Review of Literature
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Hippocrates describes epidemic jaundice but it was not until 1912 when Cockayne suggested catarrhal jaundice that result from an infection of liver. He proposed the term infective hepatitis which we now know as type A viral hepatitis.

Feinstone et al (1973) Identified hepatitis A virus in the feces of hepatitis patient by immuno electron microscopy (IEM). A process in which clarified suspensions of feces collected from patients in the late incubation period or acute phase of illness, were mixed with sera known to contain high titer of anti-HAV and the resultant virus antibody complexes detected by examination with transmission electra microscope.

Provost et al (1975) Siegl and Frosner (1978) discovered hepatitis A virion is a non enveloped, spherical particle with a diameter of about 27 nm. The particle has icoshedral symmetry.

In an attempt to overcome the limited sensitivity of IEM assay have been developed for the detection of specific viral antigens and HAV-RNA. A number of workers ( Hollinger et al 1975, Purcell et al 1976 ) developed sensitive immuno assay, radioimmuno assays (RIA) or ELISA for detection of HAAg in fecal samples.

Schulman et al (1976 ) learned about biology and pathogenesis of HAV by experimental inoculation of chimpanzees and other species of higher primates.

Ticehurst et al (1979) successfully did propagation of HAV in cell culture.
HAV is the only human Hepatitis virus that has been reliably propagated in conventional cell culture. This has allowed development of conventional vaccine for prevention of Hepatitis A that contains inactivated virus grown in cell culture.

Deinhardt et al (1981) isolated HAV in marmoset liver explant cultures and was subsequently propagated in continuous fetal rhesus monkey kidney cells.

The virus requires several days to weeks to reach maximal titers in cell culture and usually requires immunologic or hybridization techniques to detect, because of lack of virus induced cytopathic effect.

Lemon et al (1983) observed that there is only one serotype of HAV and it is antigenically distinct from all other Picorna viruses.

A major distinguishing feature of HAV among the Picorna viruses is its exceptional stability (Parry and Mortimer 1984) this physical feature is directly related to the structure of virion and obviously has important implications for the biology and epidemiology of the virus. HAV has been found to resist heating to 60°C for 60 min. At higher temperatures infectivity is lost rapidly in a physiological, isotonic environment. However in the presence of 1M magnesium chloride HAV retain its structural integrity and biological functions even at a temperature up to 80°C. Variations in pH between 3-10 has no demonstrable effect on the stability of virus. Survival of HAV is favoured at low relative humidity (Mbithi et al.) and inactivation of virus in water requires higher concentration of residual free chlorine (15-25 ppm).

Virus replicated in the liver is shed into the bile from which it reaches the intestine and exit by the stool. Considerable replication
and shedding of virus occurs in completely asymptomatic individuals or during the incubation of the disease suggesting that virus replication is by itself non-cytopathic. Studies of experimentally infected owls and monkeys indicate that infectious virus first appears in stool within first week after oral on intravenous inoculation. The quantity of virus shed in feces increases during a period of second or third week reaching maximum just before clinical onset of symptoms. Lesser amount of virus are found in blood. Symptoms of Hepatitis A usually have their onset about 4 weeks after exposure, but this time they range from 2 to 6 weeks. Acute liver cell injury is marked chemically by elevation of serum aminotransferase enzymes (ALT, AST) and is followed somewhat later by rise in serum bilirubin levels. Histopathologically, Hepatitis A is marked by hepatocellular necrosis, centrilocular cholestasis and periportal infiltration of liver with mononuclear inflammatory cells.(Lemon et al in Textbook of Infectious disease 2nd edition).

Total serum Immunoglobulins are often non specifically elevated during Hepatitis A. Virus specific antibodies of all three major isotypes IgM, IgG and IgA appears early in the course of illness. (Lemon et al 1980).

Jansen et al (1985) detected HAV-RNA in fecal samples from patients with Hepatitis A by molecular hybridization. This technique has been found to be more sensitive than ELISA or RIA for detection of HAAg.

