Serological and Molecular Characterization of Hepatitis B, C and D Viruses Infections among Health Professionals in Ras Desta and Tikur Anbessa Hospitals, Addis Ababa, Ethiopia

By

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By

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<tr>
<td>AASLD</td>
<td>American Association for the Study of Liver Disease</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine Amino Transferase</td>
</tr>
<tr>
<td>CDC</td>
<td>Center for Disease Control</td>
</tr>
<tr>
<td>CHB</td>
<td>Chronic Hepatitis B</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme Linked Immunosorbent Assay</td>
</tr>
<tr>
<td>EPI</td>
<td>Expanded Programme of Immunization</td>
</tr>
<tr>
<td>FRET</td>
<td>Fluorescence Resonance Energy Transfer</td>
</tr>
<tr>
<td>HBc</td>
<td>Hepatitis B core</td>
</tr>
<tr>
<td>HBe</td>
<td>Hepatitis B e</td>
</tr>
<tr>
<td>HBIG</td>
<td>Hepatitis B Immunoglobulin</td>
</tr>
<tr>
<td>HBs</td>
<td>Hepatitis B surface</td>
</tr>
<tr>
<td>HBV</td>
<td>Hepatitis B Virus</td>
</tr>
<tr>
<td>HCC</td>
<td>Hepatocellular Carcinoma</td>
</tr>
<tr>
<td>HCV</td>
<td>Hepatitis C Virus</td>
</tr>
<tr>
<td>HCW</td>
<td>Health care workers</td>
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<tr>
<td>HDV</td>
<td>Hepatitis D Virus</td>
</tr>
<tr>
<td>MEIA</td>
<td>Microparticle Enzyme Immunoassay</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>RIBA</td>
<td>Recombinant Immunoblot Assay</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Reverse Transcriptase Polymerase Chain Reaction</td>
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<tr>
<td>UTR</td>
<td>Untranslated Region</td>
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ABSTRACT

The risks for occupational infection with blood-borne pathogens (HBV, HCV, and HIV) have been a source of concern among health professionals because of their frequent and often substantial exposures to patient blood and body fluids and all of which are associated with significant morbidity and mortality. A cross sectional study was conducted in order to assess the prevalence of HBV, HCV, and HDV infections among HCW’s (n=267) and risk factors associated with these viral infections. After each volunteer study subject has completed a standardized questionnaire, 10 ml of venous blood was collected from each individual for serological and molecular processing. ELISA method was used to detect HBs and HBe antigens, antibodies to HBc, HBs, HDV and HCV in the serum samples. Molecular methods were used to detect HBV DNA and HCV RNA. One hundred and eight (40.4%) of the health professionals use gloves consistently, 221 (82.3%) had at least a one time history of cutaneous exposure to blood unglowed, 165 (61.8%) of health care workers had at least a one time history of cutaneous exposure to any of the body fluids, 162 (60.6%) had splashed blood or body fluids in their faces, 158 (59.2 %) had sustained needle stick injury and 83 (31.1%) sustained sharp injury. The overall prevalence of HBV infection was found to be 51.3% (95% CI: 45-57%) and the infection prevalence increases with age (p<0.01). HBsAg was detected in 9.7 % (95% CI: 6-13.2%) of HCW’s. Absence of vaccination was the only risk factor associated with hepatitis B infection (p=0.001). Though only a single individual had HBeAg (4.5%; 95% CI: 2-7%), HBV DNA was detected by PCR in serum sample from 17 of the 24 subjects (71%). The hepatitis B vaccination rate was found to be 13%. Ninety three percent of individuals who were vaccinated with a single dose of HBs vaccine had achieved protective antibody. ‘Anti-HBc only’ positive cases were detected in 6 (2.2%; 95% CI: 0.004-3.96%) individuals however HBV DNA was not detected in any of the samples tested. HCV infection was detected in 1 (0.37%) study subject. None of the HBsAg positive individuals had marker of HDV infection. Our data suggest that occupational exposures to blood and body fluids occur frequently with low universal precaution practice and vaccination coverage. Hepatitis B infection is widespread and characterized by high level of immunity through natural infection and the presence of continuous horizontal transmission. Absence of vaccination is the only risk factor associated with hepatitis B infection. In this study HCV and HDV infection prevalence is very low. Serologic response rate to a single dose of
hepatitis B vaccine was much higher than experiences from other countries. HBeAg negative serological status and low viral replication levels characterize chronic hepatitis B virus-infected health professional. ‘Anti-HBc only’ is found in the health professionals but none of the subjects had detectable HBV DNA. This suggests the need to do future study in ‘Anti-HBc only’ case.
CHAPTER I: INTRODUCTION

1.3. General Introduction
Health care workers (HCWs) are potentially exposed to blood and body fluids containing transmissible diseases and are at increased risk to acquire these pathogens (Catalani et al., 2004; Canini et al., 2002). Hospital acquired infections are a problem in both developed and developing countries and are important causes of death (Sridhar et al., 2004). Hepatitis B and C viruses are blood borne pathogens, which might be acquired occupationally. Occupational exposure to blood and body fluids occur frequently among health professionals (West, 1984; Canini et al., 2002). An exposure that might place health care professional at risk for HBV or HCV is defined as a percutaneous injury (e.g., a needle stick or cut with a sharp object) or contact of mucous membrane or non-intact skin with blood, tissue, or other body fluids that are potentially infectious (CDC, 1988). The following fluids also are considered potentially infectious: CSF, synovial fluid, pleural fluid, peritoneal fluid, pericardial fluid and amniotic fluid (CDC, 2001). The risk of HBV infection is primarily related to the degree of contact with blood in the workplace also to the HBeAg status of the source person (Werner and Grady, 1982). Although percutaneous injury is among the most efficient mode of transmission, these exposures account for a minority of HBV infection among HCWs. In several investigations of nosocomial hepatitis B outbreaks, most HCW did not reveal an overt percutaneous injury (Garibaldi et al., 1972). HBV infections that occur in HCWs with no history of non occupational exposure to or occupational percutaneous exposure injury might have resulted from direct or indirect blood or body fluid exposure that inoculated HBV into cutaneous scratches, abrasions, burns, other lesion or on mucosal surfaces. In serologic studies conducted in the United States during the 1970s, HCWs had a prevalence of HBV infection approximately 10 times higher than the general population (West, 1984).

The average incidence of anti-HCV seroconversion after accidental percutaneous exposure from an HCV–positive source is 1.8% (Willy et al., 1990). All healthcare institutions have an obligation to review occupational exposure data on an ongoing basis to determine specific factors that could be modified to decrease risk. Hospitals have to mandate educational sessions that focus on barriers to compliance identified within their institution (Jackson and Cheung, 2004).
In Ethiopia 12% of medical admission and 31% of mortality in medical wards is due to liver disease (Tsega, 2000). No other previous study in Ethiopia has characterized the serological and molecular pattern, and magnitude of HBV, HCV, and HDV infection among HCW’s. In addition, no data is available on the risk factors of HBV, HCV, and HDV infection. Therefore, this study was undertaken to address these major gabs.

1.2. LITERATURE REVIEW

1.2.1. Microbiology of Hepatitis B, C and D Viruses

a) Hepatitis B Virus (HBV)

Blumberg et al. (1965) reported the discovery of the hepatitis B surface antigen (HBsAg), also known as Australia antigen, and its antibody, hepatitis B surface antibody (HBsAb). A few years later, Dane et al (1970) visualized the HBV virion. Since then, considerable progress has been made regarding the epidemiology, virology, natural history, and treatment of this hepatotropic virus (Geller, 2002).

HBV belongs to the family *Hepadnaviridae* and has some unique properties. It is highly species specific, infecting only humans, chimpanzees, and some other primates. Attempts to grow HBV in standard cell lines have not been successful (Lee, 1997). It is an extremely resistant strain capable of withstanding extreme temperatures and humidity. It can survive when stored for 15 years at -20°C, for 24 months at -80°C, for 6 months at room temperatures, and for 7 days at 44°C (Seeger and Mason, 2000).

The viral genome of HBV is a partially double stranded circular DNA of 3200 base pairs that encodes four partially 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. The S and C genes have upstream regions termed preS and preC (Lau and Wright, 1993). The circular genome is very compact. There are no non-coding regions in the genome, so that all regulatory segments are also part of protein encoding sequences. The S-ORF, including pre-S and S regions, codes for the
viral surface proteins. The ORF precore codes for a hydrophobic peptide that directs, the translation product to the endoplasmic reticulum, where further protein cleavage forms the third HBV antigen, HBeAg. The P gene codes for the viral polymerase (pol), which overlaps the entire length of the S-ORF. The fourth ORF codes for HBX, a protein whose exact function is not known. HBX may have multiple effector pathways and may also counteract the increased proteolytic function of the infected cells, ensuring the reliability of the replication process (Lee, 1997; Kirchner and Lin, 2004).

Virus attachment to permissive cells, fusion and penetration through cell membranes and subsequent genome release, are largely a mystery (Lu and Block, 2004). The replication strategy is unique for animal DNA viruses in that they use RNA intermediate and a reverse transcription step (Seeger and Mason, 2000).

The three types of particles present in HBV infected individuals are as follows: the Dane particle, a 42-nm double-shelled particle; filamentous particles of varying length with a 22-nm diameter; and spherical 22-nm particles. The Dane particle consists of a lipoprotein envelope surrounding an inner 27-nm nucleocapsid, within which circular DNA is attached to viral polymerase. The filamentous and spherical particles lack nucleocapsid and genomic DNA and are noninfectious (Lee, 1997; Geller, 2002). The core antigen, HBcAg, is the protein that encloses the viral DNA. It also can be expressed on the surface of the hepatocytes, initiating a cellular immune response. The e antigen, HBeAg, comes from the core gene. It is a circulating peptide derived from the core gene and then modified and exported from liver cells, serves as a marker of active viral replication. HBeAg will also act as a tolerogen, since its presence in the circulation has been associated with a diminished immune response because of its close resemblance to HBcAg, the putative target of the immune response. With few exceptions, HBeAg is present only in person who has circulating serum HBV DNA (Lee, 1997).

Soon after the discovery of the HBsAg, the serological heterogeneity of the virus became apparent (Courouce et al., 1976). Although there is only one major serotype of HBV, HBsAg has five major subtype determinants: a, d, y, w, and r. These major antigenic determinants of HBs can be distinguished with antibodies that recognize different epitopes on particles formed by the
small HBsAg (SHBs). Therefore, four subtype patterns are seen: adw, ayw, adr, and ayr, although ayr is quite rare (Schaefer, 2005). All known subtypes contain the a-determinant, which is encoded between amino acid residues 124 and 147. The difference between the mutually exclusive subtype-specific determinant d/y and w/r is generated by amino acid exchanges from K to R at residues 122 and 160, respectively (Geller, 2002). Additional sub determinants allowed the differentiation of four serotypes of ayw and two of adw. Thus, according to the Paris workshop on HBV surface antigen subtypes, eight serotypes exist (adr, ayr, ayw1, ayw2, ayw3, ayw4, adw2 and adw4). By use of the determinant q+/q found in subtype adr, nine HBsAg subtypes have been distinguished (Kidd-Ljunggren et al., 2004). The subtypes have been associated with the geographic distributions of HBV, but do not seem to correlate with clinical course. Ayw predominates in N. Europe, N. America, Africa and Australia. While, adr predominates in China, and Japan (Schaefer, 2005; Geller, 2002; Kidd-Ljunggren et al., 2004).

HBV genotypes differ by more than 8% using the sequence of its complete genome or 4% using the sequence of SHBs (Wong and Chan, 2005). Early works have found a correlation between HBV genotypes and serological subtypes. Eight genotypes of hepatitis B virus (A-H) are currently recognized, and sub genotypes have recently been described in four of these genotypes (A, B, C and F). The genotypes show a distinct geographical distribution between and even within regions, and are proving to be an invaluable tool in tracing the molecular evolution and patterns and modes of spread of hepatitis B virus. Genotypes A and D are most frequently observed in Europe, Africa, and North America, while genotypes B and C are prevalent in Asia. Genotype E is restricted to West Africa, and genotype F is found in Central and South America. Genotype G was identified in France, Germany, and North America. Recently, genotype H has been described in Central America (Kramvis et al., 2005). Structural and functional differences between genotypes can influence the severity, course and likelihood of complications, and response to treatment of hepatitis B virus infection and possibly vaccination against the virus (Schaefer, 2005).

HBV has a reported mutation rate of 10 times greater compared with other DNA viruses. These mutations can occur naturally as well as due to selective pressure from antiviral therapy. Unlike cellular polymerases, the HBV Pol is a reverse transcriptase that lacks proofreading function that
would permit it to recognize incorrectly incorporated nucleotides. As a result, HBV populations exist in the host as heterogeneous mixtures known as quasi-species (Locarnini and Bartholomeusz, 2005; Locarnini, 2004). There are five clinically relevant HBV mutant types. A mutation at the 1896 nucleotide (precore/core region) processing the production of the HBeAg was identified first. The prevalence of this mutant virus varies among different areas (Pan and Zhang, 2005).

b) **Hepatitis C Virus (HCV)**

Non A-Non B hepatitis was described clinically and epidemiologically in the 1970s. The hepatitis C virus was identified in 1989 after it was partially cloned in 1988 and blood screening assays developed and implemented (Choo et al., 1989; Kuo et al., 1989). It is a spherical, enveloped, single stranded positive RNA virus belonging to a member of Flaviviridae (Busek and Oliveira, 2003).

The HCV genome consists of approximately 10,000 base pairs, which contain a single, uninterrupted open reading frame. This open reading frame is flanked by 5 and 3 non-coding regions, each of which contains conserved RNA structures essential for the translation of virus protein and genome replication (Takamizawa et al., 1991). The genome encodes a single poly protein of 3011 amino acids that are processed into 10 structural and regulatory proteins. The HCV precursor protein is processed by host-cell and virus proteases. The structural components of the virion are the core protein and envelope glycoproteins, E1 and E2. E1 and E2 proteins exhibit a high degree of genetic heterogeneity. The highly variable N-terminus of the E2 protein has been designated the hyper variable region 1 (HVR-1). HVR-1 may represent a neutralization epitope for humoral immunity (Thomson and Finch, 2005). The core protein of HCV is highly conserved with several B-cell epitopes that contributed to the development of practical serologic tests for HCV (Geller, 2002). The nonstructural components include NS2, NS3, NS4A, NS4B, NS5A, NS5B, and p7, whose proteins function as helicase, protease, and RNA dependent RNA polymerase, although the exact function of p7 is unknown (Busek and Oliveira, 2003).

HCV can produce at least 10 trillion new viral particles each day. In common with other RNA-dependent polymerases, the HCV RNA polymerase (NS5B) does not have a proofreading capability, and therefore generates considerable genetic diversity. This produces quasispecies and
represents minor molecular variations with only 1-2% nucleotide heterogeneity. HCV quasispecies pose a major challenge to immune-mediated control of HCV and may explain the variable clinical course and the difficulties in vaccine development (Rosenberg, 2001). Genome sequence comparison has revealed the existence of six major genotypes and a large number of subtypes (Simmonds et al., 1993). Molecular differences between genotypes are relatively large, and they have a difference of at least 30% at the nucleotide level. HCV genotypes and their subtypes coexist in various geographic locations but show different prevalence levels (Schreier et al., 1996). The prevalence of genotypes is also associated with the transmission route of the infection (van Asten et al., 2004). In addition, HCV types possess different biological potentials. Certain HCV genotypes are more amenable to interferon treatment and more frequently associated with severe forms of liver disease (Turhan et al., 2005).

c) **Hepatitis D Virus (HDV)**

Hepatitis D virus (HDV) infection was discovered by Rizzetto et al. (1977) in Italy. The virus was formerly called delta antigen or delta virus. It is found within certain HBsAg particles. Although closely associated with HBV it is distinct from the known antigens of HBV (Banker, 2005). HDV is the smallest known genome to infect humans, encoding just one protein the hepatitis delta antigen (HDAg). The HDV genome is a single, negative stranded circular RNA molecule nearly 1.7 Kb in pair. HDV requires the surface antigen of HBV (HBsAg) for the encapsidation of its own genome. The envelope proteins on the outer surface of HDV are entirely provided by HBV. At least three phylogenetically distinct genotypes exist, varying in geographic distribution and clinical manifestations. So far only one serotype of HDV is recognized all over the world (Polish et al., 1993).

### 1.2.2. Epidemiology
I. Prevalence and Incidence

a) HBV

Hepatitis B is one of the major diseases of mankind and is a serious global public health problem. Of the 2 billion people who have been infected with the HBV, more than 350 million have chronic (lifelong) infections. HBV infections result in 500,000 to 1.2 million deaths per year caused by chronic hepatitis, cirrhosis, and hepatocellular carcinoma (McMahon, 2005, Kidd-Ljunggren et al., 2004; Lavanchy, 2004; Margolis et al., 1991). HBV infection prevalence varies markedly in different geographic areas of the world, as well as in different population subgroups (Figure 1.1). It ranges over 10% in some Asian and Western Pacific countries to under 0.5% in the United States and northern European countries (McMahon, 2005). Overall, approximately 45% of the global populations live in areas of high chronic HBV prevalence (Lavanchy, 2004). The prevalence of chronic HBV infection worldwide could be categorized as high, intermediate and low endemicity. The age at the time of infection is associated with the endemicity of HBV (McMahon, 2005). Hepatitis B is moderately endemic in part of Eastern and Southern Europe, the Middle East, Japan, and part of South America. Between 10–60% of the population have evidence of infection, and 2-7% is chronic carriers. Acute disease related to HBV is common in these areas because many infections occur in adolescents and adults; however, the high rates of chronic infection are maintained mostly by infections occurring in infants and children. In these areas, mixed patterns of transmission exist, including infant, early childhood and adult transmission (Alter, 2003; McMahon, 2005).

