Introduction

Hepatitis is a pathologic term imploying injury of hepatocytes with a resulting inflammatory response .The hepatitis injury may be caused by a virus (Hepatitis A virus, Hepatitis B virus, Hepatitis non-A, non-B virus, Hepatitis Delta virus, Epstein Barr virus and cytomegalovirus virus), a microorganism (bacteria or protozoa), a toxin (alcohol, drugs, or chemicals) or by an immunological abnormalities. (Najarian and Delaney 1985).

Viral hepatitis is a major health problem throughout the world *(WHO , Bulletin 1982).* In Egypt , the disease is endemic and appears to be increasing in frequency . Previous studies showed that , by maturity , most of population has been infected with hepatitis A , and greater than 50 % has been infected with hepatitis B virus *(Hyam et al ., 1986).*

Once reliable diagnostic tests for hepatitis A and B infection has been developed , it was evident that there were other , presumed viral , causes of hepatitis . The cumbersome name non-A , non-B hepatitis was coined for this condition . *(Peutherer 1992).*

The name non-A ,non-B hepatitis means infection of the liver by at least one of three viruses ; hepatitis C virus (HCV) which

is responsible for most cases of non-A ,non-B hepatitis and primarily spread by a parenteral route , although familial , sexual and maternal transmission may occur rarely *(Danaet al ., 1994)* ; hepatitis E virus , which is the major etiologic agent of enteric non-A , non-B hepatitis , occur mainly in developing countries *(Ritter et al ., 1993),* and an unidentified virus which is a "Coaggulation factor transmitted virus" *(Alter et al .,1988).*

Non-A non-B hepatitis is the major cause of post-transfusion associated hepatitis *.(Shorey 1985).*It account for about 90 % of post-transfusion hepatitis in countries where blood is screened only for HBsAg *(Feinman et al., 1988 , Abbott HCV Learning Guide 1990),* and because , viral hepatitis is a global disease of major significance , both in the incidence of mortality and morbidity , So, specific laboratory tests must be used to establish the diagnosis .

Aim of work

The aim of this work is to determine the incidence of non-A, non-B hepatitis carrier state among the volunteer blood donors, from El-Gharbia and El-Kalubeia (Benha) blood banks, by examining their sera for the presence of hepatitis B surface antigen (HBsAg), by indirect haemoagglutination method, antibody for hepatitis C virus (Anti-HCV) by second generation Enzyme Linked Immuno-Sorbant Assay (ELISA) method and also for the surrogate markers of non-A non-B hepatitis which are elevation of alanine amino-transferase (ALT) by coloremetric method and the presence of hepatitis B core antibodies (HBcAb) by ELISA method .

REVIEW OF LITERATURE

Hepatitis A Virus

History :

In (1973) hepatitis A virus (HAV) was identified by the electron microscopy in the fecal specimens from patients and preparation of liver from experimentally infected marmoset monkeys. (International work shop in hepatitis A virus infection, 1983).

In (1975) Feinstone et al., identified 27 nm virus-like particals in the stool filtrates from patients with type A hepatitis. It was also reported that this agent was probably an RNA virus (*Provost et al., 1979*). Now, Hepatitis A virus (HAV) has been shown clearly to have the physicochemical properties of the genus Entroviruses within the family Picornaviridae (*Melnick, 1982*). and the International Committee on Taxonomy of Viruses in its fourth report, classified HAV as an entrovirus type 72 (*Mathews, 1982*).

Structure :

Hepatitis A virus (HAV) is a spherical RNA containing particles 27 to 28 nm in diameter without an envelope having a sedimentation coefficient of 156 to 160 and density of 1.33 to 1.34 g/ml in calcium chloride (International Workshop in hepatitis A virus infection ., 1983).

Epidemiology :

Hepatitis A virus causes a mild disease which is called hepatitis A or Infectious hepatitis. It is common in infancy and childhood. Outbreaks of type A hepatitis are common in families and institutions, summer camps, and especially among troops (*Sherlock., 1981*).

Serological tests have made it possible to study the rate of infection in different population throughout the world . Such studies confirm that , since the virus is spread by the faecal-oral route , it is prevalent in countries where sanitation is poor . Serological studies also show that , even in developed countries , more than half the population have been infected with HAV . (*Burns and Inglis 1992)*

Mode of Transmission

Hepatitis A virus is transmitted from person to person by fecal-oral route especially in family contacts, neighbors and contact persons in kindergartens, schools, prisons and other closed institutions (*Frosner et al., 1977*).

Infection with hepatitis A virus is caused either by contaminated food from an incubatory carrier dealing with preparation or handling of food e.g fruit salad, cold meats or drinking contaminated water, milk, and fruit juice (*Chaudhuri et al., 1975*).

Pathogenesis :

Like other erteroviruses , HAV probably infects cells in the gut and then spreads to the liver via the blood . The histopathology is similar to that of hepatitis B , with periportal necrosis and infiltration of mononuclear cells ; viral antigens are seen in the cytoplasm of the hepatocytes .The virus is excreted via the bile into the gut from about 1-2 weeks before the onset of jaundice , excretion then declines rapidly over the next 5-7 days . Virus is also present in the urine of clinical and sub clinical cases during the same period .*(Burns and Inglis 1992)* :

Clinical presentation :

Hepatitis A has an incubation period which ranges from 2-7 weeks (*Decker et al., 1979 and Eastwood et al., 1988*).

In clinically apparent cases , there is an abrupt onset of symptom. Jaundice , malaise , fever , anorexia , nausea and abdominal pain are frequent . In approximately 25% of the cases , hepato-splenomegaly may be noted . *(Eastwood et al ., 1988).*

Laboratory Diagnosis :

Diagnosis of HAV relies on the demonstration of specific IgM antibody to HAV , which develops very early in the course of infection and is generally present by the time the patient is investigated . It is detectable in the serum for 2-6 months after the onset of symptoms . IgG antibody usually persists for many years and is a useful indicator of immunity .*(Burns and Inglis 1992)* .

Prevention and control :

In countries where there has been improvement in socioeconomic condition and sanitation, there has been an increase in the mean age of exposure, and a decline in the total rate of infection .(*Dusheiko 1990*).

The development of vaccines against HAV is the subject of intense interest. HAV can be grown in tissue culture, and killed formaline inactivated vaccines have been developed from diploid fibroblast cultures which protect monkeys against challenge . Also by molecular cloning of HAV, it may be possible to produce vaccines through recombinant D.N.A technology. (*Ticehurst 1986. Cohen et al., 1987& Dusheiko 1990*).

Passive immunization with normal human immunoglobulin , gives protection to seronegative individuals for a limited period of up to 6 months . *(Burns and Inglis 1992)* .

Hepatitis B Virus

History :

In 1947, McCallum proposed that hepatitis B infection is caused by hepatitis B virus (HBV). It was also termed long incubation hepatitis and serum hepatitis (*WHO., 1982*).

Structure :

Dane et al., (1970). suggested that HBV found in the patient serum, has three different kinds of particles : spherical with a diameter of 22 nm, tubular with a diameter of 22 nm and length of 40-400 nm and Dane particles, with a diameter of 42 nm (Fig.1).*(Tiollais et al ., 1981).*

Spherical and tubular are composed of lipid , protein and carbohydrate ; they are not infectious and consist solely of surplus virion envelope , which carry the hepatitis B surface antigen (HBsAg). *(Peutherer 1992)*

The dane particles are the virions of HBV. The particles are surrounded by a lipoprotein coat about 7nm thick . The dane particle has an inner core with a diameter of about 27nm , which contains the viral DNA and DNA polymerase within a shell composed of hepatitis B core antigen (HBcAg) . The core has a further antigen the "e" antigen or (HBeAg) , which is derived from the core protein and is thus virus coded , although there is no separate "e" antigen . *(Peutherer 1992)* .

