Original Article

Study of Seroprevalence of Hepatitis B Virus In Routine Medico Legal Autopsies

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Abstract

Background: An autopsy may subject the prosecutors and others to a wide variety of infectious agents, including blood borne and aerosolized pathogens such as human immunodeficiency virus, hepatitis B and C viruses, and My cobacterium tuberculosis. Several studies revealed the increased prevalence of hepatitis B, C, D, G, tuberculosis, HIV, prion diseases, hanta virus, measles, HTLV-1 or bacterial infections in mortuary workers. Safety becomes an issue, both in medical and ecological aspects regarding the protection of environment with the high seroprevalence of HIV and hepatitis viruses. The Study has been carried out to find the seroprevalence of Hepatitis B virus in 515 medico legal autopsies at our centre between April 2014 and September 2014.

Materials & Methods: The samples were tested blindly that the identity of the individual was unknown. The samples were collected via cardiac chamber at the time of autopsy. The samples were tested using HbsAg ELISA kit. The results were recorded in proforma and analyzed.

Results: Out of the 515 samples tested, Males occupy predominant number of cases, accounts for about 86.6% of study samples, whereas female constitute only 13.2% of the study sample. Of the 515 samples tested, HbsAg were detected in 18 samples (3.5%) using ELISA kit. All the positive cases were not previously known to have HBV-infection. Data such as demographics, cause of death, brought dead or treated, post mortem interval and positivity for HbsAg are recorded. In this study the prevalence of HBV is 100 % in Transgender as only one case was tested and it came as positive which is followed by male population.

Conclusion: The present study concludes that testing of HBV in medico legal autopsies is a convenient and effective method in monitoring the surveillance of HBV-infection in the general population and it can be used for epidemiological studies. . In screening postmortem blood for HbSAg, the present study represents that HbSAg ELISA kit is simple, rapid, no special equipment are required, even whole blood can be used and has very high sensitivity of 99.58% and specificity of 100%.

Key Words: Autopsy; Hepatitis B; Infection; High risk Autopsy; Universal Precaution

Introduction

Autopsy examination is not only used for medico legal purpose but also in doing medical research and education by identifying new diseases or new manifestations of already existing diseases. It was mentioned in various studies by various authors that apart from establishing the cause of death it also helps in improving the quality in clinical service by evaluating the therapeutic effectiveness of various strategies[1-3]. The infectivity status of the deceased person was not known in majority of the cases subjected for autopsy, as said before. An autopsy may expose the prosecutors and other personnel to wide varieties of blood borne and aerosolized infectious agents such as retro virus, hepatitis B and C viruses, and Mycobacterium tuberculosis[4]. Several studies revealed the increased prevalence of hepatitis B, C, D, G, tuberculosis, HIV, prion diseases, hantavirus, measles, HTLV-1 or bacterial infections in mortuary workers[5].Despite the precautions taken to control the infection and availability of various vaccines, the health care professionals who had engaged themselves in the medical and medico legal practice face the risk of access to blood-borne viral infections by exposing them to body tissues or body fluids which are often loaded with infectious pathogens, irrespective of the stage of human remains. Safety becomes an issue, both in

medical and ecological aspects regarding the protection of environment with the high seroprevalence of HIV and hepatitis viruses[6]. The highest rate of laboratory- acquired air borne and blood borne infections from the dead bodies was seen in autopsy workers, which was also established by the studies conducted between 1970 and 1989 in British clinical laboratories[6,7]. However, in many forensic situations the statistical risk of hepatitis and HIV infection are markedly greater in autopsies on bodies of homosexuals and drug abusers than in the general autopsy population[8].

Aims and Objectives

1)To study the seroprevalence of Hepatitis B virus among the routine cases brought for Medico Legal Autopsies. 2) To analyze the risk ratio and create awareness among the heath care personnel who handles dead bodies. 3) To diagnose the clinically undetected HBV cases. 4) To determine whether postmortem of dead bodies which are thought to be at low risk groups, are safe or not.

Methodology (Materials & Methods)

The identity of the individual is not revealed at any part of the study. The samples were collected via cardiac chamber at the time of autopsy.

