

PREVALENCE OF DELTA ANTIGEN IN FULMINANT HEPATITIS CASES

**THESIS SUBMITTED FOR THE DEGREE OF
DOCTOR OF MEDICINE IN MICROBIOLOGY**

**SUBMITTED BY
TOSHA USGAONKAR**

**UNIVERSITY OF BOMBAY
HAFFKINE INSTITUTE FOR TRAINING, RESEARCH & TESTING
BOMBAY**

412

1993

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INDEX

1. INTRODUCTION	1
2. REVIEW OF LITERATURE	7
3. MATERIALS AND METHODS	34
4. RESULTS	44
5. DISCUSSION	47
6. SUMMARY AND CONCLUSION	52
7. REFERENCES	53

INTRODUCTION

The last two decades have witnessed an explosion in the knowledge of viral hepatitis, a major public health problem throughout the world affecting several hundreds of millions of people. Hepatitis as a clinical entity has a long history and viruses have been recognised as causative agents of hepatitis or liver diseases ever since the discovery of animal viruses themselves. The disease may occur incidentally during the course of infection with many viruses such as yellow fever, lassa fever, herpes simplex, cytomegalovirus, rubella, Ebstein Barr virus, Coxsackie, virus, marburg etc. However, the term 'Viral hepatitis' refers to a primary infection of the liver by hepatitis virus Type A or Type B or by other hepatitis viruses referred to as Non A Non B hepatitis virus.

The term infectious hepatitis introduced in the U.S.A. in 1939 (1) is now called viral hepatitis Type A. Similarly serum hepatitis so called because it was shown to be transmitted by blood transfusion, injection of serum and other blood products or through use of syringes contaminated with minute quantities of serum or tissue fluids was later termed viral hepatitis Type B. In 1963 Blumberg made the discovery of Australia Antigen while working on human serum lipoprotein allotypes (2) which later in 1968 was shown to be associated with the virus of Type B Hepatitis (3). This Antigen was subsequently shown to be the surface component of

type B hepatitis virus. Therefore, it was a chance finding that the viral antigen can be demonstrated by simple serological techniques in the sera of infected persons.

Though a lot of research work was done on infectious and serum hepatitis, in the absence of specific antigen-antibody tests, the early epidemiological observations could not prove that there were more than two types of viral hepatitis (4). Though multiple episodes of acute viral hepatitis were reported, it was only later on when serological tests for detecting the hepatitis B surface antigen (HBs Ag) made it possible to identify cases of post-transfusion hepatitis caused by Hepatitis B Virus (HBV). But again studies indicated that this virus was responsible for only 25% of such cases (5). Hepatitis A can rarely be transmitted by blood transfusion since its viraemic stage is very short and this viraemia is associated with clinical illness (5). In addition none of these cases demonstrated sero-conversion to other known human hepatitis viruses namely cytomegalovirus and Epstein Barr virus. This suggests the existence of at least one additional unrecognised human hepatitis virus which was designated as the non A non B virus. In this category of non A non B viruses, two new viruses have been identified, the hepatitis C virus which is one common form of parenterally transmitted virus and hepatitis E virus which is enterically transmitted non A non B virus (6).

In 1977 Rizzetto et al (7) reported the observation of a new viral antigen present in the nuclei of hepatocytes in patients infected with HBV. Subsequently studies confirmed the original observation and revealed that the new antigen was related to a new agent, later termed hepatitis delta virus or HDV.

This hepatitis delta virus (HDV) contains HBsAg on its surface and delta antigen (HDAg) and a small RNA genome in the interior (8). This new human pathogen is always found associated with HBV causing both fulminant hepatitis and the accelerated progression of pre-existing HBV hepatitis (9). The association of HDV with HBV is related exclusively to the supply of HBs Ag Coat from HBV which both protects the circulating virion and confers upon it, its distinctive hepatotropism (9). Therefore HDV which is invariably pathogenic replicates only in hosts who are positive for HBsAg (10) since it cannot produce HBsAg on its own.

Although a lot of literature has been written on HBV and its associated disease, HDV has recently opened a new area of investigation because of the fact that it contributes to the worsening of the underlying HBV hepatitis or that it induces acute fulminant hepatitis in a number of cases of co-infection with HBV. The HDV is predominantly a cytotoxic virus (11) and the direct cytopathic mechanism of HDV explains the failure of Conventional Immuno Suppressive therapy to alter the course of the disease (12). Also an

auto-antibody directed against microsomal membranes of the liver, kidney etc. is found in about 10% - 30% of the patients suffering from co-infection by HBV and HDV , suggesting that the virus may elicit an autoantibody cross reactive with normal human components (13). Studies with genome analysis has shown that appearance of HDAG in HDV infection co-incides with a decrease of detectable HBV-DNA in serum (14). HBV-DNA returns to the original level following disappearance of HDAG and development of antibody to it i.e. Anti HD.

The HDV can infect a person who is already HBsAg positive i.e a chronic carrier or a patient suffering from acute or chronic type B hepatitis or there may be a simultaneous infection with HBV and HDV (15). Whatever is the form of infection it leads to the worsening of the clinical illness as well as the worsening of liver cell necrosis. There is massive production of HDV in patients who are already HBsAg positive. This is more common in fulminant hepatitis which predominates in drug addicts and is characterized by extensive release of large amounts of HDV into blood (16,17).

The route of transmission of HDV is same as HBV but it shows striking geographical difference in distribution. In western communities its distribution is closely correlated with parenteral drug abusers and other high risk populations. Spread is usually horizontal and perinatal transmission is unusual. This explains some of the differences in

distribution (18).

The epidemiological picture of HDV has changed from an Italian to a global perspective. In a worldwide epidemiological and clinical survey by Saracco et al (19) on 377 cases of fulminant hepatitis in persons positive for HBsAg in Greece, Italy, U.S., U.K., Central African Republic, Taiwan, Egypt and India only 52% could be attributed to infection with HBV which was defined as presence of IgM antibody to HBeAg and absence of serum markers of infection by extraneous viruses, 30% cases were caused by co-infection with HBV and HDV or by infection with HDV superimposed on carrier of chronic HBsAg.

In India studies to find out the prevalence of delta agent in fulminant hepatitis cases were done in Chandigarh. It was found that 6% of HBsAg positive cases were positive also for the delta agent (20).

In another study in Pune it was found that acute viral hepatitis patients did not show presence of anti delta antibodies. But 13.63% of commercial blood donors and 6.8% of voluntary blood donors showed anti delta antibodies (21).

Although, a lot of epidemiological work is going on all over the world to find out the prevalence of delta agent in fulminant hepatitis which is the cause of morbidity and mortality for the human race, there is tremendous paucity in India of information on the delta agent prevalence though what is interesting is that hepatitis is not uncommon in

India. Keeping this in mind a study was performed to find out the prevalence of delta agent in fulminant hepatitis cases in Kasturba Infectious Diseases Hospital of Bombay which filters most of the hepatitis cases of coastal areas of Maharashtra.

REVIEW OF LITERATURE

HBV

Human Hepatitis B virus is a small DNA virus that causes complex clinicopathological syndrome ranging from acute and chronic hepatitis, cirrhosis of liver to hepatocellular carcinoma (22). It belongs to group of animal viruses known as hepadna viridae. Other members of this group are Woodchuck hepatitis virus (WHV), Beachy ground Squirrel Virus (GSHV), Pekinduck hepatitis virus (DHBV), (23). These viruses are mainly hepatotropic eliciting acute and chronic liver diseases of variable severity.

