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Hepatitis D Virus Infection; Iran, Middle East and Central Asia

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Introduction

Hepatitis delta virus (HDV) is a unique RNA virus that requires a helper function provided by hepatitis B virus (HBV) for replication[1, 2, 3]. Thus, HDV can replicate only in people who are also infected with HBV. Infection with HDV may result in either acute or chronic hepatitis[4].

The clinical course of hepatitis D is variable but usually more severe than that of other forms of viral hepatitis. Patients with acute hepatitis D may present with fulminant hepatitis [5], a rare sequela of the acute hepatitis caused by other hepatitis viruses. Chronic hepatitis D is a serious and rapidly progressive liver disease [6].

Virology

Hepatitis delta virus (HDV) is an incomplete defective RNA virus which is the smallest animal virus and consists of spherical particles on electron microscopy and contains RNA and delta antigen (HDAg) with a diameter of about 36 nm. It was first described in 1977. HDV particle contains delta antigen that exists in two forms: a large delta antigen (LHDAg) of 27 kDa and a small delta antigen (SHDAg). In virus-infected cells, HDAg is located exclusively in the nuclei. HDV envelope consists of all three protein species of HBsAg and as a result HDV probably utilizes the same cellular receptor as HBV. Replication of HDV is restricted to the liver. Although replication of HDV can occur within hepatocytes in the absence of HBV, HBV is necessary for coating the HDV virions and allowing their spread from cell to cell. SHDAg supports replication, whereas LHDAg acts predominantly to suppress replication. LHDAg, together with HBsAg, is necessary for assembly of HDV. HDV RNA and HDAg are encapsidated by envelope proteins derived from the pre-S and S antigens of HBV. The HDV genome is a single-stranded positive-sense RNA molecule. HDAg appears to be essential for viral replication. HDV particles consist of HBsAg, HDAg (both LHDAg and SHDAg), and HDV RNA. Assembly, thus, can only occur in the presence of the helper virus HBV. HBsAg and LHDAg are essential and sufficient for the assembly of particles[7-10].

Based on sequence relationship, HDV isolates have been suggested to group into three genotypes that have geographical distribution. Genotype 1 compromises most of the HDV isolates sequenced and includes isolates obtained from almost every part of the world, with predominance in North America, Europe, Africa, the Middle East, the South Pacific, and East Asia, which often causes aggressive hepatitis and is more frequently associated with liver cirrhosis and hepatocellular carcinoma, genotype 2 has been found only in East Asia (Taiwan and Japan) and may be associated with relatively milder diseases than genotype 1, and genotype 3 has been restricted to northern South America and is associated with a

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particularly fulminant form of hepatitis[11-13].

Epidemiology and Transmission

Since HDV is dependent upon HBV, the epidemiology of HDV infection is similar to HBV but with notable exceptions. Evidence of HDV infection has been found all around the world. It is estimated that approximately 5% of hepatitis B surface antigen (HBsAg) carriers are infected with HDV infection worldwide. HDV infection occurs worldwide but incidence and prevalence data are limited due to inaccurate reporting and delayed detection. It is more difficult to determine the number of cases of acute or fulminant hepatitis related to HDV infection, as the incidence varies among continents, countries, and regions. In general, three epidemiological patterns of HDV infection can be identified. These include the endemic pattern (such as what occurs in southern Italy and Greece), the epidemic pattern (epidemics have been reported in the Amazon Basin of Venezuela), and the occurrence of HDV infection among high-risk groups such as intravenous drug users (in developed Western countries). Infection with HDV is infrequent in populations such as the Alaskan Natives, in which HBV acquisition occurs during infancy and childhood. The epidemiology of HDV infections does seem to be changing in some regions such as Italy. Vaccination against HBV, decrease in HBV infection and thus in the pool of HBsAg carriers who may be infected with HDV are responsible for this decrease. Immigration patterns can be expected to have an impact on HDV infection. Nonetheless, it continues to represent a public health problem in some parts of the world yet [14-18].

