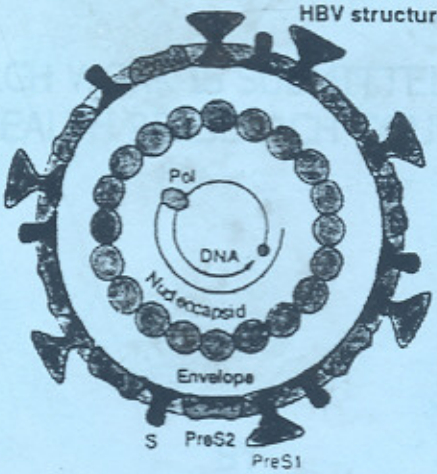
PREVALENCE OF HEPATITIS B AND C VIRUS INFECTION IN HEALTH-CARE WORKERS OF BIR HOSPITAL, KATHMANDU



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Sanjaya K Shrestha

To;

my parents Gokul and Rewa

and my brother Suman

and my wife Shruti

and son Siddartha

and someone who is expected very soon, my .....

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SUMMARY

Background

Health care personnel are at increased risk of occupational acquisition of hepatitis B virus infection, but there is only scant information in Nepal on the risk factors associated with HCWs that make them prone to this infection. I, therefore, surveyed a random sample of 145 HCWs of different categories to find out the prevalence and risk factors of HBV infection associated with them. In addition, hepatitis C virus (HCV) is a newly recognized cause of parenterally acquired hepatitis, and the risk of HCV transmission to health care personnel remains unclear.

Methods

From December 2001 to January 2002, HCWs at Bir Hospital, Katmandu, were offered testing for HBV and HCV and were asked to complete a questionnaire. Confidentiality was assured by encoding the questionnaires and serum samples. Serum samples were tested for HBV surface antigen **(HBsAg)**, antibody to HBV core antigen **(anti-HBc)**, antibody to surface antigen **(anti-HBs)**, and antibody to HCV **(anti-HCV)**.

Results

Antibodies to HBV core antigen were found in 21 (14.5%) of 145 HCWs. HBV surface antigens were detected in 2 (1.4%) HCWs. After statistical calculation, infection with HBV was associated with type of HCW (nurses and non-professionals) ($p<0.05$), years of exposure to ones profession (more than 11 years) ($p<0.05$), and lack of HBV vaccination ($p<0.05$). None of the HCWs were found to be positive for HCV.

Conclusion

These data suggest that the prevalence of HBV infection in HCWs at Bir Hospital is higher than that observed in the general population. In addition, nurses and non-professional HCWs are at significantly higher risk compared to other HCWs. Since the lack of HBV vaccination was a significant risk factor associated with HBV infection, the only way to prevent infection by HBV among HCWs is to vaccinate all categories of HCWs including strict observation of universal precautions.

INTRODUCTION

Hepatitis B Virus (HBV) infection and its sequelae are serious public health problems worldwide. HBV infection is usually clinically inapparent. Approximately, 5-10% of infected adults and more than 90% of infected neonates become chronically infected by the virus and develop chronic liver disease of varying severity (1). This chronically infected group run the major risk of developing cirrhosis and hepatocellular carcinoma (HCC).

The WHO estimates that, on worldwide basis, more than two billion people (one third of the world population) have evidence of past or current HBV infection. It is estimated conservatively that there are 350 million chronic carriers of hepatitis B (2). Many are lifelong carriers, although not all are infectious, and some will clear the virus after varying intervals of many months or years. Among the chronic carriers 50% can be expected to die prematurely, either as a result of chronic inflammatory liver disease or the development of hepatocellular carcinoma (3). The latter is among the 10 most common cancers in the world, with a particularly high incidence in the South East Asian and Western Pacific regions and sub-Saharan Africa – regions where HBV infection is highly endemic. Up to 80% of cases of HCC can be attributed to hepatitis B, which is only second to tobacco among the known human carcinogens (2).

HBV is transmitted through parental route, sexual contact, and perinatally. High risk group include health-care workers, commercial sex workers, homosexuals, heterosexuals with multiple partners, intravenous drug addicts, patients requiring multiple blood transfusions, children born to HBsAg-positive mothers, household contacts of HBsAg-positive carriers or cases, etc.

Health-care workers in contact with patients, and especially patient's blood and body fluids, usually have a higher carrier rate than the general community. HBV is an occupational risk. A number of surveys, mainly reflecting occupational exposures before the widespread availability of hepatitis B immunization (vaccine for HBV was released in 1982 by food and Drug Administration, USA), have shown an increased prevalence of

hepatitis B infection markers in workers exposed to blood and body fluids , especially HCWs. Numerous seroprevalence studies have shown that HCWs have prevalence rates of past and present HBV infection that are three to five fold higher than that of general U.S. population (4). West (1984) reviewed evidence from a number of seroprevalence studies in the USA and concluded that the overall risk to people employed in health–related fields was four times that of the general adult population; physician and dentists were at 5-10 times the risk of the general population, and groups with over 10 times the risk included surgeons, clinical workers in dialysis units and mental handicap units and laboratory workers having frequent contact with blood and body fluid samples (5).

Most of the studies conducted in Nepal and India report the prevalence of hepatitis B surface antigen (HBsAg) among HCWs, which detects only the active disease, and does not detect those who were infected in the past and became immune naturally. The present study was conducted to determine the seroprevalence and the epidemiological characteristics of Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) infection among a sample of HCWs at Bir Hospital, Kathmandu. Four seromarkers were used for the study; antibody to hepatitis B core antigen (anti-HBc), which indicates past infection, hepatitis B surface antigen (HBsAg), which detects active infection, antibody to hepatitis B surface antigen (anti-HBs), which indicates immunity against HBV infection, and antibody to hepatitis C virus (anti-HCV), which indicates infection by HCV.

AIMS

To determine the seroprevalence of HBV and HCV in health care workers and to asses the relationship between HBV and HCV infection and risk factors in health care workers.

OBJECTIVES

1) To find out the distribution of HBV and HCV infection with reference to study variables such as age, sex, type of health care worker, number of years exposed to ones profession and vaccination status .
2) To screen the anti-HBc status and then to advise whether to go for vaccination.
3) To find out the humoral imunological status of the health care workers.

REVIEW OF LITERATURE

Historical Background

The first generally accepted reference to parenterally transmitted hepatitis is that of an outbreak of jaundice in 1885 that affected approximately 15% of a group of 1289 Bremen shipyard workers. Lurmen, a thoughtful and perceptive German public health official who studied the outbreak, attributed it to the earlier receipt by these workers of smallpox vaccine containing glycerinated lymph of human origin (6).

A dramatic epidemic in 1942 of icteric hepatitis involving 50,000 US servicemen represented the final proof of the existence of parentally transmissible hepatitis. The epidemic followed shortly after the receipt of specific lots of yellow fever vaccine that had been 'stabilized' with pooled human sera. Subsequent interviews with blood donors of the pool revealed that some had had a recent bout of 'catarrhal jaundice' and that at least one was ill at the time of donation. The outbreak was proven to be of HBV origin through a serological analysis conducted 40 years later among selected individuals who had been involved in the original vaccine programme (6).

Transfusion-associated jaundice was first described in the 1940s and became increasingly recognized as a complication of haemophilia treatment following the introduction of plasma product therapy in subsequent years (6).

Blumberg et al (1965), in Philadelphia, while searching for diagnostic genetic markers in two multiply transfused haemophiliacs, unexpectedly identified a lipoprotein precipitate using an agarose gel immunodiffusion system. The significance of this precipitin line, termed the 'Australia antigen' because serum from an Australian aborigine had been its source, was at first unclear. When it became apparent within a few years time that what was being detected was, in fact, the outer shell of the HBV, the term Australia antigen was abandoned and replaced with the designation hepatitis B surface antigen (HBsAg) (7). For these achievements, Dr. Baruch Blumberg received the Nobel prize in physiology or Medicine in 1976.

Virologic characteristics

HBV belongs to a family of closely related DNA viruses called the hepadnaviruses. Included in this family are the woodchuck hepatitis virus, the duck hepatitis B virus, and several other avian and mammalian variants. All the hepadnaviruses have similar hepatotropism and life cycles in their hosts (8). The whole virion, also termed the Dane particle, is a 42 nm sphere that contains a core, or nucleocapsid, enclosing the DNA. The viral genome of HBV is a partially double-stranded circular DNA of approximately 3200 base pairs that encodes four overlapping open reading frames: S, for the surface, or envelope, gene; C, for the core gene; X, for the X-gene; and P, for the polymerase gene(8).