Patients with HBV infection contain several particulate forms like the spherical and filamentous forms which constitute the 'Australia Antigen' and the Dane particle which is the whole infectious Virion (24). The immune response in a person infected with HBV involves three antigenic systems - Hepatitis B surface antigen (HBsAg) , Hepatitis B Core antigen (HBcAg) and Hepatitis B 'e' Antigen (HBeAg). Both humoral and cellular responses occur during the infection.

The HBsAg which is recognised as a specific marker for infection with HBV becomes detectable in circulation about a month after exposure to infection and several weeks before elevation of transaminase levels and appearance of Jaundice. Peak levels of HBsAg are seen in the preicteric phase of the disease. HBsAg disappears with recovery from clinical

disease in most patients, but in a small proportion it persists for years, these are the carriers. Antibody to HBsAg appears within weeks after the disappearance of HBsAg and persists for years and it is related to resistance to infection.

HBcAg is not detectable in the serum of patients but can be demonstrated in liver cells by immunofluorescence. Antibody to core antigen (Anti-HBc) usually appears in the preicteric phase and persists for months. It is not protective but appears to be related to the amount and duration of replication of the virus. The highest titres of Anti-HBc are found in persistent HBsAg carriers. (24).

HBeAg is a soluble antigen that is not part of the virion but is specific for the infection. It appears in the serum at the same time as HBsAg, but in most cases it disappears within a few weeks followed by appearance of antibody to it; which lasts for several months. The HBeAg is believed to correlate with the number of viral particles and the degree of infectivity of carrier sera. Sera with HBeAg are highly infectious and those with anti HBe of little infectivity. (24).

There is a great diversity of clinical syndromes after exposure to HBV (22). The incubation period varies from 4 weeks to 6 months. The whole course of acute hepatitis B tends to be more insidious and prolonged than with hepatitis A. The initial symptoms develop less rapidly and the

predrome is usually longer. Arthralgias are common and duration is longer than hepatitis A. Arthritis with redness, swelling or effusions, seen to occur and may be presenting feature causing diagnostic confusion (25,26). It usually appears early in the predrome and tends to clear over days or weeks, often before the onset of Jaundice. The arthritis affects large joints and also the small joints of the hands and feet. An erythematous or urticarial rash may precede the arthritis suggesting a 'Serum Sickness' like syndrome (27). Abdominal discomfort or pain are common fever vomiting and diarrhoea are unusual.

Symptoms tend to improve with the onset of jaundice. In the icteric phase the serum bilirubin tends to rise but not very steeply as in hepatitis A but reaches higher levels (median 195 $\mu\text{m}/\text{Lit}$) and jaundice lasts longer (average 30 days) particularly in older patients (28). The rise in aminotransferase levels is slow but reaches high levels after 1-2 weeks after the onset of jaundice.

A problem in acute hepatitis B is that inspite of resolving, the disease may progress to chronic hepatitis and cirrhosis, with persistence of HBsAg in serum. The various forms of chronic liver disease are chronic Asymptomatic carrier state, chronic persistant hepatitis, chronic lobular hepatitis, chronic aggressive hepatitis and post necrotic type of cirrhosis of liver (22). Several important factors like age, sex, immunological state and co-infection with other agents

play a role in predisposing to the development of chronic hepatitis (29).

The mortality rate in acute hepatitis is less than 1% and is usually associated with fulminant hepatitis which is a syndrome of acute hepatic failure that results from massive hepatic necrosis . Coinfection with HDV on HBV acute hepatitis results very often in fulminant hepatitis (30).

Fulminant Viral Hepatitis /FHF/FVH

This is a rare but serious complication of acute viral hepatitis. It is defined as , "the onset of hepatic encephalopathy within a given time period from the onset of the clinical illness", the time interval is usually taken as within 8 weeks from first symptom (31) to 4 - 6 weeks (32,33). The first symptom is considered to be jaundice (34), although may not be invariable especially in children. Coagulopathy can also be considered as evidence of acute liver failure. Case fatality rate in HBV - FHF is 0.84% (35).

The risk factors in acute viral hepatitis predisposing to fulminant hepatitis are :

- a) Age : Old age has been cited as a predisposing factor to FHF (36).
 - b) Sex : Females are at higher risks than males (37).
- Early and enhanced anti- HBs production which forms complexes with HBsAg in hepatic microcirculation causing ischaemic

necrosis of hepatocytes (38) is sited as the cause for predisposing to FHF.

C) Pregnancy : Pregnancy in less industrialized countries due to malnutrition is a predisposition to FHF (39).

d) Malnutrition: Malnutrition by itself predisposes to fulminant hepatitis (39).

e) Immunosuppressive Therapy : Immunosuppressive therapy after being withdrawn may lead to FHF (40).

f) Simultaneous infection : Simultaneous Infection with hepatitis B virus and hepatitis delta virus has been noted to be associated with a higher incidence of fulminant viral hepatitis (41).

Co-infection with HBV-HDV accounted for a 22 - 25% of patients with HBsAg and IgM seropositive acute hepatitis patients to develop FVH in the U.S., Italy, Egypt and Ireland (18,19).

Sanchez et al (18) found that 70% of FVH in Spain was due to HBV-HDV co infection . The incidence was lesser in U.K. i.e. 16% and less than 10% from Greece, India, Taiwan and Central African Republic (19).

The spread of IV drug abuse accounts for the majority (68.4%) of cases of FHF - B/D especially in western countries (18,19).

Co-infection is indicated by the presence of one or more acute phase markers for HDV, (HDAg, HDV-RNA, IgM-anti HD) and IgM - anti HBc (42). HDV can interfere with the viral

replication of HBV and occasionally, might lead to a fall in the serum titres of HBsAg and even IgM-anti HBe to below detection levels. Total anti-delta anti bodies should be sought even in the absence of HBsAg (42).

Complications of fulminant hepatitis include :

(1) Hepatic encephalopathy which may give rise to increase in intracranial pressure due to cerebral odema. Clinical features include peaks of systolic hypertention, hyperventilation, decreased pupillary reflexes, opisthotonus, and impairment of brain stem reflexes. Papilloedema is rare (43).

(2) Bacterial and fungal infections contribute significantly to mortality and morbidity in fulminant hepatitis (44).

(3) Respiratory complications and haemodynamic disturbances are common.

(4) Renal failure leading to reduction in urinary output (less than 300 ml/day) and elevation in serum creatinine (more than 300 μ mol/l : N < 105) develops in 30% of patients metabolic acidosis and electrolyte disturbances follow.

(5) Coagulopathy in the form of Thrombocytopenia occurs which leads to increased haemorrhages.

(6) Metabolic disturbances in the form of hypoglycaemia and hypophosphatemia may occur (35).

Prognostic Indices :

(1) Asterexis, constructional apraxia, convulsions rapid reduction in liver size and bleeding diathesis are clinical indices of hepatic failure.

(2) Biochemical indices of hepatic failure are, rapid fall in transaminases, decreased albumin levels, and prolonged prothrombin time.

(3) AST/ALT ratio is raised because of damage to hepatocyte membrane and mitochondria. Hepatocyte necrosis without regeneration leads to decrease in fetoprotein level.

Serum Gc - actin complexes increase because of phagocytosis of necrotic hepatocyte.

HBc IgM titre increases because of over production by host.

(35)

Survival and outcome

Survival is greatest when the time interval between onset of encephalopathy and jaundice is less than 8 days and is seen typically in fulminant hepatic failure B and A (45 - 47). Survival based on collective data from 8 countries was 47.4% for FHF- b/D and 26.7% for FHF-B (19).

