CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1. Introduction
Hepatitis D (hepatitis Delta) virus (HDV) was detected by Rizzetto among patients with a severe form of hepatitis B virus (HBV) infection in the year of 1977(Rizzetto et al., 1977). It is estimated that 15 million people are infected with HDV world wide. The virus is a defective single-stranded RNA virus which requires the HBsAg of HBV to establish infection in humans. The antigenomic strand of HDV encodes the only protein, hepatitis delta antigen (HDAg), in two molecular-weight forms: small HDAg (S-HDAg) and large HDAg (L-HDAg) (Taylor, 2006).

Individual with previous chronic HBV mono-infection are also vulnerable to super-infection with HDV after exposure to blood from an individual infected with both HBV and HDV. This may be observed as an acute flare of hepatitis and this flare can lead the initial discovery of pre-existing HBV infection. Superinfection with HDV can be self limited and result in clearance of both viruses, also this outcome is rare. Most patients with superinfection develop a progressive form of chronic hepatitis and it often manifests as a worsening clinical illness in a previously stable chronic carrier of HBV. Clinical illness with superinfection can be rapidly progressive, leading to cirrhosis within 2 years in 10% -15% of patients (Rizzetto, 2010).

In association with HBV, HDV produces significantly more severe illness than HBV alone (Gupta et al., 2005).
The disease spectrum of HDV infection varies greatly from fulminant hepatitis, rapidly progressive disease, to a subclinical course. Persistent replication of HDV associated with continuous hepatic inflammation and elevated alanine aminotransferase (ALT) levels is a characteristic of chronic active hepatitis D (Smedile et al., 1994). There are two modes of the HDV infection: co-infection, results from acute infection with both hepatitis B virus (HBV) and HDV, whereas superinfection results from HDV infection of patients with underlying chronic hepatitis B infection. Super-infection with HDV increases higher risk of chronic HBV infection leads to progressive disease and cirrhosis in approximately 80% of cases than co-infection with HBV and HDV (Smedile et al., 1982).

The routes of HDV transmission are similar to those for HBV, including blood-borne, sexual, percutaneous, permucosal, and perinatal transmission. However, patients having HBV-HDV co-infection may have more severe acute disease and higher risks of fulminant hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) than those having HBV infection alone (Fonseca, 2002).

Also, HBV infected patients with HDV superinfection have a higher rate of progression to chronic disease and serious complications (Xiridou et al., 2009).

Based on phylogenic analysis, HDV isolates collected worldwide have been classified into 8 groups, HDV-1 (former genotype I), HDV-2 (former genotype IIa), HDV-3 (former genotype III), HDV-4 (former genotype IIb), and HDV-5 to HDV-8 (Radjef et al., 2004).
These HDV types show different geographic distribution and are associated with different disease patterns. Genotype 1 has been distributed worldwide, whereas other HDV genotypes circulate unevenly. Over 15 million people are infected with HDV worldwide and its prevalence in Italy, Eastern Europe and western region of Asia is higher than in the rest of world and appears to be endemic in the Middle East (Mumtaz et al., 2005).
1.2. Rationale

Probably at least 5% of the HBV carriers have HDV infection (Taylor, 2006). HBV-HDV co-infection may lead to more severe acute disease and higher risks of fulminant hepatitis, cirrhosis, and hepatocellular carcinoma than those having HBV infection alone. Also, HBV infected patients with HDV super-infection have a higher rate of progression to chronic disease and serious complications. In Sudan there is few data about the prevalence of HBV- HDV co-infection.
1.3. Objectives

1.3.1. General objective

To detect Hepatitis D virus Antigen among Hepatitis B positive hemodialysis patients attending Hemodialysis Centers in Khartoum State during 2015.

1.3.2. Specific objectives

a) To detect Hepatitis D virus Antigen among hepatitis B positive hemodialysis patients using serological method (ELISA).

b) To correlates the presence of Hepatitis D Virus Antigen to the other factors including: age, sex.

c) To estimate the percentage of Hepatitis D positive patients among Hepatitis B positive hemodialysis patients.
CHAPTER TWO

LITERATURE REVIEW

2.1. Discovery

The discovery of hepatitis D virus (HDV) dates back to the mid-1970s, and followed the detection of a novel nuclear antigen in patients with a severe form of chronic hepatitis B. The first report of this antigen, believed to be a hepatitis B antigen and called the delta antigen, was published in 1977 (Rizzetto et al., 1977). Three years later, experiments in chimpanzees had already demonstrated that the hepatitis delta antigen (HDAg) was a structural component of transmissible pathogen that required the hepatitis B virus (HBV) for its life cycle (Rizzetto et al., 1980).

