Estimation and epidemiological assessment of undiagnosed HIV cases and their co-positivity with hidden HBV & HCV in Lucknow and surrounding districts of northern India – a post mortem study

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ABSTRACT

Background: Undiagnosed HIV cases are continuously jeopardizing the efforts to combat HIV/AIDS. Screening of these hidden cases is necessary to sensitize our policymakers and more so our healthcare workers to this occupational hazard.

Objectives: To determine the serostatus of HIV, HBV & HCV in human corpses with unknown status and to correlate HIV infectivity with HBV-HCV infectivity, socio-demographic character, behavioural pattern, and habitat.

Methods: This was an observational cross-sectional study that included all human corpses with unknown serostatus, brought to a single institute in India for medicolegal autopsy. Serum was extracted and an ELISA kit was used to screen these cases.

Results: 929 blood samples were collected. The prevalence of undiagnosed HIV, HBV, and HCV was 1%, 1.9%, and 1.4%. This was much higher than the known prevalence in the North India region. The co-positivity of HBV & HCV cases was also found to be statistically significant. The multivariate logistic regression analysis revealed that marital status, addiction habits, and homelessness were found to be significantly associated with the prevalence of HIV.

Conclusion: There is a need for healthcare agencies worldwide to have policies for screening of undiagnosed cases since these obscure cases endanger the healthcare workers.

Keywords: Undiagnosed HIV cases, Obscure HBV-HCV cases, Occupational hazards, Autopsy room, HIV epidemiology.

INTRODUCTION

Undiagnosed HIV cases are continuously jeopardizing the efforts to combat HIV/AIDS. Gap report published by UNAIDS 2013, revealed that globally 75% of persons with HIV are from 15 countries, India securing 3rd place after South Africa and Nigeria both in PLHIV and AIDS-related deaths. They also reported that of the 2.1 million new HIV infections that occurred in 2013, India secured 4th place after South Africa, Nigeria, and Uganda. Many surveys around the world showed declining HIV incidence and prevalence. However, literature survey did not reveal any studies done to estimate the actual burden of undiagnosed disease. The problem
of undiagnosed cases is not only limited to developing and underdeveloped countries, but the rate of new HIV infections in European country continues to rise, with one-quarter of cases remaining undiagnosed\(^{2, 3}\). In North America, mainly in New York City initially, health care providers experienced that positivity rates are significantly lower than the expected national estimate as most of the residents are unaware of being infected\(^{4}\). In India, very little data regarding undiagnosed cases are available. The reason behind the low detection of cases is best explained in Manipur, a small state of India which demonstrate a low-to-moderate lifetime rate of HIV testing despite an increased prevalence rate of HIV among the people who inject drugs (PWID)\(^{5}\). As these HIV-positive individuals are either completely unaware or hide their infectivity status due to stigma, data regarding the extent of undiagnosed HIV cases and uptake of HIV testing in the Indian setting is minimal. An extensive study conducted more than 25 years ago identified the virus in 21 of 41 (51%) serum specimens or blood mononuclear cell fractions from cadavers where the longest post-mortem interval at the time of testing was 37.5 hours (The virus can be detected up to 21.5 hours post-mortem). The virus was detected on a skull bone six days and from a stored specimen of the spleen even after 14 days 6. The post-mortem room is a source of potential hazards where the staff need to minimize risk\(^{7}\). USA, in 2003, reported the first documented case of occupational HIV transmission in the world\(^{8}\). It is reported that even most experienced autopsy surgeon who is not wearing cut-resistant mesh, have a chance of percutaneous injury exposure at a rate of 1 per 53 cases and other health care workers, not wearing a cut-resistant mesh have a risk of 2.4% for occupational HIV exposure\(^{9}\). Besides HIV, the presence of hepatitis B and hepatitis C virus in human corpses, with undiagnosed serostatus, brought for autopsy, endanger health care workers and result in stigmatization. This not only make health care personnel more anxious but also reluctant towards performing their duty. Co-infectivity of blood-borne viruses with HIV is a hard blow on the efforts to reduce morbidity and mortality. This study will not only protect the healthcare worker by increasing awareness but also provide a database of undiagnosed seroprevalences of HIV, HBV, and HCV to national and international health care agencies.

**OBJECTIVE**

To determine the sero-status of HIV, HBV & HCV in human corpses with unknown status and to determine correlation of HIV infectivity with HBV-

HCV infectivity, socio-demographic character, behavioural pattern, and habitat.

**MATERIAL AND METHOD**

This was an observational cross-sectional study conducted from August 2015 to March 2019. The study was carried out on human corpses brought randomly, to the mortuary of a tertiary care centre, for medicolegal autopsy, where the serostatus was unknown. This centre caters to a large population from both urban and rural settings of Lucknow and the nearby district of northern India. It receives an average of 5000 to 6000 corpses annually for medicolegal autopsy.

