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RESEARCH ARTICLE

SEROPREVALENCE OF VIRAL BIOMARKERS FOR 'LABORATORY ACQUIRED INFECTIONS' (LAI) BETWEEN THE STAFF OF GOVERNMENT AND PRIVATE SECTOR LABORATORIES IN HOOGHLY AND BURDWAN DISTRICT, WEST BENGAL, INDIA: A CRITICAL COMPARISON

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ABSTRACT

In a pathological laboratory worker may be infected from needle stick injury or by aerosol/droplet exposure of the mucous membranes of the eyes, noses, or mouth called laboratory acquired infections (LAI). To assess the risks of LAI a cross sectional study was performed during the period of 2007 to 2012 after getting ethical clearance. It included randomly selected twenty laboratories from 'Hooghly' district out of which twelve laboratories from government and eight laboratories from private sector and from 'Burdwan' district twenty two laboratories were included, where nine laboratories from government and thirteen laboratories from private sector. Those clinical laboratories were situated within five kilometer radius surrounding the district/sub-divisional/rural hospitals of 'Hooghly' and 'Burdwan' districts. Randomly selected 126 technical personnel were of choice. From each person one blood sample was collected for serologic detection of 'Hepatitis B surface antigen', 'anti-Hepatitis C virus antibody' and 'anti HIV-1 & 2 Antibody'. By this way, sixty personnel (34 from government and 26 from private sector laboratories) from 'Hooghly' and sixty six personnel (27 from government and 39 from private sector laboratories) were included from 'Burdwan' district. Results revealed that in 'Hooghly' district HBsAg (two cases), anti-HCV antibodies (one case) were found positive in government sector. In private sector positive reports were obtained in 1 case for each of HBsAg and anti-HCV Ab. The anti-HIV antibody was found negative for both the government and private laboratory personnel. For 'Burdwan' district, in government sector positivity were found for HBsAg 3.70%, anti-HCV antibody 3.70% out of total 27 laboratory personnel where as the anti-HIV antibody was found nil. In case of the private laboratories of the said district positivity rate for HBsAg was 2.56%, anti-HCV antibody 2.56% and anti-HIV antibody was found negative. Inter district comparison showed insignificant ($p>0.05$) variation in both government and private sectors laboratories for said factors. Risks of LAI can be minimized by introducing practice of standard precautions for bio-safety.

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INTRODUCTION

Laboratory acquired infections (LAI) are defined as all infections acquired through laboratory or laboratory-related activities regardless of whether they are symptomatic or asymptomatic in nature, termed as 'laboratory associated infections' also. Codes of practice and guidelines are documented which specify safe practices for particular task or occupations (Leanne, 2001).

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Health and safety in clinical laboratories is becoming an increasingly important subject as a result of emergence of highly infectious diseases such as hepatitis, HIV etc. This is even more so in developing countries where health and safety have traditionally been regarded as low priority issues (Ejilemele and Ojule, 2004). The emphasis is on employee's training and education, use of safety equipment and the responsibility of employers to provide a work site that is maintained in clean and good sanitary condition. A laboratory safety programme should consist of commitment by top management, establishment of safe work place, collective responsibilities of management, supervisors and laboratory workers to support the programme, establishment of

appropriate on the job training and development and implementation of effective and comprehensive infection programme (Pklay, 1992). Any employee who is exposed to infectious biological agents on the laboratory is prone to (primary) infection. The transmission of pathogen(s) in the laboratory can happen by other modes also. This can be illustrated by considering the manipulation of typical blood-borne pathogens such as 'HIV' or 'Hepatitis B' viruses, 'Hepatitis C' viruses, naturally transmitted by percutaneous or mucosal exposure to infected blood or other body fluids. In the laboratory, an infection can occur via cutting injuries and through contact of the mucous membranes with aerosols that contain high titres of the virus (Cole and Cook, 1998). LAI(s) can also result in transmission of the pathogen to people outside the lab, when the infected laboratory worker infects relatives or other people who comes in contact with, also called secondary infection or transmission. In a laboratory setting contamination can take place through four different ways: Inhalation (aerosols and others through respiratory tracts); Percutaneous inoculation (Needle stick injury, cuts or abrasions from contaminated items and animal bites and scratches); Contact with mucous membrane of eyes, mouth, and nose and Ingestion (mouth pipetting, mouth contact with contaminated material via fingers or gloves, droplets, splashes etc).