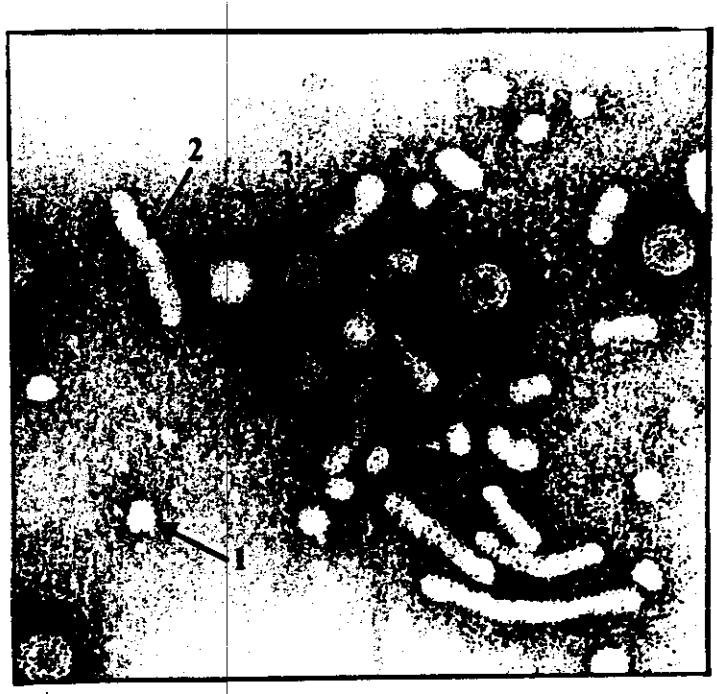

Fig. (1)

Hepatitis B virus : Electron microscopy shows three components : small spheres (1, diameter 28nm) , tubules (2), which are groups of small spheres, and Dane particles (3, 42nm), which are probably the infective virions . Photographed from , Sherlock and Summerfield (1979) (Magnification 1× 40) .

Epidemiology :

The prevalence of HBV infection varies widely in different parts of the world . Highest rates, 10 - 15 % , was found in South East Asia , China , equatorial Africa , Oceania and South America . Vertical and horizontal transmission are common . Intermediate rates 5 % was found in eastern Europe , around the Mediterranean and Middle East . Lowest rates , 0.1 - 0.5 % , was found in Western Europe , North America and Australia .

Overall , it is estimated that there are 200 - 300 million carriers in the world , with a preponderance of male carriers in all populations . (*peutherer 1992*)

Transmission :

Parenteral Route of Transmission :

Transfusion of whole blood or blood products, is one of the most common known methods of transmission of hepatitis B virus. The risk of Hepatitis B in recipient of blood, increases with increasing the number of transfusions . (*Allen and Sayman, 1962*).

It was found that hepatitis occurs in 52% of recipients of HBsAg positive blood as compared to 20% for those receiving only surface antigen negative blood (*Jawetz et al., 1987*).

Parenteral exposure of contaminated material may occur if a toothbrush or razor is shared, a small cut is contaminated or an

instrument is improperly sterilized . Syringe-transmitted infection is particularly common among narcotics addicts and where parenteral drug abuse is a serious problem . (*Howard, 1984*).

Non-Parenteral Routes of Transmission :

Non-Parenteral routes of exposure may also play a part in infectivity, as HBV has been detected in a variety of body secretion and excretion, including saliva, semen and vaginal fluid, so infection may be transmitted by kissing or sexual intercourse (*Steigmenn and Doutrdourekas., 1976*). The virus will gain entry through cuts and abrasions or across mucous membranes .

(Peutherer 1992) .

At very high risk are the sexually promiscuous, particularly male homosexuals (*McColluum and Zuckerman 1981*).

HBV can be transmitted also in tears (*Boxall et al., 1974*). breast milk (*Darrel & Jacob, 1978*) and pancreatic and biliary secretions (*Hoefs et al., 1980*).

Vertical Transmission of HBV (Perinatal) :

Vertical transmission from mother to child is one of the most important routes . Transmission probably occurs when maternal blood contaminates the newborn mucous membranes during birth. Transplacental infection is thought to be quite rare . *(Peutherer 1992)* .

Pathogenesis :

The virus itself is not directly cytopathic , and the diversity of lesions described in infected patients has been attributed to variation in the capacity of the host's immune response to eliminate or suppress the infective agent . The mononuclear cell infiltrate in the liver is composed chiefly of cytotoxic T cells . The lysis of infected hepatocytes , in association with production of virus-neutralizing antibody , is probably responsible for recovery. *(Stites et al ., 1984)*

Incubation Period :

Hepatitis B virus has a long incubation period, this period ranges from 2 to 26 weeks with an average of about 50 days (*Ockner, 1985*).

Clinical Presentation :

Acute Viral hepatitis

Acute hepatitis B infection may cause serious icteric hepatitis or even fulminant hepatitis, but the infection may also be an icteric and asymptomatic in a high proportion of cases . Symptoms begin gradually towards the end of the long incubation period. They include fever, anorexia, malaise, and fatigability. Jaundice develops in about half the cases, and about 1% have a fulminant course and

die. The acute disease, has a variable duration ranging from days to months. (*Eastwood et al., 1988*).

Chronic Viral Hepatitis

Chronic hepatitis B is defined as the presence of hepatitis B surface antigen (HBsAg) in the serum for longer than six months, There is a spectrum of disease ranging from benign to severe form of chronic hepatitis. The disease may remain clinically silent for decades but nonetheless, progress to cirrhosis and hepatocellular carcinoma (HCC) (*Beasly 1988*).

Chronic Active Hepatitis

Reactivation of quiescent chronic HBV infection may occur spontaneously or more frequently in individuals following the withdrawal of immunosuppressive drugs (e.g chemotherapy, steroids, organ transplantation), In some patients reactivation may precipitate fulminant hepatic necrosis. (*Eastwood et al., 1988*).

Super infection :

Hepatitis B virus carriers may develop hepatic super infection with other hepatotropic viruses, sudden increase in the serum transminases may represent super infection with HAV, NANB or HDV (*Eastwood et al., 1988*).

Serological Markers of (HBV)

Hepatitis B Surface Antigen (HBsAg)

Infection with hepatitis B virus leads to the appearance of hepatitis B surface antigen in the serum during the incubation period which is usually 2 to 8 weeks before either biochemical evidence of liver dysfunction or the onset of jaundice , So , it is the earliest indicator of the presence of acute infection (Fig. 2) . The HBsAg was originally refered to as Australia antigen or hepatitis associated antigen .

(*McCollum and Zuckerman , 1981, Peutherer 1992*) .

HBsAg is a general term to describe antigen material produced during the expression of the genome of hepatitis B virus .

(*Howard, 1984*)

Ahtone & Maynard (1983) reported that 905 of persons who aquire acute hepatitis B infection will have a negative HBsAg test results within 4 to 5 months after the onset of clinical illness. A person with HBsAg positive result is considered to have an infectious condition and can potentially transmit HBV to other susceptible as chronic carrier and may remain HBsAg positive for a prolonged period. Carrier state of hepatitis B virus becomes established in approximately 5 to 10% of the infected adults (*McCollum and Zuckerman, 1981*).

In cases of persistence of HBsAg for more than 6 months , and elevated serum transaminase levels, the patient is usually will pass either to chronic active hepatitis (CAH) or chronic persistent hepatitis (CPH) (*Ahtone & Maynar, 1983 and Peutherer 1992*).

Antibody to HBsAg (Anti-HBs Antibodies) :

It was found that some persons exposed to infection with HBV manifest a low level of anti-HBs antibodies as the only serological marker of exposure (*Mushahwar et al., 1981*). The presence of anti-HBs antibodies following natural infection with HBV or after vaccination with HBV vaccine is associated with immunity to future hepatitis B infection (*Ahton & Maynard, 1983*).

The transfusion of blood containing anti-HBs antibodies appears to carry no risk of hepatitis B to recipient. However, anti-HBs antibodies may not appear in about 10% - 15% of cases of acute HBV infection, in addition anti-HBs antibodies usually appears weeks or months after the disappearance of HBsAg and multiple blood samples may be necessary to document this conversion (Fig. 2) (*Perrillo et al., 1983*).

Anti-HBs antibodies may be transferred passively across the placenta or by administration of HBs immunoglobulin and are usually present for several months (*Ahtone et al., 1983*).

Hepatitis B Core Antigen (HBcAg) :

HBcAg is found within the core of the virus. which is the inner envelope of HBV, the most interesting feature of the dane particle is that the outer, and inner shells are serologically distinct (*Ahton & Maynard 1983*). It is found mostly in the nucleus of the infected hepatocytes seen by electron microscopy and immunoflourescence and its presence indicates active viral replication (Fig 2) (*Robert et al., 1984*).

Hepatitis B Core Antibody (HBcAb) :

This is the antibody produced by the body defense mechanisms against HBcAg (*Stites et al., 1982*). (*Neimark et al., 1982*). Anti-HBc antibodies will appear 10-20 weeks after exposure or about 2-10 weeks after the appearance of HBsAg in the serum. Its presence indicates either current or recent infection with the hepatitis B virus (Fig 2) (*Ockner, 1985*).