Subject Selection: Study was conducted on those cases, coming for Medico Legal autopsy to the Institute of Forensic Medicine, Madras Medical College, Chennai - 600003. The identity of the individual should not be disclosed in any part of the study.

Inclusion Criteria: All dead bodies subjected for autopsy

Exclusion Criteria: All decomposed dead bodies subjected for autopsy

Principle of the Test: Human HBsAg ELISA Kit which contains polystyrene micro well strips that are pre-coated with monoclonal antibodies specific to HBsAg is used to detect the presence of HBsAg in the given sample by using antibody "sandwich" ELISA method

a) Add serum or plasma sample of the deceased to the Polystyrene microwells whose strips are pre-coated with monoclonal antibodies specific to HBsAg

b) During incubation, if HBsAg is present in the sample, the specific Ag Ab complex formed is captured on the solid phase.

c) Then add the second antibody conjugated the enzyme horseradish peroxidase (the HRP-Conjugate) directed against a different epitope of HBsAg into the wells.

d) During the process of second incubation step, these HRP-conjugated antibodies and the anti-HBs-HBsAg complexes which are previously formed during the first incubation bound to each other. After that, washing the wells removes the unbound HRP-conjugate.

e) Chromogen solutions A and B are added into the wells.

f) The colorless chromogens which are added are hydrolyzed by the bound HRP-conjugate in presence of the antibody-antigen-antibody (HRP) "sandwich" immune complex to a blue-colored product.

g) Once the reaction with sulfuric acid stops, the blue color turns yellow.

The amount of color intensity can be measured by deter mining absorbance using 450nm as reading wavelength with 620-690 nm reference wavelength which is proportional to the amount of antigen captured in the wells, and to its amount in the sample respectively. Wells remain colorless if it contains samples negative for HBsAg. (Fig 1)

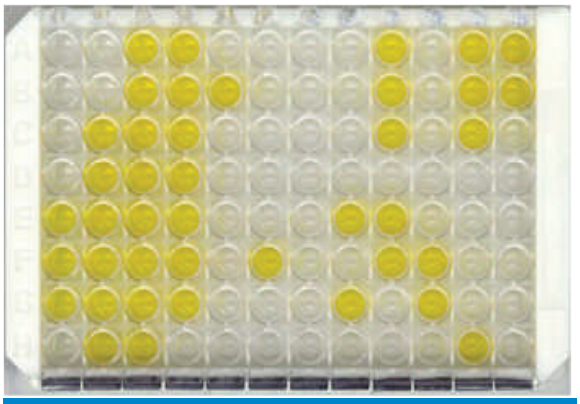

Fig 1: Diagram of Generic 'Antigen Sandwich' Elisa for Completed Generic Elisa Assay

A – Blank
B C D - Negative controls
E F - Positive controls
G - Sample 1 Positive
H - Sample 2 Negative

Limitations of the test

1. Only un-pooled human serum or plasma can be used
2. HBV infection cannot be excluded without considering the other evidences for the same by taking only the negative HBsAg obtained

Performance Characteristics

(i) Diagnostic specificity: 99.58%
(ii) Diagnostic sensitivity: 100%

Analysis and Results

Out of the 515 samples tested, Males occupy the predominant number of cases, which accounts for about 86.6% of study samples, whereas female constitutes only 13.2% of the study sample. Of the 515 samples tested, HbsAg were detected in 18 samples (3.5%) using ELISA kit. All the positive cases were not previously known to have HBV-infection. Data such as demographics, cause of death, brought dead or treated, post mortem interval and positivity for HBsAg are recorded. Out of the 18 positive samples, 13 were male and 4 were female and 1 transgender. (Fig 2)