**Sample size calculation & Sampling technique:**

\[
\text{Sample size} = \frac{(Z_{1}\alpha/2)^2 \times p \times (1-p)}{d^2}
\]

\(Z_{1}\alpha/2\)-is standard normal variate at 5% type 1 error \((p<0.005)\), it is 1.96 and at 1% type 1 error \((p<0.01)\) it is 2.58. As in most studies, \(P\) values are considered significant below 0.05 hence 1.96 is used in the formula.

\[p = \text{Expected prevalence in population-based on previous or pilot studies.}\]

\[d = \text{Precision –decided by the researcher.}\]

So, taking \(Z_{1}\alpha/2=1.96, \quad P=50\% \ (0.5)\) for infinite population, \(d=3.25\% \) absolute for considering relative power of study 80% for checking the hypothesis that prevalence of HBV/HCV among HIV positive cases

\[
\text{Sample size} = \frac{(1.96)^2 \times 0.50(1-0.50)/ (0.0325)^2}
\]

**Sample size= 910**

Based on the assumption of data loss during study, a total of 1018 samples were collected using nonprobability convenient sampling method.

The following exclusion criteria were considered:
1. Human corpse with known sero status
2. Inability to obtain consent from next to kin.
3. Haemolysed blood samples

We have collected 5 ml of blood from the right chamber of the heart under universal precautions, after obtaining consent from the next of kin. The samples were centrifuged, serum separated, labelled, and stored at \(-20^\circ\)C. Thousand eighteen blood samples were collected randomly, of which 89 had to be discarded due to poor quality serum. Nine hundred and twenty nine were analysed using...
Microlisa HIV kits of J.Mitra & Co. Pvt to detect the seropositivity status of all 3 viruses.

RESULTS

The Monte Carlo chi-square test was used to compare the categorical variables. The univariate and multivariate backward conditional binary logistic regression was carried out to determine the strength of association between the prevalence of HIV and various factors. The odds ratio (OR) with 95% confidence interval (CI) was calculated and the p-value<0.05 was considered significant. This analysis was carried out on the SPSS 16.0 version of Chicago, Inc., USA. The study results are presented in percentages. The prevalence of HIV, HBV and HCV was 1% (n=9), 1.9% (n=18) and 1.4% (n=13). The HBV was found to be positive in 44.4% of positive HIV subjects. However, HCV was positive in 22.2% of positive HIV subjects. The association was statistically significant. None of the subjects were found to be co-positive for all HIV, HBV, and HCV. Co-positivity of HBV and HCV was present in 4 subjects (0.4%). (Table 1).

Table 1: Association of HIV with HBV and HCV

| Characteristics | Study population (n=929) | Prevalence of HIV | OR (95%CI) | p-value |
|-----------------|-------------------------|------------------|------------|---------|
| Age in years    |                         |                  |            |         |
| <18             | 36 3.9                   | 0.0              | -          | -       |
| 18-30           | 367 39.5                 | 0.0              | -          | -       |
| 31-45           | 303 32.6                 | 0.7              | 0.20 (0.04-1.01) | 0.06    |
| >45             | 223 24.0                 | 7.3              | 1.00 (Ref.)|         |
| Gender          |                         |                  |            |         |
| Male            | 736 79.2                 | 6.8              | 0.52 (0.12-2.10) | 0.35  |
| Female          | 193 20.8                 | 1.6              | 1.00 (Ref.)|         |
| Place of residence |                   |                  |            |         |
| Rural           | 258 27.8                 | 4.1              | 1.37 (0.25-7.60) | 0.71    |
| Urban           | 494 53.2                 | 3.6              | 0.53 (0.08-3.22) | 0.49    |
| Semi-urban      | 177 19.1                 | 2.1              | 1.00 (Ref.)|         |
| Religion        |                         |                  |            |         |
| Hindu           | 797 85.8                 | 8.0              | 1.00 (Ref.)|         |
| Muslim          | 128 13.8                 | 1.0              | 0.87 (0.10-7.17) | 0.097  |
| Sikh            | 4 0.4                    | 0.0              | -          |         |
| Socio-economic status |           |                  |            |         |
| Lower           | 34 3.7                   | 0.0              | -          | -       |
| Lower-middle    | 235 25.3                 | 3.3              | -          | -       |
| Middle          | 97 10.4                  | 1.0              | -          | -       |
| Upper-lower     | 286 30.8                 | 5.1              | -          | -       |
| Upper-middle    | 248 26.7                 | 0.0              | -          | -       |
| Upper           | 29 3.1                   | 0.0              | -          | -       |