Middle East and Middle Asia

Delta infection is endemic in Middle East countries, but unfortunately there are not enough data regarding Central Asian countries. In Iran, the prevalence rate of HDV infection varies from 2.4 in blood donors to 10 percent in chronic liver disease patients[19-22]. In asymptomatic carriers of HBsAg from Jordan, Kuwait, Saudi Arabia, and Turkey the prevalence is 2%, 31%, 3.3%, 5.2%, respectively. Its prevalence in acute hepatitis patients from Egypt, Jordan, Kuwait and Tajikistan, is 16.94%, 16%, 4% and 9.2%, respectively. In chronic liver disease patients from Yemen, Turkey, Jordan, and Egypt, it is 2%, 32.7%, 23% and 23.53%, respectively. In patients on hemodialysis and kidney-transplant recipients from Oman, 7.7% and 22.2% had anti-HDV Ab positive, respectively. 14.7% of IDUs from Saudi Arabia were Anti-HDV Ab positive (table 1). All HBV strains sequenced and reported from Middle East countries were genotype 1[23-34].

HDV is a bloodborne virus and may thus be transmitted by parenteral contact with blood products. Common methods of transmission of HDV infection include intravenous drug abuse, transfusion, sexual contact, and nosocomial infection. In addition, inapparent parenteral spread is probably responsible for spread of HDV infection within families. HDV infection appears to be endemic in some populations, particularly in countries around the Mediterranean Sea. In endemic and Mediterranean areas, many of patients seem to acquire HDV infection early in life, possibly even in childhood. The route of spread of HDV in these populations is uncertain, as patients often do not have any of the known parenteral contact factors such as intravenous drug use and blood transfusion.

The modes of transmission of HDV are similar to those of HBV infection, and percutaneous exposures are the most efficient. Intravenous drug use is among the commonest modes of HDV transmission in areas of low prevalence, such as North America. The reported frequency of HDV infection among HBsAg-positive injection drug users varies from 31% in Ireland to 91% in Taiwan. Hemophiliacs and other persons who receive large amounts of

Table 1. Epidemiology of Delta Hepatitis: Presence of Anti-HDV Among Chronic Carriers of HBsAg in the Middle East and Central Asian Countries.

Country	Author's Name	Anti-HDV Positive
Egypt	Darwish *et al.*	Acute hepatitis 16.94% Chronic hepatitis 23.53%
Iran	Amini *et al.* Rezvan *et al.* Alavian *et al.*	Population base 2.4% Blood donors 2.5% Chronic hepatitis 5.6%
Jordan	Toukan *et al.*	Chronic liver disease 23% Acute hepatitis 16% HBsAg carriers 2%
Kuwait	Al-Kandari *et al.*	Acute hepatitis 4% HBsAg carriers 31%
Oman	Aghanashinikar *et al.*	On hemodialysis patients 7.7% Kidney-transplant recipient 22.2%
Saudi Arabia	Al-Tarif *et al.* Njoh *et al.* El-Hazim *et al.*	Blood donors 3.3% IDUs 14.7% Thallassemics 21%
Tajikistan	Iarasheva *et al.*	Acute hepatitis 9.2%
Turkey	Balik *et al.*	Chronic liver disease 32.7% HBsAg carriers 5.2%
Yemen	El-Guneid *et al.*	Chronic liver disease 2%

pooled blood products are at increased risk of acquiring HDV infection.

Sexual transmission of HDV is less efficient than that of HBV. There are many studies that prove the sexual transmission of HDV (heterosexual couples, homosexual men, and male contacts of female prostitutes) without a history of intravenous drug abuse. Perinatal transmission of HDV is rare. HDV appears to be transmitted only rarely by transfusions of whole blood (less than 1 in 3,000 transfusions) despite screening of blood for HBsAg. Another rare route of transmission is via hemodialysis. History of war injury is a risk factor for HDV infection in Iran[35-37].