One peculiar feature of HBV is the excess production of envelope protein material which aggregate to form spherical and filamentous particles with average diameter of 22nm. These particles are found in the circulation along with the complete virion, the Dane particle (9). Excess production of these particles cause neutralization of anti-HBs, hence allowing virus to spread without coming in contact anti-HBs, which is a survival strategy.

The envelope particles, the surface antigens (HBsAg) have several specific antigenic determinants. The antigenic determinant 'a' is common to all surface antigens. The Subtype determinants are d or y and w or r which are mutually exclusive. Thus four types of surface antigens that occur as adw, adr, ayw, and ayr have epidemiologic importance. The development of humoral immunity to HBsAg is protective, and recombinant HBsAg provides the basis for the HBV vaccines currently available.

Hepatits B core antigen (HBcAg) is the nucleocapsid that encloses the viral DNA. When HBcAg-derived peptides are expressed on the surface of hepatocytes, they induce a cellular immune response (cytotoxic T cell response) that is crucial for killing infected cells and clearing the virus. The core antigen is present only in hepatocytes and does not circulate in the serum.

Hepatitis Be antigen (HBeAg), a circulating peptide derived from the core gene and then modified and exported from liver cells, serves as a maker of active viral replication (8). With few exceptions, HBeAg is present only in persons who have circulating serum HBV DNA .

The long P gene encodes the DNA polymerase, which also serves a reverse-transcriptase function, since replication requires RNA intermediates. The X gene encodes two proteins that serve as transcriptional transactivators, aiding viral replication. These proteins may also play a part in the development of hepatocellular carcinoma.

The presence of HBV DNA in serum, the best indication of active viral replication, is detected by hybridization methods or by the more sensitive polymerase-chain-reaction

(PCR) technique. Quantitation of HBV DNA is helpful in predicating the response to therapy (9).

Hepatitis B surface antigen circulates at a very high serum concentrations (up to 10^{13} particles per ml) during HBV infection (10). It is detected in serum 1 to 10 weeks after infection with HBV and 2 to 8 weeks before the onset of clinical hepatitis. In less than 5% of cases of acute hepatitis B, HBsAg may be undetectable at the time of presentation, because levels of HBsAg either never reached or had already declined below the detection threshold of the assay.

Although anti-HBs appears early during acute infection, marked antigen excess prevents routine detection of anti-HBs until HBsAg disappears. Immune complex may be detected by ELISA. Solid phase coated with anti-HBs will capture the immune complex of HBsAg and anti-HBs, then anti human globulin (labeled) can be used to detect the captured complex. Thus it indirectly detects anti-HBs. Typically, with the resolution of acute hepatitis B, HBsAg becomes undetectable as anti-HBs becomes detectable (11). Antibody to HBsAg is a neutralizing antibody, which, when present, is associated with lifelong immunity and is the antibody produced in response to hepatitis B vaccination with current vaccines composed entirely of HBsAg; at present, anti-HBs levels of at least 10 mIU/ml are regarded as protective (12). Failure of acute HBV infection to resolve and the development of chronic HBV infection are suggested by persisting of circulating HBsAg and absence of detectable anti-HBs.

As the viruses get cleared surface antigens gradually decline and become undetectable. There is a period of one to two weeks after the disappearance of HBsAg and before the appearance of anti-HBs, which is known as the 'window period'. The patient may still be infectious during this period though HBsAg is negative. Antibody to core antigen (anti-HBc) is detectable during this window period.

Hepatitis core antigen is a particulate non-secreted nucleocapsid protein which, rather than circulating in the serum, remains cell associated and virion associated. The

corresponding antibody, anti-HBc, is an important marker of HBV infection, whether current or remote (11).

Antibody to the nucleocapsid protein (anti-HBc) appears early in the course of infection (just after HBsAg) and persists indefinitely. IgG anti-HBc is a particularly reliable marker of previous HBV infection and may persist even when anti-HBs titers decline to undetectable levels many years following recovery from HBV infection (13).

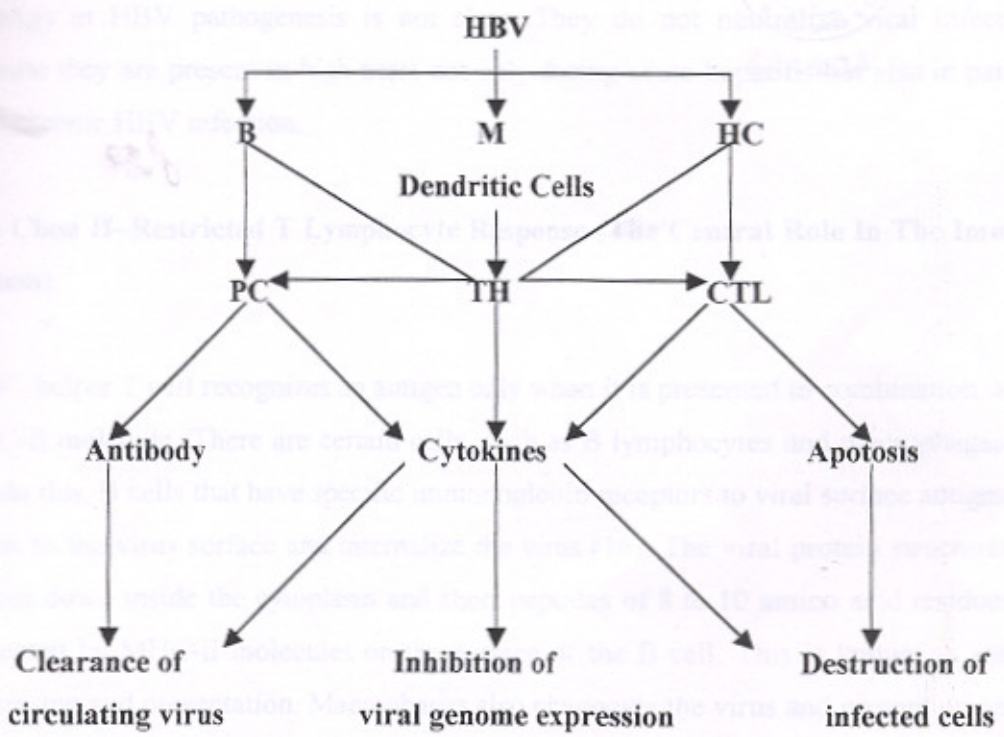
Antibody to core antigen (anti-HBc) is detected in virtually all patients who have ever been exposed to HBV. Unlike anti-HBs, this antibody is not protective, but it serves as a marker that indicates infection somewhere in the past. It is the first host induced immunological marker of HBV infection, which appears shortly after the time HBsAg is first detected in the serum (14). Its presence alone cannot be used to distinguish acute from chronic infection. Patients who have persistent HBV infection are positive for anti-HBc, as are those who have recovered from HBV infection. The IgM subtype of anti-HBc is associated with acute infection and is therefore helpful in distinguishing acute from chronic infection. IgM antibody usually disappears within four to eight months after acute infection, whereas IgG anti-HBc remains indefinitely, almost life long (13). Since some patients with chronic hepatitis B becomes positive for IgM anti-HBc during flares in their disease, its presence is not an absolute reliable marker of acute infection (15).

Antibodies to hepatitis Be antigen (anti-HBe) appears once the antigen have been cleared and the virus is no longer replicating (9).

Immunopathogenesis

The HBV is not directly cytopathogenic to hepatic cells. The host immune attack against HBV is the cause of the liver injury, medicated by a cellular response to small epitopes of HBV proteins, especially HBcAg, presented on the surface of the hepatocyte (16). Both the cellular and humoral limbs of the immune system are activated against the HBV infection, and it is the cellular response that is involved in disease pathogenesis. There are mainly three mechanisms by which the immune response acts to clear the virus from the

body (see fig 5). First, the antibodies produced against the surface antigen of the virus neutralize the free virus in the circulation , but these antibodies have no effect on the viruses that have entered into the hepatocytes, where it is actively multiplying and spreading to other hepatocytes. Second, the various cytokines produced by activated B and T cells inhibit the viral gene expression and replication within the hepatocytes, thus limiting the spread of the disease, without killing the infected hepatocytes. Third, the cytotoxic T lymphocytes (CTL) cause destruction of the infected cells by a process of apoptosis, as well as by secretion of cytotoxins. The destruction of infected cells by apoptosis, a physiological programmed cell death, does not lead to inflammation, but the cytotoxins released by T cells lead to death and inflammation of hepatocytes.