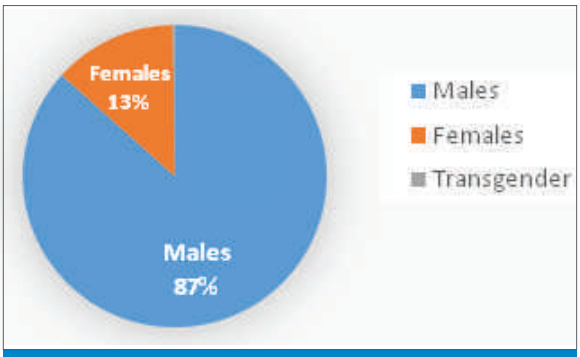

Fig 2: Sex distributions among the study sample

Among 515 cases analyzed, predominant numbers of cases are male patients (86.6%). Out of the 18 positive cases, thirteen cases were positive among the males (72.2%) with 2.9 % of overall positive cases and four cases were positive among the females (22.22 %) with 5.9 % of overall positive cases, one case was found in transgender (5.5 %) with 0.2 % of overall positive cases. In this study the prevalence of HBV is 100 % in Transgender as only one case was tested and it came as positive which is followed by male population. (Fig 3)

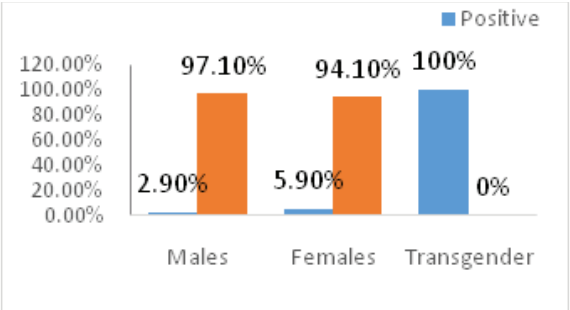

Fig 3: Positivity among both the sexes

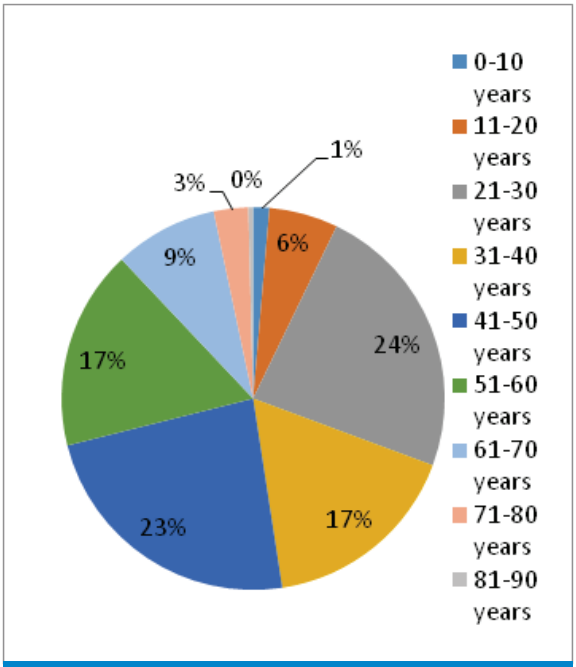

Fig 4: Age distribution among the study sample

Among 515 cases analyzed, predominant number of cases falls under age group between 21 to 30 and 41 to 50, followed by age group between 31to 40 and 51 to 60. Out of the 18 cases, six cases were positive in the age group 21-30, five cases were positive in the age group 41-50, four cases were positive in the age group 31-40 and three cases were positive in the age group 51-30 indicating that highest Seroprevalence was in the age group 21-30. (Fig 4) & (Fig 5)

