatitis C antibodies was 18·2% (95% CI 14·5-22·3) and 6·5% (95% CI 4·3-9·4) respectively among cases, and 5·2% (95% CI 3·3-7·9) and 1·0% (95% CI 0·3-2·5) respectively among controls. Overall, 7.1% mothers of cases and no mothers of controls were HIV positive. In the 6 months prior to recruitment, 226 (56·4%) cases and 32 (8·0%) controls reported >10 injections, and 291 (72·6%) cases and 78 (19·5%) controls had received an intravenous infusion. At least one blood transfusion was reported in 56 (14·0%) cases and 3 (0·8%) controls in the past two years. HIV infection was associated with history of more injections and infusions (adjusted OR (aOR) 1·63; 95% CI 1·30-2·04, p<0.0001), blood transfusion (aOR 336·8; 95% CI 23·69-4787, p<0.0001), surgery (aOR=399·75, 95% CI 13·99-11419, p=0·0005), the child’s mother being HIV positive or having died (aOR=3·13, 95% CI 1·20-8·20, p=0·0202). and increased frequency of private clinic and government hospital visits (p<0·0001), adjusting for confounders.

Interpretation: The predominant mode of transmission in this outbreak was parenteral, likely due to unsafe injection practices and poor blood safety.

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## Introduction

Pakistan is one of four countries in Asia (along with Bangladesh, the Philippines and Papua New Guinea) where the estimated number of new HIV infections has been rising since 1990, with HIV incidence projected to rise among all the defined key population groups over the next decade.[1,2] The HIV epidemic in Pakistan has been concentrated among key population groups, including persons who inject drugs (PWID), men who have sex with men (MSM) and male, female and transgender female sex workers.[3] In 2020, 205,000 people were estimated to be living with HIV with 3% being children aged below 15 years, with 11% overall estimated to be on treatment. Prior to March 2019, 1423 children had ever registered for HIV care, and 1041 were actively in HIV care in Pakistan (National AIDS Control Program data).

In April 2019, a local physician reported fourteen new HIV diagnoses among children in Ratodero, a town within Larkana district in Sindh province.[4,5] By December 2019, 881 of 21962 children (4%) tested positive in a screening facility established in response to the identification of the unusually large cluster of paediatric HIV diagnoses (Sindh AIDS Control Program data). In addition, 219 of 15927 adults (1.4%) tested HIV positive.

There have been documented outbreaks of HIV infection reported in peer reviewed publications previously (two in Larkana (Sindh) in 2003[6] and 2016,[7] one in Gujarat (Punjab) in 2008[8] and two in Sargodha (Punjab) in 2018-2019.[9] These reports did not mention the involvement of children in the outbreaks. Two outbreaks reported in newspapers in Chiniot and Dera Ghazi Khan (Punjab)[10] did mention children but were un-corroborated by the provincial AIDS Control Program.

The 2019 Larkana outbreak was the first to record a predominant involvement of children.[10] Prior to this outbreak, children were perceived to be a low-risk group and antenatal HIV screening was only offered in eight specialist centres across the country. Media reports implicated a local pediatrician as a single point source through unsafe injection practices.[11]

The aim of this study was to investigate the risk factors for HIV infection, and to understand the likely mode(s) of HIV transmission (parenteral, sexual or mother-to-child transmission).

## Methods

*Study design and participants*

An individually-matched case-control study was conducted as per published protocol.[12] Cases were individuals aged below 16 years who had HIV infection and registered for care at the public sector paediatric HIV Treatment Center at Shaikh Zayed Children's Hospital in Larkana City. This was established by the Sindh AIDS Control Program (SACP) following identification of the HIV outbreak. This hospital serves patients in Larkana city (the main city of Larkana district in Sindh province) and nearby towns including Ratodero, where the outbreak was first identified. Ratodero has a population of 331584 and 55% of households live below the poverty line.[13] Diagnosis of HIV infection at the Center was based on three positive rapid diagnostic tests (Alere HIV Combo followed by SD Bioline and Unigold) as per WHO criteria.[12]

A line-list with name, sex, age and telephone contact information of the guardians of children registered at the HIV Treatment Center by June 30, 2019 was obtained at the start of the study. The study team sought verbal permission for participation by telephoning caregiver of each consecutive child on list. If they agreed, a home visit was arranged. If an individual was not contactable, the team moved to the next individual on the list.  If there was more than one eligible case in a household, only one (selected randomly) was selected. In reality, no household with more than one eligible case was identified. Case enrolment began on 3 July 2019 and ended on 16 October 2019.

Controls were children without HIV individually matched to cases by age, sex and location. The study team approached dwellings adjacent to a case's home and enquired whether a child with matching age (+/- one year) and sex resided in the dwelling. If no control was identified or the caregiver did not agree to participate, the team approached the next dwelling along on the same street (covering both sides of the street), and then the adjacent street, until an appropriate eligible control was identified. Information about the study was provided to the caregiver and the child was invited to participate as a control. For all cases enrolled between 24 July and 17 September, the control was enrolled on the same day. Controls for cases enrolled between 3-23 July were enrolled retrospectively (3 September to 21 October) with one final control enrolled on 26 December.