The Antibody Response (The Humoral Limb)

The antibody response to HBVV envelope antigens (HBsAg) is a T cell-dependent process (17). These anti-envelope antibodies (anti-HBs) are thought to play a critical role in viral clearance by complexing with free viral particles and removing them from circulation or possibly by preventing their attachment and uptake by susceptible cells. They also contribute to the pathogenesis of the extrahepatic syndromes associated with HBV infection (glomerulonerphritis, cryoglobulinemia, polyarteritis nodosa) and to the prodromal syndromes of urticaria and arthralgias, by forming antigen-antibody complexes.

The role of the antibody response to the HBV nucleocapsid antigens (HBcAg and HBeAg) in HBV pathogenesis is not clear. They do not neutralize viral infectivity because they are present in high titers not only during acute hepatitis but also in patients with chronic HBV infection.

The Class II–Restricted T Lymphocyte Response (The Central Role In The Immune System)

$CD4^+$, helper T cell recognizes an antigen only when it is presented in combination with a MHC-II molecule. There are certain cells, such as B lymphocytes and macrophages that can do this. B cells that have specific immunoglobin receptors to viral surface antigen can attach to the virus surface and internalize the virus (16). The viral protein structures are broken down inside the cytoplasm and short peptides of 8 to 10 amino acid residues are presented by MHC-II molecules on the surface of the B cell. This is known as antigen processing and presentation. Macrophages also phagocyte the virus and present processed peptides similarly. $CD4^+$ cells specific for these peptide epitopes recognize these peptides through their T cell receptors (TCR) and become activated. The $CD4^+$ cell plays a central role in the immune system, because it further activates both the humoral and cellular limbs of the immune system. It activates the humoral limb by secreting lymphokines that activate specific clones of B cells that have immunoglobulin receptors for the virus

surface antigen. Thus the B cells differentiate into plasma cells and produce a huge amount of anti-HBs immunoglobulin antibodies against the virus. It activates the cellular limb by secreting other lymphokines that stimulate cytotoxic T cells that have been partially activated by MHCclass-1 restricted mechanism.

The class I-Restricted T Lymphocyte Response (The Cellular Limb)

Anti-viral CTL are believed to play a major role in eradication of infection by virtue of their capacity to identify and kill virus-infected cells through recognition of viral peptides presented by HLA class–I molecules (18). The patients with self–limited acute hepatitis develop a vigorous, polyclonal, HLA class I-restricted CTL response against multiple epitopes in the HBV envelope, nucleocapsid, and polymerase proteins. The dominant role played by the nucleocapsid in the class II-restricted T helper cell response to HBV does not hold for the class I-restricted CTL response, at least with respect to the HLA-A2-restricted CTL response, which is directed much more extensively against the HBV envelope and polymerase proteins.

The $CD8^+$ cytotoxic T cell recognizes endogenously synthesized HBV antigens presented by HLA class-I molecules at the hepatocyte membrane. Upon antigen recognition, these cells perform several antiviral functions (16). First, they are directly cytopathic for the antigen presenting hepatocyte by triggering it to undergo apoptosis. Second, they secrete lymphokines that recruit antigen–nonspecific inflammatory cells that amplify their cytopathic effect and worsen the severity of the disease by at least an order of magnitude. Third, they secrete other lymhokines that noncytolytically inhibit HBV gene expression and viral replication by destabilizing the viral RNA, thereby interrupting the viral cycle and possibly contributing to viral clearance without destroying the infected liver and killing the host .

If the overall response is weak, because the host does not respond efficiently to viral antigens or because the response becomes exhausted after an initial vigorous attempt to control the infection, the virus persists with an associated liver disease of varying severity, setting up the mitogenic and mutagenic environment that favors the

development of chromosomal and genetic damage that leads to malignant transformation and hepatocellular carcinoma (HCC)(16).

No specific HBV-oncogene sequence appears to be responsible for HCC. As with other forms of liver cancer, tumors associated with hepatitis B result from chronic inflammation and repeated cellular regeneration, typically occurring only after 25 to30 years of infection. One, but certainly not the only driving force for the development of HCC, is the integration of HBV into the host genome. This integration occurs at random. By chance, HBV integration can lead to activation of critical gene promoters of the host. Products of transactivators of the X-gene might induce translocations, deletions, and duplications within the host genome (19).

Epidemiology

The age at which an individual is infected determines the likelihood of a chronic infection developing. 90% of the adults infected with HBV successfully clear the acute infection and become immune naturally. 9% proceed to chronicity and become chronic carriers. This 9% also forms the human reservoir for the spread of infection in the community. 1% is seen to suffer from fulminant hepatic failure and die early in the acute stage. Fulminant hepatitis refers to a clinical condition with rapid onset and development of acute hepatic failure, with encephalopathy, coma, and death in more than 70% of cases (20). It is due to an enhanced immune response with more rapid clearing of virus (21). Whereas, 98% of babies born to mothers with chronic HBV infection become infected, and 95% of these will develop a persistent infection (1). The reason behind this is that the immune system of very young ones is less able to clear the virally infected hepatocytes.

WHO estimates that there are approximately 350 million HBV carriers globally at present (2). An individual is labeled as a chronic carrier of HBV if serologic conversion to hepatitis B surface antigen (HBsAg) is maintained for more than six months. The carrier rate of HBsAg varies worldwide from 0.1to0.2% in Britain, the USA and Scandinavia to more than 3% in Greece and Southern Italy, and even up to 10 to 15% in Africa and the

Far East. The rate of exposure to HBV in any community is even higher. Carrier rate of HBsAg is very high in some isolated communities; 45% in Alaskan Eskimos and 85% in Australian Aborigines (21).

South East Asia, China and Africa, where the prevalence is high, more than half the population is infected at some time in their lives and more than 8% are chronic carriers of the virus, the result of either neonatal transmission (vertical) or transmission from one child to another (horizontal) (22). The general population of Taiwan has 15-20% carrier rate, which is one of the highest in the world. Approximately, 80to 90% of the general population was shown to be infected by the age of thirty. In Taiwan, chronic HBsAg carrier status was significantly associated with hepatocellular carcinoma showing a relative risk ranging from 20 to 100 (23). Areas with low levels of endemicity include North America, Western Europe and Australia, where only a minority of people comes in contact with the virus, as a result of horizontal transmission among young adults (22).

In 1995, the population of India was reported to be around 900 million. The average estimated carrier rate of HBV in India was 4%, with a total pool of approximately 36 million carriers. Among the estimated 350 million HBsAg carriers worldwide, therefore, India alone contributes 9% of global pool (24). In high carriage rate areas, infection is acquired by passage from the mother to neonate. The infection is usually not via the umbilical vein, but from the mother at time of birth and during close contact afterwards (21). A study from the Philippines suggests that in that developing countries an HBsAg carrier pool is created mainly by vertical transmission from mother–to–child and not by the horizontal route (25). Studies also indicate that about one-fourth to half of the population have evidence of current or past infection. About 10% of HBsAg carriers are positive for hepatitis B e antigen (HBeAg), which indicates active virus replication (26). Different investigators have reported differences in carrier rates of HBsAg from different parts of the country, which may be explained by the wide variations in social, economic, and health factors in different regions of India. The seroprevalence of HBsAg in certain populations in India, especially of low socioeconomic group, is much higher. Tibetan refuges in northern India were found to have a HBsAg seroprevalence of 21% about five times higher than that of general population (27).

Irshad et al, conducted one study to find out the prevalence of HBV infection in healthy persons in North India (28). The seromakers they used were HBsAg and antibody to surface antigen (anti-HBs). Voluntary blood donors represented the general population, and their high-risk groups included commercial sex workers (CSW), eunuchs, truck drivers, professional blood donors and health care workers. HBsAg was detected in 2.6% and anti–Hbs in 14% of healthy voluntary blood donors with an equal distribution between both sexes. Among the high-risk groups, CSWs showed 3.6% & 19%, truck drivers 5% and 16%, professional blood donors 12% & 9%, and eunuch 0% & 18% of HBsAg and anti-HBs respectively. They had expected CSWs, eunuchs and truck drivers to have a high prevalence of HVB markers as they generally do not practice safe sex, and are exposed to multiple sex partners. However, their data showed that only truck drivers had a high HBsAg positive. This suggested that sexual contact might not be an important mode of spread of HBV in India. Rather, vertical transmission and blood transfusion from professional blood donors are important modes of spread of HBV in India. Looking at the prevalence of anti-HBs it is even clearer that there is no significant difference between the general population (represented by healthy voluntary blood donors) and the groups with multiple sex partners and unsafe sex practice. The slightly lower prevalence rate of anti-HBs in professional blood donors may amount to their low immune response due to very low nutritional status and very low socioeconomic condition. This study also shows that the prevalence of HBsAg in HCWs is lower (1.4%) than that of general population (2.6%), ($p<0.01$), which is contrary to other studies that have shown prevalence rate in HCWs.