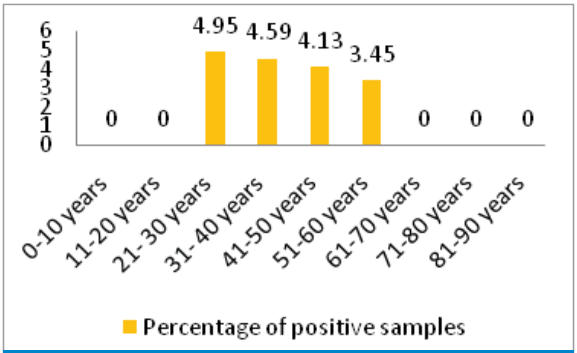

Fig 5: Positivity percentage among the age distribution in study sample

Among 515 cases analyzed, predominant number of cases falls under the category of Road Traffic Accidents (51.46%), followed by Fall from height (11.46%), Natural cause (10.3%), Poisoning (7057%) and others. Out of the 18 positive cases, four cases were positive in Natural cause, Three cases were positive in the RTA, Hanging and Murder cases, two cases were positive in TTA and Poisoning and one case was positive in the Fall from height. Even though Road traffic accidents constitute the majority of samples collected (51.46%) and Natural cause (with 7.56 % of Seroprevalence) constitutes 22.23 % of overall positive samples, highest Seroprevalence was noted in case of Hanging with 15.79% (Three cases were positive out of nineteen cases) with 16.67% of overall positive samples (Three out of eighteen cases). (Fig 6)

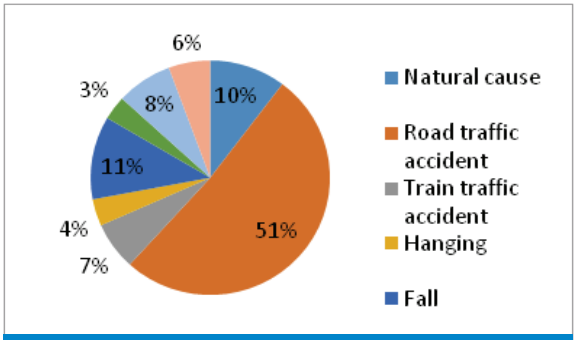

Fig 6: Distribution of manner of death among the study sample

This shows that HBV screening is of great importance among the cases we receive for autopsy irrespective of manner of death. Hanging and natural cause in our study showed a relatively higher prevalence of HBV infections compared with other manners of deaths. (Fig 7)

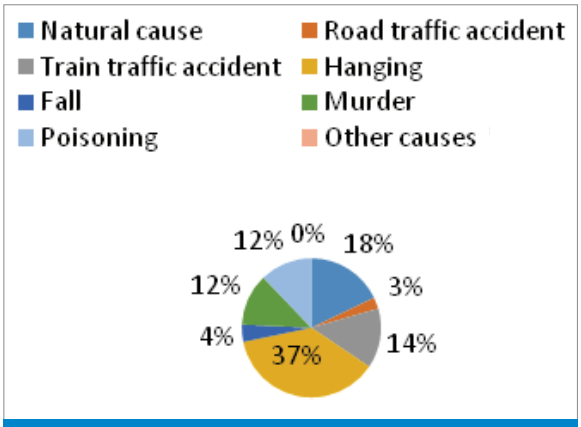

Fig 7: Distribution of percentage of positive cases of the study sample among the manner of death

Among 515 cases analyzed, predominant number of cases falls under the category of Hospital treated patients (82.33%). In this study, Out of the 18 positive cases, twelve cases were positive among the brought dead category (13.2%) with 66.67 % of overall positive cases and six cases were positive among the hospital treated category with 33.33% of overall positive cases. (Fig 8) & (Fig 9)

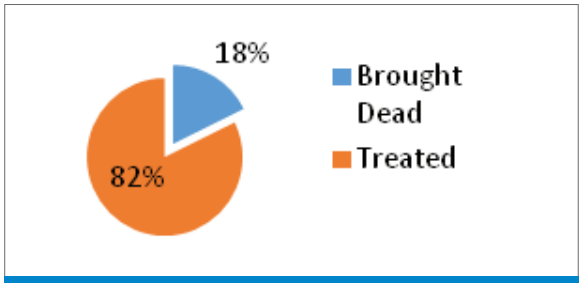

Fig 8: Distribution of brought dead and treated case among study sample

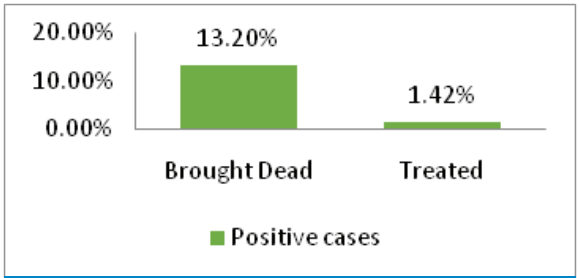

Fig 9: Distribution of Positive case among the brought dead and treated case in the study sample