There are innumerable studies in the developed countries to search out that in what extent vogue laboratory facilities are safe for their staff in prevention of laboratory acquired microbiologic infections. The highest percentages of LAI(s) in USA for typhoid fever (10.0%), brucellosis (9.4%), q-fever (6.3%), tuberculosis (9.4%) and hepatitis (4.3%). As per CDC report (CDC, 1987) occupationally acquired HIV infection is highest (24.8%) in the laboratory technician category. But there is paucity of information regarding Indian scenario. On that background, this study was designed to study the occurrence of LAI amongst the working laboratory staff being practiced in various clinical laboratories of 'Hooghly' and 'Burdwan' district of West Bengal.

MATERIALS AND METHODS

To assess the risks of LAI, investigation was conducted with 126 technical personnel in 'Hooghly' and 'Burdwan' districts. For this purpose, random sampling was of choice. From 'Hooghly' district sixty personnel (n=60; 34 personnel from government sector laboratories and 26 personnel from private sector laboratories) and from 'Burdwan' district 66 personnel (27 personnel from government sector laboratories and 39 personnel from private sector laboratories) were included as study subjects. Study protocol was explained to the working technologists and assistant category staff of the selected laboratories. Written informed consent was taken in the standardized proforma from the selected subjects before collection of blood sample to participate in the study. From each person one blood sample (total sample = 126) was collected for serologic detection of biomarkers for 'Hepatitis B surface antigen', 'anti-Hepatitis C virus antibody' and 'anti HIV-1 & 2 antibodies' according to standard methods for each test in our laboratory. Five millilitre of blood was collected from each individual randomly in plain vials and allowed to clot it.

Then the samples were brought to our investigating laboratory. The serum was collected from each sample after centrifugation at 3000 rpm for 10 minutes. The separated sera were tested for the specified three parameters of viral bio-markers using three different immunochromatography based test kit. Serum for anti HIV-1 and HIV-2 antibody was detected using test kit, 'Tri-Dot' of J. Mitra & Co., India (Sudha *et al.*, 2005). Serum for 'Hepatitis-B' surface antigen (HBsAg) was detected using kit 'SD Bioline' of Biostandard Diagnostic Ltd., India (Maity *et al.*, 2012). Serum for anti 'Hepatitis C' virus (HCV) antibody was detected using test kit 'Signal HCV' supplied by Span Diagnostic Limited, India (Pincus *et al.*, 2007). Baseline data were collected using laboratory analysis, results obtained were analyzed and comparative study was performed.

RESULTS

From the results of serological findings we did notice that in 'Hooghly' district HBsAg (two cases), anti-HCV antibodies (one case) were found positive in government sector. In private sector positive reports were obtained in 1 case for each of HBsAg and anti-HCV Ab. Anti-HIV antibody were found nil for both of the government and private laboratory personnel. For 'Burdwan' district, in government sector positivity were found for HBsAg 3.70% (1), anti-HCV antibody 3.70% (1) out of total 27 laboratory personnel where as the anti-HIV antibody was found nil. In case of the private laboratories of the said district positivity rate for HBsAg was 2.56% (1), anti-HCV antibody 2.56% (1) and anti-HIV antibody was found nil. Inter district comparison showed insignificant ($p>0.05$) variation in both government and private sectors laboratory acquired infections for 'Hepatitis B', 'Hepatitis C' and 'HIV' (Table 1- Table 4; Figure 1-Figure 2).

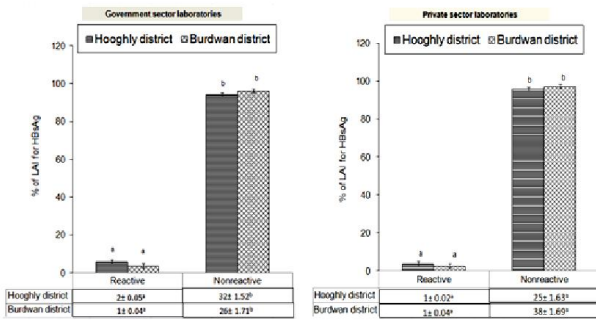
Table 1. Intra district comparison considering government and private sectors from the view point of HBsAg test results under LAI Serology

'Hooghly' district			
Nature of Serological test	LAI-Serology for HBsAg		p-value
	Reactive	Non-reactive	
Nature of governing sector			
Government sector laboratory (No. of sample=34)	5.88% (2± 0.05 ^a)	94.11% (32±1.52 ^b)	<0.001
Private sector laboratory (No. of sample=26)	3.84% (1± 0.02 ^a)	96.15% (25±1.63 ^b)	<0.001

'Burdwan' district			
Nature of Serological test	LAI-Serology for HBsAg		p-value
	Reactive	Non-reactive	
Nature of governing sector			
Government sector laboratory (No. of sample=27)	3.70% (1± 0.04 ^a)	96.30% (26±1.71 ^b)	<0.001
Private sector laboratory (No. of sample=39)	2.56% (1±0.04 ^a)	97.44% (38±1.69 ^b)	<0.001

Each column represents mean ± SEM for each group. Analysis performed by Student's two tail 't' test. Values of bars with different superscripts (a, b) differ from each other significantly at the level of $p<0.05$.