2.2. Structure

The virion particle was shown to be composed of the HBV envelope proteins surrounding a ribonucleoprotein core-like structure comprising the HDAg and a molecule of RNA (Rizzetto et al., 1980). The delta agent obtained the status of a distinct virus in 1983 with the official name of hepatitis delta virus. Nowadays, the term hepatitis D virus is preferred, even though ‘delta’ is still used. The uniqueness of this virus was confirmed in 1986, after cloning and sequencing of its genome (Wang et al., 1986). Thereafter, HDV obtained its own genus, the Deltavirus (Mason et al., 2005). Hepatitis D virus (HDV) is a defective single-stranded RNA virus which requires the HBsAg of HBV to establish infection in humans. The antigenomic strand of HDV encodes the only protein, hepatitis delta antigen (HDAg), in two molecular-weight forms: small HDAg (S-HDAg) and large HDAg (L-HDAg) (Taylor, 2006).
2.3. Replication

HDV envelope consists of all three protein species of HBsAg and as a result HDV probably utilizes the same cellular receptor as HBV. Replication of HDV is restricted to the liver. Although replication of HDV can occur within hepatocytes in the absence of HBV, HBV is necessary for coating the HDV virions and allowing their spread from cell to cell. Virus replication is helped by small hepatitis delta antigen (SHDAg) but is inhibited by large hepatitis delta antigen (LHDAg). HBsAg and LHDAg are needed for HDV assembly. Envelope proteins derived from the pre-S and S antigens of HBV encapsidate HDV RNA and HDAg. HDAg is necessary for viral proliferation. HBsAg, HDAs, and HDV RNA are the main particles of HDV. Assembly can only occur in the presence of the helper virus HBV (Alavian and Alavian, 2005).

2.4. Genotypes

Based on phylogenetic analysis, HDV isolates collected worldwide have been classified into 8 groups, HDV-1 (former genotype I), HDV-2 (former genotype IIa), HDV-3 (former genotype III), HDV-4 (former genotype IIb), and HDV-5 to HDV-8 (Radjef et al., 2004). Except for genotype 1, which is represented worldwide, all other genotypes are mostly found in specific geographical areas. Genotype 2 prevails in Japan (Imazeki et al., 1990), Taiwan (Wu et al., 1995), and Russia (Ivaniushina et al., 2001), genotype 3 in the Amazonian region (Casey et al., 1993), genotype 4 in Japan (Sakugawa et al., 1999; Watanabe et al., 2003) and Taiwan (Wu et al., 1998) and genotypes 5–8 in Africa (Le et al., 2006).

2.5. Transmission

The natural reservoir is man, even though chimpanzees infected with HBV and woodchucks infected with the woodchuck hepatitis virus can be infected by HDV. Infection with HDV is parenterally transmitted. In industrialized
countries, high-risk populations include illicit drug users and people exposed to blood or blood products. HDV does not seem to be a typically sexually transmitted disease, as the frequency of infection in sexually promiscuous heterosexual or homosexual groups is lesser than that of HBV or HIV. In socially and economically disadvantaged populations, many infections occur by inapparent intrafamilial routes of transmission, facilitated by poor hygiene. Perinatal transmission of HDV is rare (Weisfuse et al., 1989).

2.6. Clinical features

An HDV infection absolutely requires an associated HBV infection. The outcome of disease largely depends on whether the two viruses infect simultaneously (coinfection), or whether the newly HDV-infected person is a chronically infected HBV carrier (superinfection) (Hadziyannis, 1997; Purcell and Gerin, 1996).

2.6.1 Coinfection

Coinfection of HBV and HDV (simultaneous infection with the two viruses) results in both acute type B and acute type D hepatitis. The incubation period depends on the HBV titre of the infecting inoculum. Depending on the relative titres of HBV and HDV, a single bout or two bouts of hepatitis may be seen. Coinfections of HBV and HDV are usually acute, self-limited infections. The chronic form of hepatitis D is seen in less than 5% of HBV - HDV coinfected patient (Hadziyannis, 1997; Purcell and Gerin, 1996). Acute hepatitis D occurs after an incubation period of 3 - 7 weeks, and a preicteric phase begins with symptoms of fatigue, lethargy, anorexia and nausea, lasting usually 3 to 7 days. During this phase, ALT and AST activities become abnormal. The appearance of jaundice is typical at the onset of the icteric phase. Fatigue and nausea persist, clay-colored stools and dark urine appear, and serum bilirubin levels become abnormal. In patients with acute,
self-limiting infection, convalescence begins with the disappearance of clinical symptoms. Fatigue may persist for longer periods of time (Lai, 1994; Purcell and Gerin, 1996).