Tandon B.N. et al, in a study of the prevalence of HBsAg and anti–HBs in preschool children of age below 5 years, found that the HBsAg carrier rate in India was built up in early childhood. The overall carrier rate was found to be 2.1% and anti–HBs positivity of 14.7% in the preschool age children, not significantly different from that of adult population of India with a rate of 10-38 % anti–HBs (29).

The WHO estimates that the number of HBV carrier will reach 400 million by the year 2000. The numbers will continue to increase until neonatal vaccination and immunization are universally accepted (22). Approximately, 350 million chronic carriers in the world

constitute the reservoir for continuous transmission of the virus. Many of them would remain carriers for the entire life period. Humans seem to be the only natural host for HBV infection. The disease therefore, cannot be eradicated unless these people are either cured or die.

Transmission

The infection is transmitted through percutaneous and permucosal exposure to infective blood and body fluids by transfusion of blood or blood products, haemodialysis, use of contaminated needles, syringes and other sharp instruments, needle–stick injuries, oral surgery, perinatal exposure, or sexual exposure. Transmission of HBV in the United States and other developed countries primarily is through parenteral or sexual exposure, and commonly recognized risk factors include male homosexual activity, illicit parenteral drug use, occupational exposure to blood (in HCWs), sexual or household exposure to an HBV–infected contact, and heterosexual activity with multiple partners (30). In India, perinatal transmission is more important.

HBsAg has been found in various body fluids such as saliva, menstrual and vaginal discharge, seminal fluid, colostrum, breast milk, serous exudates, etc. and these have been implicated in the spread of infection. Various studies show that HBV is much more transmissible than Human Immunodeficiency virus (HIV). Risk of transmission by needle–stick injury (a common mode of transmission in HCWs) is substantially higher for HBV (10 to 30%) compared to HIV (0.3%). In five studies of occupational risk for HIV infection among HCWs that included 1673 subjects, most of whom were hospital workers caring for patients with AIDS, 770 of the subjects had parenteral needle–stick injuries or contamination of mucus membranes or open wounds by body fluids from patients with AIDS. Only 3 of the 1673 (0.18%) became infected with HIV (31-35).

Blood sucking arthropods such as mosquito such as mosquitoes or bed bugs may be important vector particularly in tropics. HBsAg has been detected in several species of mosquitoes and bed bugs trapped in the wild or fed experimentally on infected blood, but

no convincing evidence of replication of the virus inside these insects have been obtained. Mechanical transmission is also a possibility. In one study for risk factors for transmission of HBV to Gambian children, bedbugs were found to be a major route of transmission (36).

Occupational aspects of hepatitis B

Viral hepatitis by HBV is recognized as an occupational risk in health care workers and, on the other hand, individuals with hepatitis B infection may pose a risk of transmission to patients in the course of their work. It is well recognized that hepatitis B can be transmitted via infected blood and other body fluids, either during acute infection or from individuals who have become virus carriers. It is the transmission by accidental exposures to infected blood or other body fluids that is particularly relevant to occupational risk.

The prevalence of hepatitis B markers among a group of emergency physicians was found to be about five times greater than in the general population in USA (37). The excess risk of hepatitis B infection among USA oral surgeons was confirmed in a seroprevalence study of 434 individuals; 26% had evidence of previous infection, including two who were positive for HBsAg (38).

Even in countries where hepatitis B infection is endemic, there is evidence of additional occupational risk among HCWs. Among 234 dentists in the Philippines, the prevalence of hepatitis B infection markers was found to be 58%, similar to the prevalence in the general population but increasing with the number of years in dental practice (39). In Japan, a seroprevalence study found that over a third of hospital workers had evidence of previous hepatitis B infection, about the same prevalence as a group of healthy controls, but the nurses and surgeons had significantly higher seroprevalences than other staff or the controls (40). A study in Cairo revealed a higher prevalence of hepatitis B infection markers among nonprofessionals staff (60%) than among doctors and nurses, presumably as a result of non-occupational infection in early life, but still found a relationship between infection markers and blood exposures and years of practice among the physicians (41). A seroprevalence study in a hospital in Nigeria showed HBsAg

positivity in 39% among doctors and dentists, which was substantially above the 20% among the first–time unpaid blood donors ($p<0.05$) (42). A study in the Johns Hopkins Hospital found that 59 (6.2%) of 943 HCWs compared with 1879 (1.8%) of 1042339 local blood donors ($p<0.001$) were positive for antibodies to HBV core antigen (anti–HBc) (43).

In India, the HBsAg seroprevalence rate among HCWs reported by several authors shows great variation. Mahajan et al, (44) reported a high prevalence 33.3% of HBsAg among labortary technicians who acquired infection while collecting blood from HBsAg–positive cases. Thyagaraja et al, (45) detected HBsAg among 30% lady doctors , 25% nursing orderlies , 20% in technicians and 16% male doctors. Joshi et al, (46) used reverse passive haemagglutination assay for HBsAg detection and ELISA for antiHBs. The prevalence respectively of HBsAg and anti-HBs among orderlies were 15% and 10% followed by 9.1% and 27.3 in technicians, 0% and 39.3% in nurses, and 3.6% and 16.2% in doctors. Their general population was represented by voluntary blood donors (healthy university students and employs of government and private sectors), which showed 7.1% HBsAg and 17.5% anti–HBs. Pal and Prasad (47) reported HBV infection among laboratory attendants 25% and doctors 20%. Reddy et al, (48) detected the overall seropositive rate of 7.6% in different categories of HCWs at S.V. Medical College hospital, Tirupati.

Modes of occupational transmission of hepatitis B

The most important means of transmission of hepatitis B in the occupational setting is by inoculation of infected blood, either by stab injuries with blood contaminated needles (so called needle–stick injuries) or cuts with scalpels or other sharp instruments contaminated with blood (sharp injuries). The various studies done between 1978 to 1985 showed that the risk of transmission of hepatitis B after needle–stick injuries from HBsAg–positive source patients was significantly greater if the source patient was Hepatitis B e antigen (HbeAg) positive: the transmission risk from HBeAg–positive source patients was between 19% and 31%, while for HBeAg–negative source patients it was 1-6 % (49-53). HBeAg positivity implicates that active viral replication is occurring.

Contact with infectious material is necessary and the most infectious material is blood and its products. This is important in blood banking practice and in laboratories dealing with blood analysis. Even aerosols of infective serum have been suspected to transmit infective serum have been suspected to transmit infections (44). Dentists are among the health care professionals having a very high risk of hepatitis B infection. They are frequently in contact with patient's saliva and blood. Accidental parental inoculations with sharp instruments are common in dental procedures. In addition, dental professionals are at risk also for splashes and aerosolization of blood and saliva (54).

Risk of transmission of hepatitis B from infected workers

While hepatitis B is undoubtedly an 'occupational hazard' for HCWs, there is recent conclusive evidence to suggest that recipients of health care, i.e., patients, are also at increased risk of acquiring hepatitis from an infected HCW (55). HCWs have transmitted HBV to patients in clinical settings, most frequently during surgery or exposure–prone procedures (56-59). From the 1970s, HBV transmission from 42 infected HCWs to more than 375 patients has been reported in USA and other countries. Obstetricians and gynecologists, cardiac surgeons and dentists have the major share in these outbreaks. In a recent study, one cardiac surgeon transmitted HBV infection to 14% of his patients during a period of only one year (60). The source of transmission was carefully investigated and confirmed on the basis of identical HBsAg subtype and HBV DNA core sequencing in the surgeon and his patients. Rates of transmission of hepatitis B from HCWs to patients could range form 0.3% to 14%. However, these numbers may not fully represent the magnitude of transmission as majority of the infections are subclinical and in all these studies, investigations were started only when index clinical case was recognized.

Welch J et al found that three patients who had had gynecological surgery had developed acute hepatitis B (61). Non of these patients had any apparent risk factors for hepatitis B, but all had been operated on by the same surgeon, who was found to be a carrier of hepatitis Be antigen (HBeAg). Of 268 patients operated by this surgeon in one hospital, 247 were screened for markers of recent or current hepatitis B . 22 (9%) had such

markers, associated with symptoms in five. The operations carrying greatest risk of infection were hysterectomy (10/42) and caesarean section (10/51).