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Each bar represents mean ± SEM for each group. Analysis performed followed by Student's two tail 't' test. Values of bars with same superscript did not differ from each other significantly at the level of p>0.05

Figure 1. Inter district comparison considering government and private sectors from the view point of HBsAg test results under LAI Serology

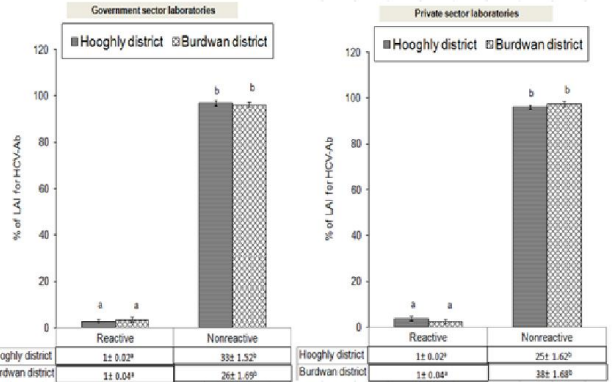
Table 2. Intra district comparison considering government and private sector from the view point of anti-HCV antibody test results under LAI (Serology)

'Hooghly' district			
Nature of Serological test	LAI-Serology for anti HCV Ab		p-value
	Reactive	Non-reactive	
Nature of governing sector			
Government sector laboratory (No. of sample=34)	2.94% (1±0.02 ^a)	97.05% (33± 1.52 ^b)	<0.001
Private sector laboratory (No. of sample=26)	3.84% (1±0.02 ^a)	96.15% (25±1.62 ^c)	<0.001
p-value	>0.05	<0.01	

'Burdwan' district			
Nature of Serological test	LAI-Serology for anti-HCV Ab		p-value
	Reactive	Non-reactive	
Nature of governing sector			
Government sector laboratory (No. of sample=27)	3.70% (1±0.04 ^a)	96.30% (26± 1.69 ^b)	<0.001
Private sector laboratory (No. of sample=39)	2.56% (1±0.04 ^a)	97.44% (38±1.68 ^c)	<0.001
p-value	>0.05	<0.01	

Each column represents mean ± SEM for each group within parenthesis. Analysis performed followed by Student's two tail 't' test. Values with different superscripts (a, b, c) differ from each other significantly at the level of p<0.01 or <0.001.

Serological findings of samples from laboratory workers revealed, for 'Hooghly' district, the existence of HBsAg and anti-HCV antibody with 7.50% and 5.00% respectively. Where as in 'Burdwan' district, the positivity were found for 1.32% HBsAg and 1.32% for anti-HCV antibody, which showed significant (p<0.001) difference with international status. LAI HBsAg and anti-HCV antibody positivity rate was 2.4 % and 2.4% respectively (Sarwar, 2008). But the anti-HIV antibody was found nil for both the study areas though the international scenario (CDC, 1992) confirmed a higher level (6.9 %) of sero-conversion for anti-HIV antibody in laboratory workers (Table 5).



Each bar represents mean ± SEM for each group. Analysis performed by Student's two tail 't' test. Values of bars with same superscript did not differ from each other significantly at the level of p>0.05.

Figure 2. Inter district comparison considering government and private sector from the view point of anti-HCV Ab test results under LAI (Serology)

Table 3. Intra district comparison considering government and private sector from the view point of anti HIV antibody test results under LAI (Serology)

'Burdwan' district			
Nature of Serological test	LAI-Serology for anti-HIV Ab		p-value
	Reactive	Non-reactive	
Nature of governing sector			
Government sector laboratory (No. of sample=27)	0	100% (27±1.67 ^a)	<0.001
Private sector laboratory (No. of sample=39)	0	100% (39±1.74 ^a)	<0.001
p-value	>0.05	>0.05	

'Hooghly' district			
Nature of Serological test	LAI-Serology for anti-HIV Ab		p-value
	Reactive	Non-reactive	
Nature of governing sector			
Government sector laboratory (No. of sample=34)	0	100% (34±1.52 ^a)	<0.001
Private sector laboratory (No. of sample=26)	0	100% (26±1.63 ^a)	<0.001
p-value	>0.05	>0.05	

Each column represents mean ± SEM for each group within parenthesis. Analysis performed followed by Student's two tail 't' test. Values with same superscript (a) did not differ from each other significantly at the level of p>0.05.