**2.6.2. Superinfection**

Superinfection of HBV and HDV (HDV infection of a chronically infected HBV carrier) causes a generally severe acute hepatitis with short incubation time that leads to chronic type D hepatitis in up to 80% of cases. Superinfection is associated with fulminant acute hepatitis and severe chronic active hepatitis, often progressive to cirrhosis (Lai, 1994; Purcell and Gerin, 1996). During the acute phase of HDV infection, synthesis of both HBsAg and HBV DNA are inhibited until the HDV infection is cleared (Lai, 1994).

Fulminant viral hepatitis is rare, but still about 10 times more common in hepatitis D than in other types of viral hepatitis. It is characterized by hepatic encephalopathy showing changes in personality, disturbances in sleep, confusion and difficulty concentrating, abnormal behavior, somnolence and coma. The mortality rate of fulminant hepatitis D reaches 80%. Liver transplantation is indicated (Lai, 1994; Purcell and Gerin, 1996). Chronic viral hepatitis D is usually initiated by a clinically apparent acute infection. Symptoms are less severe than in acute hepatitis, and while serum ALT and AST levels are elevated, bilirubin and albumin levels and prothrombin time may be normal. In chronic hepatitis D, the HBV markers are usually suppressed (Lai, 1995; Lai, 1994; Purcell and Gerin, 1996). Progression to cirrhosis usually takes 5 - 10 yrs, but it can appear 2 years after onset of infection. About 60 to 70% of patients with chronic hepatitis D develop cirrhosis. A high proportion of these patients die of hepatic failure (Purcell and Gerin, 1996). Hepatocellular carcinoma (HCC) occurs in
chronically infected HDV patients with advanced liver disease with the same frequency as in patients with ordinary hepatitis B. HCC may actually be more a secondary effect of the associated cirrhosis than a direct carcinogenic effect of the virus.

Taken together, three phases of chronic hepatitis D have been proposed: a) an early active phase with active HDV replication and suppression of HBV, b) a second moderately active one with decreasing HDV and reactivating HBV, c) a third late one with development of cirrhosis and hepatocellular carcinoma caused by replication of either virus or with remission resulting from marked reduction of both viruses (Hadziyannis, 1997). The mortality rate for HDV infections lies between 2% and 20%, values that are ten times higher than for hepatitis B (Purcell and Gerin, 1996).

2.7. Host immune response

Both humoral and cellular immunity are induced in patients infected with HDV (Lai, 1994; Purcell and Gerin, 1996). These immune responses may provide protection from HDV re-infection, or simply modulate clinical symptoms. However, second cases of hepatitis D have not been reported (Lai, 1994; Purcell and Gerin, 1996). Anti-HD antibodies do not always persist after acute infection is cleared. The serological evidence of past HDV infection is therefore not easy to demonstrate (Lai, 1994).

2.8. Diagnosis

Hepatitis D virus induces innate and adaptive immuneresponse in the infected host, which consist of immunoglobulin (IgM) and (IgG) production (Aragona et al., 1987). Therefore, in the serum, the three specific HDV markers are HDV RNA, HDAg and anti-HDV. Hepatitis D virus RNA can be detected in serum by either molecular hybridization or RT-PCR. Hybridization assays have a
detection limit of about 10^4–10^6 genomes/ml (Negro et al., 1988; Zignego et al., 1990). This technique has been superseded by RT-PCR, which is more sensitive, with a detection limit of 10 genomes/ml (Zignego et al., 1990; Wu et al., 1995). In liver samples, HDV RNA can be detected by in situ hybridization. This method is, however, not used in routine as it is very difficult and time-consuming. New automated assays are now being established to render possible the follow-up of viral RNA kinetic in the serum of infected patients during treatment (Mederacke et al., 2010, Schaper et al., 2010). Serum HDAg can be detected by two different methods, namely the enzyme-linked immunosorbent assay (ELISA) (Shattock and Morgan, 1984) and the radioimmunoassay (RIA). These assays are not available in the US for clinical diagnosis. HDAg can be detected by immunofluorescence or immunohistochemical staining of liver biopsies. As HDAg, serum anti-HDV IgM and IgG antibodies can be detected by ELISA or RIA. The diagnosis has of course to indicate whether there is an HDV infection, but it also has to distinguish among the three situations of infection: acute HBV/HDV coinfection, acute HDV superinfection of a chronic HBV carrier or HDV chronic infection. As HDV is dependent on HBV, assessing the presence of HBsAg is necessary before investigating the other markers in order to establish the diagnosis. Acute HBV/HVD co-infection is highlighted by the presence of a high titre of IgM anti-HBc, antibodies that disappear in chronic HBV infection. It bears otherwise the same characteristics as acute HDV superinfection. HDAg appears early but also disappears quickly. Repeated testing is necessary so that it does not elude detection (Buti et al., 1986).