Risk of acquiring HBV from a HCW depends on multiple factors. Frequency of events that result in percutaneous exposure to the blood of HCW, specific factor in HCW and host factors are important (55). Surgical procedure such as dental, gynecological and cardiac surgeries involve operation in more vascular area and spaces that are not easily visible or accessible. The gynecological procedures characteristically involve deep pelvic palpation of suture needles that can cause needle–stick injuries and percutaneous blood exposure (61). The risk of transmission of HBV is at least 30% after a needle–stick exposure with blood from HBeAg positive and about 6% from HBeAg negative blood (49-53).

Specific factors in HCWs include in addition to their surgical technique and dexterity in avoiding injuries, infectivity as measured by HBeAg positivity. In a recently reported outbreak, one cardiothoracic surgery resident transmitted HBV to 19 (14%) of 142 patients over a period of one year (60). He was having replicative HBV infection (HBeAg and HBV DNA positive) and had very high levels of HBV DNA (15ng/ml). The resident was asked to simulate surgical suturing. In one-hour simulation of suture tying, the resident developed paper–cut-like lesions over his fingers. Washings from his hands were HBsAg and HBV DNA positive. Hence, HCWs who have replicative hepatitis B infection (HBeAg, HBV DNA positive) have a higher chance of transmitting the infection. Host factors like protective antibodies, prior vaccination of the patients and his immune status also influence the risk of acquiring infection (55).

In the UK, proof of immunity (through vaccination or past infection) is required of all surgeons and other medical staff performing invasive procedures. Students have to show certificates of immunization and immunity on registration for a medical or dental course (21).

MATERIALS AND METHODS

Sample

Bir Hospital, a teaching hospital, the oldest healthcare institution of Nepal. It serves as a primary facility as well as a tertiary referral center .The health care workers (HCW), for the study purpose, were defined as any personnel coming in contact with patients or their blood and body–fluids as well as materials contaminated with blood and body–fluids. HCWs were approached at sites of convenience, asked to complete a questionnaire, and offered testing for hepatitis B virus. Before contacting individual HCW, the protocol of the study was presented in the Ethical Committee of National Health Research Council (NHRC) of Nepal, and got it approved. Participation was voluntary, and their written consent was taken.

5ml of whole blood was collected from each HCW. Confidentiality of the test was maintained by giving a code number to each sample. Each participant was informed of their test result by contacting them individually. The study took place between December 2001 and February 2002.

Variables

The following information were requested from the HCWs in the questionnaire: (1) demographics, including age, sex, department, type of health care personnel, and total years of exposure to ones working environment; (2) HBV vaccination status, including history of vaccination, time since vaccination, and completion of vaccination course or booster; (3) risks of hepatitis outside the workplace, including transfusions, and history of any invasive procedure undergone; and (4) knowledge of hepatitis, including a history of jaundice or serologic testing.

Controls

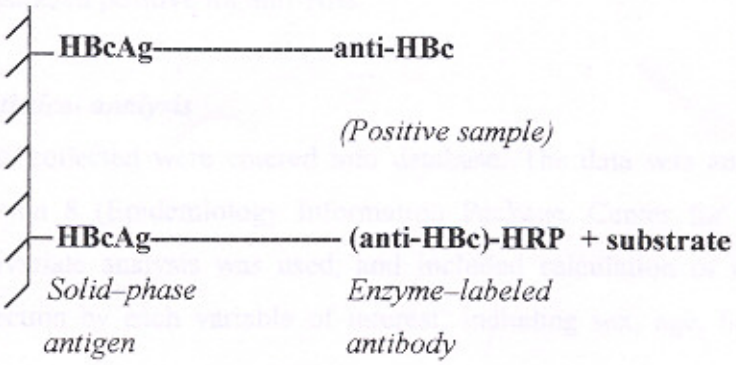
Seroprevalence rate in local unpaid blood donors were taken as control for comparison. The information was obtained from the blood bank of Kathmandu, of those blood donors who donated their blood during the same year of the study.

Serologic analysis

Serum samples were frozen and stored at –70°C. All serum samples were assayed for hepatitis B surface antigen (HBsAg), antibody to hepatitis B core antigen (anti-HBc), antibody to surface antigen (anti-HBs), and for antibody to hepatitis C virus (anti-HCV).

HBsAg was assayed using an enzyme–linked immunosorbent assay (HUMAN-ELISA). The assay is a direct immunoenzymatic method of the sandwich type in which guinea pig anti-HBs antibodies coated onto microtiter plate wells act as the capture antibody and goat anti-HBs antibodies marked with peroxidase serve conjugate antibodies. During the test procedure, the sample to be analysed is incubated in one of the antibody-coated wells. If the sample contains HBsAg, the antigen will blind to the antibody on the plate. After washing to eliminate any unbound material, goat anti-HBs conjugate is added to the well and allowed to react with the antigen–antibody complex formed in the first incubation. After a second incubation and subsequent washing, an enzyme substrate solution containing a chromogen is added. This solution will develop a blue colour if the sample is HBsAg positive. The blue colour will change to yellow after blocking the reaction with sulfuric acid. The intensity of the colour is proportional to the amount of HBsAg in the test specimens. The assay was performed according to the manufacturer's specifications. All samples with absorbency equal to or greater than the cut–off value were considered reactive and repeated in duplicate. All specimens that were repeatedly positive were considered positive for HBsAg.

Total antibody (both IgG and IgM) to hepatitis B core antigen was detected using an ELISA (HUNAN-ELISA). The test is an enzyme-immunoassay based on an inhibition principle.



The wells of polystyrene Microelisa strips are coated with hepatitis B core antigen, which constitutes the solid-phase antigen. The sample is incubated in such a well. With an anti-HBc positive sample the HBcAg will be blocked partially or completely. Subsequently, a second human anti-HBc, which has been labeled with the enzyme horseradish peroxidase is added. This labeled antibody binds to the unblocked solid-phase antigen. Incubation with enzyme substrate produces a blue colour in the test well, which turns yellow when reaction is stopped with sulfuric acid. If the sample contains anti-HBc, only a reduced colour develops in comparison with negative control samples. The assay was performed according to the manufacturer's specifications. All samples with absorbency equal or less than the cut-off value were considered reactive and repeated in duplicate. All specimens that were repetitively positive were considered positive for anti-HBc.

Antibody to hepatitis B surface antigen was detected using an ELISA (HUMAN-ELISA). The assay is a direct immunoenzymatic method of the 'sandwich' type in which the wells of microtiter plate are coated with highly purified HBsAg (ad and ay types) and the conjugate is HBsAg marked with horseradish peroxide. The sample to be evaluated is incubated in one microplate well. HBsAg bound to the well is able to specifically capture anti-HBs when it is present in the sample. After washing to remove any unbound material the conjugate is added and will bind to the antigen–antibody complex formed during the first incubation. After this second incubation and washing, an enzyme substrate containing a chromogen is added. This solution will develop a blue colour if the sample contains anti-HBs. The blue colour will change to yellow after blocking the reaction with sulfuric acid. The intensity of the colour is proportional to the amount of anti-HBs present in the test specimens. All samples at or above the minimum positive value were considered positive for anti-HBs.

Statistical analysis

Data collected were entered into database. The data was analyzed using the EPI Info version 8 (Epidemiology Information Package, Center for disease control, Atlanta). Univariate analysis was used, and included calculation of odd ratios for hepatitis B infection by each variable of interest, including sex, age, health care personnel type,

vaccination status, total number of years of exposure to their working environment, history of jaundice, household contact of jaundiced patient, invasive procedure undergone, history of blood transfusion, and practice of universal precautions. The X^2 (chi square) test, supplemented by Fisher's Exact Test if required by sample size, was used to assess the significance of differences. All available information on each variable was used. Age and total number of years of exposure to the working environment were analyzed as continuous variables in the model by grouping the data (age, <=29, 30-39, 40-49, 50-59, and >= 60 years; total years of exposure, <=5, 6-10, 11-15, and >=16 years). For all the above analysis a p (probability) value of 0.05 or less was considered to indicate statistical significance.

RESULTS

Demographics

145 health care workers participated in the study. The proportion of those approached that participated in the study was greater than 90%. Complete results from questionnaires and serology were available for analysis on all of them. The median age and total years of exposure to working environment of study participants was 38.0+/-10.4 and 16.2+/-9.9 years, respectively. 99 (68.3%) of health care workers were female. Table 1 shows the distribution of participants by the type of health care worker. Nurses constituted 35.9% of the sample.