Diagnosis

For several reasons, it is important to be able to recognize HDV infection when it is present. Knowing that HDV infection is present in a patient with HBV infection allows a more accurate prognosis. Moreover, patients with acute HDV infection are more likely to develop severe or fulminant hepatitis, and those with chronic HDV infection are significantly more likely to progress to cirrhosis and liver failure. Another reason for determining whether HDV infection is present is that the response of patients with chronic delta hepatitis to antiviral therapy and needed dosage differs significantly from that of patients with chronic hepatitis B alone. The presence of anti-HDV in serum also appears to be a reliable and specific way of diagnosing HDV infection. Anti-HDV can be detected by RIA and EIA for both IgG and IgM antibodies to HDV. Anti-HDV becomes detectable in more than 90% of cases within 1 to 2 months of acute HDV infection. In patients with acute delta co-infection, in whom the HDV infection is usually transient, anti-HDV titers may be quite low (less than 1:100), or even undetectable in some cases. In such patients, anti-HDV titers may be more easily detectable in subsequent serum samples, and several samples may need to be tested over a few weeks to confirm a suspected diagnosis of delta hepatitis. Under these circumstances, anti-HDV remains detectable beyond the acute illness, but it remains uncertain how long it may persist. IgM anti-HDV is detectable during the early phase of acute infection and therefore serves as a useful marker of acute disease. In patients with acute but self-limiting HDV infection, the IgM anti-HDV response is short-lived. In contrast, in patients with delta superinfection, in whom the HDV infection usually becomes chronic as they are already chronically infected with HBV, anti-HDV tends to appear sooner and reach higher titers. Titers of anti-HDV may exceed 1: 1,000,000 in patients with chronic delta hepatitis, and titers of more than 1: 1,000 are diagnostic. In patients with chronic HDV infection, IgM anti-HDV is present early and persists in a variable titer for long periods. The presence of IgM anti-HDV appears to correlate with levels of HDV replication. However, in the chronic setting, an IgG anti-HDV titer greater than 1:1000 correlates well with the presence of ongoing viral replication. Although the presence of anti-HDV may be a very useful screening test for HDV infection, it may not be totally reliable in immunosuppressed patients, who may not be able to mount an antibody response sufficient to be detected.

HDV infection occurs only in the presence of HBV infection (HBsAg positive) and is detected by anti-HDV (IgM for acute or IgG for chronic infection). Virtually all patients with HDV infection have HBsAg in serum. On certain occasions, the replication markers of HBV may not be evident because of a profound inhibitory effect of HDV on HBV replication or in patients with acute or fulminant hepatitis who clear HBsAg very rapidly from serum. As many as 10% to 15% of patients with acute hepatitis may not have HBsAg detectable in serum on initial presentation. In such cases, the presence of IgM antibodies to HBcAg in serum may be sufficient to diagnose acute hepatitis B. HBV replication is usually suppressed by HDV, so HBV markers may resemble a carrier state with HBsAg positive, HBeAg negative, anti-HBe positive, and HBV DNA negative [38-40].

The gold standard of diagnosis of HDV infection is the detection of HDAg in the liver by immunostaining. However, HDAg staining is available only in research laboratories. Delta antigen (HDAg) is present in serum in the late incubation period of acute infection and persists into the symptomatic phase in approximately 20% of cases. Since HDAg is often present transiently and there are no commercial assays for detection of HDAg, it is only a research test.

HDV RNA is an early marker of acute infection and a useful marker of HDV replication in patients with chronic infection. RT-PCR-based assays are more sensitive and have a lower limit of detection, as few as 10 genomic copies. In addition to diagnostic applications, levels of HDV RNA in serum may be useful for monitoring the effect of antiviral therapy. With the use of PCR, more than 90% of patients with chronic delta hepatitis have HDV RNA detectable in serum [41-42].

Clinical Features

Clinical presentation of HDV infection varies depending on whether it is a co-infection (simultaneously with acute HBV infection) or a superinfection (infect a chronic HBsAg carrier). Patients may present with typical features of acute hepatitis (jaundice, malaise, anorexia) or the disease may go undetected until it presents ultimately with cirrhosis. Acute presentation is indistinguishable from other types of viral hepatitis, but it has more morbidity rate. The epidemiological features of the patient may raise suspicions about delta hepatitis. For example, drug abusers or patients living in endemic areas should be suspected of having delta infection. Acute hepatitis usually resolves in a few weeks. In less than 5% of patients, the illness evolves into a chronic phase. Chronic hepatitis characterized by persistently abnormal transaminase levels, persistence of HBsAg, rising titer of anti-HDV, and persistent detection of HDV RNA in serum. All patients with evidence of HBV infection (HBsAg or IgM antibody to hepatitis B core antigen) should be tested for HDV infection.

Acute co-infection with HDV and HBV is characterized by a severe hepatitis with hepatocellular necrosis and inflammation. The majority of cases, however, are self-limiting, with clearance of HBV and therefore HDV. Co-infected persons are more likely to have a fulminant presentation than are patients with HBV infection alone, for unclear reasons. Co-infection is characterized by a biphasic increase in serum aminotransferase activity, a finding that is rare in acute HBV infection alone.