2.9. Treatment

The aim of treatment of hepatitis D is to eradicate or to achieve long-term suppression of both HDV and HBV. The primary endpoint of treatment is the suppression of HDV replication, which is accompanied by normalization of the
serum alanine transferase (ALT) level and amelioration of necroinflammatory activity on liver biopsy. Suppression of HDV replication is documented by loss of detectable HDV RNA in serum and of HDAg in the liver. A secondary endpoint is the eradication of HBV infection, with HBsAg to anti-HBs seroconversion. There is very little information to support that current treatment is effective in achieving this goal. Eradication of HBV infection with development of anti-HBs will protect the individual from reinfection with HBV as well as HDV. Patients who have cleared HDV but who remain HBsAg positive are still at risk of reinfection with HDV (Rizzetto et al., 1983).

2.9.1. Interferon alfa

The only drug approved at present for the treatment of chronic hepatitis D is interferon alfa (IFNa). Pegylated interferon appears to be more effective than standard interferon but the data are limited. Unfortunately, only a minority of patients treated with interferon clear HDV infection. Early attempts to treat hepatitis D with immunomodulatory drugs, such as corticosteroids or levamisole, were unsuccessful (Rizzetto et al., 1983).

2.9.2. Alternative treatment

Several drugs have been evaluated as alternatives to interferon. Overall, the results are discouraging. Ribavirin inhibits HDV replication in vitro. It has been tested in two small trials in humans without any biochemical or virological response (Garripoli et al., 1994).

2.9.3. Liver transplantation

Liver transplantation is the management of choice in fulminating hepatitis D and end-stage chronic liver disease due to HDV. Patients undergoing liver transplantation receive also passive immunoprophylaxis against HBV reinfection with anti-HBs antibodies and administration of HBV polymerase
inhibitors (Rosenau et al., 2007). This results in the complete clearance of both HBV and HDV in most patients after liver transplantation, with a survival rate at 5 years of almost 90% (Samuel et al., 1995), better than what observed in patients mono-infected with HBV (Smedile et al., 1998).

2.10. Prevention

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 HBV will prevent the transmission of hepatitis D. HBV vaccination is therefore recommended to avoid HBV-HDV co-infection (Lai, 1994).

2.11. Epidemiology

Seroprevalence studies of anti-HD in HBsAg-positive patients has shown a worldwide but not uniform distribution (Purcell and Gerin, 1996). Epidemics of HDV infections have been described in the Amazon Basin, the Mediterranean Basin and Central Africa. Two epidemiologic patterns of hepatitis D infections exist: in Mediterranean countries infection is endemic among HBV carriers, and the virus is transmitted by close personal contact. In Western Europe and North America, HDV is confined to persons exposed to blood or blood products, like e.g. intravenous drug addicts sharing unsterilized injection needles Worldwide, more than 10 million people are infected with HDV (Hadziyannis, 1997).
2.12. Previous studies

The few studies on HBV infection which conducted Hepatitis D virus seroprevalence, found this to be between 9% in eastern Sudan (McCarthy et al., 1989) and 27.8% in central Sudan (Hyams et al., 1989). Studies from neighboring Uganda demonstrated seroprevalence of anti-D antibodies in up to 30.6% of those who are HBsAg-positive (de Lall et al., 1990).

According to a study conducted from January 1994 to April 2001, the prevalence of HDV infection in Pakistani hepatitis B surface antigen (HBsAg)-positive individuals was 16.6%. A large belt with high prevalence exists in the middle of the country, which comprises rural areas (Mumtaz et al., 2005). The predominant genotype of HDV is genotype I and that of HBV is genotype D (Moatter et al., 2007).

A review of the literature shows strong variations in the prevalence of hepatitis delta virus (HDV) among Africans seropositive for hepatitis B surface antigen (HBsAg). In Senegal, West Africa, prevalence of antibodies to HDV among asymptomatic HBsAg carriers appears to be linked to regional and/or socioeconomic factors. Antibodies to HDV were found in 22 (47%) of 47 HBsAg-seropositive patients with liver disease who were hospitalized in the main hospital of Dakar and in 4% to 44% of asymptomatic HBsAg carriers. Eight (36%) of 22 patients with liver disease were seropositive for IgM antibodies to HDV, whereas none of 18 asymptomatic subjects were seropositive (P less than .01). According to studies conducted in Europe and the United States, the presence of this marker may constitute support for the argument that HDV plays a role in the development of HBsAg-associated liver diseases in Senegal and, presumably, within the African continent (Roingeard et al., 1992).
In Accra, Ghana 53 patients with hepatitis B-related liver diseases are tested for anti HDV antibodies, Six patients were reactive for anti-delta antibodies, yielding a HDV sero-prevalence of 11.3%. Anti-HDV was detected in 4(22.2%) patients with chronic hepatitis B, 1(7.6%) with cirrhosis of the liver and 1(5.3%) with hepatocellular carcinoma (Asmah et al., 2014).