Margolis et al (1988) described antigen antibody complexes in patients with acute type A Viral Hepatitis, these contain antiviral IgM antibodies and viral capsid proteins. However circulating immune complexes and antiviral antibody probably do not contribute significantly to disease development.

It is more likely that liver injury is mediated by virus specific, cytotoxic CD8 + T cells that are present in liver and less specific natural killer cells (Vallbract et al 1986). Secretory immunity appears not to play a major role in defence against Hepatitis A.

Humphrey et al (1990) have developed a solid phase form of IEM for detection of virus particles in fecal extracts which appears to increase the number of particles in immune aggregates so that they are more readily detectable. The technique uses mono-clonal antibodies to aggregate the particle which are than bound to carbon plastic grid by a protein A or anti immuno globulin. This technique has been found to be useful for detecting other enteric viruses including hepatitis E virus.

Australian antigen or HBsAg could be detected in patients with acute and chronic disease by simple assay procedures such as agar gel diffusion (AGD) or counterimmunoelectrophoresis (Gerety et al, 1978).

In 1972, a modified radioimmunoassay (RIA) called “Sandwich” RIA was developed by Overby et al, to detect HBsAg. This diagnostic test has a sensitivity 10000 times that of AGD and can detect less-than .5 ng HBsAg per ml of serum. Sandwich assays have remained the methodologies of choice for detecting HBsAg because of their long history of high sensitivity and specificity. Recent modified IEA have employed microparticles (MEIAs) and computerized
instrumentation to produce very rapid and completely automated MIAS for HBsAg (Decker 1991, Eble et al 1991). HBsAg is a non-infectious protein particle of about 22 nm in diameter that can be visualized by electron microscopy (Bayer et al, 1968). HBcAg is not directly detectable in serum because of the encapsulating HBsAg. However, HBcAg can be detected by immunofluorescent techniques in liver biopsy specimens.

HBeAg is a non-structural protein of HBV (Magnius and Espmark 1972). HBeAg can be detected by sandwich immunoassay similar to that of HBsAg. The antigen is captured by antibody affixed to a solid phase and is then detected with a second labeled antibody (Mushahwar and Overby 1981).

In less than 10% patients extra hepatic immune complex mediated manifestations may be present like polyarthritis,( which is typically symmetric involves chiefly the distal joints e.g. Proximal interphalangeal joints & subsides with development of jaundice), hematuria & proteinuria reflecting glomerular involvement (Lisker – Melman et al 1989), angioedema, urticaria, maculopapular rash, polymyalgia rheumatica, neuropathies, myocarditis etc. (Bacon et al 1975, Tabor 1987, Ussell et al 1984).

After infection with HBV, the first virologic marker detectable in serum is HBsAg. Circulating HBsAg precedes elevations of serum aminotransferase activity & clinical symptoms & remains detectable during the entire icteric or symptomatic phase of acute hepatitis B & beyond. In typical cases, HBsAg becomes undetectable 1 to 2 months following the onset of jaundice & rarely persists beyond 6 months. After HBsAg disappears, antibody to HBsAg (anti HBs) becomes detectable in serum & remain detectable indefinitely
thereafter. Because HBCAg is sequestered within an HBsAg coat, HBCAg is not detectable routinely in the serum of patients with HBV infection. On the other hand anti HBC is readily demonstrable in serum beginning with in the first one to two weeks after the appearance of HBsAg & preceding detectable levels of anti HBs by weeks to months because variability exists in the time of appearance of anti HBs following HBV infection, occasionally a gap of several weeks or longer may separate the disappearance of HBsAg & appearance of anti HBs, this gap is known as “Window period”. During this period anti HBC may represent serological evidence of current or recent HBV infection. (Jules et al in Harrison’s Principle of Internal Medicine).

Blood containing anti HBC in the absence of HBsAg & anti HBs has been implicating in the development of transfusion associated. HBeAg is a marker of active HBV replication. In most patients, HBeAg clearance occurs before clearance of HBV DNA.