Superinfection is characterized clinically as an acute hepatitis in otherwise stable chronic HBV carriers. The acute hepatitis may be severe and progress into fulminant failure. The serologic features consist of HDV RNA, IgM anti-HDV, HBsAg, and IgG anti-HBc. HBV DNA is usually suppressed. In both acute co-infection and superinfection, IgM anti-HDV is detected, and distinguishing serologic feature for co-infection is the presence of IgM anti-HBc. Chronic HDV infection is almost always the result of a superinfection because co-infection seldom leads to chronic infection. Acute superinfection of HDV on a chronic HBV patient is often clinically distinguishable. Typically a severely marked hepatitis is seen, with high levels of HDV virus. The majority of cases do not resolve but rather lead to chronic co-infection. In superinfection with HDV, the presence of established HBV infection provides the ideal substrate for HDV, and, as a consequence, chronic progressive liver disease develops in over 85% of patients. Uncommonly, HDV superinfection produces a self-limited infection followed by subsequent clearance of HDV and HBV. Fulminant hepatitis may result from HDV superinfection and is characterized by the presence of HDV markers and the absence of IgM anti-HBc in serum. HDV superinfection may be divided into the following three phases: acute phase, active HDV replication and suppression of HBV with high ALT levels; chronic phase, decreasing HDV and reactivating HBV with moderate ALT levels; and late phase, development of cirrhosis and hepatocellular carcinoma caused by replication of either virus or remission resulting from marked reduction of both viruses. The clinical features of chronic HDV infection are not specific and, in general, cannot be distinguished from chronic hepatitis of other causes on clinical grounds alone. In chronic HDV infection, IgG anti-HDV and IgM anti-HDV are present in the serum, and HDAg is demonstrable with immunohistochemical staining or in situ hybridization of the liver tissue [43-45].

Natural History

Two forms of acute delta hepatitis are recognized, depending on whether the infected individual is already infected with HBV. Acute hepatitis caused by co-infection with HDV and HBV is associated with a higher risk of severe and fulminant liver disease than is hepatitis caused by HBV alone. Acute co-infection resolves in 80-95% of cases, with elimination of HBV through humoral immune mechanisms. However, 2-20% develop fulminant hepatitis and in 30% of cases of fulminant hepatitis associated with HBV, a simultaneous acute hepatitis D infection is detected. In addition, 2-5% of acute co-infection cases result in chronic infection. A common feature of delta co-infection is a biphasic pattern of illness, with two separate peaks in serum aminotransferases thought to reflect injury caused by each of the two viruses sequentially.

Unlike acute co-infection, HDV superinfection results in chronic HDV-HBV in more than 70-80% of cases. Approximately 15% of patients who are superinfected with HDV will have a disease that is rapidly progressive, with cirrhosis developing within 12 months of infection. A further 15-20% of patients have a benign course with spontaneous remission of the histologic disease. The remaining 65-70% have a slowly progressive course leading to cirrhosis. The more aggressive disease is typically seen in adults with intravenous drug use as their risk factor for acquisition of infection. Similar to acute

coinfection, acute HDV superinfection results in fulminant hepatitis in 2-20% of cases. In some outbreaks of severe delta superinfection in populations with a high HBV carrier rate, mortality is in excess of 20%.

Chronic HBV-HDV infections are associated with severe liver disease, but there is also a chronic healthy carrier state for HDV, similar to that noted with HBV. Chronic HDV does progress to cirrhosis frequently. In Italy, 50% of chronic hepatitis B carriers with cirrhosis had HDV infection although only 3% of chronic hepatitis B carriers are infected with HDV. The development of cirrhosis is also more rapid than for chronic HBV, and there is a predominance of co-infected patients with cirrhosis who are young. Also in Italy, 10-15% of patients progress to cirrhosis and clinical liver failure within a few years after superinfection of HDV while the remainder progress to cirrhosis slowly and similarly to an isolated HBV infection. Although chronic HBV infection is a well-recognized risk factor for the development of hepatocellular carcinoma, a similar association has not been clearly demonstrated for chronic HDV infection. Overall, the pattern of disease progression appears to vary with geography, genotype, and mode of transmission. Slowly progressive, mild disease is more common in endemic areas. On the other hand, HDV disease appears to be more severe in non- endemic areas where injection drug use is the main form of transmission [46-49].