Seroprevalence of HBV and HCV

There was a high frequency (71.4%) of asymptomatic infection with HBV in the HCWs who were HBV^+. 15 out of 21 HBV^+ gave no history of hepatitis. Antibodies to hepatitis B core antigen (anti-HBc), which indicates past or present HBV infection, were detected in 21 (14.5%) of 145 HCWs. Hepatitis B surface antigen (HBsAg), which indicates active HBV infection, was detected in 2 (1.4%) HCWs. Non of the HCWs were positive for antibody to HCV. Risk factors associated with anti–HBc (HBV^+) were lack of HBV vaccination, health care personnel type (nurses and non-professional HCWs), and years of exposure to ones profession (more than 11 years).

Hepatitis B vaccination

71(49%) of 143 HCWs reported complete doses of HBV vaccination. Evidence of seroconversion (antibodies to hepatitis B surface antigen) in those who had taken complete three doses of HBV vaccination was detected in 61 (85.9%). An incomplete course of vaccination, more than five years time since vaccination, or lack of a booster was associated with lack of a seroresponse. 21 (14.5%) out of 145 HCWs had evidence of past infection with HBV. Out of these 19 (90.5%) were positive for anti-HBs indicating natural immunity against HBV in more than 90% of infected individuals.

Table 1 Distribution of study population according to health care worker type

HCW TYPE	MALE		FEMALE		TOTAL	
	No.	(%)	No.	(%)	No.	(%)
Doctors	27	(84.4)	5	(15.6)	32	(100)
Nurses	_		52	(100)	52	(100)
Lab workers	10	(55.6)	8	(44.4)	18	(100)
Non-professionals	9	(20.9)	34	(79.1)	43	(100)
Total	46	(31.7)	99	(68.3)	145	(100)

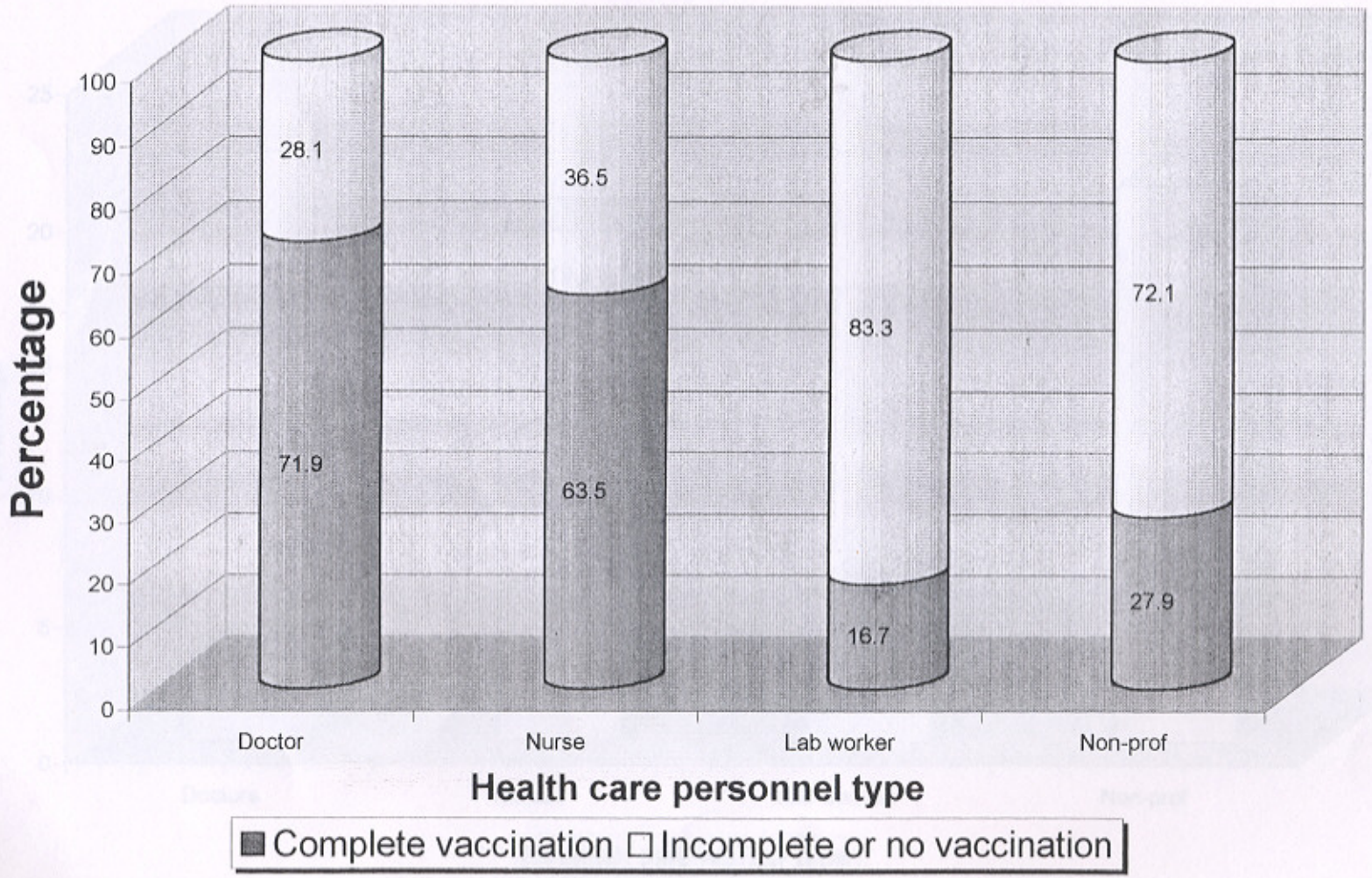
Table 2 Distribution of study population according to vaccination status

HCW type	**Complete dose**		**Incomplete or no vaccination**		**Total**	
	No.	(%)	No.	(%)	No.	(%)
Doctors	23	(71.9)	9	(28.1)	32	(100)
Nurses	33	(63.5)	19	(36.5)	52	(100)
Lab workers	3	(16.7)	15	(83.3)	18	(100)
Non-professionals	12	(27.9)	31	(72.1)	43	(100)
Total	71	(49.0)	74	(51.0)	145	(100)

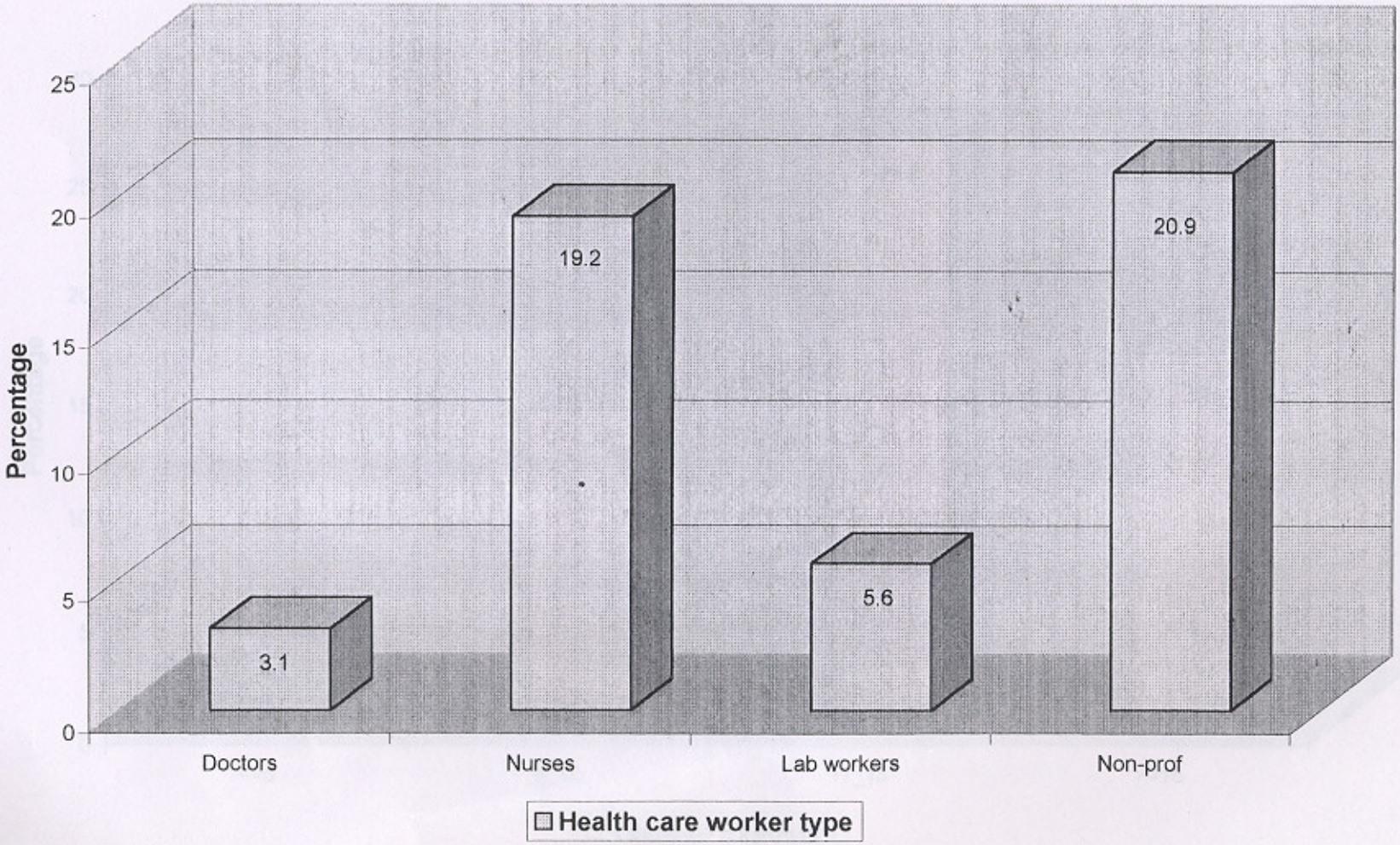
Table 3 Analysis of HBV Seroprevalence in Health Care Workers in 2002 in Bir Hospital