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The prevalence of HIV was significantly (p=0.02) higher among alcoholics (2.1%) than non-alcoholics (0.5%) (Table-III). The prevalence of HIV was higher among people with history of frequent hospitalization while assessing HIV testing status, no case was found among people who have tested for HIV within 5 years of time (Table-III). The prevalence of HIV was higher but insignificantly (>0.05) associated with migrant status and significantly (p=0.0001) associated with homelessness (Table 3).
Table 3: Prevalence and association of HIV infection according to addiction habit, health care & habitat status

| Addiction habit | Study population (n=929) | Prevalence of HIV | OR (95% CI) | p-value |
|----------------|--------------------------|-------------------|-------------|---------|
| Alcohol        |                          |                   |             |         |
| Yes            | 28                       | 30.9%             | 4.54 (1.12-18.31) | 0.02*   |
| No             | 64                       | 69.1%             | 1.00        |         |
| Drug           |                          |                   |             |         |
| Yes            | 60                       | 6.5%              | 4.24 (0.86-20.90) | 0.06    |
| No             | 86                       | 93.5%             | 1.00        |         |
| Frequent hospitalization |                   |                   |             |         |
| Yes            | 36                       | 3.9%              | 3.16 (0.38-25.96) | 0.25    |
| No             | 89                       | 96.1%             | 1.00        |         |
| HIV testing status |                   |                   |             |         |
| <5 years       | 14                       | 1.5%              | -           | -       |
| ≥5 years       | 29                       | 3.1%              | 4.23 (0.50-35.55) | 0.18    |
| Not remembered | 50                       | 5.4%              | 2.41 (0.29-20.03) | 0.41    |
| Unknown        | 83                       | 90.0%             | 1.00        |         |
| Migrant status |                          |                   |             |         |
| Migrant        | 16                       | 17.2%             | 1.37 (0.28-6.69) | 0.69    |
| Non-migrant    | 76                       | 82.8%             | 1.00        |         |
| Homelessness   |                          |                   |             |         |
| Homelessness   | 46                       | 5.0%              | 185.68 (22.64-1522.50) | 0.0001* |
| Non-homelessness | 88                      | 95.0%             | 1.00        |         |

OR-Odds ratio, CI-Confidence interval, *Binary logistic regression, Ref.-Reference, *Significant

**DISCUSSION**

The prevalence of HIV infection in human corpses brought for medicolegal autopsy is around 1.0%, while the known prevalence of HIV infection in the general population is 0.24% in this region. HBV and HCV prevalence were also found in 1.9% and 1.4% of these undiagnosed cases respectively. This is an alarming situation for both the healthcare workers and the lawmakers as this difference in prevalence may indicate a rise in undiagnosed cases like other studies. Estimation of the actual burden of this undiagnosed disease at one point of time is not possible so time to time studies have been programmed in different countries like South Africa & Peru region etc and results were alarming; these results showed a clear rise in the prevalence of HIV among corpses than HIV% in general population. Another study from the USA demonstrated that persons who are unaware of their HIV seropositivity status contribute nearly one-third of the current transmission. Co-infection with HBV and HCV virus was found to be significant in our study population, which is like many other studies, programmed in recent past. Commonly associated risk behavior and mode of transmission must be a significant player behind this.

In the general population maximum prevalence was found among the 31 to 45 age group but in our settings prevalence was maximum in age group >45 (Table 2). If we have a look at a similar study of undiagnosed HIV cases but in the emergency department, in that setting too our results regarding the prevalence of undiagnosed HIV cases among different age groups are quite different. Our study sample and demographic characteristics are different from these previous studies. So, the present study, even though not representing the general population but it provides a clear insight into undiagnosed HIV seroprevalence in the autopsy room. This often creates panic among health care worker and put them under undue risk of occupational exposure of HIV infection. It was seen in many studies that even most experienced autopsy surgeons who are not wearing cut-resistant mesh, have a chance of percutaneous injury exposure at a rate of 1 per 53. In our scenario unavailability of this cut resistance mesh increase the risk of occupational HIV exposure many folds.

Due to lack of available facilities, rural areas remain the most neglected areas hence have a higher prevalence rate of undiagnosed HIV/AIDS. Poor literacy rate, along with lack of awareness of HIV/AIDS, not only predisposes them to infection but also makes them unscreened for the disease, hence providing a major contribution to the pool of undiagnosed cases. Stigma related to HIV/AIDS is also a major setback in the diagnosis of HIV/AIDS in rural populations. So, this study indicates the importance of placing equal emphasis on HIV screening in rural areas.