Prevention

Because of its requirement for chronic HBV infection, HDV infection can be prevented by vaccinating susceptible persons with hepatitis B vaccine. There are no specific interventions to prevent HDV superinfection in chronic HBV carriers, other than counseling to avoid behaviors which increase the risk of exposure to HDV.

Treatment

The management of acute HDV infection is supportive. Patients should be monitored closely for evidence of encephalopathy, coagulopathy, and other signs of liver failure by clinical and biochemical parameters. Liver transplantation is the treatment of choice for patients with fulminant or end-stage liver disease secondary to HDV. Patients who undergo liver transplantation for chronic HDV infection have lower rates of HBV infection post-transplantation than do patients with HBV infection alone, and it has been suggested that HDV has an inhibitory effect on HBV replication [50]. HBIG administered preoperatively and postoperatively effectively reduces the rate of HBV reinfection after liver transplantation (see earlier section). Thus, the outcome of patients who undergo liver transplantation for end-stage HDV infection is comparable to that of patients transplanted for other indications. Liver transplantation, therefore, is the recommended therapy for patients with liver failure caused by HDV.

Therapy for chronic hepatitis D is problematic and options are limited. Interferon alpha is effective in only a small proportion and has to be administered at high doses for a prolonged period. Relapses on cessation of therapy are common unless the HBsAg is cleared, which occurs infrequently [51, 52]. Long-term interferon has been tried but the side effects, inconvenience and cost make this a difficult option. In pilot studies of interferon alfa in chronic delta hepatitis, high doses of the drug given for prolonged period led to a significant lessening of disease in 25 percent of patients. Randomized trials of low doses of interferon alfa given for 6 to 12 months; however, failed to find a lasting benefit [53, 54, 55]. In a multicenter trial in Italy, therapy with 9 million units of interferon alfa given three times a week for 12 months led to remission in 36 percent of the patients, but a lower dose was ineffective [56]. The only virology endpoint which can be associated with permanent clearance of HDV is HBsAg clearance. A pilot study of lamivudine monotherapy has been reported, but although HBV DNA suppression occurred, HDV RNA was not cleared. A pilot study of lamivudine and interferon-alfa combination therapy has also been reported, but neither aminotransferase nor HDV RNA levels normalized. Both ribavirin and lamivudine have proved ineffective in chronic delta hepatitis [57-58].

In conclusion, therapy in chronic delta hepatitis requires relatively high doses of interferon (5 million units daily, or 9 to 10 million units given three times weekly) for prolonged period (at least 12 months) in patients who are IgM anti-HDV and/or serum HDV RNA positive and/or liver HDAg positive, have abnormal ALT, and a histology of chronic hepatitis is recommended. Such therapy leads to sustained improvement (usually with disappearance of HBsAg from serum) in 15 to 25 percent of patients[59,60]. Patients with chronic HDV infection and decompensated cirrhosis are at risk of developing cirrhosis with portal hypertension and hepatic decompensation. When these complications occur, referral for liver transplantation is necessary.

Summary

The hepatitis delta virus (HDV) is a small defective virus. Delta hepatitis is the least common form of chronic viral hepatitis but is the form most likely to lead to cirrhosis. Delta hepatitis is serologically complex, so effective therapy is difficult. HDV is a defective RNA virus that replicates efficiently only in the presence of HBsAg. Thus, delta hepatitis occurs only in patients who are HBsAg-positive. Infection is acquired parenterally and probably also via close personal contact in endemic areas. HDV infection is strongly associated with injection drug abuse. Chronic HDV infection often results in severe liver disease. The diagnosis is made on the basis of the presence of antibodies against HDV (anti-HDV) and HBsAg in the serum of a patient with chronic liver disease and it is confirmed by the finding HDV antigen in liver or HDV RNA in serum (by reverse-transcription-polymerase-chain-reaction assay). It is important to determine whether delta hepatitis is present because the responses to therapy of patients with this disease are less satisfactory than those with hepatitis B, and the recommended regimen of interferon alfa is different.