The study team that conducted household visits included a male and female interviewer and a trained phlebotomist. The interviewers had matriculate education as minimum, were fluent in spoken and written Urdu and Sindhi and had prior experience of conducting interviews in the community. The study team was trained on the study protocol including recording GPS coordinates, informed consent and interview procedures and completion of study record forms using didactic training and role-play. Refresher training was conducted two months after the start of the study. Medical evaluation, counselling and support was available in the event of disclosure of possible sexual abuse, and the study team was trained on procedures to facilitating this. All caregivers provided written informed consent. All children aged $\geq$10 years provided written assent with caregivers signing the assent form. All paper forms were securely stored and electronic data was maintained in a password controlled database with restricted access. All study team members signed a confidentiality agreement. The study was approved by the Ethics Review Committee of the Aga Khan University (2019-1536-4200).

*Procedures*

A structured questionnaire was administered to the participants' parents/caregivers for those aged below 10 years, or to the participants directly for those aged 10-15 years. Girls were interviewed by a female study worker and boys by a male study worker. The questionnaire asked about socio-demographic characteristics (age, sex, ethnicity, schooling, marital status, and household asset ownership) and for cases only, HIV testing and treatment history. The questionnaires were administered in either Urdu or Sindhi depending on participant preference.

The vital status and HIV testing history of the participant's biological parents were recorded, and mothers of all participants were tested for HIV to assess likelihood of mother-to-child transmission. Data were collected on possible parenteral exposures including frequency of contact with different types of health care services (private hospitals or clinics, government hospitals, basic health units or rural health centres), injections (excluding vaccinations) or intravenous fluid infusions ("drips"), surgery and blood transfusions. Participants were asked about cultural practices such as circumcision (males only), head shaving, tattooing, nose or ear piercing and engaging in "zanjeer zani" (self-flagellation using knives in an annual ceremony). Participants aged ≥10 years were asked about history of consensual or non-consensual sexual intercourse and intravenous drug use including needle sharing by an interviewer of the same sex. Permission was sought from caregivers to ask these questions in privacy without the caregiver being present. Knowledge about HIV transmission and prevention was also assessed through an interviewer-administered questionnaire.

A blood sample was collected from participants for hepatitis B virus (HBV) and hepatitis C virus (HCV) serology (cases and controls) and for HIV lineage studies (cases only). ELISA assays (DIAsource, Belgium) were used for qualitative detection of antibodies to hepatitis C and for hepatitis B surface antigen detection. Control participants underwent HIV testing to confirm HIV-negative status, and a replacement control was identified in the event of a positive test. Pre- and post-test HIV counselling was provided, and HIV testing was performed using a fourth generation rapid HIV test (Alere™ HIV Combo). Those with a reactive HIV test were referred to the paediatric or adult (in the case of a mother testing HIV-positive) HIV clinic in Larkana for confirmatory testing and linkage to care. Point-of-care CD4 absolute counts (Alere Pima$^{TM}$ CD4 Analyzer) were obtained from patient records at the paediatric HIV Treatment Center at Shaikh Zayed Children's Hospital, Larkana City.

*Statistical analysis*

A sample size of 406 cases and 406 individually-matched controls would provide 80% power to detect an OR of at least 2.0 for variables with a prevalence in controls between 10% and 85%, with a correlation coefficient for exposure within pairs of 0·2 (Type 1 error=0·05).

Statistical analyses were performed using STATA version 15 (StatCorp, Texas, USA). Continuous variables were presented as median (interquartile range (IQR)). Categorical data were presented as proportions. A household wealth index was constructed from 31 variables measuring house materials and ownership of land and household assets. Factor analysis was used to combine the 31 variables into a single factor which was then divided into quintiles.

The number of intravenous fluid infusions and number of injections were combined into a single variable due to their similarity.

Conditional logistic regression (CLR) was used to estimate odds ratios (ORs) and 95% CIs for association of HIV with key exposures, to allow for the individually-matched design. We first assessed univariable ORs of HIV status with socio-economic and behavioural risk factors. Variables associated with HIV infection with p-value<0·1 were fitted to a hierarchical framework (Figure 1).[14] For each of these potential exposures, we fitted a CLR multivariate model of the association of the exposure with HIV status, adjusting for any confounding variables that were at higher levels in the framework and were associated with the exposure among the control group. Association of potential confounders with the exposure was assessed using chi square tests or non-parametric tests for trend.

To account for the possibility that association between health service use and HIV were a result of behaviour change following HIV diagnosis, a sensitivity analysis was carried out among cases who had been diagnosed within 60 days prior to interview and their matched controls.

*Role of funding source*

The study was funded by the Department of Pediatrics and Child Health, the Aga Khan University, Karachi. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication. The funder had no role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

## Results

To achieve recruitment of 406 cases, 546 caregivers were contacted (102 did not respond to calls and 38 declined participation). To recruit 406 age and sex matched controls, consent was sought from a total of 508 families with eligible children (102 did not provide consent). Of these, five were excluded (3 had age mismatch and 2 were duplicate cases) leaving 401 case-control pairs for analyses. In densely populated areas, the research team tried on average 8-10 households before finding a suitable control. In less densely populated areas, the average was 3-4 households.

The median age of participants was 3 years (IQR 2-5) and 62.1% were male (Table 1). The majority lived with their parents and 81% of participants' mothers had had no schooling. Of the 401 cases, 389 (97·0%) were on ART at enrolment into the study. The median duration from diagnosis to ART initiation was 12 days (IQR 5-27), and median duration on ART at enrolment was 62 days (IQR 40-79). All cases were alive.