Characteristic	Number HBV^+	Number of participants	Prevalence (%)	p value
Sex				
male	3	46	6.5	-
female	18	99	18.2	18.2
Age class(years)				
<=29	4	40	10.0	-
30-39	3	44	6.8	NS
40-49	14	59	23.7	NS
>=50	0	2	0	NS
Type of HCW				
Doctors	1	32	3.1	-
Nurses	10	52	19.2	0.0452
Lab workers	1	18	5.6	NS
Non-professionals	9	43	20.9	0.0371
Years of exposure to ones profession				
<=5	0	24	0	-
6-10	4	29	13.8	NS
11-15	5	19	26.3	0.0121
>=16	12	73	16.4	0.0343
HBV vaccination				
Yes	3	71	4.2	-
No	18	74	24.3	0.0010
Household contact				
Absent	16	111	14.4	-
Present	5	34	14.7	NS
Invasive procedure				
No	11	88	12.4	-
Yes	10	57	17.5	NS
Jaundice				
No	15	211	12.4	-
Yes	6	24	25.0	NS
Blood transfusion				
No	20	140	14.3	-
Yes	1	5	20.0	NS
Universal precaution				
always	0	12	0	-
sometimes	21	127	16.5	NS
don't know	0	6	0	NS

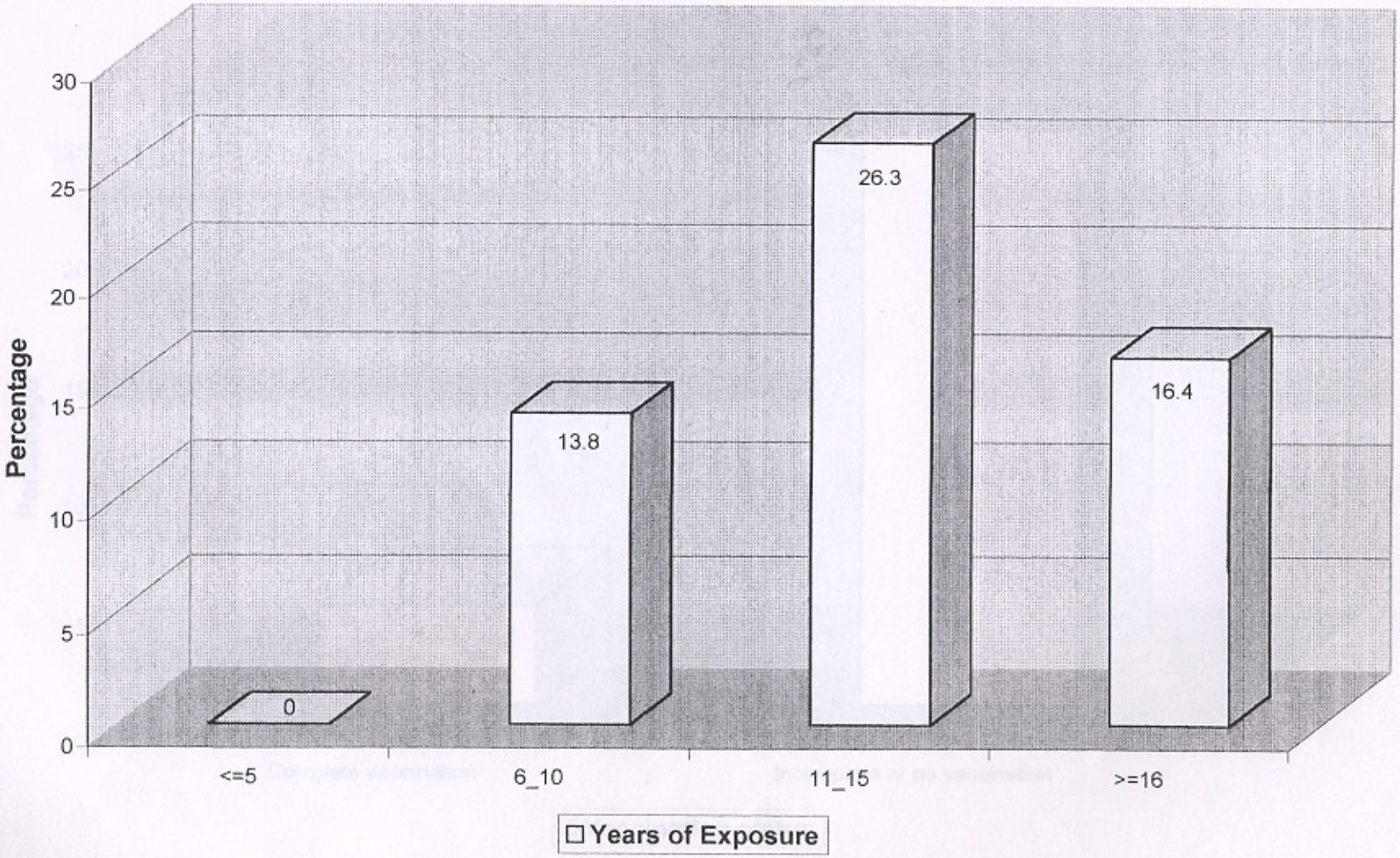
VACCINATION STATUS



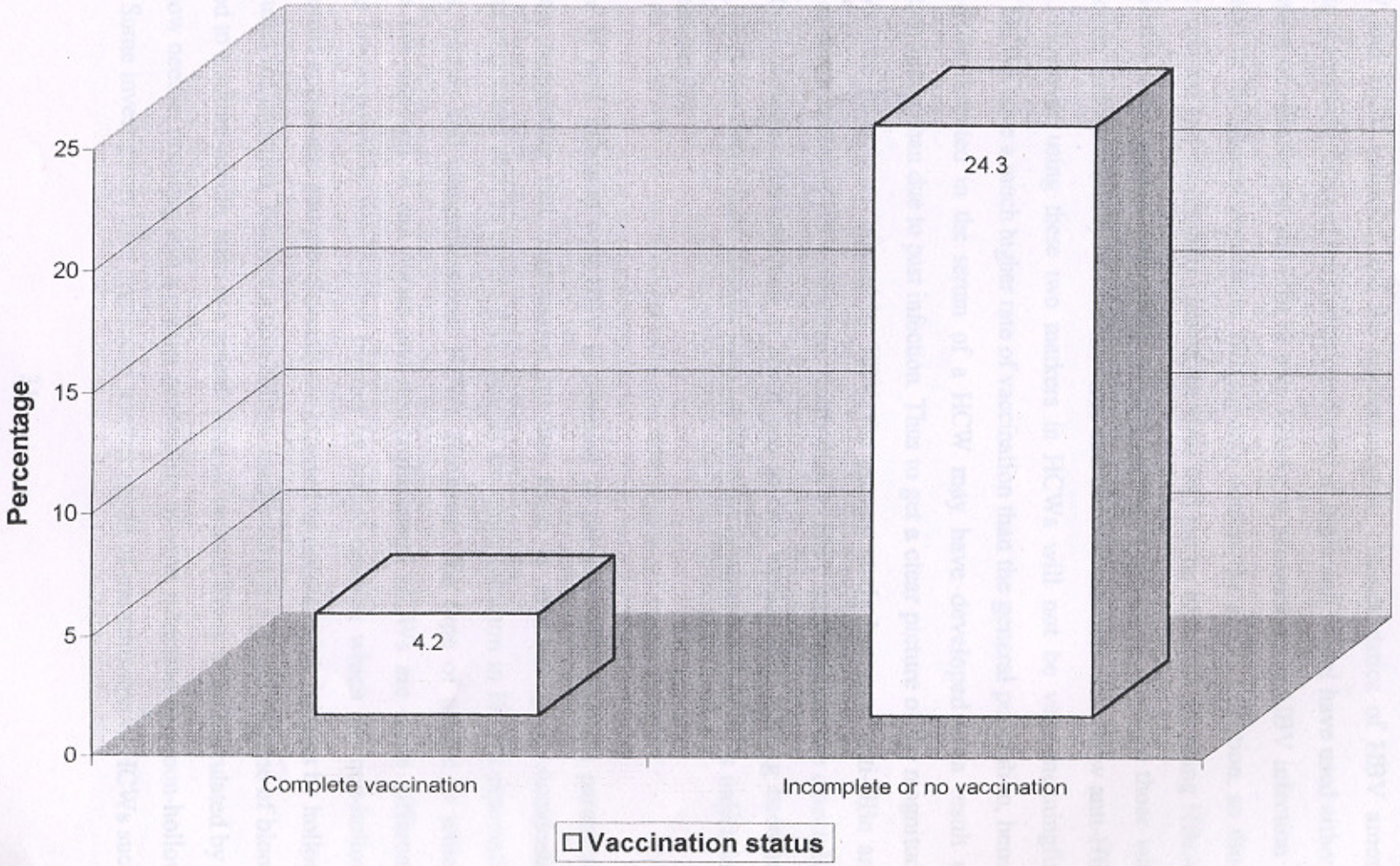
PREVALENCE OF HBV INFECTION IN DIFFERENT CATEGORIES OF HEALTH-CARE WORKERS



PREVALENCE OF HBV INFECTION RELATED TO THE NUMBER OF YEARS EXPOSED TO THE HEALTH CARE PROFESSION



PREVALENCE OF HBV INFECTION WITH RESPECT TO VACCINATION STATUS OF HEALTH CARE WORKERS



DISCUSSION

To the best of my knowledge, this is the first investigation of its kind into the prevalence of HBV and HCV infection, and the epidemiological characteristics of HBV among HCWs in Katmandu. Most of the studies conducted in India and Nepal have used either a combination of HBsAg and anti-HBs or only HBsAg as seromakers of HBV infection in HCWs and in the general population. HBsAg only detects the active infection, so those who got infected and successfully cleared the virus will not be detected. By using HBsAg and anti-HBs in the general population, a better picture is produced, because those who got infection and successfully cleared the virus and became immune will show anti-HBs positive. However using these two markers in HCWs will not be very meaningful, because HCWs have a much higher rate of vaccination than the general population, hence the anti-HBs detected in the serum of a HCW may have developed as a result of vaccination rather than due to past infection. Thus to get a clear picture of the magnitude of HCWs who have been infected by HBV, the present study has used anti-HBc and HBsAg as seromarkers of HBV infection. Antibodies to HBV core antigen are detectable two to three months after infection or about one to two weeks after HBsAg becomes positive. Ig G anti-HBc, once positive remains life-long in most persons, thus indicating past infection (72).

Evidence of past infection with HBV is common in persons with frequent parenteral exposures, suggesting that transmission by this route is efficient (71). Accidental parenteral exposures such as needle-stick injuries are quite common in HCWs especially surgeons, nurses and non-professional HCWs. However, the type of needle to which surgeons are exposed to and nurses and non-professional HCWs are quite different. Surgeons are exposed to needle-prick injuries by suture-needles which are non-hollow type whereas nurses and non-professionals are exposed to needle-prick injuries by hollow needles used for injection. Because a non-hollow needle carries a larger volume of blood compared to a suture-needle, there is a greater dose of viruses likely to be inoculated by a non-hollow needle, resulting into a greater probability of viral infection by a non-hollow needle. Some investigators have implicated another mode of transmission in HCWs such

as aerosolization of blood and other body fluids of patients, though not proved (44, 54). This mode of transmission may be particularly important to dentists and laboratory workers where aerosolization of blood, saliva, and other body fluids is quite common. Small abrasions or cuts on their hands may allow infection. Among health care personnel, the reported prevalence of HBV is generally higher than that of the general population. In India, the HBsAg positive rate among HCWs reported by several authors shows great variation, ranging from 1.4% to 33.3% (28,44-48). This study shows that prevalence rates of past or present infection (i.e. anti-HBc positive) are 14.5% and active infection (i.e. HBsAg positive) 1.4% in HCWs. This study also reveals that after more than a decade of vaccine availability, one of the risk factors of HBV infection in HCWs is the absence of HBV vaccination. The other risk factors being increased duration of employment, and nurse and non-professional HCW type.