Anti-HBc antibodies is very important since it is the only hepatitis serum marker to be detected in the serological gap period in which both HBsAg and anti-HBs antibodies are negative inspite of the patient being infectious (Fig 2) (*Zuckerman, 1980*).

Anti-HBc antibodies is a good epidemiological tool, since, when measured in conjunction with the HBsAg, it identified more cases of infection with HBV (*Sherlock et al.,1985*).

The IgM hepatitis core antibody (Anti-HBcIgM) becomes detectable in the serum at about the time of clinical onset of acute hepatitis together with rise in the serum transaminases. This makes it a good markers for the diagnosis of acute recent infection with HBV. At that time, the only other marker present is HBsAg which does not differentiate acute from chronic infection (Fig 2) .(*Ockner 1985*).

Initially it is present in high serum titer and persist for several months to one year, after the infection is cleared. Its disappearance is therefore, the most reliable index for the clearance of hepatitis B virus and its continuous presence usually means active hepatitis B virus replication (*Deinhardt et al., 1982, and Sherlock 1985*).

The quantification of IgM core antibody (Anti-HBcIgM) is useful in 2 ways. It is an indicator of the prognosis in acute infection since patients with high titer tend to progress to chronic hepatitis and it can differentiate acute from chronic hepatitis, being serum titer much higher in the acute form and low range in chronic (*Sherlock 1985*).

The IgG hepatitis B core antibodies (Anti-HBcIgG antibodies) predominate after a period of time ranging from several months to one year following the acute infection with the hepatitis B virus and persists in patients with acute and chronic for years after clearance of the hepatitis B Virus . (Fig 2) (*Picciotto et al., 1981& Ockner, 1985*)

Hepatitis B " e " Antigen (HBeAg) :

HBeAg is produced when the virus is replicating and thus , it is usually found soon after HBsAg .It is an early indicator of acute active infection , representing the most infectious period . Usually , short-lived (three to six weeks) (Fig . 2) , persistence of "e" antigen in acute stage beyond ten weeks is indicative of progression to chronic carrier state and probable chronic liver diseases .

(Peutherer 1992).

Hepatitis B " e " Antibodies (Anti-HBe Antibodies) :

This is the antibody produced by the host immune response against the "e" antigen (HBeAg) of the virus , HBeAg appears when HBeAg become negative (Eastwood et al ., 1988). It occurs after the appearance of anti-HBc antibodies . It appears late in the course of disease in the majority of the patients infected with hepatitis B virus (*Szmuness et al., 1981*). Anti-HBe antibodies can be detected in the serum 2-3 months after the onset of the disease and is detectable up to 1-5 years after acute infection (Fig.2) . Seroconversion from HBeAg to anti-HBe during acute stage is prognostic for resolution of infection. Its presence with anti-HBc can confirm the convalescence stage in absence of anti-HBs .

(peutherer 1992)

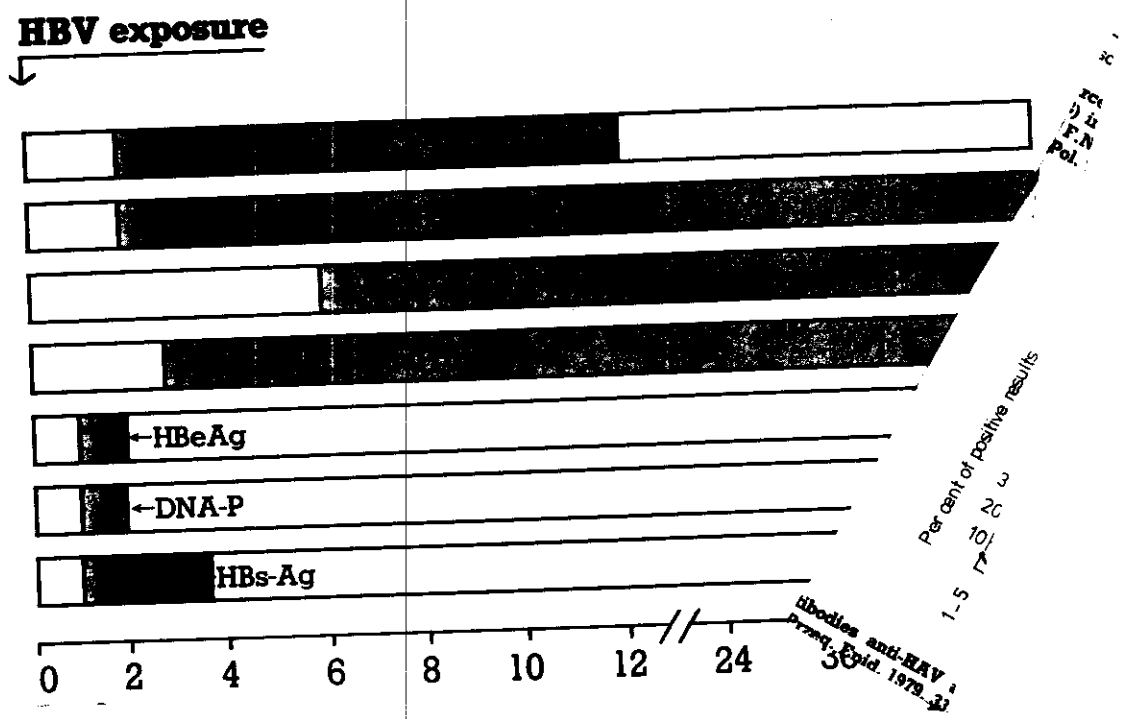

Fig. (2)

Sequential appearence and disappearance of hepatitis B antigens and antibodies in an uncomplicated course of acute type B hepatitis with transient HBs antigenemia, stippled bars indicate detectable parameters. Photographed from Arnold et al., (1983).

The DNA Polymerase Enzyme :

Alter et al., (1974). found that the DNA ploymerase enzyme is so closely associated with particular HBcAg and it may be used as a biochemical marker for the core of hepatitis B virus .

(*Alter et al., 1984*).

The DNA ploymerase is an enzyme produced by the HBV during its active replication to repair the gap present in the circular DNA. It has therefore, similar significance to HBcAg but it is more sensitive marker . (Fig 2) (*Zuckerman 1979*).

It is detectable for a short time, and disappears before elevation of transaminases. It is detected during peak surface antigenemia. In acute disease it is present for only few days to weeks (*Neimark et al., 1982*) , but in patients who developed chronic type B hepatitis , DNA , and DNA polymerase persist and accompany elevated serum transaminases even after 6 months of symptomatic disease. *(Eastwood et al ., 1988 , Peutherer 1992)*

Treatment of Hepatitis B Virus :

Treatment of acute hepatitis B virus :

The treatment is supportive and includes intravenous fluids for hydration, for correction of electrolytes abnormalities, and to provides caloric intake if nausea and vomiting are present .

(Myers et al ., 1986)

Treatment of chronic persistent hepatitis B virus :

Anti viral e.g. adenine arabinoside monophpsphate , has induced transient responses , but therapy is complicated by the toxicity of the drug . Interferon have shown promise , particularly synthetic interferon alfa - 2b (Intron A) . Ribavirin is the first drug to offer a potentially effective oral treatment .*(peutherer 1992)*

Such treatment can only inhibit viral replication , so , it is usual to select patients with evidence of chronic active hepatitis and continuing viral replication with HBeAg , viral DNA or DNA polymerase in the serum . *(Peutherer 1992).*

If patients are selected according to these criteria , a response to interferon can be expected in 50 % of patients . *(peuthther 1992)*

The response to interferon treatment is more frequent in anti-HBe positive than in the HBeAg positive patients , probably due to the lower replicative levels present in the former . However , the post treatment relapse rate was significantly higher in the anti-HBe positive patients , suggesting that , the lack of HBeAg results in difficulty in HBV eradication .*(Sotorrio et al ., 1993)* .

Prevention and Control :

To prevent the spread of HBV infection through blood transfusion, we must screen blood for evidence of HBV prior to transfusion, the use of volunteer rather than paid blood donors, substantially decreases post-transfusion hepatitis B infection. (*Barker et al., 1976*).

It has been estimated that screening all donors for anti-HBc antibodies and discarding all positive donors, would decrease the post-transfusion hepatitis by about 6% and decrease the donors pool by 5% (*Hooland et al., 1982 and Butterly et al., 1989*).

