

RESEARCH ARTICLE

# Prevalence of blood-borne viral infections among autopsy cases in Jordan

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## ABSTRACT

**Background:** Morgues are high risk areas for the spread of infection from cadavers to staff during the post-mortem examination. Infection can spread from corpses to workers by airborne transmission, by direct contact, or through needle and sharp object injuries. **Objective:** Knowledge about the prevalence of these infections on autopsy is essential to determine the risk of transmission and to further enforce safety measures.

**Methods:** This is a descriptive study. All autopsies performed in the Department of Forensic Medicine at Jordan University Hospital during the study period were tested for the serology of human immunodeficiency, hepatitis B and C viruses. Positive tests were confirmed by nucleic acid testing.

**Results:** A total of 242 autopsies were tested. Age ranged from 3 days to 94 years (median 75.5 years, mean 45.3 years (21.9 ^ SD)). There were 172 (71%) males. The cause of death was considered natural in 137 (56.6%) cases, accidental in 89 (36.8%), homicide in nine (3.7%), suicide in four (1.7%), and unknown in three (1.2%) cases. Hepatitis B surface antigen was positive in five (2.1%) cases. Hepatitis C virus antibody was positive in five (2.1%) cases and the hepatitis C virus polymerase chain reaction was positive in two (0.8%) cases. HIV antibody was not detected in any of the cases. The infection status of cases was not associated with age, sex, nationality, or cause of death.

**Conclusion:** The study findings indicated that there is a low prevalence of virus-infected autopsies in Jordan. However, the risk of transmission remains a potential threat and therefore the necessary precautions should always be taken during autopsy.

Keywords: autopsy, hepatitis B virus, hepatitis C virus, human immunodeficiency virus, Jordan

## INTRODUCTION

Morgues are high risk areas for the spread of infection from cadavers to staff during post-mortem examination. Infection can spread from corpses to workers through air, by direct contact, or through needle and sharp object injuries. The human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) are considered to be among the most important of those infections and can be transmitted by needlestick or sharps injuries from infected autopsies to staff.<sup>1-5</sup> Healthcare workers performing exposure-prone procedures frequently sustain such injuries and some of these exposures can even go unnoticed.<sup>6</sup>

The risk of occupational acquisition of a blood-borne virus by a healthcare worker is related to the prevalence of the virus in the patient population, the efficiency of virus transmission after a single contact with blood, and the nature and frequency of occupational blood contact.<sup>7</sup> Therefore, knowledge about the prevalence of these infections among autopsies is crucial to determine the risk of transmission and to further enforce infection control safety measures in such environments. Data on the rates of infection in autopsy cases are scarce in our region. To our knowledge, no data from other Arab Middle Eastern countries are available. However, studies have been reported from other countries such as USA,<sup>8</sup> Denmark,<sup>9,10</sup> Germany,<sup>11</sup> Japan,<sup>12</sup> France,<sup>13,14</sup> India,<sup>15</sup> Iran,<sup>16,17</sup> and Italy.<sup>18</sup> These studies confirmed the presence of infected autopsies with variable rates according to associated risk behaviors, such as intravenous drug use. This study determined, for the first time in Jordan, the prevalence of HIV, HBV, and HCV among examined autopsy cases in a large university hospital.

## METHODS

This descriptive study was conducted in Jordan University Hospital, which is a large teaching hospital with 540 patient beds and a forensic medicine center. In Jordan, there are a total of eight centers for Forensic Medicine; three of these, including ours, are in Amman, the capital city. The data were collected during the period from 1st July 2009 to 1st July 2010. Approval for the study was obtained from the

scientific research committee of the Faculty of Medicine at the University of Jordan (date: 14th January 2008; number: 14/67). All autopsies performed in the Department of Forensic Medicine during the study period were included. Cases comprised of medicolegal deaths. Forensic workers were not aware of the infection status of the cases. The cases were either from Jordan University Hospital or referred from the coronial system. Cases with putrefaction were excluded. Blood was drawn from the femoral or subclavian vein, and was refrigerated at 2808C until testing. Clotted blood samples were excluded. Data were collected for the following variables: age, sex, cause of death, and nationality.

## Serological tests

Antibodies against HIV-1/2 and HCV and the hepatitis B surface antigen (HBsAg) were all tested in the serum by using the enzyme immunoassay (Murex Diagnostics, Dartford, UK), according to the manufacturer's instructions. All of the positive results were subsequently confirmed by testing with polymerase chain reaction (PCR).