This shows that HBV screening is of great importance among the cases we receive for autopsy as their HBV status was not known (66.67% were brought dead with no details regarding history of exposure or blood transfusion or HBV/HIV status)

Among 515 samples analyzed, predominant number of samples was collected between 12 to 24 hours (51.1%), followed 6 to 12 hours (19.6%) and 24 to 36 hours (19.6%). Out of the 18 positive cases, thirteen cases were positive among the samples which were collected 12 to 24 hours after death (positive percentage of 4.94%) with 72.22 % of overall positive cases. (Fig 10) & (Fig 11)

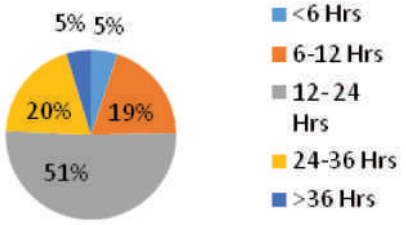

Fig 10: Distribution of Post mortem interval among the study sample

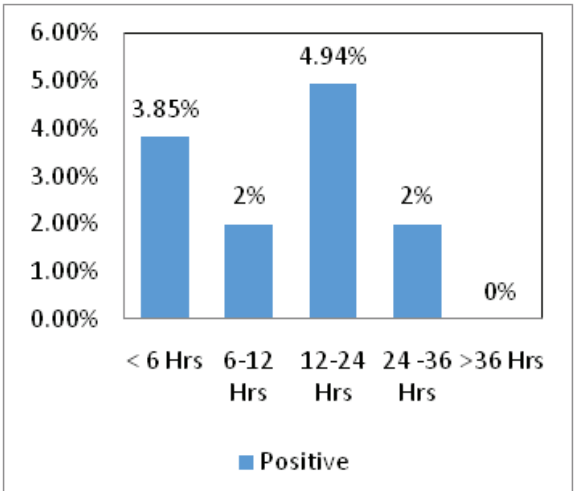

Fig 11: Distribution of Positive percentage among Post mortem interval in the study sample

The samples collected up to 36 hrs after death showed positive results for HBsAg. This shows that HBV screening is of great importance among the cases we receive for autopsy as HBsAg was detected even in the post mortem blood samples and hence consider all cadaver as a potential source of infection to health care workers (Fig 12)

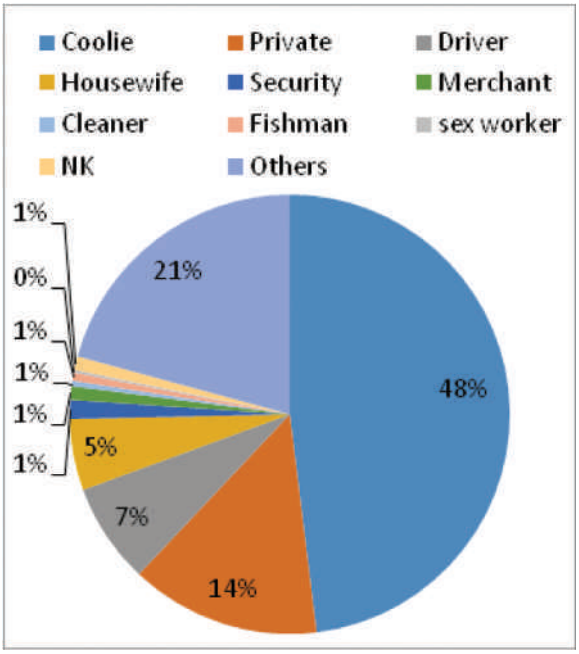

Fig 12: Distribution of Occupation among the study sample

This shows that HBV screening is of great importance among the Coolie and commercial sex workers which showed a relatively higher prevalence of HBV infections compared with other occupations (Fig 13)