Current vaccines are prepared from 22 nm viral coat particles obtained from plasma of chronic carriers or by recombinant DNA technology (*Andre 1988, Mmurray 1988*). Recombinant vaccines have gained wider acceptance in the western world because the Anti- HBs antibodies titers persist longer in initially good responders , and the risk of infection is correspondingly lower in those vaccinated peoples (*Dusheiko 1990*).

Non-A, Non-B Hepatitis

History :

Non-A, non-B (NANB) hepatitis appeared as a separate entitiy in the mid-1970., and became the leading cause of post-transfusion hepatitis (PTH) (*Sarver, 1986*).

Once reliable diagnostic tests for hepatitis A and B infection had been developed , it was evident that there were other , presumed viral, causes of hepatitis . The name non-A, non-B hepatitis was coined for these conditions . *(Peutherer 1992).*

The term non-A, non-B (NANB) viral hepatitis is used to describe hepatitis that is not caused by the commonly known hepatotropic viruses such as HAV, HBV, HDV, CMV, EBV and others (*Eastwood et al., 1988*).

The name non-A, non-B hepatitis includes infection of the liver cause by at least three viruses : hepatitis C virus, which is blood transmitted virus, hepatitis E virus, which cause the epidemic water-borne non-A, non-B hepatitis and an unidentified virus which is a "Coaggulation-Factor-Transmitted virus" (*Alter et al., 1988 , Ritter et al ., 1993 , Dana et al 1994*).

Properties of hepatitis C virus (HCV) :

Studies in primates at the Centers of Disease Control (CDC) proved the presence of a transmissible agent in blood products and sreum from blood donors (*Tabor et al., 1984*). This agent was identified as a small lipid enveloped virus with a positive-stranded RNA molecule. Filtration studies revealed that it was less than 60 nm in diameter (*Holf et al., 1987, Choo et al., 1989*).

It is sensitive to organic solvents such as chloroform and formalin and heating at 60 C for 10 hours (*Tabor et al., 1980, Bradley et al., 1983, Feinstone et al., 1983*).

HCV is the major cause of transfussion-associated non-A, non-B hepatitis. In addition, HCV appears to be a major cause of community-acquired non-A, non-B hepatitis in the United State for which no parentral exposure can be documented (*Kuo et al., 1989*).

Hepatitis C virus (HCV) is responsible for most cases of non-A , non-B hepatitis and primarily spread by a parentral route , although familial , sexual and maternal transmission may occur rarely . *(Dand et al ., 1994).*

Recently , hepatitis C virus has been subdivided into at least four genotypes , and the prevalence of each genotype has been reported to differ widely in different countries . *(Okuno et al ., 1994)*

Properties of hepatitis E virus (HEV) :

By 1987, researchers at the Centers for Disease Control had established an animal model for enteric NANB hepatitis after transmitting the human disease to primates, they recovered a disease-associated 27 to 34 nm virus-like particles. The viral agent was found to be a member of the calicivirus family but it showed characteristics similarities to that of picornavirdae, which includes the enterovirus type 72, the hepatitis A virus. The genome of HEV is a single-stranded form of RNA. (*Yoshizawa et al., 1980 , Abbott diagnostics Educational services 1990*).

Hepatitis E vieus (HEV) , the major etiologic agent of enteric non-A , non-B hepatitis , is considered to occur mainly in developing countries . *(Ritter et al ., 1993).*

Properties of coaggulation-factor transmitted non-A , non-B hepatitis virus :

A third virus of non-A, non-B hepatitis is transmitted by most lots of the coagulation factor concentrates antihaemophilic factor (factor VIII,AHF) and factor IX. (*Crask et al., 1978*). It is inactivated by heating to 60 C for 10 hours. (*Bradley et al., 1979*). It has also been reported that this agent was found to be resistant to chloroform (*Bradley et al., 1983*). This virus can be transmitted to chimpanzees. The convoluted tubules seen by electron microscope

in the liver biopsy of chimpanzees inoculated with blood transmitted virus (hepatitis C virus) are not found in association with this virus (*Tabor et al., 1984*).

Epidemiology of non-A , non-B hepatitis :

NANB hepatitis has been reported in nearly all parts of the world and approximately 100 million individual are chronic carriers of hepatitis C viruses . (After abbott Diagnostics Educational Services Feb,1990). Hepatitis Cvirus has claimed to be the cause of 10 - 50 % of all cases of acute hepatitis . *(peutherer 1992)*

Hepatitis E virus which is the major etiologic agent of enteric non-A , non-B hepatitis , is reported to occur mainly in developing countries , and around 1% of blood donors have a past exposure to HEV . *(Ritter et al ., 1993).*

The contribution of NANB hepatitis cases depends on geographical location and the patient risk group . In Europe, North America, and Australia, it may account for 20% - 40% of the reported cases of acute viral hepatitis (*Alter et al., 1982*). In Africa and Asia, NANB hepatitis may account for 50% of all viral hepatitis cases discovered (*Liaw et al., 1983*).

Modes of Transmission of NANB Hepatitis

1-Transfusion Transmitted NANB Hepatitis :

NANB hepatitis is the major cause of transfusion associated hepatitis(*Shorey, 1985*). It accounts for about 90% of post-transfusion hepatitis (PTH) in countries where blood is screened only for HBsAg (*Feinman et al., 1988*) and the frequency of transfusion-associated hepatitis is approximately 7 - 10% in multitransfused patients (*Eastwood et al., 1988*).

Blood products may also transmit NANB hepatitis, however the risk of developing NANB hepatitis after infusion with pooled products is much higher due to the bio-amplification of large blood pools (*Barbara and Tedder 1984*).

Hemophiliacs who have been exposed to unheated and/or dry heated pooled clotting factor concentrates are at high risk of chronic hepatitis C . Although the mechanism and site of hepatitis C virus (HCV) replication are not yet known , HCV is thought to replicate through a complementary negative RNA strand , as has been shown for flaviviruses . The detection of negative RNA strands has therefore been regarded as a marker of replication . *(Willems et al ., 1994).*

2-Haemodialysis :

Among 2070 patients and 1629 personnal of 13 haemodialysis units followed prospectively for up to 3 years, the average annual attack rate of acute NANB hepatitis was 5.8% (Range 0.5 - 16%) for patients and 0.8% (Range 0% - 2.3%) for staff. For patients the risk of NANB hepatitis was associated significantly with recent transfusion, while for staff the risk factor that correlated with NANB hepatitis was recent needle stick. (*Dienstage et al., 1981*).

In addition to blood transfusion , there may be other routes of hepatitis C virus infection associated with long term dialysis . Hepatitis C virus seems to be an important cause of chronic liver disease in dialysis patients .*(Nakashima et al ., 1994)*.

3- Organ Transplant Recipients :

Approximately 70% of the chronic liver disease in renal transplant recipients can be attributed to NANB hepatitis. More than 50% of these cases advanced to chronic active hepatitis and to cirrhosis, leading to liver failure in 5 - 10 year after infection. NANB hepatitis in these immunocomprimised individuals is also associated with a higher frequency of life threatening extrahepatic infections (*Eastwood et al., 1988*).

4- Inravenous Drug Users :

NANB virus is thought to account for approximately 40-50% of acute hepatitis seen in intravenous drug users. This is an under estimation because many intravenous drug users have serologic evidence of HBV infection, co-or superinfection with NANB agents cannot be accurately diagnosed (*Eastwood et al., 1988*).

In Europe and the United State, the prevalence of hepatitis C virus (HCV) antibody positive cases is generaly reported to be up to 77% in drug users infected with Humen Immuno Difficieny Virus 1 (HIV-I) (*Jackson et al., 1991*).

Hepatitis C virus infections are known to be common in injectable drug users , and additional public health measures may be required for this problem . *(Woodfield et al ., 1994).*

5-Hospital Contact :

Individuals in contact with patients infected with NANB hepatitis or their blood are at high risk. Occupational contact was observed as a risk factor in 7.6% of sporadic cases of NANB hepatitis in Japan (*Nagata et al., 1985*).

Health-care workers are known to be at risk from occupational transmission of blood-born viruses , including HCV . There may be serious implications following infection with hepatitis C virus , including possible transmission to patients *(Zuckerman et al .,1994)*

6-Sexual Transmission :

NANB hepatitis was observed to be transmitted through hetrosexual and homosexual contact, several studies were carried out to evaluate the incidence of NANB hepatitis among homosexual men. Homosexual contact had accounted for less than 10% of sporadic NANB hepatitis (*Alter et al., 1982*).