In another study by Saracco et al,(19) it was found that chronic HBsAg seropositive hepatitis with abnormal liver serum biochemistry beyond one year developed in 45% of survivors from FHF B/D Co-infection compared with 3.3% of survivors with fulminant hepatic failure B .

Based on various epidemiological studies it was found that hepatitis delta virus plays a very important role in fulminant hepatitis B and though it depends on HBV for its survival its geographical distribution is variable than that of HBV.

Since this work was based on prevalence of delta agent in fulminant hepatitis cases, the delta virus will be discussed in detail.

Delta Hepatitis

The virus and the disease

The transmission experiments in chimpanzees (48) have established that the delta antigen (HDAg) a new reactivity described in 1977 in Italian carriers of HBsAg (7) is the marker of an infectious agent, the hepatitis delta virus (HDV) (49).

The virion is a 36 nm particle containing HBsAg on the surface and delta antigen and a small RNA genome in the interior (8). The particle is an organised structure that prevents hydrolysis of the internal RNA, thus protecting the infectivity of the virus (14). The particle is labile to certain agents like detergents, repeated freeze thaw cycles, and concentrated sucrose disrupt the structure and release degraded RNA. HDV-RNA is estimated to have a size of 1.7 kb and a linear conformation with strong base pairing. The RNA genome consists of 1700 nucleotides and the delta antigen do not appear to be associated in a nucleocapsid complex. The

delta hepatitis virus has been found to have a buoyant density of 1.25 gms/cms. (50). There is no evidence of a 3' Poly-A Tail nor of a Reverse transcriptase in the particle, suggesting that HDV-RNA may have properties of a negative strand virus or behave like histone or plant RNAs (11). The genome has a high degree of self-complementarity. Analysis of the genomic sequence for its coding potential has shown the presence of several open reading Frames (ORFs) both on the genomic and antigenomic strands. One ORF is located on the antigenomic strand and has been shown to code for the delta Antigen (9).

The HDV Antigen appears to consist of a single, phosphorylated, positively charged polypeptide, with a molecular weight of about 24-27 KD, capable of binding RNA which accumulates predominantly in the nucleoplasm of the nuclei of infected hepatocytes (9). Experiments to map the immunodominant region of the delta antigen within the polypeptide have involved expressing different clones containing fragments of the coding ORF, and evaluating the antigenecity and immunogenecity of the product in competition experiments with the natural antigen and antibodies. Thus results obtained have localized the immunodominant region close to the amino end, but excluding the first twenty residues from the initiator methionine, between positions 1540 - 1364 (51).

The Association between HBV and HDV

The molecular basis for the biologically intriguing association between HBV and HDV remains unclear when HDV superinfection of a chronic HBV carrier occurs. The 'e' antigen status appears not to be a determining factor since both 'C' Ag positive and 'e' Ag negative ('e' Antibody positive) carriers appear equally susceptible. Seroconversion from HBeAg to HBe Ab and diminished HBV replication have however been reported as a result of HDV replication. By contrast in other cases marked replication of both viruses have been well documented by high levels of viral nucleic acids in serum and by double staining of hepatocytes for delta antigen and HBV core with specific antisera raised against purified bacteria expressed delta antigen. These cases which are more common in drug addicts have been noticed for the aggressive nature of the disease, which suggests a compounded effect from both viruses (9). Experimental studies using Woodchuck; hepatocyte cultures infected in vitro (52) and both human hepatoma and kidney cells transfected with appropriate HDV containing plasmids (53) suggests that the association with HBV is likely to relate exclusively to the supply of the HBsAg coat which both protects the circulating virion and confers upon it, its distinctive hepatotropism.

Molecular basis of pathogenecity

The fact remains that HDV contributes to the worsening of the underlying HBV hepatitis or that it induces acute fulminant hepatitis in a number of cases of co-infection with HBV. Recent reports of mild as well as particularly aggressive forms of hepatitis have suggested that in analogy with the plant virioids, a region of the genome may be involved in modulating pathogenecity by interacting with an essential cell constituent (9).

Apparent Inhibition of HBV synthesis by HDV infection was observed in HBs Ag carrier chipanzees expressing HDAg (48). Genome analysis has shown that appearance of HDAg coincides with a decrease of detectable HBV DNA in serum. This decrease progresses as expression of HDAg increases, often to the virtual disappearance of HBV-DNA at the peak of HDAg expression (14). HBV-DNA returns to the original level following disappearance of HDAg and development of antibody to it (Anti-HD).

Diagnosis of HDV infection is made by immunohistological identification of HDAg in liver and detection of IgM and IgG Anti-HD in serum by RIA or EIA (49,54,55). Features of patients with HDV infection indicate that delta hepatitis is a clinical entity separate from the underlying HBV infection. Cardinal for the understanding of this disease are the postulates that HDV is invariably pathogenic and under

natural conditions replicates only in hosts who are HBsAg enemic, therefore the mechanism and outcome of the infection are different in the normal individual and the individual who is already infected with HBV at the time of exposure to HDV. In normal individuals exposed to HDV (and simultaneously to HBV), the HBsAg necessary to rescue the defective pathogen is induced acutely by the HBV component of the infectious inoculum. In this context, a limiting factor to expression of HDV is the short duration of hepatitis-B Viraemia (15,16).

The serologic response to acute HBV/HDV co-infection is often abortive, represented by the transient appearance of IgM Anti HD, it can also be totally absent (49). Such hepatitis is not distinguishable clinically from classic hepatitis B. Occasionally, synthesis of HDV is massive and can result in extensive liver damage that may be additive with that caused by HBV (41). This form of severe sometimes fulminant hepatitis, predominates in drug addicts and is characterized by extensive release of large amounts of HDV into blood (16,17).

Events are different and more frequently ominous in carriers of HBV who are superinfected with HDV. The established and continuing HBV infection may predispose to persistence of the defective pathogen (15). Retrospective and prospective (15) studies indicate that superinfected carriers may be the only individuals to become carriers of HDV. As a rule, a strong serological reaction develops, characterised by IgG antibody

to HD antigen.

The ultimate clinical picture depends on whether the carrier state was previously asymptomatic or suffered from HBV-associated disease. Smedile et al (56) found that asymptomatic carrier may present with an acute HBsAg positive hepatitis with all features of classic hepatitis B except the possible lack of IgM antibody to core antigen. The illness is often severe, due to the explosive replication of HDV in HBV carrier. This form accounts for a substantial proportion of fulminant HBsAg positive hepatitis in Europe and the U.S. (41). The acute hepatitis type D has a propensity to become chronic. This poses a diagnostic problem if the prior HBsAg state of the patient is unknown, as transition to chronicity of HDV infection could simulate progressive hepatitis B (56). In carriers with prior HBV associated liver disease, the primary infection may coincide with an apparent flare up of the chronic hepatitis which may be fatal (57). Persistence of HDV adds additional damage to an already compromised liver aggravating the histological lesion and accelerating the clinical course of the underlying HBV-induced disease. A strong association between HDV infection and serious liver disease has emerged and intrahepatic HDAg connotes a chronic active hepatitis in about 90% of cases, often associated with or progressing to cirrhosis (58). The liver damage is due to direct cytopathic

mechanism thus explaining the failure of conventional Immunosuppressive therapy to alter the course of the disease (12). An autoantibody that is not organ specific but is species specific (predominantly human) and is directed against microsomal membranes of the liver, kidney and other organs, is found in about 10% - 30% of these patients, suggesting that the virus may elicit an antibody cross reactive with normal human components (13).