CD4 count was obtained for 357/406 (87.9%) cases. The median CD4 count was 999 cells/ µl (IQR 638-1479). Among participants aged 5 years and above CD4 count was available for 117/131 (89.3%), the median was 739 cells/ µl (IQR 518-1133), and 26/117 (22.2%) had a CD4 count below 500 cells/ µl.

Of 397 living mothers of cases, 91·4% had previously been screened for HIV. Maternal HIV status was positive in 7·1% (n=28) of cases and 0% of controls (Table 1), and unknown for 11 mothers who were deceased (4 cases and 7 controls). The median age of the 28 children whose mother was HIV positive was 2 years (IQR 2-5, range 0-10). Among the 8 children aged 5 years and above, median CD4 count was 710 cells/µl (1 missing count). Of the mothers living with HIV, 19/27 (70·4%) were taking HIV treatment and one had missing information. Among 787 living fathers, 322 reported previously being tested for HIV (19·4% of fathers of controls and 62·1% of fathers of cases). Among cases' fathers, 5/246 (2·0%) were living with HIV and 3/5 were taking treatment. For the five cases with fathers with HIV, four of the mothers were also living with HIV. No control had an HIV positive father.

The prevalence of HBV and HCV infection was 18·2% and 6·5% respectively among cases, and 5·2% and 1·0% respectively among controls (Table 1). HCV prevalence was similar among those with and without HBV (5·5% (4/73) and 6·7% (22/328) respectively.

At least one blood transfusion in the past two years was reported by 14·0% of cases compared to 0·8% of controls (Table 1). The most common reason reported for blood transfusion in cases and controls combined was anaemia or "weakness" (n=48/59, 81·4%), with 6/59 (10·2%) reporting thalassaemia (all 6 were cases of whom 3 also had HBV and 1 had HCV infection). In the 6 months prior to enrolment, 226 (56·4%) cases and 32 (8·0%) controls reported >10 injections, and 291 (72·6%) cases and 78 (19·5%) controls had received an infusion (Table 1).

Socioeconomic factors which showed strong association with HIV infection on univariable analyses were greater household wealth, older age of parents, higher level of father education, mothers who worked for a living, and never having attended madrassa (Table 1).

Biological factors showing strong association with HIV infection were seropositivity for Hepatitis B (OR 4·47, 95% CI 2·55-7·82) and C (OR 6·50, 95% CI 2·27-18·62), mother having died or being HIV positive (OR 5·17, 95% CI 2·16, 12·38), repeated visits to government hospitals and private clinics, greater number of injections and of infusions in the past 6 months blood transfusion or surgery in the past 2 years and number of times head was shaved in the past 2 years. (Table 1). These variables were represented with a hierarchical framework (Figure 1).

In multivariable analysis, adjusting for confounding variables listed in Table 2, there was strong evidence that HIV infection was associated with more than one visit to a government hospital (aOR=1·93, 95% CI 1·03-3·61, p<0·0001), number of visits to a private clinic (p<0·0001), at least one injection/infusion in the past 6 months (aOR=1·63, 95% CI 1·30-2·04, p<0·0001), history of blood transfusion (aOR=336·75, 95% CI 23·69-4787, p<0·0001), history of surgery (aOR=399·75, 95% CI 13·99-11419, p=0·0005) and the child's mother being HIV positive or having died (aOR=3·13, 95% CI 1·20-8·20, p=0·0202) (Table 2). After adjusting for potential confounders (private clinic visits, number of infusions/injections, household wealth and mother's occupation), there was evidence for an association of HBV infection with HIV (aOR=3·21, 95% CI 1·19-8·68, p=0·021), but no evidence that HCV infection was associated with HIV (aOR=4.64, 95% CI 0.60-36.01, p=0.14).

When the analysis was restricted to cases diagnosed within 60 days prior to enrolment (n=92) and their matched controls, number of visits to a private clinic or government hospital, and number of injections and infusions were still associated with HIV (results not shown).

Notably, there was a trend of an increasing prevalence of HCV infections with increasing number of blood transfusions among cases (Table 3). No participants reported having sex or that they had been sexually abused, but two (one case and one control) reported intravenous addictive substance use.

Enquiring into knowledge about HIV transmission and prevention, parents of cases knew less about HIV than parents of controls (Table 4). The only exception was knowledge that HIV can be transmitted by blood transfusion, which was more likely to be correctly answered by cases.

## Discussion

HIV infection in children was associated with blood transfusions, surgery, use of both private and government health services, receipt of injections and intravenous fluid infusions as well as with mother's occupation. There was a dose-dependent association of injections and infusions with HIV infections. These findings support parenteral transmission most likely in health facility settings as the predominant mode of transmission in this outbreak. HIV infection was also associated with mother's HIV status/mortality, but since 92.0% of cases had an HIV negative mother, this cannot be the main route of transmission.