Although the form of sexual transmission is not well identified , the duration of sexual activity suggests that the time needed to become infected is long . *(Garcia-Bengoechea et al ., 1994).*

7-Perinatal Transmission :

NANB hepatitis has been transmitted to infants born to mothers who had active NANB hepatitis during the third, but not the second trimester, of pregnancy (*Eastwood et al., 1988*).

Perinatal transmission from ordinary anti-HCV positive women to newborns is a very unfrequent event . Transmission of IgG anti-HCV account for transient newborn positivity of the anti- HCV ElISA test . *(Manzini et al ., 1993).*

Priliminary data suggested that HIV-I may enhance the risk of maternal HCV transmission to newborn babies. (*Jackson et al., 1991,*). Perinatal transmission was rare in those born from HIV negative mothers , frequent if mothers were HIV positive . *(Barbacini et al ., 1993).*

8-Sporadic Infections .

Tabor et al., (1979) reported that 93% of donors whose blood transmits non-A, non-B hepatitis to recipients have themselves never received a transfusion.

Acheson and his Colleagues (1988), described non-parentrally transmitted non-A, non-B hepatitis (NANB) in children in north west England suggesting that those cases might represent further evidence that NANB hepatitis observed in western countries can somtimes be sporadic instances of epidemic, waterborne NANB hepatitis . Faecal-oral transmitted diseases are commonest in Mediterranean countries, yet we know of no reports of faecal-oral transmission of sporadic NANB hepatitis in children in the Mediterranean region. Perhaps in these countries children presenting with acute HBsAg-negative hepatitis have usually been diagnosed clinically as having hepatitis A, without measurement of IgM anti-HAV antibodies, so that sporadic NANB hepatitis in children has been missed (*Careadda et al., 1988*).

Dusheilo and his colleagues (1990) recorded cases of hepatitis C virus infection after a human bite. Transmission of NANB hepatitis by saliva has been shown in champanzees this might therfore be one of the routes of transmission in sporadic HCV infection (*Wang et al., 1991*).

Incubation period :

The mean incubation period for transfusion-associated NANB hepatitis is 7-8 weeks with a range of 2-26 weeks. Shorter incubation period of 1-2 weeks have also been reported (*Eastwood et al., 1988*).

Clinical presentation :

1- Acute NANB Hepatitis :

This disease accounts for between 4% and 26% of hospital admission for acute viral hepatitis, although the relative incidance differs greatly worldwide (Medical Research Council Working Party 1974).

The acute illness is generally less severe than either acute hepatitis A or B, most patients with acute post-transfusion hepatitis are not jaundiced and suffer only vague symptoms of fatigue and malaise, with a lower peak serum Alanine amino-transferase (ALT) than encountered in hepatitis A or B . However the acute disease has an ominous propensity to progress to chronic liver disease. Biphasic elevation of serum aminotransferases in cases of acute post transfusion NANB hepatitis make the determination of convalescence in these patients quite difficult. Sporadic NANB hepatitis in particular,may lead to fulminant hepatitis in about 1-2% with a particulary bad prognosis. (*Mathiesen et al.,1980,Peutherer 1992*).

A number of other important clinical features including arthritis, rashes, aplastic anaemias and agranulocytosis, meningoencephlitis, reticulopathies and prepheral neuropathies, have been found to be associated with NANB hepatitis (*Stock et al., 1978*).

2-Chronic NANB Hepatitis :

It has been unequivocally established that a proportion of patients with acute NANB hepatitis develop chronic liver disease, in many studies the rate of progression of acute to chronic disease is disturbingly high and up to 50% of persons with post-transfusion hepatitis may have raised aminotransferases for six months post infection (*Rakela & Redeker 1979 , Peutherer 1992*).

Most chronic NANB carriers have few subjective symptoms other than fatigue. The long-term prognosis varies, in approximately 20% of patients, cirrhosis may develope insidiously within ten years (*Koretz et al., 1980*).

An increasing number of reports have drawn attention to the possibilily that hepato-cellular carcinoma (HCC) may be associated with cirrhosis due to NANB virus , and recently hepato cellular carcinoma (HCC) has developed in chimpanzees infected with NANB virus (*Kiyosawa et al., 1984, Okuda et al., 1984, Muchmore et al., 1988, Ray et al ., 1994*).

Chronic sequelae of non-A , non-B hepatitis :

The long term follow-up studies in patients with NANB hepatitis have revealed that at least 50% of patients infected with NANB agents develop chronic disease . The disease may continue to appear and to resolve both biochemically and histologically up to 2-3 year, followed by intermittent or constant elevation of serum transaminases. Persistant viremia can prevail in the presence or absence of elevated ALT activity. The total resolution of the disease may occur in only a small proportion of infected individuals (*Eastwood et al., 1988*).

NANB hepatitis will evolve into chronic hepatitis in 50% of patients, at least 20% of whom will eventually have cirrhosis Furthermore patients with chronic hepatitis C infection and cirrhosis are at high risk for hepatocelluar carcinoma (*Schiff, 1991, Ray et al ., 1994*).

The mortality rate from fulminant hepatic failure resulting from HCV infection was thus significantly higher than for fulminant hepatic failure caused by hepatitis A virus or hepatitis B virus infection alone (*Yanagi et al., 1991*).

Diagnosis of Non-A, Non-B Hepatitis

1-Diagnosis by exclusion :

The diagnosis of acute NANB hepatitis is still depend on exclusion ,when HBsAg and anti-HBcIgM antibodies are both negative, hepatitis B is not the cause of acute infection. When anti-HAV IgM antibodies is not detected so hepatitis A is not the cause of the hepatitis. By exclusion of these markers from the clinical diagnosis and with ALT levels at least 2.5 time normal , diagnosis of NANB hepatitis is suspected (*Alter et al., 1982*).

2- Using Surrogate Markers for NANB Hepatitis :

In the absence of a specific non-A , non-B antigen or antibody assay , two indirect tests have been proposed as a means of detecting non-A , non-B agents in carrier blood donors , these tests were called the surrogate markers for NANB hepatitis , which have been used primarily in U.S.A and France , these tests measure alanine aminotrasferase (ALT) and anti-HBc antibodies (*Koziol et al., 1986, Eastwood et al ., 1988*).

The first marker is ALT which is an enzyme released into the circulation after damage to the liver. Its presence is a nonspecific indicator of liver damage (*Sugg et al., 1987*). The diagnosis of chronic non-A , non-B hepatitis may be based on elevation of serum

aminotransferase (ALT) for one year or more , in the absence of evidence for infection with hepatitis B virus , and other liver diseases or taking medication which likely to cause rise of serum ALT. *(Marcellin et al ., 1991).*

The second marker, the anti-HBc antibodies is an indicator of the core antigen of HBV. As such, it is a life-long marker for post infection and a marker for active acute or chronic infection. It was found that blood products that are positive for anti-HBc antibodies correlate with transmission of NANB hepatitis at a rate two to three time higher than blood that is negative for anti-HBc antibodies (*Stevens et al., 1984*).

Koziol et al., 1986, found that, there was no correlation between the presence of anti-HBc antibodies and the presence of an elevated ALT level and these two surrogate markers for NANB carrier state appeared to act as independant variables .

It has been suggested that the incidence of post-transfusion NANB hepatitis might be reduced by up to 30% by exclusion of donated blood that is positive for anti-HBc antibodies or has ALT activity above 45 UI/L. Alter et al., 1991, predicted that 50% of donors positive for both markers would transmit NANB hepatitis by transfusion (*Contreras et al., 1991*).

3- Detection of Anti-Hepatitis C Virus Antibodies :

Hepatitis C virus (HCV) is responsible for the transfusion-associated non-A , non-B hepatitis and antibodies to the HCV, could be detected in blood .(*Stevens et al., 1990*). Also , hepatitis C virus has been recognized as a major cause of non-A , non-B viral hepatitis . *(Yap et al 1994).*

The assay for detection of the circulating viral antibodies to HCV was developed using a purified antigen prepared from recombinant yeast clones from the genome of the viruses (*Stevens et al., 1990*).

The appearance of anti-HCV was delayed, mean delay 9-21 weeks after transmssion or 15 weeks after the onset of clinical hepatitis. This prolonged delay suggestes that some donors are capable of transmitting infection with the non-A, non-B hepatitis before they will be detected by the anti-HCV antibodies assay (*Alter et al., 1989*).