In Northern India, the prevalence of hepatitis D in HBsAg-positive individuals from New Delhi was reported to be 8.1% in 1996 (Irshad and Acharya, 1996) and 10.6% in 2005 (Chakraborty et al., 2005), which was lower than in Chandigarh in Northern India, where the infection was reported endemic in 1995 and showed a prevalence of 14.2% (Singh et al., 1995). In Central India, a study in Indore showed higher prevalence of 5.7% in patients with chronic liver disease, 1.9% in those with acute viral hepatitis, 15% in those with hepatic failure, and 2.3% in those with chronic renal failure (Jaiswal et al., 1999). Study curred out in Mumbai showed a prevalence of 16% in patients with acute viral hepatitis, 17% in asymptomatic HBsAg carriers, and 19% in patients with chronic liver disease. Among the high-risk population, HDV prevalence was 20% in chronic renal failure patients, 29% in medical professionals, and 38% in recipients of multiple transfusions (Amarapurkar et al., 1992).

HDV is a major public health issue in Iran (Alavian, 2008). Anti-HDV prevalence in asymptomatic HBV carriers from Southern Iran was 14% in 1989 (Shahinsaz et al., 2006). A recently published study reported a prevalence of 9.7% in chronic HBV patients from Shiraz, which represented a decrease in prevalence from 1989 (Taghavi et al., 2008). A study conducted during 1986-1988 has estimated the prevalence of hepatitis D in various high-risk groups. The prevalence of anti-delta was found to be 2.5% (3/120) in asymptomatic chronic carriers of HBsAg, 33.33% (2/6) in hemophiliac patients, 44.5% (16/36) in HBsAg-positive hemodialysis patients. Five out of eight patients with
hepatocellular carcinoma (HCC) were also positive for anti-HDV (Rezvan et al., 1990).

In mainland China, HDV infection is not very prevalent but it does exist. The results of a study done in Sichuan Province in 1987 have suggested a low prevalence of 0.8% in HBsAg-positive patients, although the prevalence rate of HBV was high (Wang et al., 1987). In Hong Kong, hepatitis D has high prevalence rates among intravenous drug abusers (IVDAs). These finding were reported by a study that tested the sera of a large number of patients with acute or chronic HBV infection for anti-HDV between January 1988 and December 1990. HDV was detected in 13 out of 14 IVDAs, which corresponds to a prevalence of 93% in this group, whereas the prevalence was only 0.15% in 664 non-IVDAs (Lok et al., 1992).

Taiwan is considered to be endemic for hepatitis B, but as a result of effective immunization, HBV prevalence has decreased markedly. Hepatitis D prevalence in Taiwan was very high in the 1990s and before, but the prevalence has decreased greatly since then and new cases of HDV infection are now encountered rarely (Chen, 2003). In 2003, the HDV prevalence in HBsAg carriers was 15.3% (56/366) (Lu et al., 2003).

In Japan, certain areas are highly HDV prevalent, especially Mikayo Islands in Okinawa (Sakugawa et al., 1999). A study published in 1990 has assessed the prevalence of HDV infection in Japan at different time periods. Hepatitis D was first observed in 1979-1983 and the prevalence was 16% in acute hepatitis B, 6.8% in HBV carriers, and 26% in chronic liver disease. Anti-HDV was later rarely observed. The findings of this study suggest limited sporadic HDV infection in Japan (Tamura et al., 1990).

In Korea, HDV prevalence was estimated to be 0.85% in 1985. In 2003, a study was conducted in which 194 HBsAg-positive Korean patients were
tested for anti-HDV, out of which, seven (3.6%) tested positive. Six of these patients had HCC and one had cholangiocarcinoma. Therefore, HDV was mainly associated with patients with HCC with a prevalence rate of approximately 4%, which has not changed greatly during the past 20 years (Jeong et al., 2005).

In Indonesia, only a few studies are available on the prevalence of HDV, thus, this infection does not seem to be a major problem in this area unlike some other Asian countries. A study published in 1988 assessed the prevalence of hepatitis D in pregnant women in Bandung, a densely populated area of Indonesia. Out of the 926 pregnant women included in the study, only 2.8% (26) were carriers of HBsAg, among which, none tested positive for delta antibodies in spite of the fact that in this Indonesian population, HbsAg was frequent (Vranckx et al., 1988). However, in 2003, the prevalence was found to be < 0.5% in the HBsAg carriers of Surabaya, which is also very low (Lusida et al., 2003).