## DNA and RNA extraction and cDNA synthesis

DNA was prepared from serum samples by using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The extracted DNA sample was stored at 2208C until analysis. RNA was extracted from specimens using the QIAamp Viral RNA extraction kit (Qiagen, Hilden, Germany) and resuspended in 50 ml of RNase-free sterile water (Promega, USA). Ten microliters of the RNA sample was heated at 658C for 5 minutes and then cooled rapidly; the sample was then reverse transcribed at 428C for 50 minutes using 200 units of Moloney murine leukemia virus (superscript III enzyme, Invitrogen), 2 ml of 10 mM dNTPs, 0.5 mg of random hexamer, and 40 U of RNase inhibitor (Promega, USA). The reaction was stopped by performing a 15-minute incubation at 708C.

## PCR and reverse transcription-PCR

PCR amplification for the detection of the HBsAg coding region and reverse transcription-PCR (RT-PCR) for the detection of HCV and HIV-1 were performed as previously described<sup>19</sup> by using the primer sequences and concentrations listed in the same reference. In all PCR runs, appropriate positive and negative controls were integrated in parallel reactions. The HIV and HCV uniplex PCR were

Table 1. Distribution of seropositive cases by age.

Age	Sample	%	HBV n (%)	HCV Ab n (%)	HCV PCR n (%)	HIV n (%)
0–9	13	5.4	0	0	0	0
10–19	15	6.2	0	0	0	0
20–29	42	17.4	0	1 (3.1)	0	0
30–39	32	13.2	0	1 (3.1)	1 (3.1)	0
40–49	42	17.4	1 (2.3)	3 (7.1)	1 (2.3)	0
50–59	22	9.1	0	0	0	0
60–69	31	12.8	3 (9.7)	0	0	0
70–79	32	13.2	0	0	0	0
80–89	11	4.5	1 (9.1)	0	0	0
90–99	2	0.8	0	0	0	0
Total	242		5 (2.1)	5 (2.1)	2 (0.8)	0

Ab: antibody.

performed in a 25-ml total volume, consisting of 3 ml template cDNA, 0.5 ml dNTP (10 mM stock), 2.5 units Taq DNA polymerase (Promega, USA), and 5 ml 5X PCR buffer. The same concentrations of the amplification components and 5 ml of the isolated DNA samples were used for the detection of HBV DNA. The same PCR conditions were also used for the amplification of all viruses which are as follows: initial denaturation at 95°C, 5 minutes and 35 cycles of 50 seconds each at 94°C, 40 seconds at 55°C, and 35 seconds at 75°C with a final 3 minutes at 72°C. The PCR products were separated by performing electrophoresis in 2% agarose gels and visualized after staining with ethidium bromide.

### Statistical analysis

To study the association of the infectious status with variables characterizing demographics and cause of death, Fisher's exact test was conducted for categorical variables when any one cell exhibited an expected value of less than 5 or when there was a zero cell. The Mann–Whitney U test was used for the analysis of nonparametric data. The p value was considered to be statistically significant if it was less than or equal to 0.05. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS), version 16 (SPSS Inc., Chicago, IL, USA).

## RESULTS

During the study period, blood samples were collected from 344 autopsies, of which 242 were included in

the study and 102 had to be excluded because the collected blood samples had clotted.

The age range was from 3 days to 94 years (median 75.5 years, mean 45.3 years (SD  $\frac{1}{4}$  21.9)). There were 172 (71%) males and 70 (29%) females. Among autopsies, 219 (90.5%) cases were for Jordanian citizens. The number of autopsies referred from Jordan University Hospital was 93 (38.4%) and that from other hospitals was 149 (61.6%). The circumstances of death were considered natural in 137 (56.6%) cases, accidental in 89 (36.8%), homicide in nine (3.7%), suicide in four (1.7%), and unknown in three (1.2%) cases. Blood was collected within 4–24 hours of death in all patients except in four patients where the collection occurred within 24–48 hours of death. The HBsAg and HBV PCR were both present in five (2.1%) cases and HCV antibody in five (2.1%) cases with HCV PCR being positive in two (0.8%) cases. HIV antibodies were not detected in any of the cases. All positive markers occurred in Jordanians except for two non-Jordanian cases, where the HCV antibody was the only positive marker that was present. No co-infection with HBV and HCV was observed in any of the infected cases. The seroprevalence by age group is given in Table 1. Of the 242 tested cases, 10 (4.1%) cases were infected with at least one virus (considering a positive HCV antibody as a marker of infection for HCV). All five cases infected with HBV had died of natural causes, whereas all five cases infected with HCV had died of accidental causes. All positive cases had blood samples collected within 4–24 hours of death.

Table 2. Comparative analysis of the study sample.

	Not infected n / %	Infected n / %	p value
Males	162	10	0.067 <sup>a</sup>
Natural death	132	5	0.75 <sup>a</sup>
Jordanian	212	8	0.227 <sup>a</sup>
Age (years)			0.383 <sup>b</sup>
Range	1–94	24–80	
Median	42.5	46.5	

<sup>a</sup>Fisher's exact test; <sup>b</sup>Mann–Whitney U test.