Hepatitis B is highly endemic in developing regions with large population such as South East Asia, China, Sub-Saharan Africa and the Amazon Basin, where at least 8% of the population are HBV chronic carrier. In these areas, 70–95% of the population shows past or present serological evidence of HBV infection. Most infections occur during infancy or childhood particularly acquired from the carrier mothers at birth. Since most infections in children are asymptomatic, there is little evidence of acute disease related to HBV, but the rates of chronic liver disease and liver cancer in adults are high (Alter, 2003; Lavanchy, 2004).

In Africa, infections with HBV play a major role in the etiology of most liver diseases. The WHO African region includes all of Sub-Saharan Africa and Algeria. By country, estimated HBsAg
seroprevalence ranges between 5% and 19%, and the total number of carriers may approach 58 million with as many as 12.5 million likely to die prematurely due to hepatitis B-induced liver disease (Brian et al., 2004, Sobeslavsky, 1980).

Figure 1.1. Worldwide prevalence of HBV carriers (Adapted from Lavanchy, 2004)

In Ethiopia as in other Sub-Saharan Africa, the prevalence of liver disease is high. They account for 12% of the hospital admissions and 31% of the mortality in medical wards of Ethiopian hospitals (Tsega, 2000). A nationwide sero-epidemiological study of hepatitis B markers prevalence was conducted in Ethiopia on 5,270 young males from all regions of the country. Overall prevalence rates were 10.8% for HBsAg and 73.3% for "at least one marker positive"; a remarkable geographical and ethnic variability of marker prevalence was observed, reflecting the wide differences existing in Ethiopia in socio-cultural environment and activities such as tribal practices and traditional surgery. Sexual practices and medical exposure also play some role as determinants of hepatitis B marker prevalence in Ethiopia (Kefene et al., 1989). A community based seroprevalence study in the capital city of Ethiopia; Addis Ababa has shown a 7% seroprevalence of HBsAg, higher in males than females. Overall HBV seroprevalence rose steadily to over 70% in 49 year olds. The age at which 50% had evidence of infection was around 20 years (Abebe et al., 2003). Another study done in Ethiopia has shown an overall HBsAg
prevalence of 6.2% and 2.1:1 male to female ratio, infection occurring early in life and continuing to increase gradually without leveling off. Overall HBV marker prevalence (HBsAg, anti-HBc, anti-HBs) over 15 years of age was 76% (Tsega, 1991). The health status of 239 Ethiopian refugees in the United States was evaluated; over 70 per cent were males 15-30 years old and Hepatitis B surface antigenemia was detected in 9.4 per cent (Parenti, 1987). Another study which tested sera of 200 recent Ethiopian immigrants (less than 1 year in Israel) for the presence of antibodies against hepatitis B and hepatitis C viruses has shown the following result: The prevalence of the various markers was: anti-HBc 52%, and HBsAg 11.5% (Flatau et al., 1993). Screening sera of all male donors appearing at the blood bank of a regional hospital in Northwest Ethiopia (Gondar) in 1994 (n=1022) and 1995 (n=1164), for HBsAg was carried out on 549 consecutive sera. The crude seroprevalence of HBsAg was 14.4% (Rahlenbeck et al., 1997). One or more hepatitis B virus markers were found in 86% of chronic hepatitis, 88% cirrhosis and 78% hepatocellular carcinoma patients studied in Addis Ababa (Tsega et al., 1992).

b) HCV

HCV is endemic in most parts of the world. There are, however, considerable temporal and geographical variations in the incidence and prevalence of infection. The World Health Organization estimates 170 million individuals worldwide are infected with hepatitis C virus. Globally, the infection has an estimated prevalence of 3% (WHO, 1999). The prevalence rates in healthy blood donors are 0.01-0.02% in the United Kingdom and Northern Europe, 1-1.5% in Southern Europe, 1.8% in US and 6.5% in parts of Equatorial Africa (Figure 1.2.). Prevalence rates as high as 22% are reported in Egypt and are attributed to the use of parenteral antischistosomal therapy (Pellicano, 2004).

Figure 1.2. Global prevalence of antibodies to hepatitis C virus infection among blood donors, Anti-HCV antibody determined by EIA with supplemental testing (Adapted from Wasley and Alter, 2000)
Sera of 200 recent Ethiopian immigrants (less than 1 year in Israel) were tested for the presence of antibodies to hepatitis C viruses. The prevalence rate was found to be (3%) (Flatau et al., 1993). Another study which aims to assess the prevalence and possible aetiological association of HCV with chronic liver disease and hepatocellular carcinoma HCC, anti-HCV were determined by enzyme-linked immunosorbent and recombinant immunoblot assays in 500 healthy volunteer blood donors, 14 patients with chronic hepatitis, 156 cirrhotics and 68 cases of HCC in Ethiopia. The prevalence of anti-HCV was 1.4%, 21%, 36% and 46%, respectively (Tsega, 1995). Another study done in Ethiopia on various group of subjects has demonstrated 2% seroprevalence (Frommel et al., 1993) and a population based seroprevalence study revealed 0.9% prevalence (Ayele et al., 2002).

c) HDV

Hepatitis delta virus is present worldwide and in all age groups. The epidemiology of HDV closely parallels that of HBV, it has been estimated that 18 million people are infected with this virus amongst the 350 million carriers of the HBV around the world (Fonseca, 2002). HDV is endemic in many parts of the developing world, and is an important problem all over the world in terms of morbidity and mortality (Banker, 2005, Hadziyannis, 1997; Previsani and Lavanchy, 2001). HDV infects worldwide but not uniformly, with recognized high prevalence in Amazon,
Middle East, West Africa and certain Pacific islands (Polish et al., 1993; Previsani and Lavanchy, 2001; Christopher and John, 2000) (Figure 1.3).

The results of HDV antibody determinations carried on 566 HBsAg positive serum samples from a population of 5270 Ethiopian military recruits were reported. In this report the prevalence of anti-HDV among apparently healthy HBsAg carriers was 5.8%. The prevalence increases with age within the available range (18-30 years) (Rapicetta et al., 1988).

**Figure 1.3. Prevalence of HDV infection worldwide (Adapted from Polish et al., 1993)**

II. Modes of Transmission

a) HBV

HBV is spread through contact with infected blood and body fluids. Blood is the most important vehicle for transmission, but other body fluids have also been implicated, including semen and saliva (Kirchner and Lin, 2004). Currently, three important modes of HBV transmission have been recognized: perinatal, sexual and parenteral/percutaneous transmission of HBV (Ellett and Marsha, 1999). In Southeast Asia, China, and sub-Saharan Africa, HBV infection usually is acquired perinatally or in early childhood. In contrast, 80 percent of infections in the United
States, Canada, and Western Europe occur in adults via sexual contact or intravenous drug use (Atkins and Nolan, 2005). In most of sub-Saharan Africa, early life horizontal transmission is thought to be the predominant mode of transmission. Groups at increased risk for HBV infection includes persons with a history of sexually transmitted disease, household contacts of HBV-infected persons, health care workers, hemodialysis patients, intravenous drug users, infants born to HBV-infected mothers, immigrants and children of immigrants from hyper endemic areas, homosexuals, persons who have more than one sexual partner in a six-month period, sexual partners of HBV-infected persons (Kirchner and Lin, 2004).

Perinatal transmission is important mode of transmission in areas of intermediate and high prevalence (Schweitzer, 1975). Before HBV vaccine was integrated into the routine immunization program, the proportion of babies that become HBV carriers is about 10-30% for mothers who are HBsAg-positive but HBeAg-negative. However, the incidence of perinatal infection is even greater, around 70-90%, when the mother is both HBsAg-positive and HBeAg-positive. The rate of infection depends up on the maternal status of HBeAg / anti-HBe (Ellett and Marshal, 1999; Chakravarti et al., 2005). In one transmission study of HBV infection in Ethiopia, 19 of the 25 HBsAg positive mothers had anti-HBe none had HBeAg. There was only one case of vertical transmission, during the follow up period, new horizontal infection occurs in 2 infants and 2 older siblings, demonstrating the lesser importance of perinatal transmission (Tsega et al., 1989).

Sexual transmission of hepatitis B is a major source of infection in all areas of the world, especially in the low endemic areas, such as North America (Lavanchy, 2004). HBV is very efficiently transmitted sexually during heterosexual and homosexual contact. Heterosexual transmission can still be important as shown by the 40% transmission rates to non-immune partners of patients with acute or chronic hepatitis B (Van Damme et al., 1995).

The parenteral transmission includes injection drug use, transfusions and dialysis, acupuncture, working in a health-care setting, tattooing and household contact (Margolis et al., 1991). Blood and blood products are the main routes through which the virus is transmitted. Only a very small amount of blood is needed for transmission (down to 0.00004 ml intradermally). The risk for
transfusion-associated HBV infection has been greatly reduced since the screening of blood for HBV markers and the exclusion of donors who engage in high-risk activities, the transmission is still possible when the blood donors are asymptomatic carrier with HBsAg negative (Luo et al., 1993). Parenteral/percutaneous transmission can occur during surgery, after needle-stick injuries, intravenous drug use, and following procedures such as ear piercing, tattooing, acupuncture, circumcision and scarification. The nosocomial spread of HBV infection in the hospital, particularly in dialysis units, as well as in dental units, has been well described, even when infection control practices are followed. As with other modes of transmission, high vial titers have been related to an increased risk of transmission. People at high-risk of infection include those requiring frequent transfusions or hemodialysis, physicians, dentists, nurses and other healthcare workers, laboratory technicians, intravenous drug users, police, firemen, laundry workers and others who are likely to come into contact with potentially infected blood and blood products (Margolis et al., 1991). In one study of hepatitis B transmission no clear risk factor is found in 20-30% of patients (Lee, 1997).

b) HCV

HCV is chiefly contracted through parenteral exposure to infected material such as blood transfusion, or injection with dirty needles. Risk factors for HCV infection include: drug injection, history of hepatitis, health care workers who are at risk for needle sticks and other exposures history of previous surgical procedure and blood transfusion (Zaller et al., 2004). Relatively weak risks associated are sex with an infected partner, high-risk sexual behavior, and low socioeconomic status. The risk of vertical transmission is 5%. A mother's co-infection with HIV significantly increases this risk (Shehab, 2004). The accumulated evidence indicates that hepatitis C virus (HCV) can be transmitted by sexual contact but much less efficiently than other sexually transmitted viruses, including hepatitis B virus and human immunodeficiency virus (HIV). However, because sex is such a common behavior and the reservoir of HCV-infected individuals is sizable, sexual transmission of HCV likely contributes to the total burden of infection. HIV coinfection appears to increase the rate of HCV transmission by sexual contact (Terrault, 2002; Brook, 2002).

c) HDV
Transmission of HDV may occur through a) direct percutaneous exposure to contaminated blood through the parenteral use of drugs or through blood and blood product transfusion, b) horizontal non-parenteral transmission of siblings, especially between household members who are HBsAg carriers, c) sexual contact, d) through open skin lessons, needlestick injury or environmental contamination, e) perinatal transmission is uncommon (Christopher and John, 2000; Brook, 2002). Transmission of HDV is thought to occur by person-to-person contact where HDV is highly endemic, whereas it mainly occurs through parental exposure to blood or body fluids in areas of low endemicity such as many developed countries (Banker, 2005).

1.2.3. Occupational Risks for Viral Infections in Healthcare Setting

HCWs are potentially exposed to blood and body fluids containing transmissible diseases and are at increased risk to acquire these pathogens (Catalani, et al., 2004). In health care setup, risk of acquiring infection by both patients and health care worker from each other is fairly high. Hospital acquired infections are a problem in both developed and developing countries and are important causes of death. Many different microbes cause hospital-acquired infection in health care workers; these include various commensals, HIV, HBV and HCV (Sridhar et al., 2004).

Hepatitis B and C are blood borne pathogens, which might be acquired occupationally. Occupational exposure to blood and body fluids occur frequently among health professionals (Canini et al., 2002, West, 1984). An exposure that might place health care professional at risk for HBV and HCV is defined as a percutaneous injury (e.g., a needle stick or cut with a sharp object) or contact of mucous membrane or non-intact skin with blood, tissue, or other body fluids that are potentially infectious. In addition to blood and body fluids containing visible blood, semen and vaginal secretions are considered potentially infectious. Although semen and vaginal secretions have been implicated in the sexual transmission of HBV and HCV, they have not been implicated in the occupational transmission from patients to HCWs (CDC, 1988). The following fluids also are considered potentially infectious: CSF, synovial fluid, pleural fluid, peritoneal fluid, pericardial fluid and amniotic fluid. The risk for transmission of HBV and HCV from these fluids is unknown. Faeces, nasal secretions, saliva, sputum, sweat, tears, urine, and vomitus are not considered potentially infectious unless they contain blood (CDC, 2001).
Exposure to blood borne viral pathogens from sharps injuries continues to pose a significant risk to HCW. The number of sharps injuries sustained by HCW is still unclear, primarily due to under-reporting. In one review a mean rate of 4.0% (range 1.0-6.2%) sharps injuries per 10000 HCW was calculated from eight studies involving more than 7000 HCW (Trim and Elliott, 2003). Health care personnel experience an appreciable proportion of disease from blood borne pathogens acquired through “sharps” contacts. Approximately 3 million HCWs experience percutaneous exposure to blood borne viruses (BBVs) each year. This results in an estimated 16,000 hepatitis C, and 66,000 hepatitis B cases annually. More than 90% of these infections are occurring in low-income countries, and most are preventable (Kermode et al., 2005). Worldwide, it has been estimated that approximately 40% of hepatitis C virus and hepatitis B virus infections are attributable to occupational exposure to sharps and needles (Berry, 2004).

HBV is a well recognized of occupational risk for HCWs. The risk of HBV infection is primarily related to the degree of contact with blood in the work place also to the HBeAg status of the source person. In studies of health care personnel who sustained injuries from needles contaminated with blood containing HBV, the risk of developing clinical hepatitis if the blood was HBsAg and HBeAg–positive was 22%-31%; the risk of developing serologic evidence of HBV infection was 37%-62%. By comparison, the risk of developing clinical hepatitis from a needle contaminated with HBsAg-positive, HBeAg–negative blood was 1%-6%, and the risk of developing serologic evidence of HBV infection, 23%-37% (Sulkowski et al., 2002). Although percutaneous injury is among the most efficient mode of transmission, these exposures account for a minority of HBV infection among HCWs. In several investigations of nosocomial hepatitis B outbreaks, most HCW did not reveal an overt percutaneous injury (Garibaldi et al., 1972), although in some studies up to one third of infected HCW recalled caring for a patient who was HBsAg positive. In addition, HBV has been demonstrated to survive in dried blood at room temperature on environmental surfaces for at least one week. Thus, HBV infections that occur in HCW with no history of non occupational exposure to or occupational percutaneous exposure injury might have resulted from direct or indirect blood or body fluid exposure that inoculated HBV into cutaneous scratches, abrasions, burns, other lesion or on mucosal surfaces (CDC, 2001). The potential for HBV transmission through contact with environmental surfaces has been demonstrated in investigation of HBV outbreaks among patients and staffs of hemodialysis units.
Early studies suggested that the transmission rate after percutaneous exposure to HCV is 1.8% (range 0%–10%) (Puro et al., 1995). More recent surveys using larger sample sizes demonstrate that the risk of transmission of HCV after percutaneous exposure is significantly lower, approximately 0.5% (Mayo-Smith, 1987). Specific factors associated with increased risk of percutaneous transmission of HCV have not been identified, but viral titer in the source material is likely to have a significant effect.

Transmission rarely occurs from mucous membrane exposures to blood, and no transmission in health care personnel has been documented from intact or nonintact skin exposures to blood (Mikulich and Schriger, 2002). The skin or mucous membranes of operation room (OR) personnel may have contact with patient blood in as many as 50% of operations (reported range 6.4% to 50%). Cuts or needle sticks may occur in as many as 15% of operations (reported range 1.7% to 15%). Risk increases with longer, more invasive, higher blood loss procedures. Surgeons and first assistants are at highest risk for injury. They suffer as many as 59.1% of injuries in the OR. Scrub nurses and scrub technicians sustain the second highest frequency of injuries in the OR (19.1%), followed by anesthesiologists (6.2%) and circulating nurses (6%). The remainder injuries sustained by various other groups, including medical students. Although risk of injury and exposure is different for various personnel, risk in the OR is never zero (Quebbeman et al., 1991).

A study done in Turkey has shown nurses are frequently exposed to occupational exposure for HBV and HCV infection, 22.4% had received sharp injuries and 63.6% had suffered needle stick exposures. The study also found 11.2% of nurses who had worked for a period of between 0 and 5 years and 37.1% of those who had worked for a period between 16 and 20 years had evidence of HBV or HCV infection. Of nurses working in surgical clinic, 59.4% had evidence of previous HBV or HCV infection and those working in hospital clinics had an 18.2% infection rate (Kosgeroglu et al., 2004). A national survey conducted in USA among health professionals to assess the degree of occupational exposure to blood and body fluid has revealed the following results: 74% had soiled their hands with blood at least one time in the preceding six months, 51% had splashed blood or amniotic fluid in their faces and 24% reported one or more needle stick injuries during that same period (Willy et al., 1990).
In serologic studies conducted in the United States during the 1970s, HCW had a prevalence of HBV infection approximately 10 times higher than the general population (Dienstag and Ryan, 1982). A study done in Peru on seroprevalence of HCV in health personnel has revealed that there is a major risk in health workers and the group with the highest risk among the health workers is the group specialized in hemodialysis, followed by laboratory, surgery and gastroenterology (Colichon, 2004). Another study done in Italy has compared the prevalence among different groups and indicates an increasing trend in this order: blood donors, general population, clerical and nursing school attendees, health care worker group, with the highest value (3.4%) (Catalani et al., 2004). A Hungarian study on prevalence of Hepatitis C has also shown occupational acquisition of hepatitis C (Mihaly et al, 2001).