References

1. Rizzetto M, Verme G. Delta hepatitis--present status. *J Hepatol.* 1985; **1**:187-93.
2. Taylor J. Structure and replication of hepatitis delta virus. *Semin Virol* 1990; **1**:135-41
3. Purcell RH, Gerin JL. Hepatitis delta virus. In: Fields BN, Knipe DM, ed. Fields virology. 2nd ed. Vol. 2. New York: Raven Press, 1990: 2275-87
4. Hoofnagle JH. Type D (delta) hepatitis. *JAMA* 1989; **261**: 1321-1325
5. Smedile A, Farci P, Verme G, Caredda F, Cargnel A, Caporaso N, Dentico P, Trepo C, Opolon P, Gimson A, Vergani D, Williams R, Rizzetto M. Influence of delta infection on severity of hepatitis B. *Lancet*. 1982; **2**:945-7.
6. Rizzetto M, Verme G, Recchia S *et al*. Chronic hepatitis in carriers of hepatitis B surface antigen, with intrahepatic expression of delta antigen. *Ann Intern Med* 1983; **98**: 437-441
7. Bonino F, Hoyer B, Ford E, Shih JW, Purcell RH, Gerin JL. The delta agent, HBsAg particles with delta antigen and RNA in the serum of an HBV carrier. *Hepatology* 1981; **1**: 127-131.
8. Chen PJ, Kalpana G, Goldberg J *et al*. Structure and replication of the genome of the hepatitis delta virus. *Proc Natl Acad Sci USA* 1986; **83**: 8774-8778.
9. Taylor JM: Hepatitis delta virus. *Intervirology* 1999; **42**:173.
10. Wang K.S., Choo Q.L., Weiner A.J. & 7 other authors. Structure, sequence and expression of the hepatitis delta (d) viral genome. *Nature* 1986; **323**, 508-514.
11. Casey JL, Brown TL, Colan EJ *et al*. A genotype of hepatitis D virus that occurs in northern South America. *Proc Natl Acad Sci USA* 1993; **90**: 9016-9020
12. Sakugawa H, Nakasone H, Nakayoshi T, Kawakami Y, Miyazato S, Kinjo F, Saito A, Ma SP, Hotta H, Kinoshita M. Hepatitis delta virus genotype 2 b predominates in an endemic area, Okinawa, Japan. *J Med Virol* 1999; **58**: 366-372
13. Shakil AO, Hadziyannis S, Hoofnagle JH, Di Bisceglie AM, Gerin JL, Casey JL. Geographic distribution and genetic variability of hepatitis delta virus genotype 1. *Virology* 1997; **234**: 160-167
14. Hadziyannis SJ: Delta hepatitis. *J Gastroenterol Hepatol* 1997; **12**:289.
15. Hadziyannis SJ. Review: hepatitis delta. *J Gastroenterol Hepatol* 1997; **12**: 289-298
16. London WT, Evans AA: the epidemiology of hepatitis. Viruses B, C, and D. *Clin lab med* 1996; **16**:251.
17. Ponzetto A, Forzani B, Parravicini PP, *et al*. Epidemiology of hepatitis delta virus infection. *Eur J Epidemiol* 1985; **1**: 257-263
18. Rizzetto M, Purcell RH, Gerin JL. Epidemiology of HBV associated delta agent: geographical distribution of anti-delta and prevalence in polytransfused HBsAg carrier. *Lancet* 1980; **1**: 1215-1218
19. Amini S, Mahmoodi MF, Andalibi S, Solati AA. Seroepidemiology of hepatitis B, delta and human immunodeficiency virus infections in Hamadan province, Iran: a population based study. *J Trop Med Hyg*. 1993; **96**:277-87.
20. Rezvan H, Forouzandeh B, Taroyan S, Fadaiee S, Azordegan F. A study on delta virus infection and its clinical impact in Iran. *Infection*. 1990; **18**:26-8.
21. Malekzadeh R, Borhanmanesh F. Prevalence and prognostic implications of hepatitis delta (D) virus infection in a asymptomatic hepatitis B surface antigen carriers in Iran. *Ir J Med Sci* 1989; **14**: 35-38
22. Alavian SM, Asari S, Manzori-Joybari H. Prevalence and risk factors of HDV infection in HBV infected cases. *Govaresh* 2004; **9**: 217-221
23. Toukan AU, Abu-el-Rub OA, Abu-Laban SA, Tarawneh MS, Kamal MF, Hadler SC, Krawczynski K, Margolis HS, Maynard JE. The epidemiology and clinical outcome of hepatitis D virus (delta) infection in Jordan. *Hepatology*. 1987; **7**:1340-5.
24. Al-Kandari S, Nordenfelt E, Al-Nakib B, Hansson BG, Ljunggren K, Al-Nakib W. Hepatitis delta virus infection in acute hepatitis in Kuwait. *Scand J Infect Dis*. 1988; **20**:15-9.
25. el Guneid AM, Gunaid AA, O'Neill AM, Zureikat NI, Coleman JC, Murray-Lyon IM. Prevalence of hepatitis B, C, and D virus markers in Yemeni patients with chronic liver disease. *J Med Virol*. 1993 Aug; **40**(4):330-3.
26. Makhmudov OS, Inoyatova FI, Kadirov BA, Abdumadjidova SU. Delta infection in children with chronic viral hepatitis B. *Turk J Pediatr*. 1997 Jan-Mar; **39**:75-80.
27. Balik I, Onul M, Tekeli E, Caredda F. Epidemiology and