The point that needs to be made regarding the pathologic changes associated with delta hepatitis infection is that the HDV is predominantly cytotoxic rather than lymphocytotoxic (11) and there is a similarity in morphologic expressions induced by the HDV and the hepatitis non A non B virus.

Rizzetto et al (7,12) showed that in Biopsy specimens of hepatitis B carrier patients who had delta agent induced chronic hepatic disease ,the predominant findings were Oesinophilic alterations of the hepatocytic cytoplasm, sometimes progressing to acidophilic bodies and only rarely fine droplet steatosis . Lobular inflammatory cell were lymphocytes, mainly in the sinusoidal lumen as well as macrophages with PAS positive nonglycogenic granules in the perisinusoidal spaces, while some parenchmal areas with significant hepatocellular alterations were free of inflammatory cells. By contrast, portal tracts were expanded and densely infiltrated by mononuclear cells. In general focal bridging and confluent necrosis in hepatitis resembled

that of chronic hepatitis B. But severe degree characteristic microvesicular fatty degeneration, and acidophilic degeneration in chronic hepatitis B which were less conspicuous were frequently present in hepatitis D. In addition hepatocytes containing HDAg may undergo ballooning and karyopyknosis without peri-infiltration of lymphocytes (59).

Serology

The specific diagnosis of HDV infection involves the detection of delta antigen in liver biopsy specimens or of anti-HD in serum. In HBV/HDV co-infection both or either IgM and IgG anti-HD can be raised in the presence or absence of HDAg (60). IgM appears soon after the onset of the disease later to disappear whilst IgG is normally detectable during convalescence. A chronic HDV infection on the other hand is characteristically associated with high titres of both classes of anti-HD throughout the disease in association with detectable HDAg in liver and/or serum in a proportion of cases depending on the stage of the disease. Histological diagnosis of HDV hepatitis is exclusively dependent on the presence of HDAg in hepatocyte nuclei.

Recent cloning of HDV RNA has generated HDV C DNA clones which have been used in the detection of HDV RNA in both serum and liver by specific and sensitive molecular hybridization assays (61). These use C DNA probes or

Riboprobes of variable lengths both radio-actively and non-radioactively labelled. Published reports have shown good correlation (60% - 65%) between serum HDV RNA detected using this non invasive technique and the presence of delta antigen in liver biopsy specimens (61) coupled to the newly developed amplification of nucleic acids by polymerase chain reaction (PCR) which has already been applied for virological diagnosis. The sensitivity of this type assay will increase by 1,000 to 10,000 times permitting detection of virion concentrations of less than one particle per ml. Both these assays and western blot analysis of HDAg in serum, which was invaluable in showing the presence of HD antigenemia in presence of anti HD in chronic hepatitis, remain however predominantly research oriented (9).

IgM antibody to HDV is often the only serological test positive in the clinical stage of hepatitis D and repeated testing for this marker is necessary to diagnose acute HDV co-infection. The serological follow up provides important prognostic information ; Waning of IgM confirms resolution of HDV infection, persistence predicts chronicity (62).

Serological diagnosis with particular reference to hepatitis delta virus RNA in acute delta hepatitis : serological testing for HDV RNA is direct and will demonstrate HDV replication in a large number of cases with acute delta hepatitis. Testing for IgM anti HD could provide supplemental evidence for HDV infection. Sequential testing for these

markers will facilitate assessment of outcome of HDV infection (62).

Characterisation of HDV Infection

Type	HBV Markers		C/F
	Present	Absent	
Simultaneous Inf. with HBV and HDV (Co-infection)	Anti HBcIgM HBsAg	Anti-HBs	Liver enzymes raised jaundice nausea fever arthalgias
Infection of chronic HBV carriers	HBsAg	Anti HBcIgM Anti HBs	Liver enzymes jaundice nausea, fever, arthalgias
Resolved HDV Inf.	Anti-HBs Anti-HBc	HBsAg Anti HBcIgM	None

Prevention of HDV infection is by prevention of HBV infection by immunization (62).

Epidemiology

The discovery of the Australia Antigen by Blumberg et al. (63) and recognition of its close association with serum hepatitis, enabled Krugman et al (64) at the wilowbrook state school for the mentally retarded in New York to confirm the differences between type A and type B hepatitis. Furthermore it was established that prolonged carriage of HBV could occur, that it was associated with

chronic persistent hepatitis and chronic active hepatitis, and that infection was very common and more likely to lead to chronic disease and to the development of hepatocellular carcinoma (65). Also early studies by Blumberg emphasized the striking geographical variation in the frequency of the carrier state; worldwide and in different communities. It has now become clear that vertical or perinatal transmission can progress to cirrhosis (66) and is probably the commonest mode of transmission worldwide. Also the demonstration of an antigen in the liver tissue, the Delta antigen led to the identification of an RNA virus HDV which superinfects patients with HBV and modifies its epidemiology and clinical course (9). Its mode of transmission is similar to HBV. There are however certain geographical differences which are difficult to explain. In western communities its distribution is closely correlated with parenteral drug abusers and other high risk population. Spread is predominantly horizontal and perinatal transmission is unusual (18). This may explain some of the differences in the distribution of HDV infection worldwide which does not always correlate with the geographical distribution of HBV infection (18). HBV carrier rate is found maximum till the age of 40 years and then declines.

The mode of spread of HBV and HDV is as follows ;

(1) Spread by blood and blood products

HBV could be readily spread by blood transfusion and to

haemophiliacs receiving treatment with blood clotting factors VIII and IX. HDV also can spread via blood donations. Screening of blood for HBV infection could prevent this (67).

(2) Haemodialysis associated spread

The high frequency of dialysis associated hepatitis B occurs in many European countries as well as in the U.S.A. But is now uncommon in U.K., since the introduction of regular screening for HBsAg.

HDV infection in Haemodialysis units is relatively rare (68).

(3) Spread in institutions like institutions for the mentally retarded

Using HBV markers there have been many studies showing that mentally retarded carriers, particularly those with down's syndrome, are likely to have HBeAg and spread the infection to other patients and staff (67).

(4) Sexual spread

Spread of HBV infection amongst homosexuals and heterosexuals is a major problem in some communities (67).

(5) Spread among drug addicts

Spread of HBV and HDV amongst IV drug abusers largely as a result of sharing needles is well recognised. Between one and two thirds of HBV positive IV drug addicts in Europe may have markers of HDV infection with the prevalence ranging from 31% in Ireland to 64% in Italy (69).

(6) Vertical and perinatal spread

This is the commonest method of spread of HBV worldwide and accounts for the very high carrier rate in communities in South East Asia and parts of Africa (68) , though even in adjacent communities in Central Africa there are considerable variations, in one village vertical transmission is common and in another horizontal transmission is common (70).

Spread of HDV by this route is infrequent (71).

(7) Spread amongst health care personnel

Spread of HBV to patients from 'e' antigen positive staff is well documented and can result in quite large outbreaks. Health care personnel are at high risk of developing HBV from chronic carriers or patients with acute infection (67).

(8) Contact associated spread

To relatives of patients with HBV/HDV infection with clustering of delta infection in families have been found but appears to be uncommon (67).

Based on the prevalence of carriers communities have been identified as low endemic (0.1%), intermediate endemic (5%) and high endemic (10%).

India has been identified as having INTERMEDIATE ENDEMICITY for HBV carriers. The prevalence of HBsAg in the general population is less than 0.5% in the U.S. and Western Europe, 1 - 2% in South America and Southern Europe, 3 - 5% in North Africa and in many parts of USSR and 9 - 10% and higher in Sub Saharan Africa and South East Asia (72 - 73).