1.2.4. Pathogenesis and Immune response

a) HBV

The outcome of HBV infection depends on the kinetics of the virus-host interaction and in particular on the strength of the innate and adaptive, humoral and cellular immune response (Rehermann, 2003). Following, infection with HBV the period before symptomatic disease is now recognized as a time of dynamic interaction between virus and host. Recent work has shown that this period is the phase of infection during which maximal changes in virus replication and the activation of critical components of the immune system occurs. This suggests that the different outcomes following exposure might be determined during the early phase of infection, before the onset of clinical disease (Webster and Bertoletti, 2002).

Specific antibody patterns have been associated with different phases and outcomes of HBV infection and are widely used as diagnostic tools. Antibodies against HBcAg constitute the first markers of acute HBV infection and antibodies against HBeAg are an early sign of recovery from acute, self-limited hepatitis B. Seroconversion to anti-HBe is usually associated with less severe liver disease, normalization of ALT activity, and decrease of viral load unless a mutant virus with a stop codon in the precore region is present. Antibodies against the glycoproteins of the S, preS1, and preS2 region are neutralizing and appear when HBsAg is cleared during recovery.
from HBV infection. Their production is a T cell–dependent process and anti-HBs titers of more than 10 mIU/mL are associated with protective immunity both after recovery from HBV infection and after vaccination (Rehermann, 2003).

In acute, self-limited hepatitis, most HBV virions are cleared in the incubation phase prior to the onset of clinical symptoms. This process is induced by antiviral cytokines that are produced by cells of the innate and early adaptive immune response. It is followed by antigen-nonspecific amplification of the intrahepatic cellular infiltrate, which coincides with clinical symptoms and signs of acute hepatitis B and liver injury. Resolution of acute, self-limited hepatitis is indicated by clearance of hepatitis B antigens, development of neutralizing antibodies, and persistence of HBV-specific memory T cells that control remaining trace amounts of HBV DNA (Rehermann, 2003).

HBV is not cytopathic for hepatocytes, and viral hepatitis is caused by the cellular immune response to HBV-infected liver cells, mediated by cellular response to small epitopes of HBV proteins (Geller, 2002). The ability to mount a coordinated and efficient helper and cytotoxic T-cell response against different proteins of HBV seems necessary to achieve successful HBV control. The immunopathogenesis of HBV infection results from an efficient cell mediated immune (CMI) response. Liver injury during acute HBV infection is mediated by, cytotoxic T cells and natural killer cells (NK) cells that cause immune lysis of infected hepatocytes. These events are mediated by cytokines that are produced primarily by CD4 T-lymphocytes, and via the perforin and Fas lytic pathways, which induce apoptosis and, inhibit HBV expression by activating liver cells to degrade viral RNA while also inhibiting HBV replication by preventing the assembly of or disrupting the nucleocapsid particles within which replication occurs; however non cytopathic antiviral mechanisms can also contribute to viral clearance during acute hepatitis B (Locarnini, 2000; Rehermann, 1996). These patients, not only are HBV-specific T-cell responses strong and Th1-like, but they are also multispecific, with observed responses to a number of different epitopes within the HBV nucleoprotein. Analysis of HBV-specific cytotoxic T-lymphocyte (CTL) responses in acute HBV infection has demonstrated that effective control is associated with CTL specific for a number of different epitopes within the core, polymerase and envelope proteins. In addition the spread of HBV to uninfected cell is prevented by the presence
of virus neutralizing antibodies (Locarnini, 2000). Because traces of virus as well as HBV-specific CTL can persist for decades after clinical recovery, continuous priming of new CTL by minute traces of virus is thought to protect from reactivation of disease (Rehermann, 1996).

Hepatitis B virus is eliminated in almost all (98–99%) otherwise healthy infected persons, although very low levels of HB viral DNA may be detected in liver or lymphoid tissues after recovery (Mackay, 2002). All chronically infected patients studied to date, even those who acquire HBV infection during adulthood and are otherwise immunocompetent, display infrequent, narrowly focused, and weak HBV-specific T cell responses (Rehermann, 2003). A default option for the host, used in perinatal infections, is the development of immunological tolerance to the virus particle, seen as surface protein (antigen) of HBV (HBsAg) in liver cells with minimal, if any, host immune response (Mackay, 2002). In children, thymic deletion of major histocompatibility complex (MHC) class II restricted HBV nucleocapsid–specific helper T cells, which results in T-cell tolerance to HBeAg is thought to facilitate the development of the carrier state (Locarnini, 2000). In adults, the more disadvantageous outcome is a balance of tolerance and immunity with intermittent shifts one way or the other, either apparently spontaneous or due to stresses, infections, pregnancy and, particularly, use or withdrawal of immunosuppressive therapies (Mackay, 2002).

Several candidate mechanisms have been proposed to explain the development of persistent HBV chronic infection, mutations are usually demonstrable in genes for surface and capsid proteins of HBV, but it is not yet established to what degree these contribute either to immune evasion and viral persistence, or to the intensity of the damaging host immune responses (Mackay, 2002). In addition, the productions of excess HBsAg particles divert antibody neutralization of whole virus. In later stages, integration of HBV-DNA into the hepatocyte genome without HBeAg or HBcAg expression to identify those infected hepatocytes further evades immune effector (Locarnini, 2000). Immunological factors contribute to carcinogenesis. Long-standing inflammatory liver injury is an important procarcinogenic factor (Rehermann, 2003).

b) HCV
The variable outcome of HCV infection is determined by the interaction between the virus and the host's immune system. The majority of HCV-infected patients are capable of mounting polyclonal humoral immune responses directed at both structural and non-structural viral proteins (Freeman et al., 2001; Rehermann, 2000). HCV specific antibodies are generally detectable in the serum between 7 and 31 weeks after infection (Rehermann, 2000). The appearance of anti-HCV does not correlate with the resolution of either the infection or the progression of hepatitis. In the majority of cases, HCV infection progresses despite the presence of antibodies against the major viral proteins suggesting humoral response doesn’t play a central role in determining outcome of immunity or protection against HCV. In addition no data are available to show that HCV-infected patients have long lasting protective antibody response (Jackson and Cheung, 2004; Afdhal, 2004). Anti-HCV becomes detectable during the course of illness in at least 97% of HCV infected persons. Anti-HCV can be detected in about 70-80% of patients at the onset of symptoms and in approximately 90% of patients with in 3 months after onset (Afdhal, 2004).

There is increasing evidence that cellular immune responses involving both CD\textsubscript{4}\textsuperscript{+} and CD\textsubscript{8}\textsuperscript{+} T cells are involved in the initial period after acute infection and it is their effectiveness that determines the long-term outcome. A wide variety of vigorous CD\textsubscript{4} T cell responses persist for many years and memory CD\textsubscript{8} T cells may also be maintained. These responses are also more vigorous in acute hepatitis C patients who recover than in patients who evolve to chronic hepatitis, suggesting that the intensity of cellular immunity in the early stage of infection is a critical factor in limiting the spread of HCV (Tsai, 1999). In addition to the strength of the T-cell response, particular HCV antigens, including core and NS3, have been proposed as ‘protective’, although there is debate as to which epitopes within these regions are responsible. Furthermore, the ‘breadth’ or multispecificity of the T-cell response is likely to play an important role in the outcome of infection as responses against multiple epitopes, particularly those in conserved regions, reduce the likelihood of antigenic viral escape through antigenic variation (Afdahl, 2004).

As a DNA replicative intermediate has never been demonstrated in the HCV life cycle; there is no evidence to suggest that persistent HCV infection is related to viral integration into the host genome. Persistence must, therefore, relate to the inability of the host to mount an effective immune response, to viral factors that facilitate immune evasion, or to a combination of these
factors (Freeman et al., 2001). Thus, unlike HBV, immune deficiency may contribute to, but does not explain, non-elimination of HCV after acute infection. One explanation is the high mutagenicity of the HCV genome that produces multiple quasispecies of the virus that ‘out run’ the TCR repertoires of the host, but this alone seem insufficient. Other explanations include a lack of MHC (HLA) alleles that can efficiently bind and present structural peptides of HCV to host T cells; the capacity of mutant proteins of HCV to confront TCR with altered peptide ligands that engage but do not activate the TCR; replication of HCV in extra hepatic reservoirs as a source of continuing reinfection of the liver; suppression of CTL responses by core proteins of the virus; and the intrinsically tolerogenic milieu within the liver itself (Mackay, 2002).

c) HDV

Hepatic cell death may occur due to the direct cytotoxic effect of HDV or via a host-mediated immune response (Polish et al., 1993). Both humoral and cellular immune mechanisms may be involved in the pathogenesis of infection with HDV. This immune response may provide protection from HDV re-infection, or modulate clinical symptoms. However, second cases of hepatitis D have not been reported (Previsani and Lavanchy, 2001). All anti-HD antibodies do not always persist after accurate infection is cleared. The serological evidence of past HDV infection is therefore not easy to demonstrate (Previsani and Lavanchy, 2001).

1.2.5. Natural Course and Clinical Features

a) HBV

The outcome of infection depends on both properties of the virus and the host. Several studies found an association between variants of the virus and genetic variations found in the host (Thursz, 1997). Hepatitis B infection is characterized by four dynamic stages. The first stage, the “immune tolerant” phase is characterized by, high levels of HBV DNA replication, HBeAg positivity, normal serum transaminase levels, little or no symptoms, and minimal histological activity in the liver. In the acutely infected child or adult, this stage represents the incubation period before immune response to HBV. It usually lasts for 2-4 weeks and represents incubation period, but can last for decades in those who acquired the infection during the perinatal period. Individuals in this group are highly contagious and can transmit HBV easily. Active viral
replication is known to continue despite little or no elevation in the aminotransferase levels and no symptoms of illness (Lavanchy, 2004; Pan and Zhang, 2005).

When the tolerogenic effect is lost during the immune tolerant phase, immune-mediated lysis of infected hepatocytes becomes active. This stage reflects the “immune response,” which is the inflammatory process that results in the destruction of HBV-infected cells, elevating transaminase levels. The HBV DNA level decreases, HBeAg can be identified in the sera and ALT level increases. The duration of this stage for patients with acute infection is approximately 3-4 weeks (symptomatic period). For patients with chronic infection, 10 years or more may elapse before cirrhosis develops. Persistence of the immune response phase beyond six months is considered chronic HBV infection. This stage carries the highest risk of progression to cirrhosis and hepatocellular carcinoma (Schaefer, 2005).

The third stage, the “inactive carrier” state, is thought to mark the end of active viral replication. HBeAg becomes negative, anti-HBe appears (seroconversion), and transaminase levels normalize. A low level of HBV DNA still may be present. Reflecting very low or no replication of HBV and mild or no hepatic injury the majority of adults with, acute HBV infection enter this stage rapidly. An inactive carrier forms the largest group in chronic HBV infected patients. Around 300 million people are inactive carriers. The inactive carrier stage may last for years or even lifetime. They may already have progressed to cirrhosis or may have insignificant fibrosis. From 10 to 30 percent of carriers will have disease flares similar to acute HBV infection (Locarnini, 2000).

The fourth, or “immune,” stage is characterized by the clearance of HBsAg and development of HBsAb. HBV DNA is usually undetectable, and reactivation or re-infection is uncommon. Progression from the third to the fourth stage occurs in approximately 3 percent of HBV-infected persons per year (Chwla, 2005).

Clinical manifestations of HBV infection are a balance between viral and host factors (Koziel, 1998). Possible clinical outcomes of HBV infection are described in Figure 1.4. Acute HBV infection is sub-clinical in 70 percent of adults and 90 percent of children younger than five
years. The incubation period after infection lasts one to four months. Acute HBV infection leads to fulminant hepatic failure from massive hepatocellular necrosis in about 1 percent of infections (Kirchner and Lin, 2004).

HBV infection is termed as chronic if it continues to be HBsAg +ve for ≥ 6 months. Chronicity is dependent mostly upon age at exposure. Thus, 90% of children infected before their first birthday become chronic carriers compared to 5%-10% of adults (Chwla, 2005). Chronic HBV infection is a dynamic process with a wide spectrum of affliction. Median progression rate from chronic hepatitis to cirrhosis is 27.9% after 8-12 years and HCC is other sequelae (Lee, 1997). Chronic carriers often lack symptoms. Acute as well as chronic infections by HBV can be associated with extra hepatic diseases. The pathogenesis of both conditions involves the deposition of circulating immune complexes (Hartmann, 1997; Han, 2004).

Figure 1.4. Possible outcome of hepatitis B virus infection (Adapted from Szabo et al., 2004)

Epidemiologic studies have demonstrated that there is a consistent and specific causal association between HBV infection and HCC. In patients with persistent HBV infection, the risk of HCC was 100 times higher than in non-infected individuals. The global distribution of hepatocellular carcinoma correlates with the geographic prevalence of chronic carriers of HBV, which number
400 million worldwide. The highest rates are in Southeast Asia and Sub-Saharan Africa, with the HCC incidence >50/100,000 population (El-Serag, 2002).

b) **HCV**

The scope of the natural history of hepatitis C infection encompasses a spectrum of virus-host interactions that ranges from immediate viral clearance without stimulating humoral immunity; acute sub-clinical infection that resolves spontaneously; acute clinical infection that resolves spontaneously; sub-acute or acute infection that either resolves spontaneously or leads to chronic viremia without defined histologic or biochemical evidence of hepatic disease; persistent but stable hepatitis without progression; and progressive disease that leads to acute or chronic liver failure, cirrhosis (which may range from relatively stable over time to rapidly progressive), and hepatocellular carcinoma. What remains elusive is the frequency of these various outcomes and the factors that influence them (Thomas *et al*., 2000; Pagliaro *et al*., 1999).

The natural history of hepatitis C remains unclear. There have been wide variations in rates of development of chronic infection and in the progression of liver disease to eventual cirrhosis (Seeff, 1997; Shehab, 2004; Jackson and Cheung, 2004). Epidemiologic, clinical and virologic factors predicting the severity of liver disease have not been well defined. Among that have been considered are modality of infection, patient age, duration of disease, genotype, serum viral load, degree of variation of HCV, host immunity and co-factors (Hepatitis B and alcohol use). The most convincing data show that age above 40 years, male gender, and ingestion of 50gm or more of alcohol per day are factors that increase liver disease (Kryczka *et al*., 2001).

Acute HCV infection incubation period ranges from 2 weeks-6 months with an average incubation period of 6-7 weeks, icteric hepatitis occurs in fewer than 20% of acute infections, 60-70% has no discernible symptoms. Fulminant hepatitis is rare except for hepatitis A superinfection of chronic HCV disease (Brook, 2002). The percentage of patients with acute hepatitis C who develop chronic infection has ranged from 54% to 86% in various publications. Although there is no accurate estimate, it is believed that the majority of patients with acute hepatitis C will progress to develop chronic infection (Locasciulli *et al*., 1997; Wiese *et al*., 2000; Vogt *et al*., 1999). Chronic hepatitis C is asymptomatic in most patients (Zaller *et al*., 2004).
Although most patients with chronic hepatitis C infection have elevated serum transaminases, up to 30% of patients have persistently normal enzyme levels, and others have fluctuations (Shehab, 2004). Sequelae of chronic HCV infection include cirrhosis, liver failure, and hepatocellular carcinoma. The rate of progression to cirrhosis remains highly variable in most patients, in some studies of those who are chronically infected, 80% stabilize, whereas 20% evolve to cirrhosis over the course of 20 to 30 years (Jackson and Cheung, 2004; Afdhal, 2004). Common clinical manifestations of decompensated liver disease include variceal bleeding resulting from portal hypertension, ascites, and hepatic encephalopathy (Shehab, 2004; Thomson and Finch, 2005).

While HCV is hepatotropic, it is also found in other tissues including peripheral blood mononuclear cells and lymph nodes and is associated with extra hepatic manifestations (Hartmann, 1997; Walsh and Alexander, 2001).

c) HDV

HDV can be first acquired concurrently with acute HBV infection or as a superinfection of a chronic HBV carrier (Tepper and Gully, 1997). In acutely co-infected patients the incubation period is 3–7 weeks and there may be two bouts of clinical hepatitis due to each virus. The acute infection is often quite severe and fulminant hepatitis is 10 times more likely than with other types of viral hepatitis with an 80% fatality rate but decrease the risk of becoming an HBV carrier. Chronic infection occurs in only 5% of such patients. Superinfection of HDV in a HBV carrier causes an acute, severe, icteric hepatitis, which again is associated with a high rate of fulminant disease and also leads to chronic infection in 80%. Chronic infection leads to a high rate of subsequent cirrhosis (up to 70%), which is usually more rapid in onset (40% in 6 years) than with HBV and can occur as little as 2 years after infection. The rate of progression to liver cancer in cirrhotics with HDV is also trebled compared to HBV (Geller, 2002; Brook, 2002).

1.2.6. Laboratory Diagnosis

HBV-specific assays are routinely used for the assessment of disease activity in persistent infection, for monitoring therapeutic regimens with antiviral agents and most importantly, for
evaluating the infection in a donor’s blood to prevent recipient’s contamination (Tsitsilonis et al., 2004). In clinical practice diagnosis of HBV infection is established by the serological detection of HBV protein products antigens (Ags) as well as host-produced antibodies (Abs). Serological markers are key elements in diagnosing acute HBV infection and determining its possible evolution towards chronicity (Sablon and Shapiro, 2005). HBVAg and anti-HBVAb detection is often simultaneously carried out in the same serum or plasma specimens, using enzyme-linked immunosorbent assays (ELISAs), highly specific for HBsAg, anti-HBs, anti-HBc, and HBeAg or anti-HBe (Sablon and Shapiro, 2005).