DISCUSSION

Acquisition of blood borne pathogens is a potential occupational health hazard for 'Health Care Workers (HCWs)' across the world (Sharma *et al.*, 2010). 'Hepatitis B', a global but preventable disease, is estimated to affect at least 2 billion individuals worldwide, and 350 million among them are suffering from chronic 'Hepatitis B' virus (HBV) infection. Transmission of HBV occurs through percutaneous or per mucosal exposure to infective body fluids (Schenkel *et al.*, 2008).

In addition to sexual contact and drug injection, nosocomial transmission should not be neglected as a risk factor, even in hospitals with high hygiene standards. In India, the exact incidence of nosocomial HBV transmissions is unknown, but various measures have been implemented in recent years to reduce nosocomial HBV infections such as improved hygiene,

Table 4. Inter district comparison considering government and private sector from the view point of anti-HIV Ab test results under LAI (Serology)

'Hooghly' Vs 'Burdwan' district		
Nature of Serological test	LAI-Serology for anti-HIVAb	
	Reactive	Non-reactive
Nature of governing sector		
Government sector laboratory (No. of sample=34)	0	100% (34±1.52 ^a)
Government sector laboratory (No. of sample=27)	0	100% (27±1.67 ^a)
p-value	>0.05	>0.05

'Hooghly' Vs 'Burdwan' district		
Nature of Serological test	LAI-Serology for anti-HIVAb	
	Reactive	Non-reactive
Nature of governing sector		
Private sector laboratory (No. of sample=26)	0	100% (26±1.52 ^a)
Private sector laboratory (No. of sample=39)	0	100% (39±1.74 ^a)
p-value	>0.05	>0.05

Each vertical column represents mean ± SEM for each group. Analysis performed by Student's two tail 't' test. Values of column with same superscript (a) did not differ from each other significantly at the level of p>0.05.

Table 5. Comparison of LAI (serology) data considering viral biomarkers of 'Hooghly' and 'Burdwan' districts with International scenario of developed and developing countries

LAI (Serology)	International %	Hooghly District	Burdwan District
HBsAg	2.4±0.11 ^a	7.50±0.28 ^b	1.32±0.14 ^c
Anti-HCV-Ab	2.4±0.11 ^a	5.00±0.19 ^b	1.32±0.13 ^c
Anti-HIV-Ab	6.9±0.19	0	0

Each row represents mean ± SEM for each group. ANOVA followed by multiple comparison two tail 't' test. Values of rows with different superscripts (a, b, c) differ from each other significantly at the level of p<0.05.

increased vaccine coverage, increased awareness of medical staff, and highly sensitive testing of blood products (Webster, 2000). Table 1 showed that a higher percentage of incidence of HBsAg reactivity in government sector laboratories than in private sector in 'Hooghly' district. Where as the rate of reactivity is slightly lower in both the sectors of 'Burdwan' district for the same which may be a reflection of either higher percentage of laboratory staff immunized with 'Hepatitis B' vaccination or a better follow up of safe laboratory procedures influenced by the existing medical and paramedical institutions in the district.

From the records of table 2 it was evident that HCV-laboratory acquired infection rates were almost same in both the district though that is also alarming for the laboratory staff as the preventive measures were not always provided by the authorities. During handling of the potential infectious biological samples laboratory bio-safety protocol should be followed as far as possible (NCCLS, 1991; DHHS, 1993). Inter district comparison for reactive and non-reactive HBsAg and anti-HCV Ab results showed an insignificant p-values (>0.05), which are mentioned in Figure 1 and Figure 2.

Laboratory staffs are at high-risk of acquiring infection via the contact with blood and other body fluids in the course of their work. HBV and HCV are transmitted by the skin prick with an infected, contaminated needles and syringes or through accidental inoculation of minute quantities of blood (Sewell and Goldvogl, 1981; Sewell, 1995). The infection can be transmitted as a nosocomial infection also. The result of our study for both the districts echoes the same picture of international and national scenario of LAI, where it has been estimated that 14.4% and 1.4% of health workers are infected with HBV and 'Hepatitis C' virus (HCV), respectively (Polish *et al.*, 1993) for the above said reasons (Samuel *et al.*, 2009). The risk to laboratory workers is approximately 10 times that to the general public (Hadler *et al.*, 1985) and almost 3 times that to other hospital employees (Dienstag and Ryan, 1982). The respondents demonstrated low knowledge of 'Hepatitis B' and 'Hepatitis C' infection also. The results of present study were almost similar to study done by others (Shaheen *et al.*, 2007).