The overall prevalence of markers of infection varies between 7% and 10% in the U.S., 90% and 80% in S.E. Asia and Africa (73) . Even in countries like those of Northern and Western Europe and other highly developed countries with a relatively low prevalence, certain populations are at high risk of acquiring the disease and have cumulative infection rates of upto 70%. In countries with a high prevalence rate, the entire population is at risk and infection tends to occur in childhood (73).

Hepatitis B is hyperendemic among Pacific Islanders with carrier rates ranging from 5 - 50% and virtually all adults showing serologic evidence of current or past infection (74).

In Melbourne hepatitis B continues to be a substantial problem among IV drug users and their sexual partners (7). Holman et al (74) in Australia showed that the Aboriginal population of western Australia has an intermediate to high risk of infection with hepatitis B virus. Another study on HBV infection in hospital staff in Spain showed that the most likely incidence is as high as 265/100,000 (75).

The worldwide prevalence of chronic HBsAg carrier states has been estimated to be approximately 200 million persons or 5% of earth's population.

The NICD reports on HBsAg positivity in India is as follows: In blood donors general population the rate is 5 - 6%. In institutions for mentally retarded it is 10 - 15% . In

dialysis units of hospitals it is 20%. In prostitutes around 45 - 50% and the staff of hospitals is 10 - 12% (67). HDV , the delta agent, which superinfects carriers of HBsAg patients with acute or chronic HBV infection which has same mode of transmission as HBV shows striking geographical differences in distribution. In western communities it's distribution is closely correlated with parenteral drug abusers and other high risk populations spread is predominantly horizontal and perinatal transmission is unusual. This may explain some of the differences in distribution of HDV infection world wide which does not always correlate with the geographical distribution of HBV infection.

The epidemiologic picture of HDV has changed from an Italian to a global perspective. Large surveys in Europe have confirmed that HDV predominates in the mediteranean basin but occurs rarely in the general HBsAg positive population of northern Europe (68). In Anglo Saxon countries, HDV is virtually confined to parenteral drug addicts and their contacts. The trend is similar in the U.S. with limited spread also in homosexuals (69,70).

The most consistent endemic reservoir of the HDV infection extends from the Balkan peninsula through the Eastern mediterranean basin to the middle east (76).

Arab countries at present appear to have the highest

prevalence of HDV infection in the world (76). Other major sites exist in Africa that too with irregular distribution high being in Egypt, Kenya, Gabon and low in Nigeria and Southern Africa (76).

HDV is also present in several areas of South America as documented by an outbreak of fulminant and progressive hepatitis in Venezuela. Seroepidemiological analysis disclosed that the severe illness resulted from superinfection of HBsAg carriers by HDV (77).

No epidemic sites have been documented in the far-east. Transmission of HDV is similar to that of HBV i.e. direct parenteral inoculation with contaminated blood and its constituents. Therefore accounts for epidemic spread of the infectious agent in drug using communities. Hence screening of blood for HBsAg provides safety in transfusion associated HDV infection.

From analysis of transfusion associated HBsAg positive hepatitis in Italy, the relative risk of HBV/HDV hepatitis versus HBV hepatitis alone can be estimated to be 1 to 50. The risk however becomes excessive to HBsAg positive individuals given blood products manufactured from pools of thousands of units of plasma, as a single unit containing HDV may contaminate the entire pool (78, 79).

Approximately 50% of Haemophiliacs with HBsAg given commercial coagulation factors were found to have anti HD in

serum from Italy and the U.S. (79). Evidence for significant infection with HDV was not found in East Germany (78) and also in HBsAg positive haemophiliacs who had been given locally produced coagulation factors.

Transmission of HDV in endemic areas is likely to occur by inapparent percutaneous or transmucosal exposure to body fluids containing the infectious agent as postulated for nonpercutaneous transmission of HBV (80).

Lot of epidemiological work has been done all over the world to find out the prevalence of delta infection because of its importance in fulminant hepatitis which is a major cause of morbidity and mortality in HBV infection.

Studies carrier out by Fonseca et al (81) in Brazil showed that the prevalence of HBsAg carriers is 16.7% and among these 34.4% are positive for anti delta prevalence was found to be more in young adults.

The prevalence in Eastern Amazon was shown to be less than Western Amazon (82).

In Taiwan the overall prevalence of HDV infection was 7.2% in general population. Males are equally susceptible as females to HDV. Prevalence in different age groups showed no significance (83). In another study in Taiwan it was found that HBsAg positive rate was 22.1% among parenteral drug addicts and was similar to that found in general population of Southern Taiwan. The HBV infection rate was 99.2% and anti HDV positive rate 78.5% among HBsAg poositve subjects

which was higher than in general population (84). Yet in another study on HDV infection in Taiwan it was shown that the natural course of either acute or chronic HBV infection in childhood may be more closely related to HBV itself than to HDV (85).

In Spain parenteral transmission seen to play a major role in spread of HDV than sexual route which is of relevance (86). Also HDV infection in Spain was restricted mainly to HBsAg positive drug abusers and multitransfused haemophiliacs while in general population 6.7% of chronic HB show delta superinfection (87). Anti-HD is associated with more severe forms of chronic hepatitis such as chronic active hepatitis with cirrhosis (88). In a survey by Genesea et al (88) in Spain it was shown that although delta virus may inhibit the replication of HBV among chronic HBsAg carriers, this effect is not apparent among patients with HBV/HDV co-infection. Serological surveys carried out in U.K.

(Oxford) on blood samples from 13 locations in Pacific and South East Asia tested for evidence of infection with HBV and HDV showed that serological markers of HBV were found in all locations, maximum prevalence being 88% in Majuro Micronesia. Antibodies to HDV in HBsAg positive persons were found in 6 locations with maximum prevalence of 81% in Kiribati. Other places where such surveys were carried out were Belgium where antibody to delta agent in 218 hospitalized HBsAg carriers

were found out between oct.83 and Oct.86 out of these 52 patients were of Italian nationality and residing in Italy 9.4% had positive results for anti-delta. Among HBsAg carriers residing in Belgium, 4 patients were of S. European origin, 1 African and 2 Belgium IV drug abusers and positive results for anti delta (88).

It has been found that genetic factors also play an important part in the acquisition and clearance of HBV and HDV.

Saracco et al (19) did a world wide epidemiological and clinical survey on 377 cases of fulminant hepatitis in persons positive for HBsAg in Greece, Italy, U.S., U.K. Central African Republic, Taiwan, Egypt and India. Only 52% could be attributed to infection with HBV which was defined as presence of IgM antibody to HBeAg (IgM Anti HBe) and absence of serum markers of infection by extraneous viruses, 30% cases were caused by co-infection with HBV and HDV or by infection with HDV superimposed on carrier of chronic HBsAg. The surveys done in India for finding out the prevalence of delta antigen among HBsAg positive population are as follows: In Pune it was seen that viral hepatitis patients did not show anti-delta antibodies. 13.63% of commercial donors, and 6.8% of voluntary donors showed anti-delta anti bodies. Anti delta positive donors were also positive for HBV DNA (21). In Chandigarh during 1986 - 87, 6% of HBsAg positive serum

samples were found positive for presence of delta antigen as per NICD report (20).

A lot of epidemiological work is going on all over the world to find out the prevalence of delta virus in fulminant hepatitis cases but there is tremendous paucity of the same in India hence this study was performed to find out the prevalence of delta agent in fulminant hepatitis cases in Kasturba Hospital for Infection Diseases, Bombay which filters most of the hepatitis cases of coastal area of Maharashtra.