HBsAg is the primary diagnostic marker used for screening blood products in hospitals and health-care facilities. The envelope protein of HBV, HBsAg, is a transmembrane glycoprotein usually shed in large amounts in the serum of infected individuals, where it is found as spherical particles with a diameter of 22 nm or filaments of similar diameter (Weber, 2001). HBsAg is considered to be the sentinel marker for the confirmation of acute infection. Its presence can be detected as early as 6 weeks after exposure, and should therefore be assessed when or more classical symptoms are observed. HBsAg is also the key marker in determining whether hepatitis B infection has become chronic (Sablon and Shapiro, 2005). Serological detection of HBsAg involves the use of either monoclonal or polyclonal anti-HBs bound to solid-phase or second labeled anti-HBs to detect the captured antigen. Among the many commercially licensed HBsAg assays offered, enzyme-linked immunosorbent assays are the most commonly used. Despite the high performance of third-generation enzyme-linked immunosorbent assay in the detection of HBsAg, a high incidence of false-negative results has been reported (Ismail, 2004). Antibodies to HBsAg indicate recovery from infection and found in those immunized with HBV vaccines, but it may become undetectable in patients who have recovered fully from infection (Kidd-Ljunggren et al., 2004; Ismail, 2004). Individuals who have resolved their HBV infection usually demonstrate both anti-HBs and anti-HBc in their serum. Anti-HBs testing is useful for identifying HBV-susceptible individuals in pre- and post vaccination screening programs, where absence of these antibodies is indicative of susceptibility to HBV infection. This could be an indication for vaccination against HBV (Ismail, 2004).
As the immune system begins to mount its response to infection, an initial rise - then decline of anti-HBc IgM, is observed. By contrast, anti-HBc IgG rises but persists even after acute infection has resolved (Sablon and Shapiro, 2005). Antibody against hepatitis B core antigen (anti-HBc) is found in individuals who have experienced natural infection with HBV. Conventionally, the presence of anti-HBc in the absence of HBsAg is interpreted as evidence of a past HBV infection (Alhabab et al., 2003). Recently, there has been concern about a subgroup of individuals with the serological pattern of ‘anti-HBc only’ in which anti-HBc is the only detectable HBV marker in the absence of HBsAg or anti-HBs. It was reported that ‘anti-HBc only’ was found in 10-20% of all individuals with HBV markers in areas of low HBV endemicity and about 10% of these individuals had detectable DNA. Other studies, however, have found a higher HBV DNA prevalence of up to 40 % (Alhabab et al., 2003). Molecular methods have demonstrated the presence of the virus in patients with anti-HBc alone with a frequency varying from 0 to 90% (Shih et al., 1990; Wang et al., 1991). The significance of anti-HBc in the absence of the surface antigen is somewhat controversial such finding could represent 1) a situation where anti-HBs and anti-HBe being undetectable, loss of detectable anti-HBs, the patient is immunized; 2) a case where HBsAg levels are very low to be detected with routine assays; mutations at the antigenic ‘a’ determinant region of HBsAg or other regions of the surface gene and mutations in promoter and enhancer sequences 3) false-positivity or cross-reactivity of anti-HBc; and 4) an immunological window period, in which HBsAg is already undetectable and the surface antibody is not yet detectable (Kleinman et al., 1997; Alhabab et al., 2003). ‘Anti-HBc only’ has been described frequently among individuals infected with HIV or HCV. It is possible that co-infection with these viruses could lead to down regulation or interference of HBsAg production. The phenomenon of ‘anti-HBc only’ is not rare in diagnostic settings. The significance of this phenomenon is unknown and it is not clear how this serological profile should be interpreted, it is also unclear whether all individuals with such serological pattern need further molecular investigations (Alhababi et al., 2003).

HBeAg is considered as a better marker of viral blood infection, whereas the development of host Abs to HBe (anti-HBe) indicates the assessment of immunity and the reduction of viral replication in the infected individual (Sablon and Shapiro, 2005). Serum HBV DNA is indicative of active viral replication. It may be present at levels exceeding $10^5$ to $10^6$ copies/ml and can be
identified some 6-12 weeks after exposure to the virus, or even earlier if PCR-based methods are used (Sablon and Shapiro, 2005). Over the past decade, improvements in molecular technology, permitting detection of as few as 10 copies/ml of HBV DNA in serum have led to redefinitions of chronic HBV infection, as well as thresholds for antiviral treatment (Servoss and Friedman, 2004). HBV DNA assays are not presently recommended for the routine evaluation and management of patients with chronic HBV infections, they nevertheless provide very useful adjunct information concerning viral replication – especially in situations when patient serological profiles fall outside of classical patterns (Kimura et al., 2003). Monitoring of serum HBV DNA levels is a consistent method for the assessment of potency of antiviral therapy (Ismail, 2004). Several assays for the quantitative measurement of HBV DNA have been developed, such as the branched-chain DNA signal amplification assay and transcription-mediated amplification (TMA)-based or PCR-based nucleic acid amplification assays (Sablon and Shapiro, 2005). Signal amplification assays have sensitivities approaching 1 pg of DNA ($10^5$-$10^6$ genome copies) or even to $10^3$ genome copies. Alternatively, HBV DNA detection based on a nested PCR approach can detect as few as $10^2$-$10^3$ genome copies. At such low titers, problems with contamination and reproducibility may lead to false-positive results, thereby necessitating the use of internal or external standards. Commercial assays that make use of semi-automated systems can overcome these limitations (Sablon and Shapiro, 2005). Attempts to grow HBV in standard cell lines have not been successful (Kidd-Ljunggren et al., 2004).

b) HCV

Diagnostic assays for HCV are now well established and commercially available. Four key markers of HCV infection can currently be assessed utilizing virological tools: anti-HCV ab, HCV RNA, HCV genotype, and HCV core Ag. The diagnostic algorithm depends on the clinical context (Pawlotsky, 2003). The diagnostic algorithm with various tests done in series is described in Figure 1.5.

Many tests are commercially available for demonstrating the presence of anti-hepatitis C antibodies, and most laboratories generally classify this group of tests as “the hepatitis C antibody test (Shehab, 2004). Anti-HCV antibodies are determined by means of ELISA assays that detect a mixture of antibodies directed to different epitopes located on various viral proteins.
Tests to detect anti-HCV were first licensed by the Food and Drug Administration (FDA) in 1990. Since that time, new versions of these and other FDA-approved anti-HCV tests have been used widely for clinical diagnosis and screening of asymptomatic persons (CDC, 2003). The serologic tests identify only the presence of anti-HCV antibody, which indicates exposure to the virus, but they cannot differentiate between acute disease, resolved disease, or chronic infection (deMedina and Schiff, 1995). Three generations of EIA with varying sensitivity and specificity for anti-HCV have been developed over the past decade (Table 1.1). The first commercially available assay incorporated recombinant C100-3 antigen derived from the nonstructural region of the virus and detects only antibodies against nonstructural region 4 (NS4). It became quickly apparent that this test had a number of serious limitations. There were numerous false-positive reactions, particularly among low risk groups, the test was insensitive and, there was a considerable delay between acute HCV infection and the first evidence of anti-HCV (Fried, 1999). Two complementary approaches were taken to overcome these limitations: development of newer EIA tests with better sensitivity and specificity and supplemental tests to augment EIAs for anti-HCV (Carithers et al., 2000). The second-generation assay introduced in 1992, incorporated recombinant antigens from nonstructural regions (NS3 and NS4) like core region (c22-3), the NS3 region (c33c) of the putative HCV genome (Feucht, 1995; Ismail et al., 2004). Many limitations of the first-generation EIA were overcome by the second generation EIA for anti-HCV (EIA-2). EIA-2 continues to be the test routinely used by most clinical laboratories (Krajden, 2000). To increase both sensitivity and specificity, a greater number of HCV-encoded antigens are now included in the third-generation EIA, allowing an increase in the specificity. The addition of core and NS5 region-encoded antigens on the solid phase of serological assays also resulted in earlier detection of anti-HCV during acute infection, a marked increase in the sensitivity, and a dramatic reduction in the incidence of post transfusion hepatitis in blood banks (Ismail et al., 2004). Although this test is used by some clinical laboratories for routine screening of high-risk populations for hepatitis C, the positive predictive value of the EIA-3 assay is not well defined. As a result, the benefit of replacing EIA-2 with EIA-3 assays for routine testing in clinical laboratories is unclear (Carithers, 2000). Although third generation anti-HCV EIAs are very effective for testing high prevalence populations, confirmatory testing is still necessary when these tests are applied to populations with a low HCV prevalence to exclude false positive results. Limitations of third generation anti-HCV EIAs include: the relatively prolonged time...
between acute infection and detection of seroconversion (which typically requires at least 5-6 weeks); delayed seroconversion in immunocompromised hosts (requiring months to years); and the inability of serological tests to confirm active HCV infection (Colin et al., 2001).

The diagnosis of HCV infection can be supported by or confirmed by RIBA. A strip immunoassay developed by the Chiron Corporation (Emeryville, Calif) is being used to help differentiate true positive from false-positive EIA results. The Food and Drug administration approved the second-generation RIBA in 1993 followed by approval of the third-generation RIBA in 1999 (Kelly and Skidmore, 2002).

Table 1.1. Sensitivity and Positive Predictive Value of EIA for Anti-HCV (Adapted from Colin et al., 2001)

<table>
<thead>
<tr>
<th>Assay</th>
<th>Sensitivity (%)</th>
<th>Low Prevalence</th>
<th>High Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>EIA-1</td>
<td>70 – 80</td>
<td>30 – 50</td>
<td>70 – 85</td>
</tr>
<tr>
<td>EIA-2</td>
<td>92 – 95</td>
<td>50 – 61</td>
<td>89 – 95</td>
</tr>
<tr>
<td>EIA-3</td>
<td>97</td>
<td>25</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

*Based on detection of HCV RNA by PCR

The strip immunoassays include the EIA antigens and human superoxide dismutase (hSOD). The RIBA, in which HCV antigens derived from different regions of the HCV genome are embedded within a strip of nitrocellulose membrane. RIBA detects antibodies to individual antigens and confers increased specificity compared to EIA. However; detection of specific antibody does not differentiate between acute and chronic infection, previous exposure, or passive antibody transfer (Kelly and Skidmore, 2002). RIBAs are standardized and reproducible. However, they are more difficult to perform than EIAs, time consuming, and relatively expensive. Their primary utility has been in excluding false-positive results in blood banks. They have limited if any usefulness among patients clinically suspected of harboring a chronic HCV infection (Carithers, 2000). The RIBA is considered positive if there are reactions with at least two antigens with intensities greater than or equal to that for the weak immunoglobulin G (IgG) control and no reactivity with hSOD. Indeterminate RIBA are those in which there are reactions with only one antigen or with
the hSOD plus one or more HCV antigens. A positive reaction is denoted by, black bands that appear on the strip after incubation with a patient’s serum (Alter, 2003). The RIBA 2.0 assay, which contains the same antigens as the EIA-2 assay, has been the most commonly used supplemental assay for anti-HCV. In the low prevalence blood bank setting, 40–50% of EIA-2 positive test results are RIBA 2.0 negative, indicating a false-positive result. RIBA 3.0 uses both HCV-encoded recombinant antigens and synthetic peptides (CDC, 2003). A RIBA 3.0 assay has been approved for use by blood banks as a supplemental test for EIA-3-positive test results. This test has the advantage over the RIBA-2 assay of fewer indeterminate results and a better correlation with the presence of viremia. Other supplemental assays are under evaluation (Alter, 2003).

HCV RNA, one of the two specific markers for HCV infection, can be detected in blood within 1 to 3 weeks after exposure (Jackson and Cheung, 2004). The mainstays of laboratory testing for HCV are the molecular approaches. There are basically two kinds of virologic tests: the polymerase chain reaction (PCR) test and the branched-chain DNA (bDNA) test. The PCR assays available to date are either qualitative or quantitative. The qualitative PCR tests are quite sensitive and may detect as little as 100 to 1,000 copies per milliliter, but they report only the absence or presence of HCV-RNA. The quantitative PCR and bDNA tests attempt to define the number of HCV RNA copies per milliliter of serum (Fried, 1999). Qualitative molecular tests are useful for confirmation of positive screening tests and the so called "window period" between infection and a serologically positive antibody test can be addressed by detecting HCV RNA in serum, quantitative molecular tests provide prognostic information regarding the likelihood of response to therapy, and allow the monitoring of treatment efficacy. Similarly, genotyping assays predict response to therapy, and allow rational decisions regarding duration of treatment (Jerome and Gretch, 2004; Ismail, 2004). Commercial nucleic acid testing assays are now highly sensitive, specific, and reproducible (Krajden, 2000).

**Figure 1.5. Laboratory Algorithm for Antibody to Hepatitis C virus Testing and Result Reporting (Adapted from CDC, 2003)**
* Interpretation of screening Immunoasay test results based on criteria provided by the manufacturer,
£ Signal-to-cut-off; € Screening-test-positive results are classified as having high s/co ratios if their ratios are at or above a predetermined value that predicts a supplemental test-positive result 95% of the time among all populations tested; screening-test-positive results are classified as having low s/co ratios if their ratios are below this value; ¶ Recombinant immunoblot assay.

**c) HDV**
The diagnosis of HDV is based on the detection of HDV antigen and IgM and IgG HDV antibodies. Molecular techniques to identify HDV RNA are being developed (Kelly, and Skidmore, 2002).

**1.2.7. Treatment**
Success in HIV drug development in the 1990s revolutionized treatment of hepatitis B. Several antiviral agents that were developed for the treatment of HIV infection proved to be effective in inhibiting HBV replication (Lok, 2003). It is well accepted that antiviral therapy for chronic hepatitis is effective to improve prognosis of patients with HBV by preventing development of hepatitis state and HCC (Keeffe et al., 2005). It is then obvious that the primary goal of treatment is to eliminate or suppress HBV; that is, to decrease pathogenicity and infectivity, and thereby to stop or reduce hepatic necro-inflammation (Liaw, 2002). The need for treatment of hepatitis B depends on the natural history of the disease. The decision to treat chronic HBV generally is based on a combination of clinical, laboratory, and histologic factors (Gish, 2005). Selection of appropriate patients for antiviral therapy depends on identification of HBV replication and an elevated alanine aminotransferase level or histologic liver injury (Lee, 1997; Mailliard and Gollan, 2005). The aims of treatment of chronic HBV infection are to achieve sustained suppression of HBV replication and to induce remission of liver disease before cirrhosis and HCC develop (Tsai, 2004). The management of patients with chronic HBV infection should be tailored according to the patient’s age, comorbid conditions, and extent of HBV replication and liver disease (Tsai, 2004). Approved drugs and agents in development for the treatment of chronic HBV infection fall into two categories: immune modulators, namely recombinant interferon alfa-2b (Intron A); and direct inhibitors of HBV replication, including lamivudine (Epivir) and adefovir dipivoxil (Hepsera) (Gish, 2005). Selecting the optimal therapy for the individual patient is not simple. All three treatments have limited long-term efficacy. Although there have been few direct comparison trials, the short-term efficacy for both HBeAg-positive and HBeAg-negative chronic hepatitis B appears to be comparable with all three medications. Also, factors predictive of response are similar. Thus, the advantages and disadvantages of each treatment, the durability of response, and the patient’s preference must be carefully weighed before a decision is made (Lok, 2003). Pegylated interferon alpha offers potent immunomodulatory and antiviral activity. The advantages of interferon include a finite duration of treatment, durable response, and lack of drug resistance; the shortcomings include parenteral administration, frequent side effects and cost. The advantages of lamivudine and adefovir include ease of administration, infrequent side effects, and applicability to patients with decompensated cirrhosis, but the optimal duration of therapy is unknown as relapse is common even when the
therapeutic end point is achieved prior to treatment discontinuation (Mailliard and Gollan, 2005). Approaches using a combination of agents have promise, but have yet to be proven superior to individual drugs alone (Lok, 2003).

The therapeutic endpoints for hepatitis B treatment are a) sustained suppression of HBV replication, as indicated by HBsAg and HBe loss (with seroconversion to anti-HBe), b) decrease of serum HBV DNA of undetectable level by a non-PCR method, c) remission of disease, as shown by normalization of ALT, and d) improvement in liver histology and reduction of the acute exacerbation, cirrhosis, or hepatocellular carcinoma (Liaw, 2002; Keeffe et al., 2005)

b) HCV

Treatment of acute HCV infection is a unique opportunity to prevent the evolution to chronic infection, particularly as several studies suggest that acute infection is very much more sensitive to therapy than established disease (Thomson and Finch, 2005). However treatment is expensive and is associated with potential adverse events. Most experts recommend treatment of patients with acute hepatitis C, but with the caveat that patients be informed of their chances for spontaneous recovery (15%), and the expected side effects and required duration of therapy (McHutchison, 1999).

Management of chronic hepatitis C is complex. Some of the difficulties stem from the wide individual variability that exists in the degree and rate of disease progression and the lack of reliable prognostic markers of disease progression. The decision to treat patients with chronic hepatitis C is not based strictly on symptoms or serum ALT levels, because these features correlate poorly with the extent of liver disease. Therefore, the decision to treat patients poses a complex clinical dilemma because of the lack of definitive, non invasive diagnostic tools for tissue pathology (mainly fibrosis), and the absence of symptoms in many patients during the initial years of HCV infection (Shehab, 2004). Guidelines from AASLD (American Association for the Study of Liver Diseases) recommend that therapy for chronic hepatitis C be based on the results of a liver biopsy (McHutchison, 1999). Goals of therapy in patients with chronic hepatitis C infection include biochemical improvement (normalization of serum transaminases), virologic control and eradication, and hepatic histologic improvement. The main definition of viral control
is the achievement of a sustained virologic response, defined as negative tests for the hepatitis C virus via PCR testing 6 months after the end of treatment (Shehab, 2004). The ideal measures of response to therapy should be histological remission, prevention of progression to cirrhosis, and reduction in mortality but these long-term data are not yet available (Walsh and Alexander, 2001).