Also, sequential studies of samples indicate that , in acute stage , no more than 20 % of patients have seroconverted , this rises to 60% by 6 months and almost 100% by 1 year . Thus , the test in its present form can be used for epidemiological studies and for the diagnosis of chronic infection , but may have limited use in the early stages of acute infection .*(Peutherer 1992).*

First Generation ELISA Test :

The first generation test to detect human anti-HCV antibodies utilizes the nonstructural protein c-100-3 . This proteins prepared by recombinant technology using yeast fermentation and a fusion protein to SOD (Super Oxide Dismutase) .Some yeast proteins co-purify alongside the viral protein c-100-3 during the purification process . This fact leads to some false positive results with the first generation HCV assays . *(Alberti 1990).*

The first generation (ELISA) test for antibodies to hepatitis C virus (anti-HCV) is positive in about 75% of patients with chronic NANB hepatitis .*(Marcellin et al ., 1991).*

Second Generation ELISA Test :

The aim of the second generation ELISA test for detection of HCV anibodies is to provide means to discriminate between true and false positive results of the firist generation tests*(Dawson 1990)*

In the second genaration ELISA test, two alternate assay formats have been designed to confirm the presence of antibodies to HCV in blood or plasma . The first one is a neutralization (blocking) immuno-assay which uses a recombinant antigen produced in E-coli. The second alternate assay utilizes four synthetic peptides representing sequences within the protein c-100-3. *(Dawson 1990).*

Second Generation Recombinant immunoblot assay(RIBA Test) :

The second generation , Chiron .RIBA test is a recombinant immunoblot assay which includes the c100-3 antigen used in the ELISA and three other antigens (5-11, c-33-c, c-22-3). *(Skidmor 1990)*

In patients with Chronic NANB hepatitis, the Chiron RIBA for detection of anti-HCV antibodies was found to be much sensitive than the first generation ELISA. (*Marcellin et al., 1991).*

The second generation recombinant immunoblot assay (RIBA 2) is used routinely to confirm enzyme linked immunoassay (ELISA) 2 results . *(Van der Poel et al 1991).*

The prevalence of serum samples that remain as indeterminate using the second generation RIBA test (RIBA 2) , is estimated to 3.5% in a recent study . *(Li et al ., 1993).*

Third Generation RIBA Test :

The new immunoblot assay , RIBA 3 , has been developed , in order to reduce the high rate of indeterminate serum samples by using RIBA 2 . It uses two recombinant antigen (c-33-c and one NS5- derived protein) and three synthetic peptides from the NS5 region of the HCV genome . In addition the coating concentrations of these antigens was increased in relation to RIBA 2 *(Buffet et al., 1993).*

In spite of these improvements , 38 % of sera with indeterminate RIBA 2 results , remain yet indeterminate with the RIBA 3 . This results shows the limits of a such confirmatory test.*(Buffet et al., 1993)*

4- Detection of viral RNA by polymerase chain reaction (PCR):

Viral RNA can be detected by reverse transcription (RT) , followed by amplification of the DNA by the polymerase chain reaction (PCR) using synthetic primers derived from the known base sequence . This test has established that most patients who have antibody to the virus, have viral RNA in their plasma . This suggests that some patients eleminate the virus or that not all who are infected are viraemic . Another possibility is that some antibody results are not specific . *(Peutherer 1992).*

The sera , with indeterminate RIBA results , should be tested by HCV PCR in order to demonstrate definitive HCV infection . HCV infection , was confirmed by positive PCR in 75% of the cases . *(Buffet et al ., 1993).*

A quantitive competitive RNA polymerase chain reaction (QC PCR) assay was developed for measuring absolute levels of HCV RNA in the serum , this may be valuable for monitoring HCV infection status and selecting individuals for therapy . *(Gretch et al ., 1994).*

Treatment of non-A , non-B hepatitis :

Interferon alfa-2b (Intron A) , still remains the standard therapy for chronic HCV infection . *(Dana et al ., 1994).* HCV PCR and ALT appear valuable tests for monitoring patients with chronic HCV infection undergoing antiviral therapy . *(Capalbo et al 1993).*

It was found that Interferon alpha in a dose of 3 million units given three times weekly for a period of six-months , proves to be beneficial in approximatily 50% of patients with chronic hepatitis C, resulting in normal or nearly normal aminotransferase levels as well as histological improvement of periportal and lobular necrosis and inflammation However, the relapse rate in successfully treated patients with chronic hepatitis C was at least 50 % . (*Schiff et al., 1991*).

Administration of an attack dose of interferon (6 million units for the 3 first months) , could be one of the factors associated with a long term response rate in chronic hepatitis C in patients without cirrhosis . *(Ouzan et al ., 1993).*

On the other hand , Craxi et al ., 1993 , found that prolonging interferon treatment beyond six months in patients with chronic hepatitis C who have normalized ALT does not enhance long term response .

Treatment with oral ribavirin 1000-1200 mg per day in two divided doses for 12 weeks, showed that ribavirin is the first drug to

offer a potentially effective oral treatment for chronic hepatitis C. Also combination therapy with interferon alpha may cure individuals of chronic hepatitis C virus infection but this combination should be furtherly evaluated in controlled trials (*Reichard et al., 1991*).

A trial of intravenous acyclovir had no long-term beneficial effect. (*Pappas et al., 1985*) , but , in non-responding patient to interferon, they may be respond to the combination of interferon with acyclovir and ribavirin in the treatment of chronic hepatitis C infection . *(Sambataro et al 1993).*

Prevention and control :

The exclusion of paid, commerical blood donors profoundly reduced the incidence of post-transfusion NANB hepatitis in the USA. The use of affinity-purified, or wet-heated and particulary genetically engineered factor VIII preparations will limit the occurrence of new cases of NANB hepatitis in haemophiliacs (*Colombo et al., 1983*).

Screening donors for ALT and anti HBc surrogate testing have been introduced in the USA and some Western European countries to control the transmission of NANB hepatitis (*Aach et al., 1981, Sugg et al., 1985, Koziol et al., 1986*).

Passive immunoprophylaxis by administering IgG has been considered a possible mean for preventing post-transfusion NANB hepatitis, but the results of these controlled trials have been conflicting (*Sanckez Quijano et al., 1988*).

Hepatitis D Virus

History :

Hepatitis D (Delta) is the hepatitis caused by the hepatitis D virus (HDV) (*Rizzetto 1989*).

The delta agent or the hepatitis D virus (HDV) was discovered in Italy by Mario Rizzetto in (1977) during an investigation of the distribution of the HBV antigens in liver biopsy specimen of patients chronically infected with HBV. He discribed a new antigen in the nuclei of the infected hepatocytes that was obligatory associated with HBsAg .This new antigen was named delta. (*Eastwood et al., 1988*).

Structure :

Hepatitis D virus (HDV) is a defective hepatotropic RNA virus that requires the presence of HBV as a "helper virus" for its pathogenicity. It replicates only in hosts who have a concomitant HBV infection. HDV is a 36-nm spherical particle containing the D antigen and a single-stranded RNA molecule in the interior,which is coated by HBsAg on the exterior. (Eastwood et al., 1988). The structure of HDV is shown in (Fig 3) .

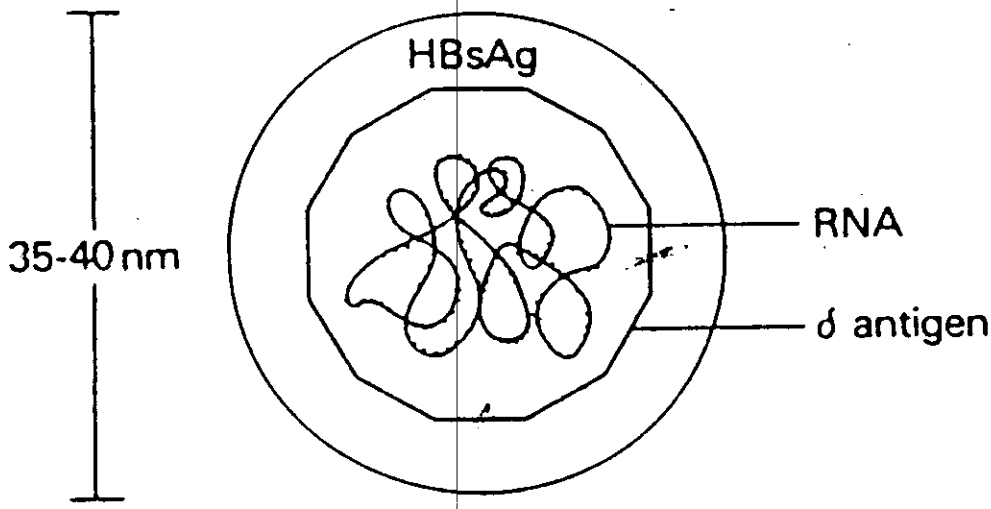

Fig. (3)

Diagramatic structure of the Delta agent . Photocopied from Hoofnagle , 1983 .