Infection in the cases was not significantly associated with sex, age, cause of death (when separated into natural and non-natural causes), or nationality (when separated into Jordanian and non-Jordanian) (Table 2).

## DISCUSSION

The prevalence of HBV, HCV, and HIV among autopsy cases in this study was 2.1%, 0.8%, and 0% respectively. Similar data from countries in the region are scarce. A study of forensic autopsies from Iran has shown rates for HBV of 5.8%, HCV 4%, and HIV 0%.<sup>16</sup> Another study from India shows rates for HIV of 0.6%.<sup>20</sup> However, higher rates were generally observed in studies from Milan, Northern France, Denmark, Baltimore, and Tokyo with rates of up to 35% for HBV,<sup>9</sup> 51% for HCV,<sup>9</sup> and 5% for HIV.<sup>18</sup> However, an accurate comparison between studies is difficult due to method-related variations, such as using a population of autopsies with high risk behaviors like intravenous drug users or using other markers for infection like nucleic acid tests.

Testing for HBV, HCV, and HIV in cadavers is usually carried out using serological methods. However, hemolysis, autolysis, and bacterial contamination of post-mortem blood samples can cause changes leading to false serological results or weaken serological sensitivity, especially in samples with delayed removal time.<sup>21</sup> Nucleic acid tests in cadavers, although their use is still controversial in this regard, have shown high sensitivity<sup>14</sup> and benefit the safety of transplantation.<sup>22</sup> In fact, the use of these tests is becoming increasingly common in many organ procurement organizations.<sup>23</sup> The majority of cases within this study were sampled

within 24 hours of death as there is correlation between hemolysis with increasing time between death and sampling.<sup>14</sup>

In view of the fact that there are only a few prevalence studies in Jordan for the tested viruses, this study provides valuable data on the prevalence of these infections. For HBV, the only large population study conducted was in 1987 and reported a prevalence of 9.9%.<sup>24</sup> Since then, three studies have been conducted on special population groups, which showed a prevalence of 5.9% among patients undergoing hemodialysis,<sup>25</sup> 4.3% in pregnant women,<sup>26</sup> and 7.3% in patients with schizophrenia (when compared with 2.6% in controls).<sup>27</sup> For HCV, the prevalence was 0.42% among all age groups in a recent large population-based study.<sup>28</sup> For HIV, the prevalence is very low with only around 200 detected cases and an estimated total number of less than 2000 cases.<sup>29</sup> These rates are similar to the rates observed in this study. This similarity is intriguing since rates in autopsies tend to be higher than rates in the community because of the over representation of high risk groups, such as people who inject drugs.<sup>3,18</sup> However, these similar rates probably occurred due to the high percentage of natural death (56.6%) among the autopsies in this study.

All five cases that were found in this study to be infected with HBV died as a result of natural causes, all five cases infected with HCV suffered accidental deaths, and all cases with HCV were younger than those infected with HBV. These accidental deaths might be partly related to risky drug use behaviors since infection with HCV in many cases is considered a bio-marker of drug injection use. On the other hand, cases infected with HBV were older and all had died due to natural causes. This might predominantly be due to sexual transmission of HBV, instead of parenteral for HCV.

Our study did not show statistical significance between infection and sex, age, cause of death, or nationality. However, the absence of statistical significance results should be interpreted with caution, particularly the results related to sex; it is difficult to conclude that cases were not associated with sex as all those infected were males. The failure to detect an association despite being present might be due to the small sample size.

In other countries, high risk autopsy cases such as drug-related deaths are identified and tested prior to

the autopsy procedure.<sup>10</sup> However, in Jordan, to the best of our knowledge, routine post-mortem testing of autopsies is not practiced. Moreover, the infection status in most autopsies is obscured for cultural reasons, such as hiding information due to the associated stigma or having insufficient time to carry out tests due to pressing for quick burial procedures. In such circumstances, it becomes more prudent to follow universal precautions and to vaccinate healthcare workers in the morgue against HBV. In Jordan University Hospital, workers in the forensic department take the HBV vaccine and follow the universal precaution measures, especially those concerning the use of personal protective equipment. However, the exact compliance rate with these measures in other centers in Jordan is unknown.

The main limitation of this study is that it is based on data from a single center. However, since the center also receives autopsy examination requests from

other hospitals, we believe our results could be a good representation of autopsies in Jordan. Nonetheless, further larger studies could provide more accurate results.

## CONCLUSION

We conclude that in Jordan, although the prevalence of HBV, HCV, and HIV in autopsies is relatively low, the risk of transmission to healthcare workers in the morgue is a potential threat. Pretesting of autopsies could also be considered in high risk conditions.

## CONFLICTS OF INTEREST

The authors report no conflicts of interest in this work.

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