IFN alpha-2b and ribavirin, peg interferon alpha-2a (40 KD) and pegylated IFN alpha-2b have been approved for use, alone or in combination with ribavirin in patients with chronic hepatitis C, in the US and in the European (Moreno-Otero, 2005). The current standard of care in previously untreated patients with chronic hepatitis C is combination therapy with pegylated interferon alpha and ribavirin (Patel and McHutchison, 2004). Standard IFN-α monotherapy results in only 10% sustained virological response while pegylated IFN-α plus ribavirin combination therapy improves the sustained virological response to 54–56% (Walker et al., 2003).

c) **HDV**

Delta virus related chronic hepatitis is difficult to treat. Concomitant infection with an RNA (HDV) and a DNA (HBV) virus makes chronic hepatitis D more difficult to treat than chronic hepatitis B alone (Niro et al., 2005). IFN-α is the only drug with proven benefit in patients with chronic HDV infection; there is no evidence of benefit for treating acute HDV infection. Indications for treatment with IFN-α include elevated serum transaminases, histopathologic evidence of chronic hepatitis, and HDV antigen in the liver (Carpenter and Ticehurst; 2000). The response to α-IFN varies widely and occurs at different times from the beginning of treatment. Treatment should be prolonged for 12 months as response-clearance of HDV RNA and normalization of ALT levels can be delayed and sometimes occur after the end of the treatment. A sustained response is accompanied by the clearance of the HBsAg from serum (Niro et al., 2005). If HBsAg becomes repeatedly undetectable IFN-∀ treatment can likely be stopped because the risk of relapse is low. Recommendations regarding evaluation, monitoring, and follow-up of patients with HDV infection are otherwise identical to those for treatment of chronic hepatitis B (Carpenter and Ticehurst, 2000).
1.2.8. Prevention and Control

a) HBV

Three main strategies are available for the prevention of HBV infection: (1) behavior modification to prevent disease transmission, (2) passive immunoprophylaxis, and (3) active immunization.

I) Behavior Modification

Improved screening measures of blood products have reduced the risk of transfusion-associated hepatitis. Behavior modification is thought to be more beneficial in developed countries than in developing countries, where neonates and children in early childhood are at the greatest risk of acquiring infection. In these groups, immunoprophylaxis, both passive and active, will be more effective (Alter, 2003).

II) Passive Immunoprophylaxis

Hepatitis B Immune Globulin (HBIG) is a sterile solution of ready-made antibodies against hepatitis B. HBIG is prepared from human blood from selected donors who already have a high level of antibodies to hepatitis B and used in passive immunoprophylaxis (Lavanchy, 2004). Passive immunoprophylaxis is used in five situations: (1) after needle stick exposure, (2) newborns of mothers infected with hepatitis B; (3) after sexual exposure, and (4) after liver transplantation, and 5) after contamination of the eye (Banatvala and Van Damme, 2003).

III) Active Immunization

Immunization against HBV, introduced almost 20 years ago, has made this infection a vaccine preventable infectious disease (Lavanchy, 2004). The first vaccines to be licensed (1981) were plasma derived, but these have largely been replaced by recombinant derived ones, which were introduced in 1986. In 1991, the WHO recommended that HB vaccine should be introduced into the Expanded Programme of Immunization (EPI). The objectives of vaccination against hepatitis B are primarily to prevent infection, thereby reducing the incidence of persistent HBV infection and chronic liver disease, and in addition eliminating the pool of chronic carriers, thus limiting transmission of infection to susceptible contacts (Banatvala and Van Damme, 2003). HBV vaccine is the first vaccine that has been proved to prevent cancer. A significant reduction in
hepatocellular carcinoma among children was observed 10 years after the introduction of universal HBV vaccine to newborns in Taiwan (Chang et al., 1997). Vaccination is recommended for all children and adolescents, adults in certain ethnic groups, HCWs, and other high-risk groups (Kirchner and Lin, 2004). Hepatitis B vaccine has an outstanding record of safety and effectiveness. The only contraindication to vaccine administration is hypersensitivity to yeast or to a component of the vaccine (Walsh and Alexander, 2001). The vaccine is given in three stages, at 0, one to two months, and at six months. Another schedule has been recommended if more rapid protection is required, e.g. for travelers or following exposure to HBV. In this case, each of the three doses is separated by a month, with a booster at 1 year (Bock et al., 1995).

Based on the currently available data there is no scientific evidence for giving booster doses of hepatitis B vaccine to fully immunized individuals (Fitzsimons, 2005). Recommendations for HCWs in European countries and the USA vary considerably as to whether or not a booster dose is recommended and whether this relates to anti-HBs levels as well as the interval between the primary course and the booster dose. The results of long-term follow-up studies, together with assessment of the role of immunological memory among vaccinees, now question the necessity of providing booster doses following a successful course of primary immunization (Banatvala and Van Damme, 2003).

Protective anti-HBs titres of >10 mIU/ml develop in 95%-99% of children and young adults who receive the series of three intramuscular doses. Those who fail to respond may be re-vaccinated; in this group, 30 to 50 percent will achieve protective levels (Walsh and Alexander, 2001). The vaccine has been shown to protect against HBV in all high risk groups including HCW, homosexuals, intravenous drug abusers and infants born to HBsAg positive mothers (Banatvala and Van Damme, 2003).

Despite successful vaccination in terms of satisfactory anti-HBs levels, some children have developed HBV infection due to an escape mutation in the "a" determinant of the HBsAg epitope driven by HB Ig given concurrently. This mutant may become more common in the next decade (Walsh and Alexander, 2001). Antibody titres decline with time elapsed since the first
vaccination, to below the putative protective level of 10 mIU/ml in up to 50% of vaccinees at 10 years, but vaccinated responders have persisting anti-HBs and/or cellular immunity and can mount a rapid and powerful an amnestic response to a vaccine challenge (Fitzsimons et al., 2005). One study has reported the importance of vaccinating those with positive anti-HBe (Halota et al., 2002).

b) HCV
It does not appear that a vaccine for hepatitis C will be available any time soon. Therefore, primary prevention, always the best public health approach, takes a center stage. Primary prevention includes strict infection control techniques, universal precautions when handling needles, sharp objects, and body fluids, and screening all blood, blood products, tissues, organs, and semen donations for the presence of anti-HCV (Jackson and Cheung, 2004). Development of an effective vaccine is hampered by the extensive genetic and antigenic diversity among different HCV strain (Walsh and Alexander, 2001).

c) HDV
Since HDV is dependent on HBV for replication, control of HDV infection is achieved by targeting HBV infections; all measures aimed at preventing the transmission of hepatitis B will prevent HDV transmission. Immunoprophylaxis against HDV is achieved by vaccination against HBV because HDV uses the envelope proteins of HBV. This mode of prevention is possible only for co-infections in HBV susceptible individuals (Previsani and Lavanchy, 2001). Immunoglobulin (Ig) and HB vaccine don’t protect HBV carriers from infection with HDV. Prevention of HBV-HDV super infection can only be achieved through education to reduce risk behaviors (Carpenter and Ticehurst, 2000).

1.2.9. Prevention of Occupational Transmission
Adherence to universal or standard precautions remains the primary means of preventing occupational exposures and thus of reducing occupational risk of acquiring infection with blood borne pathogens. Education of staff is a major component of primary prevention. All healthcare workers should understand the presence and magnitude of occupational risks, as well as methods of preventing exposures; this information needs to be periodically reinforced and updated.
(Beekmann et al., 2005). All healthcare institutions have an obligation to review occupational exposure data on an ongoing basis to determine specific factors that could be modified to decrease risk. Hospitals have to invest wisely in protective equipment and other engineering controls, provide active administrative support in terms of emphasizing the importance of safety within the institution, ensure adequate infection control personnel staffing as well as reasonable workloads, and mandate educational sessions that focus on barriers to compliance identified within their institution (Jackson and Cheung, 2004). Management commitment to occupational health is important for prevention. The commitment to safety can be demonstrated through allocation of necessary resources and delegation of authority to a needle stick prevention committee charged with monitoring the exposure control plan and the evaluation and selection of control measures including safer needle devices (Wilburn, 2004).

Health care personnel should be educated to report occupational exposures immediately after they occur, particularly because HBIG and hepatitis B vaccine are most likely to be effective if administered as soon after the exposure as possible (Mikulich and Schriger, 2002). Health care personnel who are at risk for occupational exposure to blood borne pathogens should be familiarized with the principles of post exposure management as part of job orientation and ongoing job training. Post exposure prophylaxis (PEP) remains the second line of defense in instances in which primary prevention fails to prevent occupational exposures (Beekmann et al., 2005). For susceptible HCWs who are exposed to HBV-positive blood, HBIG should be administered within 24 hours for passive prophylaxis, and the hepatitis B vaccine should be offered for active immunity (CDC, 2001). Post exposure management for HBV is individualized based on several parameters (Table 1.2.)

Individuals with an occupational exposure to hepatitis C should seek immediate medical attention. In the setting of a significant exposure (e.g., an accidental needle stick or mucosal exposure), the source should be tested for hepatitis C via the aforementioned tests. Source patients with positive antibody screens should have confirmatory testing for antibodies to the hepatitis C virus. Individuals exposed to a source with a positive hepatitis C status should have baseline testing for hepatitis C antibodies and serum transaminase levels, with repeat assays 6 months later. There are no data to suggest that treatment during this 6-month window period.
leads to any long-term improvement morbidity or mortality benefit (Shehab, 2004). No clinical trials have been conducted to assess post exposure use of antiviral agents (e.g., interferon with or without ribavirin) to prevent HCV infection the US Food and Drug Administration do not approve antiviral drugs for this indication (Mikulich and Shriger, 2002).
Table 1.2. Post Exposures Management of HBV (Adapted from Mikulich and Schriger, 2002)

<table>
<thead>
<tr>
<th>Vaccination and Response Status of Exposed Workers*</th>
<th>Source HBsAg Positive</th>
<th>Source HBsAg Negative</th>
<th>Source Unknown or Not Available for Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unvaccinated</td>
<td>HBIG† H 1 and initiate hepatitis B vaccine series</td>
<td>Initiate hepatitis B vaccine series</td>
<td>Initiate hepatitis B vaccine series</td>
</tr>
<tr>
<td>Previously vaccinated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Known responder‡</td>
<td>No treatment</td>
<td>No treatment</td>
<td>No treatment</td>
</tr>
<tr>
<td>Known nonresponder§</td>
<td>HBIG H 1 and initiate revaccination or HBIG H 2</td>
<td>No treatment</td>
<td>If known high-risk source, treat as if source were HBsAg positive</td>
</tr>
<tr>
<td>Antibody response unknown</td>
<td>Test exposed person for anti-HBs¶</td>
<td>No treatment</td>
<td>Test exposed person for anti-HBs</td>
</tr>
<tr>
<td></td>
<td>1. If adequate,‡ no treatment is necessary</td>
<td></td>
<td>1. If adequate, no treatment is necessary</td>
</tr>
<tr>
<td></td>
<td>2. If inadequate,§ administer HBIG H 1 and vaccine booster</td>
<td></td>
<td>2. If inadequate, administer vaccine booster and recheck titer in 1 to 2 months</td>
</tr>
</tbody>
</table>

Persons who have previously been infected with HBV are immune to re-infection and do not require PEP. † Dose is 0.06 mL/kg intramuscular. ‡ A responder is a person with adequate levels of serum antibody to HBsAg; § A non-responder is a person with inadequate response to vaccination; The option of giving 1 dose of HBIG and reinitiating the vaccine series is preferred for non responders who have not completed a second 3-dose vaccine series. For persons who previously completed a second vaccine series but failed to respond, 2 doses of HBIG are preferred. ¶ Antibody to HBsAg.
1.2.10. Relevance of the Study

The risks for occupational infection with blood-borne pathogens have been a source of concern among health professionals because of their frequent and often substantial exposures to patient blood and body fluids (Willy et al., 1990). Assessing the level and type of exposure is important to establish, a strategy to prevent or reduce occupational exposures, to devise a protocol for management of such exposures including post exposure management. This focusing policy on health care worker health and safety has the additional benefit of improving quality of patient care and reducing risks to patients who are cared for in and suffer from the same environmental contaminants (Wilburn, 2004). Occupational exposure to blood and body fluid by cutaneous and mucocutaneous contact and percutaneous injury among health professionals has received little attention in Ethiopian setting. Besides, the frequency with which adverse exposures to blood and body fluids are experienced by health professionals have not been documented in Ethiopia. It becomes therefore essential for this study to determine the level and type of adverse exposure to blood and other body fluids.

Transmission of at least 20 different pathogens by needle stick and sharps injuries has been reported including HIV, HBV, and HCV all of which are associated with significant morbidity and mortality (Beltrami et al., 2000; Dement et al., 2004; Ippolito et al., 1999). Infection surveillance, which defines the distribution and intensity of exposure within the health professionals, will help determine priorities for allocation of resources and measures to be taken in tackling occupation related infections. The results might also be helpful for the establishment of a scientific, reasonable and economic vaccination program against HBV infection in the Health professionals. Since there was no previous study conducted in Ethiopia, which describes the intensity and distribution of HBV, HCV and HDV among health professionals, this study was undertaken to address these gaps.

There has been concern about a subgroup of individuals with the serological pattern of ‘anti-HBc only’ in which anti-HBc is the only detectable HBV marker in the absence of HBsAg or anti-HBs (Grob et al., 2000; Weber et al., 2001). Currently, limited studies have been performed concerning the epidemiology of individuals with "anti-HBc alone" and suspected chronic HBV infection. The result will have implications in the diagnosis and treatment of individuals
furthermore it will have an input in the screening process of blood for transfusion. Hence this study aims to have baseline data on the prevalence of anti-HBc alone for future investigation.

During the long course of chronic HBV infection, a large proportion of individuals achieve significant reduction in HBV replication associated with loss of the HBeAg. Nevertheless, several HBeAg negative subjects have been found to have persistent or intermittent high HBV replicative activity, which is constantly associated with chronic liver necro-inflammation and progressive fibrosis. This form of chronic hepatitis B (CHB) is also referred to as HBeAg-negative CHB and has been shown to be mostly associated with the selection of replication competent pre-core HBV mutants that, contrary to the wild type HBV, are unable to produce HBeAg (Papatheodoridis and Hadziyannis, 2001). This study aims to generate baseline data on HBe negative chronic hepatitis since this knowledge regarding mutant species will aid us in better understanding the nature of HBV infection and in delivering better care for patients.
1.3. OBJECTIVES OF THE STUDY

General objective

3. To determine the seroprevalence of hepatitis B, C and D viruses infection among health professionals in Addis Ababa, Ethiopia.

Specific objectives

4. To assess the magnitude, frequency and type of adverse exposure to blood and other body fluids
5. To assess the different serologic and molecular profile of HBV infection
6. To measure the level of protective antibody to HBV
7. To identify risk factors for hepatitis B, C and D virus infections
CHAPTER II: MATERIALS AND METHODS

2.1. Study Design and Area
A cross sectional study was conducted from March 2005 through December 2005 to assess the magnitude of Hepatitis B, C and D virus infections and identify risk factors among health professionals at Tikur Anbassa University Hospital and Ras Desta Damtew Memorial Hospital in Addis Ababa, Ethiopia. Tikur Anbassa hospital is a University based teaching hospital that serves as referral center for hospitals all over the country and Ras Desta Damtew memorial hospital is a regional hospital serving the population of Addis Ababa.

2.2. Study Subjects
Different groups of health care workers (n=267): physicians (118), nurses (111), health assistants (13), laboratory technicians (13), pharmacists (5), and radiographers (7), working during the study period in the different department of Tikur Anbassa University Hospital and Ras Desta Damtew Memorial Hospital were approached at sites of convenience, notification of the study is given, volunteer subjects were asked to complete a questionnaire, and offered anonymous testing for HBV, HCV and HDV infections. The hospitals were selected based on convenience from teaching and non-teaching hospitals in Addis Ababa. Sample size was calculated by taking overall Hepatitis B infection prevalence among health professionals as 76% (Tsega et al., 1986), 5% level of significance and 5% margin of error

\[
n = \frac{NZ^2 P(1 - P)}{W^2 (N-1) + Z^2 P(1-P)} = 267
\]

Where: n=sample size required
Z= the standard normal deviation corresponding to 95% confidence level
P=proportion in the target population to have HBV
W= margin of error
N= sampling population

2.3. Data Collection
Objectives and procedural details of the study were explained to each participant and written informed consent was obtained before enrollment (Appendix II). Study subjects were assured that
all information obtained would be treated with utmost confidentiality. A standardized questionnaire containing all appropriate demographic and medical information was self administered and checked for completeness by the investigator. The questionnaire that anonymously queried health care personnel includes demographic data and risk assessment data (Appendix I)

2.4. Collection, Handling and Transport of Specimens
Ten ml of venous blood were collected from peripheral vein using aseptic technique from each informed and consented health care worker. The sample blood was then labelled with unique identification number. The sera were separated from clotted blood by centrifugation at 4,000 RPM (Revolution per minute) for 10 minutes. Serum samples were kept frozen in duplicate aliquots and stored in vials at -20\(^\circ\)C until tested for serology and molecular characterization.

2.5. Processing of Specimens
An aliquot of sera were thawed and tested for the presence of HBsAg, anti-HBc, anti-HCV, anti-HDV, HBV DNA, HBeAg, anti-HBs, RIBA and HCV RNA. The tests are done in series and parallel (Figure 2.1). All serological tests were performed using commercially available ELISA and MEIA. Positive and negative controls were included in each serological assay to ensure reagent stability and correct performance of the assay.