The strong association of HIV infection with visits to both government and private health facilities refute the widespread media reports that this was a single-source outbreak. Awareness and observation of universal precautions among health care workers is low[15], and reuse of syringes, needles and infusion packs[16] is widespread in clinical practice both in the public and the private sector. The rates of unsafe injection practices in Pakistan are among the highest in the world and have remained unchanged over the past decade.[17,18] In addition, segregation, handling, storage, transportation and disposal of medical waste is well below national and World Health Organization bio-safety standards.[19,20]

Notably, receipt of injections was common with half of the controls having had at least one injection in the past six months. Poor injection practices are well documented including the re-use of needles and/or syringes in patients,[16,21] use of the same syringe and needle for intravenous fluid and medicine vials in the same patient and reusing needle or syringe to access the intravenous port separated from the patient by fluid tubing.[17] Parenteral treatment is widely perceived to be more effective than oral therapies in Pakistan, which drives the demand for and supply of injectable treatments for relatively minor illnesses.[22] This is particularly problematic given that the majority of healthcare in the country is delivered by the private sector with little regulation of price, quality or standards.[23]

Having had a blood transfusion was associated with being HIV positive. The most common reason for blood transfusion was anaemia. Notably, six participants who had thalassemia (and therefore likely to have had multiple transfusions) were all HIV-positive. Only an estimated 15% of blood products in Pakistan are provided by voluntary unpaid donations, remaining comes from family members and largely, paid donors, the latter having been shown to have a higher prevalence of HIV infection than the general population.[24] The unmet demand for blood has resulted in the establishment of private blood services, that are poorly regulated.[25] Infection control practices in blood banks are poor and screening for bloodborne viruses in blood banks is erratic.[26]

Hepatitis B and C infection were common independent of each other, with the prevalence being significantly higher in cases than in controls. Hepatitis C infection was associated with number of transfusions with a dose-dependent relationship among cases.

The prevalence of blood borne viruses in Pakistan, and contaminated blood products as well as unsafe injection practices have been consistently implicated in ongoing transmission of both Hepatitis B and C.[27,28]

Seven per cent of mothers of cases tested HIV-positive but all mothers of controls were HIV-negative. It is possible that mother to child transmission (MTCT) may also play a role in transmission of HIV infection. The majority of the children with mothers who were HIV-positive were aged over two years and in children aged five years and above, the CD4 counts were within normal range, which would imply recent transmission. It is possible that both the mother and child could have acquired HIV infection through unsafe injection practices. Viral lineage studies in the mother-child pairs is underway to interrogate the possibility of MTCT and will be reported separately. We found no evidence of increased risk from traditional practices such as circumcision, ear/nose piercing, head shaving, tattooing or zanjeer zani.

The majority of cases (62·1%) was male and, aged below five years. This is consistent with the demographic profile of children infected in this outbreak reported previously.[10] Similarly, the cross-sectional study of the outbreak found 64% of children with HIV were male. It is possible that there was more frequent healthcare utilization in younger children and in boys, but this could not be investigated in this study due to the matched design of the study.

There was a strong association of mother's occupation with being a case. Among controls 95% of mothers were either housewives or farmers, whereas the mothers of controls often did skilled or unskilled manual work. This could indicate a selection bias among controls. During data collection a household could only be assessed for eligibility as a control if an adult was at home during the day to speak to the research team. This selection bias could also have been the cause of the univariate association of HIV with wealth index and father's education. These variables were no longer associated with HIV after adjusting for mother's occupation.

Cases on average had a higher household wealth index than controls, and in univariate analysis, higher level of household wealth was associated with HIV infection. One possible explanation is higher levels of healthcare utilization by wealthier households and therefore higher risk of HIV acquisition. However, wealthier households had more government hospital visits but there was no difference in use of private clinics by wealth index. Furthermore, wealthier households reported fewer injections and there was no association between wealth index and number of intravenous infusions. Another possible reason for the difference in wealth quintile between the cases and controls could be that the recruitment of cases relied on ownership of a mobile phone so that the research study team could contact families of children with HIV to determine eligibility and invite them to participate. Five percent of controls in the lowest quintile had no mobile phone. The recruitment of controls required a guardian to be at home, and poorer households may have had higher unemployment levels.

There was high level of awareness that HIV could be transmitted through needles sharing which could be a consequence of the widespread media reporting of the outbreak. However, there was very limited knowledge about other routes of HIV transmission particularly mother-to-child transmission, and how to prevent transmission. In contrast, there was much misinformation about how HIV could be transmitted e.g. through sharing food and toilets, which can result in stigma and discrimination against people with HIV. Notably, despite nearly all cases being on HIV treatment, only about half of parents of cases affirmed that HIV was treatable and 40% assumed HIV was curable. The distinction between curable and treatable may not have been clear, but lack of knowledge about treatment may significantly affect adherence to treatment.

The difference between cases and controls with respect to levels of knowledge about HIV may possibly reflect a calendar effect. Cases were mainly interviewed in July 2019, and controls in August-October 2019. During those months, HIV education and awareness sessions were conducted by the Sindh AIDS Control Program which could have improved knowledge and made the controls appear more knowledgeable than cases. However, interview date was not associated with knowledge among cases or controls.

The strengths of the study were that it was well-powered and there was a comprehensive assessment of parenteral, sexual and perinatal modes of HIV transmission including HIV testing of biological mothers to investigate likelihood of mother-to-child transmission. Testing for Hepatitis B and C testing was also performed. Questions about the likely parenteral sources of transmission were informed by observations of primary and secondary level health facilities conducted as part of the outbreak investigation by the Sindh Healthcare Commission. Limitations of the study include selection bias which likely resulted in an association between maternal occupation and HIV status. The refusal rate was relatively high which may have also introduced selection bias. Data on postulated risk factors were self-reported and therefore subject to recall bias. We enquired about receipt of injections and infusions and clinic visits over the past six months to minimize recall bias, but it is possible that cases may have had more injections and clinic visits because they were more frequently unwell because of being HIV-positive. It was not possible to establish a temporal relationship between the postulated risk factors and timing of HIV infection. Injections given as part of the Extended Programme of Immunisation (EPI) were not investigated. EPI is a well-established and regularly monitored programme. Reuse of needles has not been reported from EPI services, but suboptimal waste disposal has been noted.[29] There may have been underreporting of certain behaviours such as intravenous drug use and sexual activity, although it is unlikely that this would differ between case and controls. Finally, CD4 percentages were not available for children below five years.