MATERIALS AND METHODS

Patient Selection :A total of 229 indoor patients suffering from fulminant hepatitis were selected during a period of 10 months , (Dec.91-Sept.92) from the hepatitis ward of Kasturba Hospital for Infectious diseases (KIDH) Bombay. Out of the total 229 patients 145 were Males and 77 females and their ages varied from 19 years to 74 years. 7 patients were below the age of 12 years . Out of the total 229 patients, 17 patients had history of blood transfusion, none were Haemophiliacs ,8 were professional blood donors,4 patients had history of drug abuse,67 patients gave history of intake of Alcohol (30 gms/ day for more than 5 - 8 years), and 66 out of these were males and one was a female patient.

A thorough history taking and clinical examination was done on all patients and diagnosis of fulminant hepatitis was confirmed on the basis of clinical and biochemical profiles. The clinical profiles included increased intracranial pressure manifesting as systolic hypertension, irregularity in breathing, impairment of brain stem reflexes, opisthotonus, decreased pupillary. Reflexes etc. symptoms were Asterexis, Constructional Apraxia and Convulsions. Out of 229 patients 159 were in precoma or coma.

Bacterial and fungal infections were seen in 65% of patients, some had renal failure (urinary out put of less than 300 ml/day, and a rise in serum creatinine levels of upto 1.4

mgs% and more.) Arterial hypoxaemia due to respiratory failure, haemorrhages predominantly gastrointestinal due to thrombocytopaenia were also common, Metabolic disturbances in the form of hypoglycaemia and hypophosphatemia were seen.

All patients had highly elevated levels of Serum bilirubin of more than 12 - 14 mgs% (Direct 8 - 9 mgs% and indirect 4 - 5 mgs%) which manifested as Jaundice on inspection of patients. Other physical findings were hepatomegaly , splenomegaly, ascitis, joint effusions and skin rashes which were inconsistent except in 4 patients.

Biochemical profile showed rapid fall in transaminases, a lowered Albumin level in Serum, a rise in AST/ALT Ratio, a rise in serum Alkaline phosphatase levels and prolonged prothrombin time. Electrolyte disturbances were common. Metabolic and respiratory alkalosis were seen more than metabolic acidosis.

After a thorough examination 8 - 10 cc of venous blood was drawn from each of these 170 patients in a plain bulb, the serum was separated and after proper labelling this serum sample was refrigerated at 2° - 8° C. On a small portion of each serum sample the test for detection of Hepatitis B surface antigen (HBsAg) was done by the Auszyme monoclonal enzyme immunoassay procedure. Out of 229 patients 146 were found to be reactive for HBsAg in their sera. The remaining test serum of the HBsAg positive patients was stored at the

same temperature as before for determination of total antibody to delta antigen (Anti-delta) by the Abbott anti-Delta Enzyme Immuno assay. The serum of the patients who were negative for HBsAg was discarded.

Before starting the assay procedure the test samples and also the contents of the Assay kit were brought to ambient temperature.

ELISA FOR HBsAg

Biological principles of the procedure :

The auszyme monoclonal enzyme immunoassay procedure is an enzyme immunoassay based on 'Sandwich principle'. Beads coated with mouse monoclonal antibody to hepatitis B surface antigen (Anti-HBs) which forms the solid phase, are incubated with serum or plasma, appropriate controls, and mouse monoclonal Anti-HBs peroxidase (horse radish) conjugate (Anti-HBs : HRPO). During the incubation period, any HBsAg present, is bound to the solid phase antibody and simultaneously bound by the Anti-HBs: HRPO. Unbound material is then aspirated and the beads washed. Next O-phenylenediamine (OPD) solution containing hydrogen peroxide is added to the bead and after incubation, a yellow orange colour develops in proportion to the amount of HBsAg which is bound to the bead. The enzyme reaction is stopped by the addition of the acid. The absorbance of controls and specimen is determined using a spectrophotometer with

wavelength set at 492 nm. Specimens giving absorbance values equal to or greater than the absorbance value of the negative control mean plus a factor are considered initially reactive for HBsAg.

Method:

The wells of reaction trays were numbered with the serum specimen numbers and five wells were labelled for controls, three negative and two positive controls. The kit contents and serum specimens were brought to Ambient temperature before starting the test. 200 µl of the test specimens each and the negative and positive controls were dispensed to their appropriate wells. 50 µl of conjugate was then added to each of the wells containing the specimens and controls. After adding this Horseradish conjugate gently the tray was tapped. Then one bead which formed the solid phase was added to each well and cover seal applied to the tray. After again tapping the tray it was incubated at 40 for 3 hours. After incubation the cover seal was removed and all the liquid was aspirated and the bead was washed three to five times with 4 - 6 ml of distilled water. Immediately the beads were transferred to properly identified assay tubes. 300 µl of freshly prepared OPD substrate solution (Read at the end of this chapter) was pipetted into two empty tubes and then into each tube containing a bead, covered and incubated at room temperature for 30 minutes. Then 1 ml of 1 N sulfuric acid was added to each tube to stop the enzyme reaction. The

results were read on a blank spectrophotometer with a substrate blank at 492 nm. Determination of absorbance of controls and test specimens at 492 nm was done immediately within 2 hours after addition of Acid.

Determination of the test results :

S - Absorbance of test sample

N - Absorbance of the negative control

P - Absorbance of the positive control

The mean absorbances of the controls were calculated and any outliers in the values found for the positive and negative controls were eliminated before the results were determined.

The negative control values should be more than or equal to 0.5 times the negative control mean and less than or equal to 1.5 times the negative control mean.

N or P were recalculated after eliminating the outliers. Elimination procedure was repeated until no further outliers were obtained.

The test run was valid only if $P - N$ value is 0.400 or greater. If not Run must be repeated. The cut off value was calculated by adding the factor 0.50 to the negative control mean. The test sample was reactive for HBsAg if its value is equal to or more than the cutoff value.

ELISA for anti delta antibody

The Abbott antidelta EIA is a competitive enzyme immuno assay in which beads coated with delta antigen are incubated

with serum or plasma or appropriate controls and human antidelata conjugated with horseradish peroxidase (antidelata conjugate). After incubation the unbound material is removed by washing the bead. The presence of anti delta in the specimen will compete with the antidelata conjugate for a limited number of delta antigen binding sites on the bead. Next O - phenylenediamine (OPD) solution containing hydrogen peroxide is added to the bead and after incubation a yellow colour develops. The enzyme reaction is stopped after adding 1N sulfuric acid. The absorbance of controls and specimens is determined using a spectrophotometer with wavelength set at 492 nm. Within limits the greater amount of anti-delta in the specimen, the lower the absorbance. The absorbance values of specimens are compared with a cutoff value ($4/10$ th the negative control mean plus $6/10$ th the positive control mean) specimens with absorbance values greater than the cutoff value are considered negative for anti delta and need not be tested further. if the absorbance value of a specimen is less than or equal to the cutoff value, the specimen is reactive for anti delta.