**Figure 2.1. Flow chart of Serological and Molecular Diagnosis of HBV, HCV, and HDV Infections**
2.6. Serology

2.6.1. HBV

a) HBsAg

An aliquot was thawed and serum was assayed for HBsAg by ELISA using a commercial kit (Hepanostika HBsAg Uni-form II, Biomerieux bv, The Netherlands). HBsAg Uni-form II is an ELISA based on one-step "sandwich" principle. Each well is coated with anti-HBs (murine) monoclonal against HBsAg (subtypes ad and ay) and contains a lyophilized horseradish peroxidase (HRP) labeled anti-HBs (ovine) conjugate sphere. The samples (100:l) and positive and negative controls (100:l) were transferred into the wells, agitated on micro shaker for 15 seconds and incubated for 1 hour at 37°C. The contents of the well were soaked and washed with diluted washing solution. Substrate tetramethylbenzidine (TMB) solution was added to each well and incubated at room temperature for 30 minutes. After incubation 1M sulphuric acid was added to stop the reaction. The absorbance values were read at 450/655 nm using a Bio-rad spectrophotometer. At the end of the test, samples with absorbance values greater or equal to the cut-off were initially considered reactive. If positive results were obtained, retesting was done in duplicate. A positive result obtained on retesting of the sample was considered as positive for HBsAg.

b) Anti-HBc

All serum samples were subjected to anti- HBc Ab detection test. Serum was assayed for anti-HBc by a commercial ELISA test (Enzygnost Anti-HBc monoclonal, Dade Behring Inc, USA). Enzygnost Anti-HBc monoclonal is competitive one-step enzyme immunoassay. The wells of the micro titration plate are coated with HBcAg. 25 :l of sample serum and controls (positive and negative) were pipetted into micro well plate followed by adding of 100 :l peroxidase-conjugated monoclonal anti-HBc. The microplate was then incubated for 1 hour at 37°C. The contents of the well were washed and soaked with diluted washing solution. There after, the microplate was blotted on absorbent tissue to remove any excess liquid. After adding 100:1 of substrate chromogen TMB, the plate was again incubated for 30 minutes in dark at room temperature. To stop the reaction the same volume of stopping solution, 0.5N sulphuric acids was added. Absorbance was measured with a photometer at 450 nm and reference wavelength of 655 nm. A
sample with absorbance \(<\) Cut-off-10\% was interpreted as positive. Cut-off-10 \% \(\leq\) absorbance of sample \(\leq\) cut-off+10\% was interpreted as equivocal. Equivocal results were re-tested in duplicate.

c) **Anti-HBs**

Quantitative testing for anti-HBs was performed by use of Abbott AxSYM® AUSAB MEIA test kits (Abbott, Diagnostics Division, Germany). Samples, calibrators, positive and negative controls and all AxSYM® AUSAB reagents required for one test were pipetted by the Sampling Probe to various wells of a reaction vessel (RV) and in the Sampling center. The RV is immediately transferred into the processing center and further pipetting was done in the processing center by the processing probe. Samples and recombinant Hepatitis B virus surface antigen (Subtypes ad and ay) coated microparticles were combined in one reaction vessel. A portion of the reaction mixture was transferred to the matrix cell as a result the microparticles bind irreversibly to the glass fiber matrix. There after, biotinylated rHBsAg was dispensed on the matrix cell. The anti-biotin; alkaline phosphatase conjugate was dispensed on to the matrix cell followed by washing. After washing the substrate, 4-Methyl umbelliferyl phosphate was added. The product of this reaction (fluorescence) was measured by, the MEIA optical assembly. The concentration of anti-HBs was extrapolated from the OD reading and samples with anti-HBs concentrations greater than or equal to 10.0 mIU/ml, were considered as reactive (positive).

d) **HBeAg**

HBeAg detection was done according Abbott AxSYM® system (AxSYM HBe 2.0, Abbott) and the test was based on MEIA technology. The test utilizes the principle of direct binding of the HBeAg in the sample to the anti-HBe coated on the micro particle followed by the detection of the bound HBe by the anti-HBe alkaline phosphatase conjugate. Samples, positive and negative controls and all AxSYM HBe 2.0 reagents required for one test were pipetted by the Sampling Probe in to various wells of RV. Samples, Antibody to HBeAg (mouse monoclonal) coated microparticles, and specimens diluent were combined in one RV well. Then the reaction was immediately transferred into the Processing Center and the Processing Probe did further pipetting in the Processing Center. Inside the Processing center the matrix cell was treated with wetting solution and following this a portion of the reaction mixture is transferred to the matrix cell. The microparticles bind irreversibly to the glass fiber matrix. Alkaline phosphatase conjugated
antibody to HBeAg was added on the matrix cell forming ab-ag-ab complex. Then the matrix cell was washed with the matrix cell wash. The substrate, 4-Methylumbelliferyl phosphates was added and the MEIA optical assembly measured fluorescence. The presence or absence of HBeAg in the sample was determined by calculating the cut-off value. The sample is considered to be reactive for HBeAg if the fluorescence is greater than or equal to the cut-off value. Reactive samples were repeated in duplicate, if either of the duplicate is reactive it is interpreted as positive.

2.6.2. HDV
A commercial ELISA kit (Murex anti-Delta, Abbott murex, UK) was used to detect antibody to hepatitis D virus. The test is a competitive enzyme immunoassay. Wells are coated with hepatitis delta antigen. 25:1 of sample diluent was pipetted in the beginning followed by dispensing of 75:1 of samples, controls (positive and negative) to each well. The well was incubated for 1 hour at 37°C after this HRP labeled conjugate was added and incubated for another 1 hour at 37°C. At the end of incubation the sample and any excess conjugate was washed away and immediately solution containing tetramethylbenzidine and hydrogen peroxide was added. The well was further incubated for 30 minutes at 37°C. Finally 50 :1 of stopping solution was added (sulphuric acid). Absorbance was read at 450 nm using 620 nm as reference wavelength. Samples with an absorbance greater than the cut-off value were considered non-reactive and samples giving an absorbance less than or equal to the cut-off were considered reactive.

2.6.3. HCV
a) Anti-HCV
Commercial ELISA kit (Monolisa Anti-HCV plus version 2, BIO-RAD, France) was used to detect Anti-HCV Ab. Monolisa Anti-HCV plus version 2 is based upon the use of a solid phase prepared with purified antigens: 3 recombinant proteins produced by E. coli from clones selected in the non structural area (NS3 and NS4) and in the structural area of the hepatitis C virus genome. Detection is with goat anti-human IgG antibody purified by affinity chromatography and coupled to peroxidase. The performance of the test includes the following reaction steps: After the diluents were pipetted samples and controls (positive and negative) were added to the wells. The microplate was then incubated for an hour at 37°C. Following incubation the contents
of all wells were aspirated. Conjugate solution were added and incubated for 30 minutes at 37°C then followed by repeat washing. TMB substrate solution was quickly added and incubated in dark at room temperature. To stop the reaction 1N sulphuric acid was dispensed to all wells. The absorbance values were read at 450/655 nm using a Bio-Rad spectrophotometer. Samples with an optical density higher than or equal to the cut-off value were considered to be initially reactive. Initially positive samples were retested in duplicate and defined as positive if at least one of the re-test was positive.

b) Recombinant Immunoblot Assay (RIBA)

All specimens that were repeatedly positive for antibodies against HCV by ELISA were confirmed using RIBA (recomBlot HCV IgG 2.0, MIKROGEN GmbH, Germany). This test is a qualitative test for the determination of IgG antibodies against HCV. The test principle is based on five different purified recombinant antigens separated by molecular weight via SDS polyacrylamide gel electrophoresis (SDS-PAGE). These antigens are subsequently transferred to nitrocellulose membrane electrophoretically (western blotting). Free binding sites on the membrane are saturated with a solution of proteins. Following this matrix is washed and cut into strips. The procedure was done as follows, to facilitate detection of HCV–specific antibodies; the strips were incubated for 1 hour with the diluted serum sample (20:1), where by the antibodies bind to the antigens on the strips. Unbound antibodies were washed away and the strips were incubated in a second step with anti-human IgG coupled with HRP. The strips were incubated for another 45 minutes followed by another washing step. Specifically bound antibodies were detected by means of a colour reaction catalyzed by the peroxidase after adding a substrate solution, TMB. If a reaction against one of the HCV- specific proteins had taken place, a dark band appears at the corresponding locus on the strip. As reaction control, a band with anti-human immunoglobulin was also applied, at the upper ends of the strips that must show a reaction to every serum. Weak positive and negative controls were included in the test run. The test result was obtained by, assessing the band intensities and comparing with a weak positive control. This result was obtained by the addition of the point values of the separate bands, that were determined as $\pm$, + or ++. Results were interpreted as questionable, positive or negative.
2.7. Molecular Methods

2.7.1. HBV DNA Extraction and Detection

The HBV DNA test was a quantitative test. The detection and quantitation of HBV DNA was performed by real-time PCR using the LightCycler (Roche Diagnostics, Germany) based on fluorescence resonance energy transfer (FRET) principle. Nucleic acid was extracted from 200:1 serum and Digene Calibrator 5 (HBV plasmid DNA) using the QIAamp DNA Blood Mini kit (Qiagen) according to the manufacturer's protocol as described by others (Ho et al., 2003). A set of primers for the HBV core region was used which yielded a 130 bp product. The donor fluorescein probe and the acceptor LightCycler-Red 640(LCRed) probe directed to the 130 bp product were used. A reaction mix was made of water, BSA, nucleotides, 10 fold reaction buffers, magnesium chloride, primer sense, primer antisense, a pair of hybridization probe (labeled with fluorescein and LC-Red 640 Roche), and HotStart Taq polymerase and to these 15ul, 5ul of DNA (or water as negative control) were added to each capillary. The PCR protocol consisted of an initial step at 95°C for 10 min for the activation of the Taq DNA polymerase, followed by 45 cycles denaturation at 95°C, 12 s annealing at 60°C and 12s extensions at 72°C. In each cycle, the hybridization probes will bind to the amplified target. If the two hybridization probes specifically bind to the target, they will bind adjacent to each other. Only the fluorescein labeled probe can be excited by an external light source, the LC-Red probe cannot be excited by the external light. If the probe binds adjacent to each other FRET (fluorescence resonance energy) transfer takes place. The fluorescein was excited by the external light source. It transfers the energy to the LightCycler Red probe that itself excites a specific light that can be measured by the Light cycler. The amount of excited light is “equivalent’’ to the amount of PCR-Product, so that it enables absolute quantification. Fluorescence data were acquired once each cycle at the end of the annealing phase. The probe was designed to be 100% specific. It detects as low as 250 copies/ml.

2.7.2. HCV RNA Extraction and Detection

A real time RT-PCR based on TaqMan technology (fluorescence- based real-time PCR) was employed (m2000rt, Abbott). Viral RNA was extracted from 140 :1 of each serum sample by the QIAamp viral RNA purification protocol (Qiagen) and was dissolved in 50:1 of RNase-free water. The assay utilizes two distinct set of primers, a set specific for HCV and another set
specific for the internal control. The HCV primers target 5’ UTR region of the HCV genomes. The IC primer sequences are unrelated to the analyte sequences. Short single stand probes, anti-HCV specific probe and an internal control specific probe each labeled with a different fluorophore at the 5’ end and a fluorescent quencher at the 3’ end were used to detect amplified products at each cycle. Internal control was added to each sample including control and calibrators at the start of sample preparation. Internal control was used to evaluate sample validity and function as a check for recovery and inhibition. Armored RNA was used as the calibrator and control material. Assay calibrators were processed through the same sample preparations as specimens. One negative control and two positive controls were run on each run and processed through the same sample procedure as specimens. The reaction mixture for RT-PCR was prepared in a single tube as follows: reaction buffer, magnesium chloride, primer sense, primer antisense, each deoxynucleotide triphosphate, reverse transcriptase, Taq polymerase, and fluorogenic probe. Forty microliters of the reaction mixture was added to the PCR tubes containing 10:1 of RNA from serum or calibrators. The PCR profile involves four stages: reverse transcription, low stringency PCR, high stringency PCR and detection. HCV was reverse transcribed into cDNA (30 min at 59°C) and amplified by PCR in a single tube.

2.8. **Statistical Analysis**

Data were anonymously coded and entered into a standard spreadsheet program before being analyzed by statistical software. Basic statistics like proportion, prevalence were calculated. HBsAg, anti-HBc, anti-HBs, and anti-HCV were taken as dependent variable, with various other items (such as gender, duration of service, and type of profession) as the independent variables. A univariate logistic regression model showing likelihood of detecting either HBsAg or anti-HBc seropositive status was analyzed to detect variables predicting significant differences. Univariate analysis included calculation of odds ratios (ORs) with 95% confidence intervals (95% CI), for hepatitis B infection by each variable of interest, including sex, age, health care worker type, vaccination status, duration of service, number of needle stick and sharp injury. Chi-Square or Fisher’s exact test was applied to test whether differences between values were significant. A multiple logistic regression model was built using those variables that were found to be both significant by univariate analysis and potentially meaningful confounders of association. Results were expressed as odds ratios (OR), and probability (P) values. A two sample t-test was also
used to compare the mean age of hepatitis infected and non-infected subjects. P values < 0.05 were considered as statistically significant. Epi info version 2000 (CDC, Atlanta, Georgia, USA) was used for statistical analysis.

2.9. Ethical Consideration
This M. Sc research project has been approved by the Department Graduate Committee, the Faculty Research Publications Committee and endorsed by the Faculty Academic commission and has been ethically cleared. Written informed consent was obtained from all study subjects participating in the study (see Appendix II). The study did not have any harm except the minimal risk associated with blood drawing. The result of the research was kept confidential. Confidentiality was maintained by numeric coding of serum samples and questionnaires. Results were communicated to the respective individuals with explanations of their meaning, and an offer of proper counseling.
CHAPTER III: RESULTS

3.1. Study Subjects
A total of 267 health care workers’ (HCW’s) were investigated for HBV, HCV and HDV infections between March and December 2005 in Tikur Anbassa University Hospital and Ras Desta Damtew Memorial Hospital, Addis Ababa, Ethiopia. The age and sex distributions of 267 HCW’s and other parameters are shown in Table 3.1. The mean age of HCW’s was 32± 9.2 years (range 19-59 years). The majority of HCW’s (67.8%) were between the age of 19 and 34 years. Males account 143(53.6%) and females account the remaining 124 (46.4%) of the study subjects, resulting in overall male to female ratio of 1.15:1. Of the 267 HCW’s, 227(85%) were from Tikur Anbassa University Hospital, and the remaining 40(15%) were from Ras Desta Damtew Memorial Hospital. Of the 267 HCW’s 156 (58.4%) were married, and 107(40.1%) were single, whereas the remaining 4(1.5%) were divorced and/or widowed. The professional distribution of the 267 HCW’s is summarized in Table 3.1. Physician, nurses, health assistant, laboratory technicians, pharmacists and radiographers accounted for 118 (44.2%), 111(41.6%), 13(4.9%), 13(4.9%), 5(1.8%), and 7(2.6%), respectively. The duration of service varied between less than one year and more than 20 years. Physical examination was not performed on the study subjects. Past and present history of sexually transmitted history was not enquired and documented and 18 (6.7%) of the study subjects were jaundiced previously.
Table 3.1. Demographic Characteristics of 267 HCW’s Investigated for HBV, HCV, and HDV Infections

<table>
<thead>
<tr>
<th>Variables</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age group</strong></td>
<td></td>
</tr>
<tr>
<td>19-24</td>
<td>63 (23.6)</td>
</tr>
<tr>
<td>25-29</td>
<td>67(25.1)</td>
</tr>
<tr>
<td>30-34</td>
<td>51(19.1)</td>
</tr>
<tr>
<td>35-39</td>
<td>25 (9.4)</td>
</tr>
<tr>
<td>40-44</td>
<td>28 (10.5)</td>
</tr>
<tr>
<td>45-49</td>
<td>12 (4.5)</td>
</tr>
<tr>
<td>50-54</td>
<td>18 (6.7)</td>
</tr>
<tr>
<td>55-59</td>
<td>3 (1.1)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>143(53.6)</td>
</tr>
<tr>
<td>Female</td>
<td>124(46.4)</td>
</tr>
<tr>
<td><strong>Marital Status</strong></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>107(40.1)</td>
</tr>
<tr>
<td>Married</td>
<td>156(58.4)</td>
</tr>
<tr>
<td>Divorced</td>
<td>2(0.75)</td>
</tr>
<tr>
<td>Widowed</td>
<td>2(0.75)</td>
</tr>
<tr>
<td><strong>Type of Profession</strong></td>
<td></td>
</tr>
<tr>
<td>Physician</td>
<td>118 (44.2)</td>
</tr>
<tr>
<td>Nurses</td>
<td>111(41.6)</td>
</tr>
<tr>
<td>Health assistant</td>
<td>13 (4.9)</td>
</tr>
<tr>
<td>Laboratory Technician</td>
<td>13(4.9)</td>
</tr>
<tr>
<td>Pharmacist</td>
<td>5 (1.8)</td>
</tr>
<tr>
<td>Radiographer</td>
<td>7 (2.6)</td>
</tr>
<tr>
<td><strong>Duration of Service (in years)</strong></td>
<td></td>
</tr>
<tr>
<td>&lt; 1</td>
<td>49 (18.4)</td>
</tr>
<tr>
<td>1-5</td>
<td>85 (31.8)</td>
</tr>
<tr>
<td>6-10</td>
<td>55 (20.6)</td>
</tr>
<tr>
<td>11-15</td>
<td>22 (8.2)</td>
</tr>
<tr>
<td>16-20</td>
<td>19 (7.1)</td>
</tr>
<tr>
<td>&gt;20</td>
<td>37 (13.9)</td>
</tr>
</tbody>
</table>

3.2. Exposure to Risk Factors

Of the 267 HCWs, 53 (19.9 %) had either a history of minor or major operations and, 10 (3.7%) had received blood transfusion. Among 267 health care workers only 108(40.4%) of them used glove consistently. Frequency of occupational exposure to risk factors in HCW’s is summarized in Table 3.2. Of the 267 HCW’s, 221(82.8%) had at least a one time history of cutaneous exposure to blood, 165(61.8 %) of health care workers had at least a one time history of
cutaneous exposure to any of the body fluids, and 162 (60.7%) had splashed blood or body fluids in their faces. One hundred and fifty eight (59.2%) and 83 (31.1%) had a history of needle stick injury and sharp injury respectively.