In Malaysia, hepatitis D was first described in 1986 in some population groups. The HDV prevalence was found to be 12.5% in cases of acute hepatitis B, 6.7% in homosexual individuals, and 17.8% in drug abusers who were positive for HbsAg (Sinniah et al., 1986). In 1989, the HDV prevalence was found to be 4.9% (Tan et al., 1989). In 1996, 0.9% of the 923 jaundiced patients were found to be positive for anti-HDV [Saat et al., 1999]. In 1985, surveillance results for the detection of anti-HDV in IVDAs showed an absence of anti-HDV. However, in 1986, a prevalence of 17.8% was observed in the same group. The prevalence increased to 20% in 1989 and in 1994, 34% of the drug addicts tested positive for anti-HDV (Duraisamy et al., 1994).

Vietnam is considered to be highly endemic for hepatitis B, which is one of the most important public health concerns. In order to estimate the prevalence of
HBV and HDV in this area, a cross-sectional seroprevalence study was conducted in Thai Binh Province in 2007. Nineteen percent of the samples were HBsAg-positive, out of which, 1.3% were positive for HDV (Nguyen et al., 2007).

HDV prevalence in Saudi Arabia was 8% (3/36) in HBsAg carriers in 1986 (Ashraf et al., 1986). The prevalence in the Riyadh area in the same year was 22.2% in patients with chronic hepatitis B, 7.9% in those with acute hepatitis B, and 6.7% in HBsAg carriers. In Najran, the prevalence was 9.6% in HBsAg carriers, and 5.3% in Al-Hafouf. In the areas of Khaiber and Jaizan, no anti-delta was found in the tested HBsAg carriers (el-Hazmi and Ramia, 1986). In 1998, a study conducted in Jeddah showed an HDV prevalence of 13.6%. The HDV prevalence in IVDAs/carriers of HBsAg was 14.7% while there was 0.0% prevalence in non-IVDAs positive for HbsAg (Njoh and Zimmo., 1998). In 2004, the HDV prevalence among HBsAg-positive healthy donors was 3.3%, while that in clinic- and hospital-based HBsAg patients was 8.6%. HDV infection is expected to decrease in Saudi Arabia with decreasing HBV prevalence due to global vaccination (Al-Traif et al., 2004).

In Oman, according to a study conducted in 1991, the HDV prevalence was 7.7% (1/13) in HBsAg-positive dialysis patients and 22.2% (2/9) in HBsAg-positive renal transplant patients who have been previously transfused (Aghanashinikar et al., 1992). In 1994, HbsAg was detected in 11% of patients with kidney transplants and 12.7% of patients on dialysis. Anti-HDV was detected only in one HBsAg-positive patient on dialysis and two HbsAg carriers with renal transplants, which shows a low HDV prevalence among these patients (Al-Dhahry et al., 1994).

Hepatitis D was first detected in Lebanon in 1987 when a study reported 57% HDV prevalence in patients with chronic active hepatitis (Farci et al., 1987). In
2007, the results of a study in which 258 HBsAg-positive patients from 10 health centers were included showed 1.2% (3/258) HDV prevalence, which shows a decrease in prevalence since 1987 (Ramia et al., 2007).

In Turkey, like many other countries, a decline in HDV infection has been observed, but it is still a significant public health concern (Yurdaydin, 2006). HDV prevalence is much higher in the southeast of the country, being 27% in chronic hepatitis B patients and 46% in hepatitis-B-induced cirrhosis patients. This is in comparison to the HDV prevalence in the West of the country of 5% in chronic hepatitis B patients and 20% in hepatitis-B-induced cirrhosis patients. The analysis also compared prevalence of HDV before and after 1995. It was observed that HDV prevalence in chronic hepatitis B patients decreased from 38% to 27% in Southeast Turkey and from 29% to 12% in Central Turkey. HDV prevalence in cirrhosis patients has decreased from 66% to 46% in Southeast and from 38% to 20% in West Turkey (Değertekin et al., 2008).
CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

3.1.1. Type of study

Descriptive cross-sectional study.

3.1.2. Study area

The study was conducted in four renal dialysis centers in Khartoum State. These were Alnow Hospital, Ibn Sina Hospital, Tropical Disease Hospital and Salma Center. The practical part of this study was done in the Research Laboratory College of Medical Laboratory Science, Sudan University of Science and Technology (SUST).

3.1.3. Study duration

This study was conducted during the period from March to August 2015.

3.1.4. Study population

Hepatitis B positive hemodialysis patients who attend hemodialysis centers both males and females were included.

3.2. Sample size and sampling technique

A total of ninety patients (n=90) known as hepatitis B positive were enrolled.
3.3. Ethical consideration

This study was approved by the College of Medical Laboratory Science ethical committee, SUST. Verbal consent were taken from patients.