Method : The position of each control or specimen in the reaction tray was recorded. The kit controls and serum specimens were brought to ambient temperature before starting the test. 100 ul of antibody conjugate solution was pipetted into each well then 100 ul of controls or specimen was

pipetted into their assigned wells. (3 negative controls and 2 positive controls). The tray was tapped to thoroughly mix the contents of the well. One bead was added to each well and cover seal applied followed by gentle tapping of the tray. The trays were then incubated on a level surface at room temperature for 20 ± 2 hours. After removing the cover seal, the liquid was aspirated and the beads washed in 4 - 6 ml of distilled or deionized water. The beads were immediately transferred to properly labelled assay tubes. 300 μ l of OPD substrate solution was pipetted into two empty tubes (substrate blanks) and then into each tube containing a bead. Substrate must not touch metal. Then it was covered and incubated for 30 ± 2 minutes at room temperature. 1 ml of 1 N sulfuric acid was added to each tube.

The results were read on a blank aspectrophotometer with a substrate blank at 492 nm within 2 hours after addition of acid.

Determination of the test Results :

N - Absorbance of the negative control.

P - Absorbance of the positive control.

The mean absorbances of the controls were calculated and any outliers in the values for positive and negative control were eliminated before the results were determined. All negative control values should fall within 0.5 to 1.5 times the negative control mean.

The test run was valied only if N - P value was greater than or equal to 0.300. The cutoff value was calculated using the following equation.

0.4 (negative control mean) + 0.6 (positive control mean).

Samples with absorbance values less than or equal to the cutoff value are considered reactive and samples with values greater than the cutoff value are considered negative for Antidelta.

I. Reagents of Auszyme Monoclonal Diagnostic

- a) Auszyme (mouse) monoclonal conjugate antibody to hepatitis B surface antigen (mouse monoclonal) : peroxidase (Horse radish) minimum concentration : 0.2 µg / ml in TRIS buffer with protein stabilisers. Preservative used is gentamicin sulfate and thiomersal dye : Red, No. 33.
- b) Positive control is human HBsAg, 9 ± 2 ng/ml in TRIS Buffer with protein stabilizers. preservatives :gentamicin sulfate and thiomersal. Dye. Bromophenol blue.
- c) Negative control is recalcified human plasma, non reactive for HBsAg and Anti-HBs. Preservative is gentamicin Sulfate and thiomersal.
- d) OPD Substrate ; The OPD (O - phenylenediamine . 2 Hcl) tablet must be dissolved in diluent for OPD immediately before use; Diluent for OPD : Citrate phosphate buffer contains 0.02% hydrogen peroxide. 5 - 10 minutes prior to colour development, prepare the OPD substrate solution as

follows ; using a clean pipette, transfer into a suitable container, 5 ml of diluent for OPD for each tablet to be dissolved. Transfer. OPD tablets from bottle into container with diluent for OPD using a non metallic forceps. Allow tablets to dissolve. Do not use tablet that is not intact. Just prior to dispersing for the final incubation of the assay, swirl gently to obtain a homogenous solution.

e) IN sulfuric Acid, no. 7212.

Store kit reagents at 2 - 8°C. OPD tablets and in sulfuric acid may be stored at 2 - 30°C. before use all reagents must be brought to room temperature (15° - 30° C) and returned to storage conditions indicated above immediately after use.

II. Reagents of abbott Antidelata EIA :

a) Beads which constitutes the solid phase in abbott Anti Delta EIA are coated with Woodchuck delta antigen.

b) Conjugate means antibody to delta antigen (human) conjugated with horse radish peroxidase. Min concentration : 0.05 ug ml preservatives used are 0.01% gentamicin sulfate and 0.01% thiomersal.

c) Positive control anti-delta : Recalcified human plasma, positive for anti-delta and HBsAg. Anti-delta min. titre : 1 : 200 $\pm$ 2 log₂ dilutions.

Preservative : 0.01% gentamicin sulfate.

d) Negative control antidelata ; Recalcified human plasma, non reactive for anti-delta and HBsAg. Preservative 0.01%

gentamicin sulfate.

e) Preparation of OPD substrate and storage instructions are same as for Auszyme monoclonal diagnostic kit.

RESULTS

229 patients from the hepatitis ward of Kasturba Infectious diseases hospital, Bombay were diagnosed as fulminant hepatitis cases based on their clinical and biochemical profiles. During a period of 10 months (Dec. 91 - Sept. 92). Their sera were tested for presence of HBsAg using an Auszyme monoclonal enzyme immuno assay. Out of the total of 229 patients, 146 patients were positive for HBsAg. On these a further testing for presence of anti-delta antibody was done using 'Abboatt Anti-Delta' EIA. Out of the 146 tested, 16 patients were positive for presence of Anti delta antibody. (see table - I) Since Kasturba hospital filtered most of the patients of Coastal areas of Maharashtra, it could be said that the prevalence rate of delta agent is 10.95% (16/146).

Out of these 16 patients positive for anti-delta 11 were males and 5 were females. All belonged to the age group 24 - 57 yrs. (see table - II) Two patients had history of blood transfusions, one was a professional blood donor who had donated blood once in six months for last 5 years. From the history taken none were haemophiliacs, none had history of Immunosuppressive therapy. No one gave history of drug abuse. 4 male patients out of 11 were chronic alcoholics (alcohol intake 30 g/day for 8 years and more). In a span of 10 months (Dec '91 to Sep '92) the HBsAg and Anti delta

positive cases showed no seasonal variation (see table III).

On clinical examination 9 out of 16 patients had signs of increased intracranial pressure like irregularity in breathing, impairment of brain stem reflexes, decreased pupillary reflexes, systolic hypertension and opisthotonus. Symptoms were asterexis, constructional apraxia and convulsions off and on. 6 out of 16 patients were in coma. Bacterial or fungal infections were seen in all 16 patients. 11 out of 16 patients had renal output < 300 ml/day indicating renal failure. All had severe Jaundice. Hepatomegaly was seen in 4 patients, 3 had splenomegaly though not of severe degree. Ascitis was seen in 4 patients, 2 patients had joint effusion, urticarial skin rashes seen more on abdominal area were seen in 2 patients.

Biochemical profiles: Serum bilirubin was highly elevated in all 16 patients that were having HBV/HDV coinfection. Direct: 8 - 9 mgs% Indirect : 4 - 5 mgs% Total bilirubin of more than 13 mgs%, thrombocytopenia and prolonged prothrombin time were seen in all patients. Serum creatinine values were more than 1.3 mgs% in all 16 patients positive for antidelata. AST/ALT ratio was raised, serum albumin level showed a fall in its value, there was raised serum alkaline phosphatase in all of these 16 patients. Hypoglycemia and hypophosphatemia was seen in 7 out of 16 patients. (see table IV)

These 16 patients who were antidelta reactive were followed up. 5 patients out of 16 died in a course of 1 - 2 months (mortality rate 31.25%). 3 out of the 5 were females and 2 were males.