Table 3.2. Frequency of Occupational Exposure to Risk Factors in 267 HCW’s from Tikur Anbessa and Ras Desta Damtew Memorial Hospitals, Addis Ababa, Ethiopia

<table>
<thead>
<tr>
<th>Type of exposure</th>
<th>All categories No. (%)</th>
<th>Phys. n=118</th>
<th>Nurse n=111</th>
<th>HA n=13</th>
<th>LT n=13</th>
<th>RG. n=7</th>
<th>Phar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Needle stick injury</td>
<td>158 (59.2)</td>
<td>79 (67)</td>
<td>59 (53.2)</td>
<td>12 (92.3)</td>
<td>7 (53.8)</td>
<td>1(14.3)</td>
<td>-</td>
</tr>
<tr>
<td>Sharp injury</td>
<td>83 (31.1)</td>
<td>32 (27)</td>
<td>38 (34.2)</td>
<td>7 (53.8)</td>
<td>5 (38.5)</td>
<td>1(14.3)</td>
<td>-</td>
</tr>
<tr>
<td>Cutaneous blood exposure</td>
<td>221(82.8)</td>
<td>111 (94)</td>
<td>86 (77.5)</td>
<td>13 (100)</td>
<td>9 (69.2)</td>
<td>2(28.6)</td>
<td>-</td>
</tr>
<tr>
<td>Cutaneous body fluid exposure</td>
<td>165 (61.8)</td>
<td>92 (78)</td>
<td>56 (50.5)</td>
<td>10 (76.9)</td>
<td>7 (53.8)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mucocutaneous exposure blood or body fluid</td>
<td>162 (60.7)</td>
<td>83 (70.3)</td>
<td>66 (59.5)</td>
<td>9 (69.2)</td>
<td>2 (15.4)</td>
<td>2(28.6)</td>
<td>-</td>
</tr>
</tbody>
</table>

Phys: Physician; HA: Health assistant; LT: Laboratory Technician; RG: Radiographer; Phar: Pharmacist

3.3. HBV and HDV

3.3.1. Magnitude and Distribution of Hepatitis B Infection by Age, Sex and Occupation

The assessment of hepatitis B infection was made by detecting either anti-HBc antibody or HBsAg. The presence of either of these two markers was taken as measure of infection. One hundred and twenty nine (48.3%) of the study subjects were positive for anti-HBc and 8(3%) of the study subjects had HBsAg as the only marker of Hepatitis B infection. The overall seroprevalence of HBV infection was found to be 51.3% (95% CI: 45-57). The sex specific prevalence rate was higher in males (56.6%; 95% CI: 51-62.5) than females (45.2%; 95% CI: 39-51) but the difference did not reach statistical significance (p= 0.079). Hepatitis B infection by specific age group was highest in the age group 55-59(100%) and lowest in the age group 19-24 (Table 3.3).
Table 3.3. Seroprevalence of HBV Infection among HCW’s (n=267) by Age and Sex

<table>
<thead>
<tr>
<th>Age group (in years)</th>
<th>Sex</th>
<th>Total No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males No. (%)</td>
<td>Females No. (%)</td>
</tr>
<tr>
<td>19-24</td>
<td>11/17 (64.7)</td>
<td>13/46 (28.3)</td>
</tr>
<tr>
<td>25-29</td>
<td>17/41 (41.5)</td>
<td>9/26 (34.6)</td>
</tr>
<tr>
<td>30-34</td>
<td>23/43 (53.4)</td>
<td>5/8 (62.5)</td>
</tr>
<tr>
<td>35-39</td>
<td>10/14 (71.4)</td>
<td>6/11 (54.5)</td>
</tr>
<tr>
<td>40-44</td>
<td>14/18 (77.8)</td>
<td>7/10 (70)</td>
</tr>
<tr>
<td>45-49</td>
<td>4/5 (80)</td>
<td>4/7 (57.1)</td>
</tr>
<tr>
<td>50-54</td>
<td>3/5 (60)</td>
<td>10/13 (76.9)</td>
</tr>
<tr>
<td>55-59</td>
<td>0/0 (0)</td>
<td>3/3 (100)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>82/143 (57.3)</strong></td>
<td><strong>57/124 (46)</strong></td>
</tr>
</tbody>
</table>

The hepatitis B infection prevalence increased with age (p<0.01) (Table 3.3 and Figure3.1). The mean age of Hepatitis B infected subjects was significantly different from non-infected subjects. HBV infection among the 6 group of health professionals showed the highest rate among health assistants (85.7%) followed by radiographers (71.4%), pharmacists (60%), physicians (50%), nurses (46.5%) and the lowest rate was found among laboratory technicians (42.9%).

Figure 3.1. Percentage Seropositive to HBV Infection by Age Group
3.3.2. Hepatitis B Surface Antigen Distribution by Age, Sex, and Occupation

Among the 267 HCW’s screened for HBsAg, 26 (9.7%; 95% CI, 6-13.2) were found to be positive. Of these 17(65%) were males and 9(35%) were females. The prevalence was higher in males than females but the difference did not reach statistical significance (11.9% vs. 7.3%, OR=1.72, P=0.2). The hepatitis B surface antigen prevalence increases from 6.3% in the age group 19-24 to reach a peak in the age group 40-44 years (18%) then progressively decline to 0% in the age group 55-59 years. There is statistically significant difference in HBsAg prevalence across various age groups (p<0.000) (Figure 3.2). The hepatitis B chronic carrier rate varies across occupational category; 13.4%, 7.9%, 0, 0, 14.3%, 7.1% of physicians, nurses, laboratory technician, pharmacists, radiographers, and health assistants were found to be positive for HbsAg, respectively.

Figure 3.2. Hepatitis B surface Antigen Prevalence in Different Age Groups

3.3.3. Prevalence of HBeAg, Anti-HDV and HBV-DNA among HBsAg positive subjects

Of the 26 serum samples positive for HBsAg, 2 samples were not adequate for detection of anti-HDV antibody and HBV DNA and 4 were not adequate for HBeAg detection. HBsAg positive samples were subjected to HBeAg and HBV DNA detection tests, which assess the level of viral replication and infectivity. HBeAg was detected in 1/22 (4.5%; 95% CI: 2-7%) of HBsAg positive subjects. HBV DNA was detected in 17/24 (70.8%) of HBsAg positive cases. The median HBV DNA concentration was $1.45 \times 10^4$ copies/ml and it ranges from $3.75 \times 10^3$-
8.73x10^7 copies/ml. The highest HBV DNA viral load was detected in HBeAg positive sample (8.37x10^7 copies/ml). None of the HBsAg positive HCWs was positive for anti-HDV.

### 3.3.4. Hepatitis B Immunity and Vaccination Status of Health Professionals

We chose to define immunity as the presence of anti-HBs \( \geq 10\text{mIU/ml} \), although we realize that the population with this level of antibody may not represent the entire population that is protected against disease. Among 267 subjects only 35(13%) were vaccinated to HBV. Among vaccinated, 30/35 (85.7%) received vaccination without screening for HBV exposure and immunity, while the remaining 5/35 (14.3%) were screened for HBV infection before vaccination.

Three samples were not sufficient for anti-HBs screening. Two individuals from the group vaccinated only once and in 1 individual from the group vaccinated three times. Ninety three percent of the group who were vaccinated once had protective immunity whereas only 25% of individuals vaccinated three times had protective antibody. Markers of exposure to hepatitis B virus (anti-HBc antibody) were detected from 2 individuals who were vaccinated three times and from another 6 individuals who were vaccinated once. However, none were found to be positive for HBsAg.

A total of 102 unvaccinated HCW’s positive for anti-HBc and negative for HBsAg were screened for anti-HBs. Of these, 82/102 (80.4%) had protective antibody to hepatitis B virus (anti-HBs \( \geq 10\text{mIU/ml} \)), 14(13.7%) had anti-HBs but less than 10mIU/ml and 6(5.9%) did not have any measurable anti-HBs. Of the 82 unvaccinated HCWs positive for anti-HBs, 51(35.7%) males and 31(25%) females were found to be positive for anti-HBs. The highest level of immunity was found in the age group 55-59 years (100%) where as the lowest level was in the age group 19-24(19.3%) (Table 3.4).
Table 3.4. Prevalence of anti-HBs in the Unvaccinated Study Population

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>82/232 (35.3)</td>
</tr>
<tr>
<td>Age group ($n = 82$)</td>
<td></td>
</tr>
<tr>
<td>19-24</td>
<td>13 (20.6)</td>
</tr>
<tr>
<td>25-29</td>
<td>15 (22.3)</td>
</tr>
<tr>
<td>30-34</td>
<td>16 (31.4)</td>
</tr>
<tr>
<td>35-39</td>
<td>10 (40)</td>
</tr>
<tr>
<td>40-44</td>
<td>14 (50)</td>
</tr>
<tr>
<td>45-49</td>
<td>2 (16.7)</td>
</tr>
<tr>
<td>50-54</td>
<td>9 (50)</td>
</tr>
<tr>
<td>55-59</td>
<td>3 (100)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sex ($n = 82$)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>51 (35.7)</td>
</tr>
<tr>
<td>Female</td>
<td>31 (25)</td>
</tr>
</tbody>
</table>

3.3.5. Anti-HBC only Positive Subjects

Among the 267 HCW’s screened for different serological markers (HBsAg, anti-HBs) of HBV infection, 6 (2.2%; 95% CI: 0.4-3.96) were found to be positive only for anti-HBc and negative for other serological markers. Further investigation was done for HBV DNA (only for 3 sufficient samples) among anti-HBc positive subjects, and none of them had detectable HBV DNA in their samples.

3.3.6. Risk Factors for HBV Infection

In the univariate analysis HBsAg positive or anti-HBc positive subjects were included. Among the demographic characteristics, hepatitis B infected subjects were significantly older than hepatitis B unexposed subjects ($p<0.001$). The crude odds ratio of being hepatitis B infected indicates a greater risk in male than females (OR=1.580, 95% CI: 0.97-2.6) and a lesser risk in single individuals than married (OR=0.561, 95% CI: 0.340-0.924). Occupation was also subjected for univariate analysis and there was no statistical significant association between occupation and hepatitis B infection (Table 3.5).
Table 3.5. Univariate and Multivariate Analysis of Risk Factors for HBV Infection

<table>
<thead>
<tr>
<th>Variables</th>
<th>Odds ratio  (95% CI)</th>
<th>Adjusted Odds ratio‡</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>-</td>
<td>-</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>0.561</td>
<td>1.461</td>
<td>0.282</td>
</tr>
<tr>
<td>Married</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1.58 (0.97-2.6)</td>
<td>1.5</td>
<td>0.078</td>
</tr>
<tr>
<td>Female</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physician</td>
<td>0.18 (0.04-0.84)</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>Nurses</td>
<td>0.15 (0.03-0.7)</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>Lab Technicians</td>
<td>0.13 (0.02-0.78)</td>
<td>0.28</td>
<td>0.771</td>
</tr>
<tr>
<td>Pharmacist</td>
<td>0.25 (0.024-2.58)</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>Radiographer</td>
<td>0.42 (0.05-3.83)</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Health assistant</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sharp injury</td>
<td>1.523 (0.926-2.505)</td>
<td>0.674-1.941</td>
<td>0.097</td>
</tr>
<tr>
<td>Vaccination</td>
<td>0.259 (0.117-0.575)</td>
<td>0.151-0.788</td>
<td>0.001</td>
</tr>
<tr>
<td>Duration of service</td>
<td>0.941 (0.913-0.971)</td>
<td>0.925-1.082</td>
<td>0.321</td>
</tr>
<tr>
<td>Consistent Glove use</td>
<td>0.563 (0.342-0.925)</td>
<td>0.425-1.207</td>
<td>0.023</td>
</tr>
<tr>
<td>Operation</td>
<td>1.383 (0.753-2.542)</td>
<td>0.407-1.601</td>
<td>0.296</td>
</tr>
<tr>
<td>Blood transfusion</td>
<td>1.644 (0.470-5.753)</td>
<td>0.331-4.534</td>
<td>0.437</td>
</tr>
<tr>
<td>Jaundice</td>
<td>3.198 (1.015-10.078)</td>
<td>0.704-7.667</td>
<td>0.047</td>
</tr>
<tr>
<td>Tattooing</td>
<td>1.193 (0.578-2.460)</td>
<td>0.281-1.438</td>
<td>0.633</td>
</tr>
</tbody>
</table>

‡ Adjusted for age

On univariate analysis being vaccinated (OR=0.259, 95% CI: 0.117-0.575), consistent glove use (OR=0.563; 95% CI: 0.342-0.925), and history of jaundice (OR= 3.198; 95% CI: 1.015-10.078), were found to be significantly associated with hepatitis B infection, however, sharp injury (OR= 1.523; 95% CI: 0.93-2.51), operation (OR= 1.38; 95% CI: 0.753-2.54), blood transfusion (OR=1.64; 95% CI: 0.47-5.75), and tattooing (OR= 1.19; 95% CI: 0.58-2.46) were not found to be statistically significantly associated with hepatitis B infection. After these factors were adjusted for age, only being vaccinated remained a statistically significant risk factor for hepatitis B infection (Table 3.5.).
3.4. HCV Infection Prevalence among the Study Subjects

Samples were initially tested with second generation ELISA and only 2 individuals (0.7%; 95% CI: 0.3-1.7) were found to be positive with this screening assay. The positive sera were further analyzed by RIBA and RT-PCR in parallel to confirm the initial screening results. Only one sample was found to be positive by both RIBA and RT-PCR test. The Hepatitis C positive subject (positive to the three tests) did not have any history of needle stick, sharp injury, blood transfusion or operation. The only possible risk factor identified was a long time history of tattooing.
CHAPTER IV: DISCUSSION

Hepatitis B is one of the major diseases of mankind and is a serious global public health problem. Of the 2 billion people who have been infected with the HBV, more than 350 million have chronic (lifelong) infections. HBV infections result in 500,000 to 1.2 million deaths per year caused by chronic hepatitis, cirrhosis, and hepatocellular carcinoma (McMahon, 2005, Kidd-Ljunggren et al., 2004; Lavanchy, 2004; Margolis et al., 1991). The World Health Organization estimates 170 million individuals worldwide are infected with hepatitis C virus. Globally, the infection has an estimated prevalence of 3% (WHO, 1999). Hepatitis delta virus is present worldwide and in all age groups. It has been estimated that 18 million people are infected with this virus amongst the 350 million carriers of the HBV around the world (Fonseca, 2002). HCW’s are potentially exposed to blood and body fluids containing transmissible diseases and are at increased risk to acquire these pathogens (Catalani, et al., 2004). The risks for occupational infection with blood-borne pathogens have been a source of concern among health professionals because of their frequent and often substantial exposures to patient blood and body fluids (Willy et al., 1990). Assessing the level and type of exposure to blood, body fluid and these blood borne pathogens is important to establish a strategy to prevent or reduce occupational exposures, to devise a protocol for management of such exposures including post exposure management.

A cross sectional study was conducted from March, 2005 through December, 2005 to assess the magnitude of Hepatitis B, C and D virus infections and identify risk factors of these infections among health professionals at Tikur Anbassa University Hospital and Ras Desta Damtew Memorial Hospital in Addis Ababa, Ethiopia. Different groups of health care workers (n=267): physicians (118), nurses (111), health assistants (13), laboratory technicians (13), pharmacists (5), radiographers (7) were investigated. The study has generated a detailed and comprehensive epidemiological, serological, and molecular data for various groups of health professional for the three parenterally transmitted hepatitis viruses: Hepatitis B, C, and D viruses. The sample population has encompassed all groups of health care workers representing the various age groups (mean age 32±9.26), a good representation of the sex composition (M: F; 1.15:1), and greatly differing duration of service (0-36) years.
It was found out that 18(6.7%) subjects had a one-time history of jaundice but it was very difficult to find out to which hepatitis viruses they were exposed as none of them had any laboratory result for the jaundice. Effort was exerted to identify whether this history of jaundice was work related but no clue could be found. Exposure to blood and body fluid in this group of health care personnel is quite high reaching 82.8% for ungloved cutaneous exposure to blood. This is higher than a 64% report from Turkey (Azap et al., 2005). The 158 (59.2%) and 83 (31.1%) overall prevalence of needle stick injury and sharp injury respectively involving all health professionals shows needle stick and sharp injuries are quite common work place accidents. This is consistent with other reports, which were done previously from different regions including Sub-Saharan African countries (Talaat et al., 2003; Kermode et al., 2005; Smith and Leggat, 2005; Nsubuga and Jaakkola, 2005). This investigation suggests that exposure to blood and body fluid and percutaneous injury is a considerable burden for health care workers. Compliance to universal precaution was assessed by consistent use of glove and only 108 (40.4%) consistently use glove. This suggests the low rate of compliance, which is in agreement with other reports from Turkey, (Ayranci and Kosgeroglu, 2004) and India (Kermode et al., 2005). No published data was found in Ethiopia, which assesses compliance to universal precaution, level of exposure to blood and body fluid, needlestick and sharp injury to make comparison with the present findings. Detailed analysis on possible risk factor for blood and body fluid exposure is not done here. Previous reports from other countries has put several possible reasons for the wide spread presence of exposure to blood and body fluid; lack of training, long working hours, working habits, and experience are some of the reasons mentioned (Nsubuga and Jaakkola, 2005). Some of these factors might also operate here but further study to investigate the possible risk factors operating in Ethiopian health care set up is appealing.

Enrollment to participate in the study was on voluntary basis removing the need of random sampling. Volunteer sampling has the potential of introducing selection bias where subjects who knew their status and high-risk group may refrain from participating in the study. However, the participation of 85% of subjects who were contacted at their place of work, the presence of excess risk factors argues against an elevated error due to selection bias. Therefore, the prevalence estimates in this study are a very likelihood estimate of HBV, HCV and HDV
infection in this group of health professionals. Comparisons of HBV infection prevalence with various studies should take into account; the variation in hepatitis B prevalence among different countries in the general population, and the difference in serological markers employed to assess infection rate. The serological markers used to assess hepatitis B infection in this study included HBsAg and anti-HBc. Any subject who was found to be positive to either marker was considered as infected. Excluding anti-HBs as a marker of exposure slightly underestimates the prevalence (Tsega et al, 1989; Abebe et al., 2003).