The HIV epidemic is well-established among key populations, particularly among PWID, with a prevalence of more than 35% among PWID in cities.[3] Over the past two decades, there have been sporadic HIV outbreaks, but the 2019 Larkana outbreak is the first documented outbreak in Pakistan to predominantly involve children. There is significant potential for an HIV epidemic expansion beyond the key populations, as exemplified by the outbreak in children- the largest ever reported among children.[10] A multi-tier approach targeting health systems as well as communities is needed to address the root causes, if further outbreaks are to be prevented and the HIV epidemic in Pakistan is to controlled (Panel 1). Safe blood and injection practice cannot be enforced by the health sector alone when health literacy in communities is poor.[22,30] Health care providers and communities need to be educated on risks of unnecessary transfusions. Lastly, there is a pressing need to establish robust and sustainable care pathways for the children and their families who have been affected by this tragic and avoidable outbreak.

Contributors: FM, SFM, ARS and RAF conceived the study. FM, AAN, SHA, AH, SAM, JS, SAS provided the necessary data. AAN and VS accessed and verified, and cleaned the data. VS did the data analysis. FM, AAN, ARS, SFM, SHA, SAM, JS, SAS1, SAS2, BA, SF, QS, PK and RAF were involved in outbreak investigation. FM and AAN wrote the initial draft. HAW provided analysis overview. RAF was senior author and advisor. All authors contributed to the report and approved the final draft for submission. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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Data Sharing Statement: The de-identified data, analytic code and study protocol underlying this article will be shared publicly on Mendeley Data, V1, doi: 10.17632/dk4j7nhwdg.1 after an embargo period of 6 months.

**Panel 1: Recommendations for reducing HIV transmission**

**Policy and Regulation**
- Enforcement of legislation on mandatory blood screening
- Prohibit paid blood donations
- Regulation of health care providers
- Regulation of health facilities and blood banks

**Health systems**
- Implementation of Infection Prevention and Control (IPC) e.g. provision of sharps bins, universal precautions
- Proper disposal of medical waste
- Availability of low-cost, single use syringes
- Availability of oral medication alternatives to injectables
- Avoidance of unnecessary use of blood or blood products and parenteral treatments
- IPC training in medial and nursing curricula at under-graduate level

**Communities**
- Improve health literacy on HIV
- Media campaign to encourage volunteer blood donations
- Media campaign about risks of unnecessary transfusions and parenteral treatments

**Key populations**
- Needle exchange programs and/or provision of single use syringes
- Rehabilitation for PWID
- Provision of condoms, testing for sexually transmitted infections and HIV screening for sex workers
- Support for non-governmental organisations working with sex workers, PWID, men who have sex with men and transgenders
- Targeted antenatal screening policies for HIV (and other blood borne viruses)
- Epidemiological surveillance

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## Research in context

### Evidence before this study

The HIV epidemic in Pakistan has been concentrated among key population groups, including persons who inject drugs (PWID), men who have sex with men (MSM) and male, female and transgender sex workers with little evidence of spillover into the general population. We searched PubMed (restricted to English) from January 1, 2000 to Jan 1, 2020, using terms “HIV”, “Hepatitis C”, “Hepatitis B”, “blood transfusions”, “injection use”, “outbreak” and “Pakistan”. We similarly explored Google search engine to identify media reports of HIV outbreaks. We found 250 publications, including 7 news media items, 61 letters/perspective articles and 182 research studies. The studies highlight the association of blood-borne viruses (Hepatitis B, C and HIV) with poor infection control practices and unscreened blood from unregistered blood banks. There have been six documented prior outbreaks of HIV infection in Pakistan in adults but the outbreak in Ratodero in Sindh province was the first time that predominantly children were affected.

### Added value of this study

We conducted a case control study to investigate the risk factors associated with HIV infection in children identified in the outbreak in Larkana, Sindh in 2019. We examined the association of positive HIV maternal status, history of injection use, blood transfusions, history of health facility visits, co-prevalent Hepatitis B and Hepatitis C infection with HIV infection. In children aged over 10 years, potential sexual exposures were also explored through interview by trained interviewers. We found a strong association of HIV infection in children with multiple attendances to private clinics and government hospitals, number of injections/infusions in the past 6 months and history of blood transfusion or surgery as well as mother’s HIV status. This study provides more robust evidence, as compared to previous anecdotal reports, that parenteral transmission is a potentially important route of HIV transmission resulting in outbreaks in Pakistan.

Implications of all the available evidence

We have documented the most extensive outbreak of paediatric HIV infection ever reported in Pakistan and using the case-control study design have identified that parenteral route was the predominant mode of transmission in this outbreak. Poor infection control, lack of safe sharps disposal and incineration, unregulated blood screening have been repeatedly reported as contributing to Pakistan's Hepatitis B and C epidemic and this study reiterates the need to take stringent action to implement safe injection and blood screening practices across the country. Strict and urgent implementation of safe blood in registered blood banks, vigilant regulation of unregistered medical practitioners and health facilities, provision of sharps disposal facilities and incineration, and community engagement and education on hazards of unnecessary injections and transfusions are essential to prevent further onward transmission of blood-borne viruses in Pakistan.