In this study, enrollment was offered to any health care personnel encountered while visiting different departments and wards of the hospital, obviating random selection of participants and introducing the potential for biased sampling. Reluctance on the part of HCWs who already knew that they had been infected, and some HCWs knowing that they fall under a very high risk group, and refusing to participate in this study would lead to underestimation of the prevalence of HBV infection in HCWs. However, efforts to recruit as many HCWs as possible and the enrollment of more than 90% of those approached should have minimized this bias.

The significantly higher prevalence, 19.2% and 20.9% of HBV infection in nurses and non-professional HCWs, respectively, can be attributed to several factors. Two most important factors are the lower rate of vaccination in non-professionals, and non-implement of universal precautions in nurses and non-professionals. The reasons behind the lower rate of vaccination in non-professionals are lack of awareness, illiteracy and financial constraints. Other important factors are the low socioeconomic status of this group, and the lack of knowledge on the modes of transmission of the disease. Frequent contact with sharp instruments including hollow-needles during their disposal and spillage contaminated with blood and other body fluids occur in the course of their work. In case of nurses, recapping of hollow-needles after injecting a patient, using both hands

is likely to prick the hand holding the cap of the needle if the needle misses the hole of the cap. Thus, one-hand technique of needle capping is recommended.

Non-professional HCWs included ward attendants and cleaners who are all from the low socioeconomic strata. Their socioeconomic status is low not only compared to other HCWs, but also compared to general population. There are a number of studies in India and abroad showing higher prevalence of HBV infection in the low socioeconomic group. Tibetan refugees in northern India were found to have a HBsAg seroprevalence of 21%, about five times higher than that of general population (27). A study in Cairo revealed a higher prevalence of hepatitis B infection markers among non-professional staff (60%) than among doctors and nurses (41).

Some of the reasons attributed to low socioeconomic group for having higher rates of HBV infection are their poor living conditions and unsafe sexual practices. Poor living conditions, which are crowed and unhygienic, enhance the transmission by close contact with carriers. Moreover, due to their financial constraints and living conditions they are less able to protect themselves from bug-bite and mosquito-bite which are also suspected to be vectors for HBV transmission (36), though not yet proved. This mode of transmission can particularly be important in tropics.

In this study, a large percentage of HCWs were vaccinated (49%), and only two (1.4%) active cases of HBV infection (HBsAg positive) were detected. These data support those population-based studies that suggest a decrease in HBV among HCWs since the advent of vaccination programs (73). However, the temporal coincidence of other factors, such as universal precautions, which at least in some studies have been associated with decreased high-risk exposures, and the observation in some centers of a decreasing incidence of HBV predating the availability of HBV vaccine, make it impossible to attribute the lower rates solely to HBV vaccination programs (74).

Recent data elsewhere (75) has suggested that the risk of HBV infection among HCWs has decreased with the availability of HBV vaccination (and the implementation of universal precautions). This reduced risk has not yet been fully assessed in hospital-based

studies in developing countries where this policy has not been vigorously adopted. Though a large percentage (49%) of our HCWs were vaccinated, most of them were doctors and nurses. The vaccination in the non-professional group was very low. A significant factor impeding universal implementation of HBV vaccination in all categories of HCWs is the cost of the vaccine, which is prohibitively expensive on a large scale. Of those vaccinated, who showed negative for antibody to surface antigen (anti-HBs), an incomplete course of vaccination or more than five years and absence of a booster since the last dose were often reported. Hence this study also demonstrates the importance of three complete doses of vaccination and a booster every five years.

In conclusion, I would like to emphasize that the only way to prevent this occupational hazard in health care workers, is by **creating awareness** on the risk of HBV infection especially to the illiterate and non-professional health care workers, following strict compliance with **universal precautions** (especially using **one-hand technique for recapping needles**, and **decontamination** of sharp instruments before cleaning them by the non-professional HCWs), and a mandatory vaccination against HBV to all categories of health care workers.

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