Epidemiology :

Transmission of HDV, as with HBV, may occur by parenteral or inapparent parenteral means. Current estimates, based on the prevalence of anti-HDV antibodies in HBsAg carriers, suggest that approximately 5% of HBsAg carriers worldwide are infected with HDV. High-incidence areas include the Amazon basin, Equatorial Africa, Middle East, Asiatic Russia and Mediterranean basin. Epidemics of severe hepatitis related to HDV infection have been documented (*Dusheiko et al., 1989 , Peutherer 1992*).

In the developed countries, infection occurs mainly in drug addicts, prisoners, haemophiliacs and institutionalized persons *Novick et al., 1989*) . The disease, for some reasons is not very common in homosexuals (*Mele et al., 1988*).

Clinical manifestations :

Hepatitis D virus can only infect simultaneously with HBV or as a superinfection of a chronic HBV carrier . The symptoms are similar to those of acute and chronic hepatitis B . There is agreement that the presence of HDV may increase the severity of the clinical features compared with those seen with HBV alone . This is reflected in a 10% risk of fulminant hepatitis with simultaneous HBV and HDV infection and a 20% risk in superinfections . *(Peutherer et al ., 1992).* A biphasic pattern can be observed with two distinct aminotransferase peaks.

Superinfection , in known HBV carrier, may present with an intercurrent hepatitis, which may be mistaken for an exacerbation of underlying hepatitis B infection. In a person not known to have previous chronic HBV infection, the disease may masquerade as classical acute hepatitis B. During the acute phase of the infection, HDV not infrequently represses HBV expression and may even terminate the HBV carrier state (*Chin et al., 1988, Buti et al., 1989*).

Chronic hepatitis D infection usually appears as a progressive chronic active hepatitis, with a histological picture of severe disease, or even cirrhosis. Usually such patients have low levels of hepatitis B replication and are anti-HBe positive antibodies (*Negro et al., 1988*).

Diagnosis :

The initial antibody to hepatitis D virus is HDV IgM . During the episode of acute hepatitis , there may be a drop in the HBsAg titre and , although it is usually still detectable , it may disappear temporarily in a few cases . *(Peutherer 1992)*.

HDV infection may be diagnosed directly by the presence of serum or liver HDV Ag or HDV RNA (*Buti et al., 1989*). Interahepatic staining by immuno histochemical techniques for HDV Ag currently represents the most widely available test for the diagnosis of persistent HDV infection (*Negro et al., 1989*).

Treatment :

Interferon therapy for the treatment of chronic HDV has met with only limited success . Relatively high does are required to obtain suppression of HDV, this can be achieved in approximately 50% of patients, but patients tend to relapse when the treatment is stopped, even after prolonged courses of interferon treatment. Liver transplantation for advanced cases carries the risk of recurrence of hepatitis D, and unusual patterns of HDV infection without expression have been noted in patients without HBsAg recurrence . (*Rizzetto et al., 1986 , Peutherer 1992*).

Prevention and contron :

HDV infection is prevented by preventing the spread of hepatitis B and by vaccination against HBV. (*Rizzetto et al., 1986*) .

MATERIALS AND METHODS

Materials and Methods

I. Materials :

1) Subject study:

They were volunteer blood donors , who coming to EL-Gharbia and EL-Kalubeia (Benha) blood banks. Collection was enrolled between January 1993 and December 1993.

The volunteer blood donors had no jaundice , and had not taking any medication which are likely to cause elevation of serum alanine aminotransferase (ALT) .

2) Reagents used in the determination of Alanine Amino-transferase (ALT) by colorimetric method (Boehringer Manheim Company)

A) Solution No. 1 : Consisting of phosphate buffer, DL. alanine and α - Oxoglutarate.

B) Solution No. 2 : Consisting of 2,4-Dinitro-Phenyl-hydrazine.

3) Reagents used in the determination of hepatitis -B-surface Antigen (HBsAg), by Indirect Haemo-agglutination test (IHA) (Hochest Company) :

1) Cellognost HBsAg IHA lyophilized reagent consisting of human O-erythrocytes sensitized with antibodies from rabbit to HBsAg.
2) Suspension medium for cellognost HBsAg consisting of tris-buffer solution supplemented by rabbit serum.

4) Reagents used in the determination of Hepatitis B core antibody (HBcAb) by Enzyme linked Immuno-Sorbant Assay (ELISA) method :(Hochest Company) :

1) 96 wells, coated with genetically-engineered synthetic HBcAg.
2) Conjugate : The vial contains 0.5 ml human IgG anti-HBc conjugated to horse-redish peroxidase.
3) Negative control ; The vial contains 2 ml human serum negative for all hepatitis B virus markers.
4) Positive control ; The vial contains 2 ml human recalcified plasma reactive for anti-HBc and non-reactive for HBsAg .
5) Conjugate diluent: The vial contains 15.5 ml human plasma negative for all HBV markers .
6) Washing buffer concentrate: The vial contains 40 ml phosphate buffer saline (PBS), and Tween-20 .

7) Chromogen : The vial contains 0.8 ml Tetra-Ethyl-Benzidine.

8) Substrate : The vial contains 10 ml 0.005% H_2O_2-Citrate buffer.

9) Stop Solution : The vial contains 55 ml 2 N sulphuric acid

5) Reagent used in the determination of Hepatitis C Virus antibody (anti-HCV) by second generation Enzyme Linked Immuno-Sorbant Assay (ELISA 2), (General Biological Corp.):

1. HCV peptides plate : One 96-well microtiter plate coated with synthetic oligopeptides .
2. Conjugate : One bottle (2 ml) with more than 0.1 ug/ml of monoclonal anti-human IgG conjugated with horse-radish peroxidase (HRPO) , dissolved in a tris buffer with bovine serum , and protein stabilizers . Preservative : 0.001 % Gentamycin .
3. Conjugate diluent : One bottle (20 ml) of tris buffer containing protein stabilizers . Preservative : 0.001 % Gentamycin .
4. Anti-HCV positive control : One bottle (0.5 ml) human serum contains Anti-HCV with inactivation . Preservative : 0.001 % Gentamycin
5. Negative control : One bottle (0.5 ml) of normal human serum. Preservative : 0.001 % Gentamycin .

6. Specimen diluent : One bottle (30 ml) of tris buffer contains bovine serum , surfactant , and protein stabilizer . Preservative : 0.001 % Gentamycin .
7. O-Phenylenediamine (OPD) diluent : One bottle (30 ml) of Citrate-Phosphate buffer contains 0.02 % H_2O_2 .
8. O-Phenylenediamine (OPD) tablet : One bottle (5 tablet) . Each tablet contains 10 - 15 mg of OPD .
9. Washing solution : One bottle (10 ml) of concentrated phosphate buffered saline solution with Tween-20 (100X) .
10. Stopping solution : One bottle (10 ml) of 2N sulfuric acid .

II. Methods :

Sample Collection :

**** Sera from Selected volunteer blood donors obtained as follow:***

- Three ml random venous blood were taken aseptically from donated blood by disposable plastic syrings.

- The blood was drained in a sterile tube labeled with the donor's name and number, and left for one hour at 37°C, the blood was firmly clotted and the clot started to retract.

- The serum was separated by centrifugation for 3 min, and transfered into another sterile tube labeled with the donor's name

and number. The serum was examined for ALT then kept at –20°C until needed .

A) <u>Detemination of Alanine Amino-transferase (ALT) by colorimetric method, according to Boehringer Mannhiem manufature instructions :</u>

<u>Test Principle :</u>

1) The test principle depends on the photometric determination of the concentration of the pyruvate hydrazine.
2) The concentration of the pyruvate depends on the amount of ALT present in the serum that will react with α-Oxoglutarate + L-alanine (Solution 1).

   α-Oxoglutarate + L-alanine $\xrightarrow[\text{in... the.. serum}]{\text{ALT}}$ - glutamate + Pyruvate.
3) Pyruvate will react with 2,4 - dinitro-phenly-hydrazine to produce pyruvate hydrazine which will be detected photometrically.

<u>Procedure :</u>

- One tube was used for each specimen and another one for the reagent blank.

- One ml of solution No(1) was pipetted in all specimen tubes and also in the reagent blank tube.

- 0.2 ml of serum was dispensed into respective tube and 0.2 ml distilled water was added into the reagent blank tube. All tubes were mixed and incubated in water bath at 37 C for exactly 30 min.