Table 1: Comparison of characteristics of cases and controls, and univariate associations with HIV infection

| | | Controls, n (%) | Cases, n (%) | OR (95% CI) | p-value |
|---|---|---|---|---|---|
| N | | 401 | 401 | | |
| Sociodemographic characteristics | | | | | |
| Sex | Male | 249 (62·1) | 249 (62·1) | | |
| | Female | 152 (37·9) | 152 (37·9) | | |
| Age (years) | 0-1 | 56 (14·0) | 58 (14·5) | | |
| | 2 | 79 (19·7) | 75 (18·7) | | |
| | 3-4 | 139 (34·7) | 138 (34·4) | | |
| | 5-8 | 89 (22·2) | 91 (22·7) | | |
| | 9-15 | 38 (9·5) | 39 (9·7) | | |
| Household wealth quintile | 1 (poorest) | 90 (22·4) | 71 (17·7) | 1 | <0.0001* |
| | 2 | 94 (23·4) | 66 (16·5) | 0·99 (0·62, 1·57) | |
| | 3 | 91 (22·7) | 71 (17·7) | 1·27 (0·76, 2·10) | |
| | 4 | 67 (16·7) | 92 (22·9) | 3·07 (1·76, 5·37) | |
| | 5 (richest) | 59 (14·7) | 101 (25·2) | 5.03 (2·65, 9·54) | |
| Ethnic group | Sindhi | 252 (62·8) | 260 (64·8) | 1 | 0·95 |
| | Baloch | 74 (18·5) | 70 (17·5) | 0·80 (0·42, 1·52) | |
| | Brahvi | 31 (7·7) | 31 (7·7) | 0.95 (0·33, 2·74) | |
| | Saraiki | 43 (10·7) | 40 (10·0) | 0·82 (0·41, 1·63) | |
| | Hindkowan | 1 (0·3) | 0 | - | |
| Mother's age, (years) | 19-24 | 25 (6·3) | 54 (13·5) | 1 | <0.0001* |
| | 25-29 | 102 (25·6) | 108 (26·9) | 0·45 (0·25, 0·80) | |
| | 30-34 | 100 (25·1) | 125 (31·2) | 0·52 (0·29, 0·93) | |
| | 35-39 | 97 (24·3) | 67 (16·7) | 0·24 (0·13, 0·46) | |
| | 40-44 | 45 (11·3) | 30 (7·5) | 0·24 (0·11, 0·47) | |
| | 45-65 | 30 (7·5) | 17 (4·2) | 0·19 (0·09, 0·44) | |
| Father's age | 19-24 | 16 (4·0) | 25 (6·3) | 1 | 0·0011* |
| | 25-29 | 44 (11·1) | 68 (17·0) | 0·94 (0·42, 2·11) | |
| | 30-34 | 88 (22·1) | 99 (24·8) | 0·65 (0·31, 1·38) | |
| | 35-39 | 101 (25·4) | 105 (26·3) | 0·60 (0·27, 1·29) | |
| | 40-44 | 62 (15·6) | 52 (13·0) | 0·42 (0·19, 0·94) | |
| | 45-75 | 87 (21·9) | 51 (12·8) | 0·30 (0·13, 0·68) | |
| Mother's education (N=798) | No schooling | 330 (82·9) | 313 (78·3) | 1 | 0·14* |
| | Primary | 44 (11·1) | 51 (12·8) | 1·26 (0·81, 1·98) | |
| | Secondary & above | 24 (6·0) | 36 (9·0) | 1·73 (0·96, 3·13) | |
| Father's education (N=795) | No schooling | 206 (51·9) | 161 (40·5) | 1 | 0·0004* |
| | Primary | 75 (18·9) | 73 (18·3) | 1·37 (0·91, 2·06) | |
| | Secondary & above | 116 (29·2) | 164 (41·2) | 2·09 (1·45, 3·03) | |
| Mother's occupation (N=802) | Housewife/farmer | 380 (94·8) | 195 (48·6) | 1 | <0.0001 |
| | Skilled/unskilled manual | 2 (0·5) | 189 (47·1) | 103·3 (25·4, 419·6) | |
| | Other | 19 (4·7) | 17 (4·2) | 3·00 (0·90, 9·97) | |
| Living with | Both parents | 394 (98·3) | 397 (99·.0) | 1 | 0·33 |
| | Not with both parents | 7 (1·8) | 4 (1·0) | 0·50 (0·13, 2·00) | |
| Ever attended school | No | 336 (83·8) | 336 (83·8) | 1 | 1·00 |
| | Yes | 65 (16·2) | 65 (16·2) | 1·00 (0·54, 1·86) | |
| Ever attended | No | 347 (86·5) | 362 (90·3) | 1 | 0·022 |