HBV infection prevalence was estimated to be 51.3% (95% CI: 45-57). Anti-HBc positive and HBsAg positive as, the only marker of infection accounted for 129 (48.3%) and 8(3%) of the study subjects respectively. This prevalence estimate is comparable to a population-based study done in Addis Ababa where the hepatitis B prevalence in adults was 55.7% (95% CI: 53-58.3%) (Abebe et al., 2003). However, this is in contrast to the result of another nationwide seroprevalence study where 76% prevalence rate was reported for adult population (Tsega et al., 1986). This study, however, was a hospital-based study; the setting of which might explain this very high prevalence. Another study done in Ethiopia where the study populations were blood donors in Addis Ababa found a prevalence rate of 22% which is much lower than the present finding. Markers employed in this study were HBsAg and anti-HBs, which might slightly have lowered the infection rate because it failed to include anti-HBc only positive subjects (Gebreselassie, 1983b). In general, comparison of prevalence rate is made further difficult by the difference in demographic characteristics of the study subjects in the various studies. Two reports from Ethiopia on HBV infection among health professionals are available. Tsega and his colleagues (1989) reported a hepatitis B seroprevalence rate of 72%, higher than our report. It is very difficult to have an accurate and complete explanation for this big difference in prevalence. A different demographic characteristics (higher mean age), difference in serological technique employed (radioimmunoassay) and difference in seromarkers used to measure infection, and probable change in trend of hepatitis infection alone or in combination are possible potential explanations. It was not possible to get information on the age specific prevalence rate.
Another study on the same year in Ethiopia has reported a 60% prevalence rate of infection among health professionals and has concluded that health professionals are at a higher risk of HBV infection (Kefenie et al., 1989). The slightly higher prevalence in this study might be explained by incorporation of anti-HBs as marker of infection. In one study in Ethiopia, 5% of the subjects had anti-HBs as the only marker of infection (Abebe et al., 2003). Many other reports from the different parts of the world have consistently shown health professionals are at increased risk of HBV infection (Ganju and Goel, 2000; Thomas et al., 1993; Al-Sohaibani et al., 1995; Kosgeroglu et al., 2004; Kershenobich et al., 1990; Vardas et al; 2002; Romieu et al., 1989). Assessing excess risk of hepatitis B infection would have been better achieved if comparison group were available in this study design.

The higher sex specific prevalence rate of HBV infection in males than females (57.3% vs. 46.0%) is consistent with other reports (Abebe et al., 2003; Coursaget et al., 1987) but it fails to reach statistical significance in this study. Several explanations have been forwarded for this male preponderance of HBV infection (Coursaget et al., 1987). HBV infection prevalence showed a linear rise with age from 35% in 19-24 year olds to peak at 100% in 55-59 year olds. This trend in HBV infection prevalence emphasizes the continuous horizontal transmission of HBV infection among this group of HCW’s. Nevertheless, our result fails to clearly demonstrate the place of occupation in the horizontal transmission of HBV infection. A progressive rise in HBV infection with age was reported by, other investigators (Abebe et al., 2003; Kefene et al., 1988; Shidrawi et al., 2004; Dienstag and Ryan, 1982). Conversely, 65% of 19-24 year olds have never been exposed to HBV and are susceptible. The presence of a high percent of susceptible persons at younger age (19-24 years), where most join the work force of health care service at this age, and the ongoing horizontal transmission needs due attention. This might be an ideal age to intervene and protect them from acquiring HBV.

HBV infection prevalence rate across different categories of health care worker did not show any statistically significant variation after adjustment for age. The absence of adequate sample size for some of the health care worker categories makes comparison less accurate. The absence of variation in hepatitis B infection rate by occupational category is demonstrated by other studies.
(Tsega et al., 1989; Thomas et al., 1993; Shidrawi et al., 2004) but this is in contrast to other studies done in Mexico and USA (Kershenobich et al., 1990; West, 1984).

The HBsAg seroprevalence (9.7%) observed among HCWs in this study is similar to that reported previously for the Ethiopian general population (Abebe et al., 2003; Kefene et al., 1988) but higher than the 6% prevalence rate among health care workers reported by Tsega et al. (1989). Comparison of our result with other studies from other countries on health professionals showed a variable result. A study from Yemen, a highly endemic country, has shown similar result (9.9%) to our report (Shidrawi et al., 2004). However, our finding is higher than that reported from Mexico (1.2%) (Kershenobich et al., 1990) and Turkey (2.7%) (Korsgeroglu et al., 2004) but lower than that reported from Senegal (17.8%) (Romieu et al., 1989). The difference in demographic characteristics of the study population and the difference in hepatitis epidemiology in these countries might explain these discrepancies. There is more male HBsAg carrier (11.9%) than females (7.3%) but this did not show statistical significance. Previous studies from Ethiopia and India have reported a statistically significant difference between male and female HBsAg prevalence rate (Gebreslassie, 1983; Abebe et al., 2003; Joyee et al., 2005). But our finding is in agreement with a study from Senegal (Romieu et al., 1989). HBsAg prevalence increases with age then falls down after a peak age of 40-44%. A partly similar HBsAg prevalence pattern has also been reported by Abebe et al. (2003). However, the peak age reported was 30-34 years. The possible explanation for the initial rise could be the ongoing horizontal transmission and subsequent decline after peak age could be explained by the pathogenesis of the disease. Hepatitis B pathogenesis is marked by different stage where ultimately some will clear the virus and others die as a result of complications (Pan and Zhang, 2005).

HBeAg prevalence rate (4.5%) is relatively lower than previously reported results from Ethiopia (6.2%) (Tsega et al., 1986), (23%) (Abebe et al., 2003), and (18.9%) (Rapicetta et al., 1989) but comparable to a result reported by Tsega et al. (1989). A 0.04% HBeAg prevalence among Senegalese health professionals is lower than our report (Romieu et al., 1989). The highest amount of HBV DNA (8.37x 10^7 copies/ml) was detected from the HBeAg positive individual, however, further study with large sample size should be done to look if there is any correlation
between HBeAg positivity and HBV DNA level. Rapicetta et al. (1989) had reported a correlation between HBeAg positivity and amount of HBV DNA. It is not possible to tell whether the HBeAg positive individual is in the immunotolerant phase or immunoreactivation phase (HBeAg positive chronic hepatitis). HBV DNA was also detected from 16 (76.2%) of HBeAg negative individuals and all except one individual had <10^5 copies/ml. This result demonstrated that HBeAg testing for the clinical assessment of infectivity couldn't replace HBV DNA detection. HBV-DNA level <10^5 copies/ml implies a high possibility of chronic inactive carrier, however, further classification of individuals as HBe negative chronic hepatitis and chronic inactive carriers requires liver biopsy and liver function test (Fattovich, 2003; McMahon, 2005). A more detailed and comprehensive study is required to evaluate the presence of HBeAg negative chronic hepatitis, precore or core mutant HBV DNA as this will help in the management and follow up of patients with chronic HBV infection (Hu, 2005). The following are the probable explanation for discordant result between HBsAg (positive) and HBV DNA (negative): subjects could be inactive hepatitis carrier (Sharma et al., 2005), destruction of DNA during storage or transportation, resolved hepatitis B infection where infectious particles still present in the blood and false positive result for HBsAg.

Subjects who were found to be positive for HBsAg were further tested for anti-HDV. HDV infection marker is not detected in any of this group of HBsAg positive HCW’s. It is very fortunate not to have a single case of HDV superinfection or coinfection as this is known to cause serious disease and rapid progression (Fattovich, 2003). This is in contrast to a 5.8% prevalence rate detected among Senegalese health professionals (Romieu et al., 1989) but consistent with a report from India (Jaiswal et al., 1999). The absence of HDV infection marker could be due to the very low prevalence rate of HDV infection among the general population of Ethiopia (Tsega et al., 1986; Tsega et al., 1987) and the dependent nature of the virus (infected only individuals with hepatitis B virus).

The HBV vaccination coverage in this group of HCW’s (13%) is lower than reports from other countries (Silva et al., 2005; Vards et al., 2002; Techasathit et al., 2005). This result is not representative of Ethiopian HCW’s since 30(85.7%) of the vaccinees are from one private college
and the other 5 vaccinees (14.3%) also got the vaccine as part of research project 17 years back. The result is not surprising as there is no policy by Ministry of Health to vaccinate HCW’s. Moreover, cost and knowledge about the vaccine may be possible factors for the low rate of vaccine coverage. The finding of a 93% protective antibody with a single dose of vaccination is larger than previously reported results (CDC, 2002; Ricci and Olson, 2005). The reason is not clear for this high level of protective antibody level and awaits properly designed study as this result makes only one dose of vaccination a possibility in a country where resource is a meager. However, protective level of anti-HBs found in those vaccinated three times was only 25%. The possible explanation for this relatively low rate is the long period of time elapsed since the time of vaccination. This result is consistent with other reports, which have demonstrated the waning of anti-HBs antibody level with time (Tsebe et al., 2001; Janbakhsh et al., 2005; Jain et al., 2005; Edmunds et al., 1996). Nevertheless, the absence or low level of anti-HBs does not exclude the existence of protective immunity (Banatvalal and Van Damme, 2003; Janbakhsh et al., 2005; Jain et al., 2005). The presence of anti-HBc marker in the two individuals who were vaccinated after initial screening could be because of break through infection; however, it is not possible to define whether anti-HBc antibody marker in the 6 individuals who were vaccinated without initial screening is due to breakthrough infection or preexisting marker before vaccination. The level of protection through natural infection (anti-HBs ≥ 10mIU/ml) was found to be 82/232 (35.3%; 95% CI: 28.8-41.1) but this will underestimate the level of protection because individuals with anti-HBs as the only marker were not identified. The result of our study is lower than the report of similar studies (Tsega et al., 1989; Kefenie et al., 1989; Abebe et al., 2003) but higher than that reported by Gebreselassie et al. (1983a) and Kershenobich et al. (1990). In contrast, similar protection level was reported by other studies (Tsega et al., 1986; Vardas et al., 2002). The difference in the level of protection may be due to the difference in the level of HBV infection. Measuring the level of protection is important in deciding whether prevaccination screening of health care worker for anti-HBs is necessary. Also understanding the level of immunity and infection will be helpful in devising a policy for post-exposure management of HCW’s in our health care system.
In this study we found 6 (2.2%) individuals with anti-HBc antibody as the only marker of HBV infection. The presence of anti-HBc alone positive individuals is demonstrated by other studies in Ethiopia Abebe et al. (2003), which have found a progressive increase in anti-HBc alone positive cases with age, particularly in the 40+ years of age. However, all the anti-HBc alone cases in this study were found in the 23-33 year old individuals. In addition, 3.9% prevalence among HCW’s was reported by Tsega et al. (1989). A nationwide hospital based study in Ethiopia has also reported a comparable prevalence rate of 3.2% (Tsega et al., 1986). The result of a study on male military recruits from all over Ethiopia has also reported a 2.5% prevalence rate and HBV-DNA was detected in 2.9% of individuals with anti-HBC alone (Rapicetta et al., 1989). In our study, HBV-DNA was not detected from the 6 individuals with anti-HBc alone, which is in agreement with a study performed by Silva et al. (2005). The reports of other studies from different countries have a variable prevalence (Arraes et al., 2003; Alhababi et al., 2003).

Risk factor analysis of hepatitis B infection found only absence of hepatitis B vaccination, which was statistically significant on univariate analysis and multi-variate analysis. This is consistent with our observation of a high level of protective anti-HBs antibody in those vaccinated. Reports of other studies support that hepatitis B immunization decreases the incidence of HBV infection (Jefferson et al., 2000). However, unlike reports from other countries, (Thomas, et al., 1993; West, 1984) needle stick injury, sharp injury, occupation and duration of service were not found to be risk factor for HBV infection. The difficulty of avoiding confounding factors in this kind of study design, the high prevalence of hepatitis B infection and protective antibody in our country and the sampling methods are possible explanation for absence of association of these risk factors with HBV infection.

Among 267 subjects, only one case of HCV infection (0.37%), which was confirmed by both recombinant immunoblot assay and RT-PCR, was found. Our study has demonstrated a very low rate of HCV infection among this group of HCW’s. The infected individual does not have any risk of occupational exposure to blood and body fluid; no needle or sharp injury and very unlikely to be work related infection. The results of several studies are in agreement with our result (Thomas et al., 1993; Al-Sohaibani et al., 1995; Ganju and Goel, 2000; Campello et al.,
1992); however other reports demonstrated an elevated risk for health professionals (Catalani et al., 2004; Mihaly et al., 2001). HCV infection prevalence rate of 1.2% from Italy (Campello et al., 1992), 0% from India (Ganju and Goel, 2000), 0.7% from America (Thomas et al., 1993) and 3.5% from Yemen (Shidrawi et al., 2004) has been reported. The prevalence in the current study (0.37%) is lower than previously reported findings of population based studies done in Ethiopia, 0.9% (Ayele et al., 2002) and 2% (Frommel et al., 1993). The very low rate of HCV infection despite the presence of a large number of needle stick and sharp injury is striking. A possible explanation could be the low amount of the virus in the blood of HCV carriers (Kiyosawa et al., 1991) in combination with the very low prevalence rate of HCV infection in the general population of Ethiopia (Ayele et al., 2002; Frommel et al., 1993).

**LIMITATIONS OF THE STUDY**

Selection of the study subjects on voluntary basis introduces the possibility of selection bias this in turn affecting representativeness. The other limitation of the study involves the design. Direct comparison group was not available to the HCW, the absence of which makes an accurate estimate of relative risk difficult. Comparing our result to other previously published results has many limitations. The seromarkers used for assessment of HBV infection is not complete. Failure to use anti-HBs antibody test in the primary screening has underestimated some of the findings: hepatitis B infection rate, and level of hepatitis B immunity through natural infection. An accurate classification of HBe negative chronic hepatitis into chronic active hepatitis and chronic inactive carrier was not possible on the investigations undertaken.

**CONCLUSIONS AND RECOMMENDATIONS**

Our data suggest the presence of a high rate of exposure to blood and body fluid, large number of needlestick and sharp injuries, low universal precaution practice and low vaccination coverage. HBV infection is widespread in the HCW’s with continuous horizontal transmission as age advances and the lowest prevalence being in the youngest (19-24) age group. HBeAg negative serological status and low viral replication levels characterize chronic HBV infected HCW.
Protective antibody to HBV through natural infection is available in significant fraction of health professionals. Serologic response rate to a single dose of hepatitis B vaccine was much higher than other experiences. ‘Anti-HBc only’ is found in the health professionals but none of the samples had detectable HBV- DNA. HCV and HDV infection in health professionals seems to be rare. The only risk factor associated with hepatitis B infection is the absence of vaccination and HBV vaccination reduces HCW infection with HBV.

In conclusions, based on this study the following recommendations are made: -

• Prevention of occupational infection with blood borne pathogens should be a priority to the national program for promotion of infection control.
• Exposure prevention should be the primary strategy to reduce the risk of occupational blood borne pathogen infections. All preventive efforts should be made to reduce the risk of occupational exposures.
• Training of HCW’s on safe handling and collection of needles and sharps, use of safe devices along with a better understanding of factors that influence healthcare worker behaviors that result in injury or protection from injury is required to reduce transmission. Moreover surveillance and analysis of exposures in the health care setting is urgently needed.
• The need and cost effectiveness of screening of health professionals for evidence of immunity before hepatitis B immunization should further be studied.
• A national study, where a larger number of blood donors from different blood donation centres across the country will perhaps determine whether screening for anti-HBc in addition to HBs Ag detection is needed in Ethiopian blood donors.
• A well-designed study is required to confirm the high level of protection achieved with a single dose of hepatitis B vaccine.
• A cohort study is required on identifying the specific mode of transmission for the continuous horizontal transmission of hepatitis B virus.
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Appendix I

Questionnaire for Investigation of HBV, HCV and HDV Viral Infections in Healthcare Workers at Tikur Anbassa University and Ras Desta Damtew Memorial Hospitals, Addis Ababa, Ethiopia

Code no ----------------

I. Identification
1. Age________
2. Sex________
3. Marital status ______
4. Occupation __________
5. Duration of Service ______
6. Parity (if female) _______

II. Risk assessment
1. History of Occupational exposure to blood ungloved: 1. Yes 2. No
2. Occupational exposure to blood ungloved. 1. Yes 2. No
3. If yes how many times          once □ twice □ three times □ several □
5. This exposure in the last 6 months. 1. Yes 2. No
6. If yes how many times? Once □ twice □ three times □ several □
7. Use of Gloves during exposure prone work. 1.Consistently □ 2. Intermittently □
8. Splash of blood or body fluid to exposed face 1. Yes 2. No
10. If yes how many times          once □ twice □ three times □ several □
11. History of sharp injury 1. Yes 2. No
12. If yes how many times          once □ twice □ three times □ several □
13. Have you ever taken care of Hepatitis Patient 1. Yes 2. No
15. History of blood transfusion 1. Yes 2. No
16. History of Jaundice or Diagnosed liver disease.  
   1. Yes [ ]  2. No [ ]

17. History of tattooing  
   1. Yes [ ]  2. No [ ]

18. Previous history of hepatitis B vaccination  
   1. Yes [ ]  2. No [ ]

### III. Laboratory Results

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. HBs Ag</td>
<td>Positive</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>2. Anti-HBs</td>
<td>Positive</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>3. Anti HBc</td>
<td>Positive</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>4. HBe Ag</td>
<td>Positive</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>5. Anti HDV</td>
<td>Positive</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>6. Anti HCV</td>
<td>Positive</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>7. RIBA</td>
<td>Positive</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>8. HBV DNA</td>
<td>Positive</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>9. HCV RNA</td>
<td>Positive</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
</tbody>
</table>

### IV. Comments

__________________________________________________________

__________________________________________________________
Appendix II

I have been requested to participate in a research project that aims to determine seroprevalence and risk factors of Hepatitis B, C and D virus infections. I have been informed that all information I will be giving will be kept confidential. I understand that the risks I am exposed to will be the risk from blood drawing. I confirm my agreement by putting my signature below.

I hereby give my consent for giving of blood specimens.

Signature:_________________________ Date:_________________________