TABLE - I

ELISA Results for presence of HBsAg and Anti Delta			
Total No. of pts. tested	HBsAg positive patients	Anti delta positive patients	
229	146	16	

TABLE - I

AGE & SEX	DISTRIBUTIONS IN HBsAg ND ANTI DELTA POSITIVE PATIENTS.						
AGE IN YEARS.	1 - 10	11 - 20	21 - 30	31 - 40	41 - 50	51 - 60	61 plus
No. of pts positive for HBsAg.	4	16	31	48	19	21	7
Sex							
Male	3	11	20	32	13	14	5
Female	1	5	11	16	6	7	2
Anti delta							
Male	0/3	0/11	4/20	5/32	1/13	1/14	0/5
Female	0/1	0/5	1/11	3/16	1/6	0/7	0/2

TABLE III

Seasonal Variation

	Dec '91	Jan '92	Feb '92	Mar '92	April '92	May '92	June '92	July '92	Aug '92	Sept '92
Pts. positive for HBsAg	10	12	19	12	22	20	12	15	14	10
Pts. positive for Anti-delta	1/10	0/12	2/19	1/12	3/22	2/20	1/12	2/15	2/14	2/10

TABLE - IV

Fulminant hepatitis cases
positive for

Clinical Symptoms	HBsAg	Anti-delta
Jaundice	146	16
Nausia / Vomiting	146	16
Altered Conciousness	138	6
Renal failure	121	11
Haemorrhages	109	5
Opportunistic Infections	111	3
Skin rashes	62	2
Hepato splenomegaly	125	4
Ascitis	60	4

Biochemical Profiles

† Serum Bilirubin	146 (>13 mgs%)	16 (>13 mgs%)
† SGOT	146 (>60 U/L)	16 (>60 U/L)
† SGPT	146 (>65 U/L)	16 (>65 U/L)
† Serum Albumin	146 (M <450 µM/L) (F <400 µM/L)	16 (M <450 µM/L) (F <400 µM/L)
† Serum Alkaline Phosphatase	140 (>210 U/L)	16 (>210 U/L)
† LDH	138 (>360 U/L)	16(>360 U/L)

All the biochemical Profiles
hospital,Bombay (KIDH).

were done in Kasturbaa Infectious disease

DISCUSSION

The hepatitis delta virus has gained a lot of attention in recent years due to the fact that the infection of HBV carriers with the delta virus is associated with chronic progressive and fulminant hepatitis in a significant number of cases (30). In surveying large populations, most studies have concentrated on the prevalence of antibodies to delta antigen in hepatitis B surface antigen positive individuals. This antidelta positive population may transmit HDV infection since HDV associated RNA has been found in their serum. The determination of antibody to hepatitis D antigen (anti-HD) in serum or plasma is the assay of choice in the assessment of HDV infection. Hepatitis D antigen is generally detectable only in the liver and is transient or nondetectable in serum. though HDV has same mode of transmission as HBV its distribution has striking geographical differences. In western communities its distribution is closely correlated with parenteral drug abusers and other high risk populations. Spread is predominantly horizontal and vertical transmission is unusual (18).

The epidemiologic picture of HDV has changed from an Italian to a global perspective. The most consistent endemic reservoir of HDV infection extends from Balkan Peninsula through the eastern mediteranean basin to the middle east

(76). Arab countries at present have the highest prevalence of HDV infection in the world (76). HDV is also present in several areas of south America as documented by an outbreak of fulminant hepatitis in Venezuela (77) due to superinfection of HBsAg carriers by HDV.

From analysis of transfusion associated HBsAg positive hepatitis in Italy the relative risk of HBV/HDV hepatitis versus HBV hepatitis alone can be estimated to be one in fifty (78, 79). Transmission of HDV in endemic areas is likely to occur by inapparent percutaneous or transmucosal exposure to body fluids containing the infectious agent (80).

In a world wide clinical and epidemiologic survey carried out by Saracco et al (19) on 377 cases of fulminant hepatitis in persons positive for HBsAg in India, Greece, Italy, U.S., U.K. Central African Republic, Taiwan and Egypt only 52% could be attributed to infections with HBV and 30% cases were caused by co-infection with HBV-HDV or by infection with HDV superimposed on carrier of HBsAg .

Although a lot of surveillance is done all over the world for finding the prevalence of hepatitis delta virus infection in hepatitis cases there is tremendous paucity of literature available on HDV infection rates in India. As per NICD report a survey was done during 1986 - 87 in Chandigarh, On HBsAg positive serum samples and only 6% were found positive

for presence of delta antigen (20). Also Arankalle et al (21) from Pune found that 13.63% of commercial blood donors and 6.8% of voluntary blood donors showed anti delta antibodies . In an attempt to find out the prevalence of delta agent in fulminant hepatitis cases in Bombay a study was carried out on patients suffering from fulminant hepatitis, in Kasturba infectious disease hospital in Bombay prevalence was 10.95% (16 positive for antidelta out of 146 positive for HBsAg). The age of the HDV infected patients varied from 24 years to 57 years. Whereas Fonseca et al (81) in Brazil showed that prevalence was more common in young adults . In Western countries, HDV is virtually confined to parenteral drug addicts and their contacts (69). Spread of HBV and HDV amongst IV drug Abusers is largely due to sharing of needles. Between one and two thirds of HBV positive IV drug addicts in Europe may have markers of HDV infection with the prevalence ranging from 31% in Ireland to 64% in Italy (69). The trend is similar in the U.S. (70). Whereas in our study none out of the 16 patients positive of anti-delta had history of drug abuse. In Spain HDV infection was restricted mainly to HBsAg positive drug abusers and multitransfused haemophiliacs while in general population 6.7% of chronic hepatitis B patients show delta superinfection (87). In our study out of the 16 positive for Anti-delta none were haemophiliacs, but two patients had history of blood

transfusions given in the past due to the surgical procedures they had undergone. One patient was a professional blood donor. this suggests that in our country regular screening of blood for HBsAg before transfusion should be reinforced and also proper aseptic precautions should be practiced before any transfusion procedure.

Approximately 50% of Haemophiliacs with HBsAg given commercial coagulation factors were found to have antidelata in Serum from Italy and the U.S. (79). Evidence for significant infection with HDV was not found in East Germany, (78) and also in HBsAg positive haemophiliacs who had been given locally produced coagulation factors.

in TAIwan the overall prevalence of HDV infection was 7.2% in general population Males were equally susceptible as females to HDV and prevalence in different age groups showed no significance (83). Whereas in our study out of the patients positive for Anti delta, two thirds were males and one third were females, the reason for which is unclear.

It was shown that survival was greatest when the time interval between onset of encephalopathy and Jaundice was less than 8 days and seen typically in FHF-B (45 - 47). In another study survival based on collective data from 8 countries was 47.4% for FHF B/D and 26.7% for FHF-B (19).

In our study out of the 16 (10.95%) patients positive for

412

anti delta 5 (31.25%) expired in a course of one month. 3 out of 5 patients (60%) were females and 2 out of 5 patients (40%) were males, thus showing that mortality in females was higher. This may be due to early and enhanced production of Anti - HBs which forms complexes with HBsAg in hepatic microcirculation causing ischaemic necrosis of hepatocytes (38).

Unlike Western Countries where major cause of HDV infection is parenteral drug abuse, in our country the major problem may be due to improper screening of blood for HBsAg thus infecting patients who are given blood transfusions.

If proper screening of blood is carried out with correct aseptic precautions in the hospital procedures, a part of the problem may be corrected.

SUMMARY AND CONCLUSION

The serum of 229 hepatitis patients from hepatitis ward of Kasturba Infectious Diseases Hospital, Bombay was collected during the period of 10 months (Dec. 1991 to Sept. 1992) and tested for the presence of HBsAg by Auszyme monoclonal enzyme immunoassay. Out of the 229 serum samples 146 serum samples were positive for presence of HBsAg. On these 146 serum samples positive for HBsAg a further testing for anti-delta antibody was done by the Abbott anti-delta EIA. 16 out of 146 were found positive for anti delta antibody (10.95%).

Since the Kasturba Infectious Disease Hospital (KIDH) filters most of the hepatitis cases of coastal region of Maharashtra, it could be said that 10.95% of HBsAg positive fulminant hepatitis cases were positive for antidelta antibody. Male were found to be more susceptible than females to HBV / HDV Coinfection and the HDV infection showed no seasonal variation. The mortality rate was found to be 31.25% for HBV / HDV Coinfection.

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