Socioeconomic status represents the standard of living, income & educational status of a person. All cases in our study were found in the lower-middle, middle, and upper-lower class, no case was found in the upper-middle and upper class. Socioeconomic status is a strong predictor of mental and physical health-related problems. It was shown that youth...
having low social living, have more chance to indulge in unprotected sex and getting infected with HIV\textsuperscript{18}. In comparison to developed countries, underdeveloped, and developing countries are likely to have a greater chance of developing HIV epidemics. The utilization of different facilities to combat HIV/AIDS also depends upon socioeconomic strata. Many studies reported that HIV/AIDS-related stigma is also more concentrated among people with lower socioeconomic strata than upper\textsuperscript{25}. Low education and poverty are some important reasons behind this stigma concept and because of them, these people are unaware of different mass media campaigns held by Government to combat HIV/AIDS. Marital status is also a determinant risk factor associated with HIV infection. Like other studies, the prevalence was significantly greater among divorcee/widows\textsuperscript{20}. These separated or divorced people are more prone to indulge in prostitution and promiscuity.

Alcohol, drugs, and frequent hospitalization present with higher prevalence in our study sample (Table III). Alcohol has a disinhibiting effect with increased sexual desire and expression of masculinity, thus increasing the risk\textsuperscript{21, 22}. The underdeveloped and developing areas of the world cater higher prevalence of HIV infection among the drug abusers and this was estimated by United Nations Office on Drugs and Crime (UNODC)\textsuperscript{23}. Similarly, in our study, we found a higher prevalence of HIV seropositivity among drug users. It was also seen that unsafe injection practices are closely associated with unsafe sexual behaviors\textsuperscript{24}. These people often indulge in compulsive and promiscuous sexual activity. Awareness-raising programs for possible risk of blood-borne viruses and other preventive measures such as installation of street condom vending machines and maintenance therapy like methadone should be vigorously introduced in these neglected areas. Like other studies, hospitalization rates are higher in people with HIV than the general population due to immunodeficiency\textsuperscript{25}. Frequent hospitalization is a marker of advanced disease, associated with the ostracized behavior of relatives & family. We found in our study that the prevalence of HIV is significantly higher in subjects who either did not remember their testing time or had been tested more than 5 years ago. The study suggests that a high level of repeat HIV testing is a need of the hour to minimize an unsatisfactorily high percentage of undiagnosed cases. This also minimizes the time between the onset of infection and diagnosis and thus we can reverse the global AIDS epidemic\textsuperscript{26}.

In our study prevalence of HIV among migrant people is greater than among non-migrant. Migrants report more unprotected sexual encounters. The results of the studies have suggested that migrant man after being separated from their partner, often indulge in frequent and potentially riskier sexual activity\textsuperscript{27}. Similar results are also valid for female migrants too for having a higher number of sexual partners than female non-migrants\textsuperscript{28}. Homelessness is also a separate risk factor in HIV epidemiology. In India, an exponential rise in population lead to the migration of people towards metro cities, which are not fully equipped to accommodate the migrants. These people with very poor means of survival are often involved in risky sexual behaviour to fulfil their sexual desire hence have higher susceptibility and prevalence of HIV/AIDS\textsuperscript{29, 30}. These people are psychologically and physically unfit and destitute, have higher morbidity and mortality in comparison to people at home. So, some interventional programs should also be introduced to strengthen socio-emotional coping strategies in these street people as well as to address contextual risk factors such as stigma and discrimination by the public. So, like other studies, our study also suggests that tracing risk areas detect a higher percentage of undiagnosed cases than outreach-based testing or respondent-driven sampling\textsuperscript{31}. A major strength of this study is that it has a large sample size that provides a clear insight into the higher prevalence of these undiagnosed HIV, HBV & HCV cases in the general population brought to autopsy rooms. Due to its only limitation, this study may not represent the true picture of the general population but surely it gives a clear insight of increased undiagnosed cases & associated risk in an autopsy room.

The result of this study will sensitize all healthcare workers to these hidden cases enabling them to protect themselves against occupational hazards.

**CONCLUSION**

There is a high prevalence of hidden HIV, HBV & HCV cases in the general population. Significant co-positivity with HBV and HCV is also an independent risk factor for the community and more so to our health care worker. Prevalence of undiagnosed HIV infection is found to be significantly associated with addiction habit & habitat status.

**RECOMMENDATIONS**

A regular screening prior to autopsy is required not only to get an idea of the prevalence of HIV, HBV, and HCV in the community but also to protect those involved in the autopsy process. Screening of addict,
migrant and homeless population is also recommended. Regular KAP (Knowledge Attitude and Practice) sessions are recommended for health care worker and for the people visited in mortuary to create awareness and also sensitize them against this blood borne viruses.

CONFLICTS OF INTEREST

The authors have no conflicts of interests to disclose

ETHICAL ISSUES

None

AUTHOR CONTRIBUTIONS

RS: concept and design of the study, results interpretation, preparation of first draft and critical version of the manuscript, AKV: final approval of the version to be published, RK: drafting, concept and coordination of the overall study, HS: statistically analysed the interpreted, reviewed the literature, manuscript preparation and drafting, MS: preparation of the manuscript, analysed language and interpretation, RR: preparation and revision of the manuscript

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