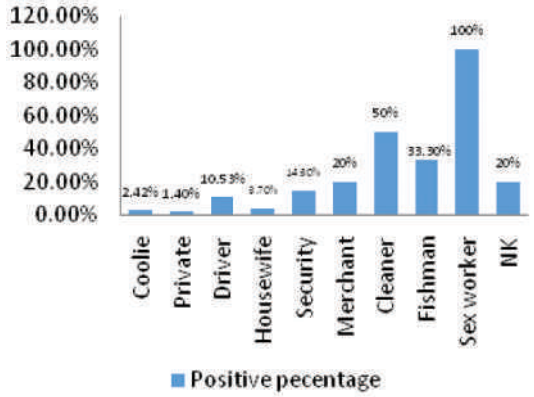

Fig 13: Distribution of Positive percentage among the various occupational groups in the study sample

Among 515 cases analyzed, predominant number of cases falls under the Married category (78.1%) followed Single (21%). Out of the 18 positive cases, thirteen cases were positive in married population and four cases were positive in Singles. Even though Married population constitute the majority of samples collected (78.1%) with 72.33 % of overall positive samples, Both Married and Single constitutes Seroprevalence of about 3% positive percentage with unknown category having highest Seroprevalence of 20% (One in five cases positive) with 5.56% of overall positive samples (One out of eighteen cases). (Fig 14) & (Fig 15)

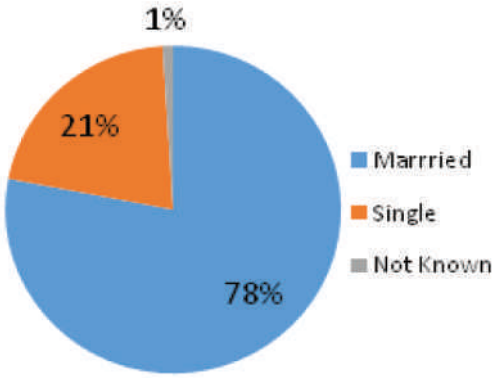

Fig 14: Distribution of Marital status among the study sample

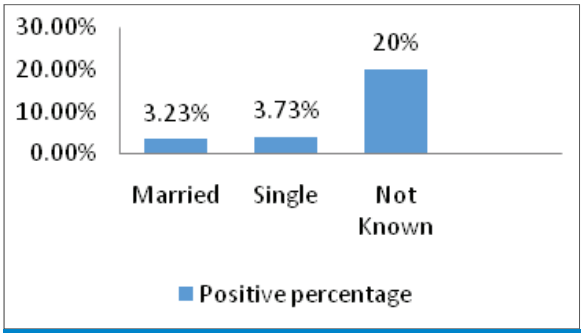

Fig 15: Distribution of positive percentage among the marital status in the study sample

This shows that HBV screening is of great importance in the Spouse and children when the deceased was found to be positive in autopsy samples. Dead bodies brought as unknown without no relatives showed a relatively higher prevalence of HBV infections compared with other category. Hence unless clearly indicated, unknown bodies should not be subjected for autopsy as a routine procedure.

Discussion

The high frequency of the Hepatitis B virus infection among the deceased and longevity of the virus in their body tissues and fluids leads to an increased morbidity and mortality among the mortuary workers[9]. Amongst all the parenteral viruses, Hepatitis B virus has the highest transmissibility rate with a rate of about 100 times greater than HIV[5,6,10]. Carolin et al[11] suggested that post-mortem blood samples should be collected within 24 hours. Challine et al recommended 12 h maximum time for drawing post-mortem blood samples.[11] In my study HBsAg was detected in the post mortem blood samples up to 36 hours. In the year 2012 about 119,000 cases of viral hepatitis due to varied etiologies were reported in India[12]. In South-East Asia, the burden of chronic HBV infection was estimated as 100 million[12]. After HEV, HBV is the second most common cause of acute viral hepatitis in India with over 40 million HBV carriers[12]. In India, every year one million populations are at risk and about 100,000 die from HBV infection[12]. Though uncommon in India, HDV infection is observed in 10% to 20% of HBV positive patients[12]. In India, the epidemics due to the unsafe practice of sharing injection among IV drug users and healthcare personnels caring the infected people 46% of hepatitis B carriage and 38% of HCV infection were documented[12]. In India, perinatal transmission rate of HBV infection increases to 90% if the mother is positive for both HBsAg and hepatitis B e-antigen (HBeAg) rather than only HBsAg positive in which case it is only 10%[12]. Universal immunization against hepatitis B was introduced in India in 10 states in the year 2002 and in 2011 countrywide. A pentavalent vaccine which was introduced recently in some states gives protection against HBV also[12].