| | | Controls, n (%) | Cases, n (%) | OR (95% CI) | p-value |
|---|---|---|---|---|---|
| | N | 401 | 401 | | |
| madrassa (N=802) | Yes | 54 (13·5) | 39 (9·7) | 0·46 (0·24, 0·90) | |
| Mother's vital status | Alive | 394 (98·3) | 397 (99·0) | 1 | 0·33 |
| | Dead | 7 (1·7) | 4 (1·0) | 0·50 (0·13, 2·00) | |
| Hepatitis B and C prevalence and parenteral risk factors (Exposures) | | | | | |
| HBsAg | Negative | 380 (94·8) | 328 (81·8) | 1 | <0·0001 |
| | Positive | 21 (5·2) | 73 (18·2) | 4·47 (2·55, 7·82) | |
| Anti-HCV | Negative | 397 (99·0) | 375 (93·5) | 1 | 0·0005 |
| | Positive | 4 (1·0) | 26 (6·5) | 6·50 (2·27, 18·62) | |
| Maternal HIV status (N=791) | Negative | 394 (100) | 369 (93·0) | | |
| | Positive | 0 | 28 (7·1) | | |
| Injections in past six months | Never | 204 (50·9) | 47 (11·7) | 1 | <0·0001* |
| | 1-2 times | 104 (25·9) | 50 (12·5) | 2·47 (1·32, 4·63) | |
| | 3-5 times | 43 (10·7) | 38 (9·5) | 4·67 (2·24, 9·74) | |
| | 6-10 times | 18 (4·5) | 40 (10·0) | 13·27 (5·24, 33·59) | |
| | >10 times | 32 (8·0) | 226 (56·4) | 70·49 (29·05, 171·0) | |
| Infusions ('drips') in past six months | Never | 323 (80·6) | 110 (27·4) | 1 | <0·0001* |
| | 1-2 times | 49 (12·2) | 73 (18·2) | 5·17 (2·83, 9·42) | |
| | 3-5 times | 20 (5·0) | 58 (14·5) | 7·61 (3·88, 14·91) | |
| | 6-10 times | 5 (1·3) | 54 (13·5) | 27·06 (8·59, 85·26) | |
| | >10 times | 4 (1·0) | 106 (26·4) | 90·89 (21·39, 386.2) | |
| Blood transfusion in past two years | No | 398 (99·3) | 345 (86·0) | 1 | <0·0001 |
| | Yes | 3 (0·8) | 56 (14·0) | 27·50 (6·71, 112·74) | |
| Any surgery in past two years | No | 399 (99·5) | 389 (97·0) | 1 | 0·022 |
| | Yes | 2 (0·5) | 12 (3·0) | 11·00 (1·42, 85·20) | |
| Zanjeer zani if male (N=498) | No | 238 (95·6) | 244 (98·0) | 1 | 0·12 |
| | Yes | 11 (4·4) | 5 (2·0) | 0·40 (0·13, 1·28) | |
| Tattoos | No | 397 (99·0) | 400 (99·8) | 1 | 0·22 |
| | Yes | 4 (1·0) | 1 (0·3) | 0·25 (0·03, 2·24) | |
| Circumcised if male (N=497) | No | 177 (71·1) | 173 (69·8) | 1 | 0·73 |
| | Yes | 72 (28·9) | 75 (30·2) | 1·08 (0·69, 1·70) | |
| Nose/ear pierced if female (n=304) | No | 42 (27·6) | 36 (23·7) | 1 | 0.63* |
| | 1-2 times | 82 (54·0) | 87 (57·2) | 1·28 (0·72, 2·26) | |
| | 3-5 times | 24 (15·8) | 27 (17·8) | 1.34 (0·60, 3·02) | |
| | 6-15 times | 4 (2·6) | 2 (1·3) | 4·09 (0·04, 4·14) | |
| Head shaved in past 2 years | No | 40 (10·0) | 23 (5·7) | 1 | 0·013* |
| | 1-2 times | 123 (30·7) | 118 (29·4) | 2·29 (1·11, 4·75) | |
| | 3-5 times | 193 (48·1) | 193 (48·1) | 2·42 (1·19, 4·95) | |
| | >5 times | 45 (11·2) | 67 (16·7) | 3·66 (1·66, 8·03) | |
| Visited government hospital | No | 287 (71·6) | 312 (77·8) | 1 | <0·0001* |
| | Once | 87 (21·7 | 11 (2·7) | 0·13 (0·07, 0·25) | |
| | More than once | 27 (6·7) | 78 (19·5) | 2·61 (1·63, 4·17) | |
| Visited private clinic | No | 112 (27·9) | 75 (18·7) | 1 | <0·0001* |
| | 1-2 times | 101 (25·2) | 19 (4·7) | 0·29 (0·15, 0·55) | |
| | 3-5 times | 119 (29·7) | 56 (14·0) | 0·73 (0·45, 1·18) | |
| | 6-10 times | 49 (12·2) | 72 (18·0) | 2·39 (1·32, 4·31) | |
| | >10 times | 20 (5·0) | 179 (44·6) | 29·93 (11·72, 76·41) | |