3.4. Data collection

Data were collected by study questionnaire (Appendix 4).

3.5. Laboratory methods

3.5.1. Collection of blood specimens

A volume of 5 ml blood were collected from each patient through venipuncture technique then displaced into a plain container.

3.5.2. Sample processing

Each blood sample was centrifuged at 3000 RPM (round per minutes) for 20 minutes., then serum was gently collected into epindorff tube and stored at −20°C until the serological analysis.

3.5.3. Sample analysis

The samples were analyzed for the presence of HDVAg by a commercially available enzyme -linked immunosorbent assay “HDVAg ELISA” kit (AccuDiag™ HDV-Ag, USA). The assays were performed following the instructions of the manufacturer (Appendix 3). Positive and Negative controls were included in each assay. According to the information included in the kit’s insert, the immunoassay used has sensitivity and specificity determinate to be 100 %.
3.5.4. Principle of HDVAg ELISA kit

The HDV-Ag ELISA employs the solid phase, two-step incubation double antibody sandwich method. The patient's serum/plasma is added together with extraction solution after the polystyrene microwell strips are pre-coated with purified antibodies specific to HDV. If the HDV virus is present, the HDV particles are disrupted and what's captured in the wells is the specific HDV antigens. At this stage, unbound serum proteins must be washed off the microwells. What is added next is Horseradish Peroxidase (HRP) which is conjugated with a secondary antibody. Again, after washing, unbound conjugates are removed. Added to the wells after this are both the chromogen solutions containing Tetramethylbenzidine (TMB) and urea peroxide. During this stage of a combined presence of antibody - antigen - antibody (HRP) sandwich immunocomplex, a blue-colored product appears, which is the result of colorless chromogens hydrolyzed by the bound HRP conjugate. After stopping the reaction with sulfuric acid, the blue color turns yellow. The color intensity can be gauged proportionally to the amount of antigen in the sample. Colorless wells appear when samples are negative for HDV antigens.

**Assay principle scheme:**

**Double antibody sandwich ELISA**

\[
Ab(p) + Ag(s) \rightarrow [Ab(p) - Ag(s) + ENZ] \rightarrow [Ab(p) - Ag(s) - ENZ] \rightarrow \text{blue} \rightarrow \text{yellow} \ ( + )
\]

\[
Ab(p) \rightarrow [Ab(p) + ENZ] \rightarrow [Ab(p)] \rightarrow \text{No Color} \ ( - ), \ (\text{appendix 1}).
\]
3.5.5. Procedure

All reagents and specimens were settled to the reach room temperature, then washing buffer was prepared according to manufacturer instruction (wash buffer diluted 1 in 20 with distilled water). The strips was set in strip holder and numbered including one Blank (A, neither sample nor HRP-conjugate was added to blank well ), three negative controls (B,C and D), two positive controls (E and F). 50ul of positive control, negative control and specimen was added into their respective wells. 50ul of extraction solution supplied with kit was added to each well except blank well using separated disposable pipette tips, and then slightly mixed. The plate was covered by plate cover and incubated for 30 minutes at 37°C. At the end of incubation plate cover was removed and each well was washed five times with diluted wash buffer. Each time microwells were allowed to soak for 60 seconds. After final washing cycle the plate was turn on blotting paper tap out any reminders. 100ul of HRP-conjugate reagent was added to each well except the blank and then gently mixed. And then the plate was covered by plate cover and incubated at 37°C for 30 minutes. At the end of incubation plate cover was removed and each well was washed five times with diluted wash buffer. Each time microwells were allowed to soak for 60 seconds. After final washing cycle the plate was turned on blotting paper tap out any reminders. 50ul of chromogen A and 50ul of chromogen B was added to each well including blank well and then gently mixed. Then the plate was incubated at 37°C for 10 minutes. The enzymatic reaction between chromogen solution and HRP-conjugate produce blue color in positive control and HDV-Ag positive sample wells. And then the absorbance was measured at dual filter 450/620nm. And then the cut-off point was calculated (appendix 2).
3.5.6. Quality control and calculation of the results

Reagent, standard and control were checked for storage, stability and preparation before starting work. Each microplate was considered separately when the results was calculated and interrelated; the results were calculated by relating each specimen absorbance (A) to the cut off (C.O.) value of the plate.

Calculation of cut off value (C.O.) = NC* × 2.1 (NC* is mean of the three negative controls). If the mean OD value of negative control less than 0.05 take it as 0.05.

The OD value of the blank well must be less than 0.080 at 450nm.

The OD value of the positive control must be more than 0.80 at 450nm.

The OD value of the negative control must be less than 0.1 at 450 nm.

3.5.7. Interpretation of results

Positive more than cut off value.