Before commencing the postmortem, due to various reasons including social and cultural restrictions it will not be practically possible to collect detailed and reliable information about the risk factors which will help in assessing the HBV status of the deceased. Even if the history obtained from the relatives lacks the existence of risk factor it doesn't means its non existence. Hence, a reliable bed side test which can be performed easily and rapidly to detect HBV infection in the mortuary will be very useful. Hence routine testing in all medico-legal autopsies irrespective of their previous HBV status will help in identifying the carriers. Li et al reported 23% prevalence of hepatitis B in forensic autopsy performers[13]. Plessis et al reported 8% prevalence of hepatitis B in forensic autopsy performers[14].

In this study, the eighteen positive cases out of 515 cases were not known to have HBV which means they are clinically undetected for HBsAg. This indicates the presence of high risk people in the routine

medico-legal autopsies. The post mortem survival of the HBV was estimated in many studies. HBV survive outside the human body for up to 7 – 10 days and can withstand drying for at least a week[5]. When stored at 30-32 degree Celsius, HBV retains its infectivity for at least 6 months and when frozen at -15 degree Celsius, its infectivity will be retained for up to 15 years. The presence of very high viral load in blood and other body fluids during the early phase of infection suggests the need for practicing universal precaution which ensures efficient protection of autopsy personnel during routine medico legal autopsies.

The presence of HBsAg and anti - HBsAg in the postmortem serum which is stored for relatively long periods of time makes its detection a most reliable measure of antemortem HBV infection. Furthermore, it has been demonstrated that by assessing the post mortem changes in the serological parameters Kitchen and Newham established the post mortem stability of serological markers up to 24 hours after death [11, 15]. Schuller demonstrated that the post mortem stability of the stability of the antibodies was similar when compared to the ante mortem screening done in live donors[16].

The post mortem hemolysis, decomposition and bacterial contamination in dead bodies may not affect proteins such as the globulins that comprise antibodies [8, 13]. In this study, it was estimated that the postmortem interval was ranging from 6 hours to 36 hours for detection of HBsAg. The viral load of the cadaver, viral strain, anti viral therapy taken when deceased is alive and the temperature of the cold storage in which the body is stored will influence the postmortem viral level. HBV is transmitted from person to person when they come in contact with body fluids such as blood, Cerebro spinal fluid, vaginal secretions, seminal discharges, breast milk, amniotic f luid, pericardial fluid and synovial fluids. Others body fluids like saliva, lachrymal secretions and urine unless they found to be contaminated with blood in adequate volume are not implicated in the transmission of HBV.

Weston and Lober in his study have recognized that nearly one third of the surgical glove puncture which occur with incidence of 8% during post-mortem remains undetected by the forensic pathologist who is performing the autopsy for a longer duration which cause preexisting injuries in his hand to be bathed with the infectious blood for a prolonged period of time[17].

In my present study, the samples were tested blindly by not disclosing the identity of the individual in each and every stage. This study includes all types of cases from the general population which are brought for medico legal autopsy. Blood samples from heart were collected from 515 cases which are brought to Madras Medical College and Rajiv Gandhi Government General Hospital for medico legal autopsy. All the blood samples which were collected were tested for HBsAg using standard Human HBsAg ELISA kits which has a sensitivity and specificity of 99.58% and 100% respectively.

Out of 515 cases which were tested for HBsAg, eighteen cases were positive and among which thirteen were male, four were female and one was transgender. The positivity of 18 cases among 515, though look statistically insignificant, is higher than voluntary screening programs conducted by many organisations. Dr. Nirali shah et al in his study on the samples of voluntary blood donors demonstrated the Seroprevalence of HIV, HBV, HCV and syphilis which was found to be 0.154%, 0.887%, 0.101%and 0.22% respectively[18]. There are people who abstain from doing this screening blood test in many voluntary screening programs, which does not affect in this study. Thus, epidemiologically, routine screening for the presence of HBV in all dead bodies brought for medico legal autopsies is a sensitive indicator.