- After the incubation period, 1ml of solution No (2) were added into all tubes, and left to stand at room temperature for exactly 20 min.

- After that, 10 ml of 1.6% sodium hydroxide solution was added into all tubes, and left to stand for 5 min at room temperature, then each tube was read againest the reagent blank at a wave length of Hg 546 nm.

- Reading of the results was done according to the table provided by the manufacture within the kit, Table (1).

Table (1) : Levels of ALT by photometric evaluation of serum according to Boehring Mannheim company .

Absorbance	U/L	Absorbance	U/L	Absorbance	U/L
0.025	4	0.200	34	0.375	67
0.050	8	0.225	38	0.400	72
0.075	12	0.250	43	0.425	77
0.100	17	0.275	48	0.450	83
0.125	21	0.300	52	0.475	88
0.150	25	0.325	57	0.500	94
0.175	29	0.350	62		

N.B.: Normal values : up to 12 U/L .

B) Determination of hepatitis B surface Antigen (HBsAg) by indirect haemagglutination method (IHA) , according to the Hochest manufacture instructions :

Principle of the test :

- If hepatitis B surface antigen (HBsAg) is present in the sample. it react with the specific antibodies in the HBsAg IHA reagent to produce a pronounced agglutination of varying intensity.

- If the antigen is absent the sensitized erythrocytes in the reagent are deposited in the form of sharply outlined bottom.

Preparation of the reagent :

- The cellognost HBsAg reagent was reconstituted in 0.2 ml of distilled water and diluted with 0.8 ml of suspension medium.

- The reagent became ready for use after 30 min.

Procedure :

- A V-shaped microtitration plate was used.

- 25 ul of the suspension medium was added into each of the wells of the microtitration plate, one well was used for each donor's sample.

- 2 ul of the test sera were added into each corresponding wells.

- After that, 25 ul of the HBsAg IHA reagent was added in all wells.

- Lastly the plate was gently shacked manually, covered and let to stand in a vibration free place at room temperature protected

from heat and sun light for 2 hours, then the agglutination pattern was determined.

Interpretation of results :

- The evenly distributed agglutination pattern over the entire bottom was a positive result , and a sharply defined bottom was a negative result .

C) Determination of hepatitis B core antibody (anti-HBc)by Enzyme Linked Immuno-Sorbant Assay (ELISA) method , according to the Hochest manufacture instructions :

Principle of the test :

- The anti-HBc antibodies present in the sample, compete for a fixed quantity of HBcAg bound to the well. The quantity of enzyme tracer bound to the well and consequently the enzyme activity are inversely proportional to the anti-HBc concentration present in the sample or control .

- Measurement of enzyme active is performed by adding a chromogen / substrate colourless solution, the enzyme action produces a colour which could be detected with ELISA reader at a wave length of Hg 405 nm.

Preparation of the reagent :

- The conjugate was diluted 1:50 with conjugate diluent just before use.

- The washing buffer was diluted with distilled water to reach a volume of one liter.

- The chromogen was diluted 1 : 50 with substrate buffer, just before use

Procedure :

- All the reagents, used in this test were left to stand at room temperature (20-25 °C) before performing the test.

- Sufficient wells were selected and marked to run 1 blank, 3 negative controls and 2 positive controls, together with the required test samples.

- 50 ul negative control was dispensed into wells B1, C1 and D1.

- 50 ul positive control was dispensed into wells E1 and F1.

- 50 ul of each testing serum was dispensed into their respective well, well A1 was reserved for blank.

- 100 ul of conjugate, diluted 1:50, was dispensed into all wells.

- A cover sealer was applied in order to prevent evaporation, and incubation overnight (18-22 hours) was done at room temperature.

- The chromogen / substrate solution was prepared just before the end of the incubation period.

- At the end of the incubation period, the cover sealer was removed and discarded, the liquid was aspirated, then each well was rinsed 5 times with 0.3 ml of washing buffer.

- After rinsing, the wells was turned mouth down onto blotting paper and were tapped to remove any residue of washing buffer.

- After that, 100 ul of chromogen/ substrate solution was dispensed into all wells and incubated for 30 min at room temperature, protected from intense light.

- After the end of the second incubation period, 200 ul of stopping solution was dispensed into all wells in the same order as for chromogen / substrate solution.

- The absorbance of each of the negative control, positive control and testing samples was read against the reagent blank present in well A1, by ELISA reader at a wave length of Hg 405 nm.

The presence or absence of HBcAb was determined by comparing the absorbance of the unknown sample with the cut-off-value .

Determination of the Cut-off-Value :

The cut-off-value , was calculated as follow :

Cut-off-value = (NCx + PCx) X 0.2

NCx = mean absorbance of the negative control values .

PCx = mean absorbance of the positive control values .

Interpretation of results :

Samples with an absorbance less than or equal to the cut-off-value , were considered reactive for HBcAb (Positive) .

Samples with an absorbance greater than the cut-off-value ,were considered non-reactive for HBcAb (Negative) .

D) Determination of anti-body to hepatitis C virus by the second generation Enzyme Linked Immuno-Sorbant Assay (ELISA 2) , according to General Biologicals Corp. manufacture instruction :

Principle of the assay :

The HCV second generation ELISA test is an enzyme linked immunoassay which employs synthetic HCV peptides for the detection of antibodies to HCV in human serum or plasma . these peptides , which are reactive with the predominant antibodies of HCV , constitute the solid phase antigenic absorbent . When human serum or plasma is added to the well , the HCV-peptides and anti-HCV will form complexs on the wells , if anti-HCV is present in the specimen . After washing , the well is filled with a solution containing conjugate of purified monoclonal anti-human IgG and horse radish peroxidase (HRPO) , to allow formation of (HCV)-(AntiHCV)-(Monoclonal anti-human IgG)-(HRPO) complex . After washing out the unbound conjugate , a peroxidase substrate solution is added for colour development . The absorbance of the colour development is the measure of the anti-HCV content in the sample .

Test procedure :

1. All the reagent and specimen , used in this test , were left to stand at room temperature (20° - 25° C) before beginning the assay.

2. The needed number of wells were prepared , including three wells for positive control , two wells for negative control , two wells for blank and one well for each specimen .

3. The well numbers for the controls and specimen were written down on the data sheet .

4. A clean micro-titer plate were prepared for specimen and controls dilutions . 10 µl of specimen , positive control , and negative control were added to each well respectively , according to the assigned well numbers stated above . 200 µl of specimen diluent were dispensed into wells , and the plate were tapped to mix .

5. 100 µl of the diluted specimens and controls were transferred to their corresponding wells in the HCV peptide coated micro-plate

6. The plate were sealed with an adhesive slip ,and incubated in a 37° C humidified incubator for one hour .

7. At the end of incubation period , the concentrated washing solution was diluted with distilled water to 1:100 dilution . The adhesive slip were removed and discarded . The plate was washed , using an automatic microplate washer . (6 cycles of 0.5 ml of diluted washing solution were used).

8. During the washing process , the concentrated conjugate were diluted with 10 times its volume of the conjugate diluent .

9. After the washing process was completed , 100 µl of the diluted conjugate were added in each well except the two blank ones . the plate was sealed with an adhesive slip , and incubated in a 37° C humidified incubator for one hour .

10. At the end of the second incubation period , the adhesive slip were removed and discarded , the plate was washed , using an automatic microplate washer . (6 cycles of 0.5 ml of diluted washing solution were used)

11. The OPD solution was prepared 5 - 10 minutes , before the end of the second incubation period , by adding one OPD tablet to each 5 ml. of OPD diluent , in a clean non-metalic container .

12. 100 µl of OPD solution were added into each well including the two blank wells . The test wells were covered with a black cover and incubated at room temperature for 30 minutes .

13. 100 µl of 2N Sulfuric Acid were added to each well , including the two blank wells , to stop the reaction .

14. The reaction was measured by a photometer with automatic printer . The instrument was blanked , using the lighter one of the two blanks . The absorbance was read at a wave length of 492 nm .

15. The presence or absence of Anti-HCV antibodies was determined by comparing the absorbance of the unknown sample with the cut-off-value .

Determination of the Cut-off-Value :

Cut-off-Value = NCx + 0.15(PCx)

NCx : mean absorbance of the negative control values .

PCx : mean absorbance of the positive control values .

Interpretation of results :

* Specimen with an absorbance value less than the cut-off-value, are considered " Negative " .

* Specimen with an absorbance value greater than or equal to the cut-off-value are considered " Positive " .