Negative less than cut off value.

3.5.8. Data analysis

The data that collected from questionnaire and laboratory results were analyzed by SPSS Version 15 computerized program.
CHAPTER FOUR
RESULTS

A total of ninety blood samples (n=90) were obtained from Hepatitis B positive patients attend four renal dialysis centers in Khartoum State. The samples were collected from Alnow Hospital 25(28%), Ibn Sina specialized Hospital 22(24%) and Salma Center 26(29%), Tropical Disease Hospital 17(19%) (Table 1). The ninety samples involved 74(82%) males and 16(18%) females (Table 2). All specimens were examined for the presence of HDVAg using ELISA Kit. The result showed that out of 90 blood samples investigated, 6 (7%) were positive for HDVAg, the rest 84(93%) were negative (Table 3). From the positive blood samples 5(83%) were males, and 1(17%) were females (Table 4). Out of 74 male samples examined 5(7%) were positive for HDVAg, while the rest 69(93%) were negative. Moreover, out of 16 female samples examined 1(6%) were positive for HDVAg while the rest 15(94%) were negative (Table 5).
Table 1. Distribution of haemodialysis patients according to the hospitals

<table>
<thead>
<tr>
<th>Hospital</th>
<th>Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Alnow Hospital</td>
<td>25</td>
</tr>
<tr>
<td>Salma Center</td>
<td>26</td>
</tr>
<tr>
<td>Ibn Sina Hospital</td>
<td>22</td>
</tr>
<tr>
<td>Tropical Disease Hospital</td>
<td>17</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
</tr>
</tbody>
</table>

Table 2. Distribution of haemodialysis patients according to the Gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Males</td>
<td>74</td>
</tr>
<tr>
<td>Females</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
</tr>
</tbody>
</table>
Table 3. Frequency of HDVAg among hepatitis B positive haemodialysis patients

<table>
<thead>
<tr>
<th>Results</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Positive</td>
<td>6</td>
</tr>
<tr>
<td>Negative</td>
<td>84</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
</tr>
</tbody>
</table>

Table 4. Frequency of HDVAg according to the Gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Males</td>
<td>5</td>
</tr>
<tr>
<td>Females</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
</tr>
</tbody>
</table>
Table 5. Frequency of HDVAg in each gender separately

<table>
<thead>
<tr>
<th>Gender</th>
<th>Result</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>Positive</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>69</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>74</td>
<td>100</td>
</tr>
<tr>
<td>Females</td>
<td>Positive</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>15</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>16</td>
<td>100</td>
</tr>
</tbody>
</table>
CHAPTER FIVE
DISCUSSION

5.1. Discussion
HDV infection absolutely requires associated HBV infection. Studies revealed that in association with HBV infection, HDV produces significantly more severe illness than HBV alone. The disease spectrum of HDV infection varies greatly from fulminant acute hepatitis to rapidly progression to liver cirrhosis and hepatocellular carcinoma (Fonseca, 2002).

HDV remains a major health problem in under developed areas with uncontrolled HBV spread. This present study aimed at detection of HDVAg in hepatitis B positive patients in Khartoum State. Out of 90 blood samples investigated, only 6(7%) were positive for HDVAg. This result is similar to that obtained by McCarthy et al.,(1989) in Eastern Sudan who reported that 9% of hepatitis B positive patients infected with HDV. Also agrees with that obtained in Oman by Aghanashinikar et al., (1992) who reported that according to study conducted in 1991, the HDV prevalence was 7.7% in HBsAg-positive dialysis patients and 22.2% in HBsAg-positive renal transplant patients who have been previously transfused (Aghanashinikar et al., 1992). But disagree with that reported in central Sudan by Hyams et al.,(1989) who reported that about 27.8% of hepatitis B positive patients infected with HDV. This differences may be due to hepatitis B vaccination campaign which resulted in decline in hepatitis D virus prevalence, also this current study aimed at detection of HDVAg. This present study also showed that the prevalence of HDV is equal in both males and females (7% vs 6%).
5.2. Conclusion

1. The current study shows low prevalence of HDV among hepatitis B positive hemodiaysis patients.

2. The level of infection is equal in both males and females.

5.3. Recommendations

1. Screening of the blood before transfusion for both HBV and HDV and extensive vaccination against HBV is recommended.

2. Further studies with large number of samples and more advanced technique are required to validate the results of the present study.

3. Hepatitis B positive hemodialysis patients should regularly screen for HDV.
REFERENCES


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54. Rosenau J, Kreutz T, Kujawa M, et al. (2007). HBsAg level at time of liver transplantation determines HBsAg decrease and anti-HBs increase
and affects HBV DNA decrease during early immunoglobulin administration. *J Hepatol*; 46: 635–44.