There are two varied opinions regarding the handling of the dead bodies harboring HBV in routine medico legal autopsies. One school of thought says that the universal precaution should be followed in all cases which are brought for autopsies which is practically difficult to follow in the developing country like India as it is not cost effective. The second thought says that universal precautions should be followed if the test done before doing autopsy in mortuary is positive which will reduce cost of burden to the management by reducing the need for the supply and use of personnel equipment kit for the autopsy personnel.

Limitation of The Study

If the post mortem time interval is prolonged, the various factors such as lysis of RBC's, autolysis, contamination by bacteria and loss due to decomposition which will affect the postmortem testing of blood and body fluids are enhanced, but immunoglobulins, are considered as less likely to be affected by such factors mentioned before. This screening test for the HBsAg may not be sensitive if the person was in the window period.

Conclusion

The health care professionals who are involved in the postmortem work are exposed to a greater risk of occupational health hazard due to higher prevalence of various infectious diseases among the general population[6]. It is wise to practice the Universal Precautions in almost all dead bodies considering it as infectious as it becomes practically impossible to know the medical status of each and every body subjected for autopsy. The autopsy based occupational health hazards can be reduced by focusing on improvement in assessment, training, education, personal protection, autopsy techniques and autopsy room. Health care professionals involved in postmortem practice should be aware of the biohazards and radiation risks associated with the cadaver, instruments they handle and the atmosphere in which they work and take necessary steps to minimize the risks. Careful practice avoids many accidents in mortuary. The present study concludes that routine testing of HBV in all cases which are brought for medico legal autopsies is not only a convenient and effective method which is used to monitor the prevalence of HBV-infection among the general population, but also as a safety measure for utopsy personnel and as a study tool in epidemiological studies too[19]. There is no need for a

aspecial equipment to do this screening in postmortem blood samples for detection of HBsAg. The HBsAg ELISA kit which was used in this study has not only 99.58% sensitivity and 100% specificity but also simple to use and rapid in getting the result, can be used for doing the routine screening test in medico legal autopsies. Even whole blood can be used in this kit.

HBV positivity rate in my study population is 3.49%. The HBV status of all the eighteen seropositive post mortem blood samples was not known prior to autopsy testing. Even with a needle stick injury during autopsy, rate of infection appears to be less than 1%. Taking universal precautions with every autopsy is not practical and economical in developing countries like India. Therefore, screening of postmortem samples and knowing the deceased HBV serological status prior to autopsy may be worthwhile in cases at high risk of HBV infection, preferably when the HBV status of the deceased is not known at the time of doing postmortem.

To conclude with universal precautions should be followed during routine autopsy procedures. Only experts and health care professionals those who are skilled in handling the infected materials are allowed to enter in to the postmortem examination room. Only those experienced professionals should conduct the autopsy examination as it is proved through many studies that the risk of accidental infection was more common, when the post mortem procedure was done by an inexperienced person[5]. Studies reported that the occurrence of lacerated wound in the body of the performer in 1 in every 11 autopsies conducted by inexperienced persons [6,20]. Postmortem examination should not involve those persons with breach in the skin or mucosa or body immunity. The internal atmosphere of the postmortem examination room should be in such a way with sufficient space to avoid overcrowding and provide proper ventilation. Protective measures such as wearing gloves, headwear, masks, eyewear, shoes and full-covered gown should be given to and used by the mortuary staff while they are doing the postmortem work. Wearing double gloves during the autopsy or any surgical procedure will help us to minimize the risk of exposing themselves to the infected blood.

As all know that prevention is always better than cure, the preventive measures such as screening and immunization of the high risk group persons should be adopted. The group is considered as high risk or not based on the various factors such as history of exposure to infected samples and infected personnel, risk involved in their routine day to day practices and risk involved in the occupation in which they are engaged. Recommended safety measures should be followed in all areas wherever we comes in contact with blood and body fluids such as Delivery, Surgeries, Blood Transfusion, Intramuscular or intravenous injections, unprotected Sex and in autopsies.

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