clinical outcome of hepatitis D virus infection in Turkey. *Eur J Epidemiol.* 1991; **7**:48-54.
28. Iarasheva DM, Favorov MO, Iashina TL, Shakhgil'dian IV, Umarova AA, Sorokina SA, Kamardinov KhK, Mavashev VI. The etiological structure of acute viral hepatitis in Tadzhikistan in a period of decreased morbidity Vopr Virusol. 1991 Nov-Dec; **36**:454-6.
29. Al-Traif I, Ali A, Dafalla M, Al-Tamimi W, Qassem L. Prevalence of hepatitis delta antibody among HBsAg carriers in Saudi Arabia. *Ann Saudi Med.* 2004 Sep-Oct; **24**:343-4.
30. Njoh J, Zimmo S. Prevalence of antibody to hepatitis D virus among HBsAg-positive drug-dependent patients in Jeddah, Saudi Arabia. *East Afr Med J.* 1998 Jun; **75**:327-8.
31. el-Hazmi MA, Ramia S. Frequencies of hepatitis B, delta and human immune deficiency virus markers in multitransfused Saudi patients with thalassemia and sickle-cell disease. *J Trop Med Hyg.* 1989 Feb; **92**:1-5.
32. Ramia S, Bahakim H. Perinatal transmission of hepatitis B virus-associated hepatitis D virus. *Ann Inst Pasteur Virol.* 1988; **139**:285-90.
33. Aghanashinikar PN, al-Dhahry SH, al-Marhuby HA, Buhl MR, Daar AS, Al-Hasani MK. Prevalence of hepatitis B, hepatitis delta, and human immunodeficiency virus infections in Omani patients with renal diseases. *Transplant Proc.* 1992; **24**:1913-4.
34. Darwish MA, Shaker M, Raslan OS, Abdel-Raouf T. Delta virus infection in Egypt. *J Egypt Public Health Assoc.* 1992; **67**:147-61.
35. Duraisamy G, Zuridah H, Ariffin Y, *et al.* Hepatitis delta virus in intravenous drug users in Kuala Lampur. *Med J Malaysia* 1994; **49**: 212-6
36. Rosenblum L, Darrow W, Witte J, *et al.* Sexual practice in the transmission of hepatitis B virus and prevalence of hepatitis delta virus infection in female prostitutes in the United States. *JAMA* 1992; **267**: 2477-2481
37. Alavian SM, Manzori-Joybari H, Asari S, Moghani-Lankarani M. War injury is a risk factor for HDV infection. *Iranian Military Medicine* 2005; **7**: 95-99
38. Negro F, Rizzetto M. Diagnosis of hepatitis delta virus infection. *J Hepatol* 1995; **22**:136-9.
39. Aragona M, Caredda F, Lavarini C, *et al.* Serological response to the hepatitis delta virus in hepatitis D. *Lancet* 1987; **i**: 478-480
40. Jardi R, Buti M, cotrina M, *et al.* Determination of hepatitis delta virus RNA by polymerase chain reaction in acute and chronic delta infection. *Hepatology* 1995; **21**: 25-29
41. Shahinsaz L, Karimi M, Alavian SM. Development of RT-PCR inorder to diagnose HDV infection in HBsAg positive patients. 3th congress of physiology &pharmacology. P 256-258. Sep-18-20, 2003- Mashhad, Iran
42. Huang YH, Wu JC, Sheng WY: Diagnostic value of anti-hepatitis D virus (HDV) antibodies revisited: a study of total and IgM anti-HDV compared with detection of HDV-RNA by polymerase chain reaction. *J Gastroenterol Hepatol* 1998; **13**:57.
43. Moestrup T, Hansson BG, Widell A, Nordenfelt E. Clinical aspects of delta infection. *Br Med J* 1983; **286**: 87-90