** = test for trend*

Table 2: Multivariate conditional logistic regression models for factors associated with HIV infection

| | | Control, n (%) | Case, n (%) | aOR (95% CI) | p-value |
|---|---|---|---|---|---|
| **Visited government hospital**[a] | No | 287 (71·6) | 312 (77·8) | 1 | <0·0001 |
| | Once | 87 (21·7 | 11 (2·7) | 0·09 (0·04, 0·22) | |
| | More than once | 27 (6·7) | 78 (19·5) | 1·93 (1·03, 3·61) | |
| **Visited private clinic**[b] | No | 112 (27·9) | 75 (18·7) | 1 | <0·0001 |
| | 1-2 times | 101 (25·2) | 19 (4·7) | 0·46 (0·19, 1·13) | |
| | 3-5 times | 119 (29·7) | 56 (14·0) | 1·04 (0·52, 2·05) | |
| | 6-10 times | 49 (12·2) | 72 (18·0) | 3·77 (1·62, 8·74) | |
| | >10 times | 20 (5·0) | 179 (44·6) | 38·28 (11·86, 123·49) | |
| **Injections/ infusions in past 6 months**[c] | Increase of 1 category | | | 1·63 (1·30, 2·04) | <0·0001 |
| **Had blood transfusion**[c] | No | 398 (99·3) | 345 (86·0) | 1 | |
| | Yes | 3 (0·7) | 56 (14·0) | 336·75 (23·69, 4787·01) | <0·0001 |
| **Had surgery**[c] | No | 399 (99·5) | 389 (97·0) | 1 | |
| | Yes | 2 (0·5) | 12 (3·0) | 399·75 (13·99, 11419) | 0·0005 |
| **Mother HIV positive or died**[d] | No | 394 (98.3) | 369 (92.0) | 1 | |
| | Yes | 7 (1.8) | 32 (8.0) | 3.13 (1.20, 8.20) | 0.0202 |

[a] *adjusted for household quintile and mother's occupation*
[b] *adjusted for father's age, household quintile and mother's occupation*
[c] *adjusted for visits to private clinic, visits to government hospital, household quintile and mother's occupation*
[d] *adjusted for father's age, household quintile and mother's occupation*

Table 3: Relationship of Hepatitis B and C with number of transfusions

| Number of transfusions | Prevalence of HBV surface antigen | Prevalence of HCV antibody |
|---|---|---|
| 0 | 61/345 (17·7%) | 17/345 (4·9%) |
| 1 | 8/45 (17·8%) | 6/45 (13·3%) |
| 2 | 2/7 (28·6%) | 2/7 (28·6%) |
| 3 or more | 2/4 (50·0%) | 1/4 (25·0%) |
| $Chi^2$, p | Chi2=3·3, p=0·35 | Chi2=12·8, p=0·005 |

Table 4: Knowledge of HIV among parents of cases and controls, and univariate association with HIV infection

| | | Control | Case | OR (95% CI) | p-value |
|---|---|---|---|---|---|
| HIV can be transmitted by: | | | | | |
| Mother to baby | Yes | 4 (1·0) | 3 (0·8) | 0·75 (0·17, 3·35) | 0·71 |
| Sex | Yes | 358 (89·3) | 319 (79·6) | 0·40 (0·25, 0·63) | <0·001 |
| Sharing needles | Yes | 396 (98·8) | 369 (92·0) | 0·13 (0·05, 0·37) | <0·001 |
| Blood transfusion | Yes | 310 (77·3) | 341 (85·0) | 1·97 (1·29, 3·01) | 0·002 |
| Mosquito bites | No | 371 (92·5) | 337 (85·5) | 0·24 (0·12, 0·50) | <0·001 |
| Sharing food | No | 401 (100·0) | 397 (99·0) | - | |
| Saliva of person with HIV | No | 401 (100·0) | 396 (98·8) | - | |
| Touching person with HIV | No | 401 (100·0) | 399 (99·5) | - | |
| Sharing a toilet | No | 401 (100·0) | 398 (99·3) | - | |
| HIV transmission can be prevented by: | | | | | |
| Abstaining from sex | Yes | 303 (75·6) | 254 (63·3) | 1 | |
| | No | 4 (1·0) | 19 (4·7) | 6·99 (2·30, 21·28) | 0·001 |
| | Don't know | 94 (23·4) | 128 (31·9) | 2·17 (1·45, 3·26) | <0·001 |
| Using condoms | Yes | 233 (58·1) | 176 (43·9) | 1 | |
| | No | 10 (2·5) | 20 (5·0) | 2·83 (1·28, 6·22) | 0·01 |
| | Don't know | 158 (39·4) | 205 (51·1) | 2·01 (1·44, 2·81) | <0·001 |
| Screening blood | Yes | 300 (74·8) | 263 (65·6) | 1 | |
| | No | 1 (0·2) | 15 (3·7) | - | |
| | Don't know | 100 (24·9) | 123 (30·7) | 1·59 (1·10, 2·29) | 0·01 |
| Giving drugs to mother with HIV | Yes | 160 (39·9) | 54 (13·5) | 1 | |
| | No | 27 (6·7) | 27 (6·7) | 3·67 (1·83, 7·37) | <0·001 |
| | Don't know | 214 (53·4) | 320 (79·8) | 5·65 (3·63, 8·78) | <0·001 |
| Is HIV treatable? | Yes | 264 (65·8) | 222 (55·4) | 1 | |
| | No | 36 (9·0) | 40 (10·0) | 1·43 (0·83, 2·47) | 0·20 |
| | Don't know | 101 (25·2) | 139 (34·7) | 1·94 (1·34, 2·82) | <0·001 |
| Is HIV curable? | Yes | 179 (44·6) | 159 (39·7) | 0·65 (0·42, 1·00) | 0·05 |
| | No | 70 (17·5) | 87 (21·7) | 1 | |
| | Don't know | 152 (37·9) | 155 (38·7) | 0·77 (0·49, 1·22) | 0·26 |

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