Poor compliance of health workers to 'Hepatitis B' vaccination and lack of knowledge and misconception of existence of 'Hepatitis C' vaccine is an issue that deserves serious attention (Saffar *et al.*, 2005). Absence of satisfactory behaviour towards methods of preventing the transmission and cross infection of the 'Hepatitis B' and 'Hepatitis C' infection was found from this study. When they were asked about the reason for not being vaccinated against 'Hepatitis B' or 'Hepatitis C' infection most of the staff stated that they were too careful to avoid the infection. A similar lack of knowledge, attitude and practices (KAP) and incidence of exposure to needle stick injury may be the another cause of spreading hepatitis among them suggested in other studies too (Vaz *et al.*, 2010).

Awareness programmes for the laboratory personnel to follow the preventive measures during handling of any potential biological sample including its disposal would be immense helpful to combat the chances of LAI (Saleem *et al.*, 2010). Those programmes can refresh the previously acquired knowledge of senior staff and could spread ideas to the new comers into the laboratory. Repeated awareness programmes may lead to change the whimsical attitude of non-practicing of bio-safety procedures related to bio-medical laboratory. Laboratory acquired infection in respect of HIV, a hopeful and healthier result obtained in our study (Table 3), which is unexpected for the developed countries. Though in our study we have found zero percent reactivity for HIV tests but there is no scope to remain careless in respect of HIV-LAI when the reactivity rate is increasing day by day among general people.

'Hooghly' district showed the highest prevalence rate for both HBsAg and anti-HCV antibody regarding LAI-serology followed by international and 'Burdwan' district's results (Table 5). That indicated occurrence of 'LAI' for those specific diseases were lower in 'Burdwan' district even in respect to the international scenario which favoured a better laboratory practice in 'Burdwan' district. Only the incidence of LAI for HIV seroconversion was zero percent (0%) in both the districts of our study, where as the international HIV-LAI rate of developed countries was found higher with a significant difference (Singh, 2009).

However this finding may be resulted from the routine retrospective follow up study for LAI and risk assessment in the laboratories of the developed countries, which is a lacuna in our laboratory management system. Provision for risk assessment and regular immunological tests of biological samples of the laboratory staff should be created under health policies of us. Though the HIV reactive report is found a zero percentage amongst the laboratory workers in two districts of the study, there was no point of self satisfaction with this result (Table 5). As there is no system of risk assessment and follow up study for the exposed staff to the potential infected biological samples into the laboratory (Osterholm *et al.*, 2000), the actual scenario perhaps may be remained unveiled in our study. The increasing number of persons being treated for HIV-associated illness made it likely that more laboratory workers would encounter patients infected with HIV. The risk of transmission of HIV can be minimized if the laboratory workers use care while performing all invasive procedures and adhere rigorously to universal precautions (Baron and Miller, 2008). Employees should instruct laboratory workers on the need for routine use of universal precautions, provide quality equipments and clothing, necessary to minimize the risk of infection and monitor whether workers are abiding by the laboratory safety precautions or not.

The higher incidents of 'LAI' serology in our study (Table 5) reflected the increasing trend of 'HBV' and 'HCV' infection among the laboratory workers in our country, which may be also a result of inadequate knowledge, mentality of giving less importance to follow the laboratory ethics and guidelines of good laboratory practices (Fitzpatrick *et al.*, 2000; Baron and Miller, 2008).

Conclusion

Today the greatest concern among laboratory workers is that of becoming infected by HIV from exposure to contaminated blood and body fluids in the workplace. While it is difficult to assess the precise risk of occupationally acquiring HIV without a long term prospective study, a number of reports have estimated the risk. But in our study we have found that in laboratories of study areas the prevalence of seropositivity of HIV antibody amongst the laboratory staff was zero percent. Transmission of HIV via this route has not been documented, possibly because the viral inoculum in blood is lower than in HBV infection and because HIV appears less stable than HBV in the laboratory environment. Desiccation of the virus causes a rapid decline in the number of viable virions. Stringent follow up of bio-safety guidelines in laboratory should be maintained during handling of potential contaminated biological samples including its disposal to prevent the laboratory acquired HIV, HBV and HCV infections. Beside that there is an urgent need to increase the level and quality of training among laboratory staff to prevent the spread of HIV, HBV and HCV infections.

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