44. Rizzetto M, Durazzo M. Hepatitis delta virus (HDV) infections, Epidemiological and clinical heterogeneity. *J Hepatol* 1991; **13** (suppl4): S116-S118
45. Buti M, Esteban R, Jardi R *et al.* clinical and serological outcome of acute delta infection. *J Hepatol* 1987; **5**: 59-64
46. Huo TI, Wu JC, Chung-Ru L: Comparison of clinico-pathological features in hepatitis B virus-associated hepatocellular carcinoma with or without hepatitis D virus superinfection. *J -Hepatol* 1996, **25**:439.
47. Fattovich G, Boscaro S, Noventa F *et al.* Influence of hepatitis delta virus infection on progression to cirrhosis in chronic hepatitis type B. *J Infect Dis* 1987; **155**: 931-935
48. Hadler SC, De Monzon MA, Rivero D, *et al.* Epidemiology and long-term consequences of hepatitis delta virus infection in the Yuca Indian of Venezeula. *Am J Epidemiol* 1992; **136**: 1507-1516
49. Wu JC, Chen TZ, Huang YS, Yen FS, Ting LT, Sheng WY, Tsay SH, Lee SD. Natural history of hepatitis D viral superinfection: significance of viremia detected by polymerase chain reaction. *Gastroenterology*. 1995; **108**: 796-802.
50. Reynes M, Zignego L, Samuel D *et al*. Graft hepatitis delta virus reinfection after orthotopic liver transplantation in HDV cirrhosis. *Transplant Proc* 1989; **21**: 2424-2425
51. Di Bisceglie AM, Martin P, Lisker-Melman M, *et al.* Therapy of chronic delta hepatitis with interferon alfa-2b. *J Hepatol* 1990; **11**: Suppl 1: S151-S154
52. Lau JYN, King R, Tibbs CJ, Catterall AP, Smith HM, Portmann BC, Alexander GJM, Williams R. Loss of HBsAg with interferon-a therapy in chronic hepatitis D virus infection. *J Med Virol* 1993; **39**: 292-296
53. Farci P, Mandas A, Coiana A, *et al.* Treatment of chronic hepatitis D with interferon alfa-2a. *N Engl J Med* 1994; **330**: 88-94.
54. Hadziyannis SJ. Use of alpha-interferon in the treatment of chronic delta hepatitis. *J Hepatol* 1991; **13**: Suppl 1: S21-S26
55. Madejon A, Cotonat T, Bartolome J *et al.* Treatment of chronic hepatitis D virus infection with low and high doses of interferon-alpha 2a: utility of polymerase chain reaction in monitoring antiviral response. *Hepatology* 1994; **19**:1331-1336
56. Rosina F, Pintus C, Meschievitz C, Rizzetto M.A randomized controlled trial of a 12-month course of recombinant human interferon-a in chronic delta (type D) hepatitis: a multicenter Italian study. *Hepatology* 1991; **13**:1052-6.
57. Lau DTY, Doo E, Park Y *et al*. Lamivudine for chronic delta hepatitis. *Hepatology* 1999; **30**: 546-549
58. Lau DT, Doo E, Park Y: Lamivudine for chronic delta hepatitis. *Hepatology* 1999; **30**:546.
59. Gaudin JL, Faure P, Codinot H *et al*. The French experience of treatment of chronic type D hepatitis with a 12 month course of IFN alpha-2b. Results of a randomized, controlled trial. *Liver* 1995; **15**: 45-52
60. Niro GA. Rosina F, Rizzetto. Treatment of hepatitis D. *J Viral Hepat* 2005; **12**:2-9