The detection of HBV DNA in peripheral mononuclear cells of blood (i.e. T cells, B cells, monocytes), in bone marrow stem cells, & in other extrahepatic tissues suggests that replication of HBV may not be limited to the liver. (Zeldis et al 1986).

In less than 1% of adult patients, hepatic encephalopathy & striking prolongation of the prothrombin time, the clinical hallmarks of fulminant hepatitis develop within a few days to 8 weeks after the onset of HBV infection.

Extra hepatic manifestations of HCV include sialadenitis resembling Sjogenn syndrome; membraprolifitative glomeralonephritis; non Hodgkin’s lymphoma, lichen planus &
Moosen corneal ulcer an extremely rare form of chronic ulcerative keratitis (Koff & Deinstag 1995).

The first identifiable marker of acute HCV infection is the appearance of HCV RNA in serum, which may be detected by PCR amplification within few weeks of exposure (Kato et al 1993). However PCR amplification to detect HCV RNA remains expensive and investigational tool. In addition to PCR assays, a less sensitive branched signal amplification assay has been widely used to quantitate HCV RNA levels. (Martinot et al 1994).

Serologic diagnosis of acute HCV infection rests on detection of antibodies to recombinant HCV antigens. These antigens have included C22-3 from core region, C33-c from NS3, C100-3 from NS4 and NS5 region. (Garson and Tedder). First generation immunoassays measuring antibodies to C100-3 antigen of HCV, were imperfect because antibodies develop slowly and because of high false positive reactions. Second and Third generation enzyme immunoassays and recombinant immunoblot assays have increased sensitivity and specificity. (McHutchinson 1992).

The existence of the hepatitis D (delta) virus (HDV) as an agent of viral hepatitis has been recognized only since 1977. The HDV antigen was initially detected in the liver of patients with chronic HBV infection. HDV is a defective, transmissible, RNA containing satellite virus of HBV. It requires the helper or rescue function of HBV or other hepadna viruses for its expression, assembly and pathogenicity. Diagnosis of HDV infection in clinical practise rests on the detection of antibody to HDV antigen by commercial EIA or RIA. Total anti-HDV, however, generally in low titer (<1:100), is undetectable in over 90% of cases of acute HDV infection. (Aragona et al 1987).
Antibodies to HDV develops about 2 to 4 weeks after co-infection and about 10 weeks after superinfection. PCR assays or viral RNA are available only as a research tool. HDAg can be detected by Western blot in over 70% of cases of chronic HDV infection. (Buti et al 1989), it is techically difficult cumbersome so remains as a research tool.

Hepatitis E virus (HEV) is an RNA virus, with features most closely resembling those of calciviruses, which causes acute, but not chronic, hepatitis. Similar in some ways to HAV (Hoofnagle and Di Bisceglie 1991). HEV spreads by faecal-oral route and is an important cause of sporadic, endemic and epidemic hepatitis in certain third world countries (Gust and Purcell 1990).

HEV antigen can be identified in faeces, bile and liver by immune electron microscopy (Balayan et al 1983). Antibodies to HEV antigen can also be detected in liver by immune electron microscopy (Gust and Purcell 1990). Recombinant DNA technology has resulted in development of antibodies to HEV particles, and IgM and IgGM assays are available to distinguish between acute and resolved infections. Viral RNA can be detected in stool and serum by PCR.

Rakela et al (1975) undertook a study to determine the incidence of HAV infection as an etiological agent among patients with fulminant hepatitis and chronic active hepatitis. They retrieve serum samples of 42 cases of fulminant hepatitis that were HBsAg negative and for comparison they also took samples of 30 HBsAg positive cases with fulminant disease. Out of 42 HBsAg negative cases 10 survived and 32 died in hepatic coma. Three out of ten survivors showed seroconversion by immune adherence hemagglutination (IAHA) for antibody to HAV as well as increasing anti
HAV score by immunoelectron microscopy (IEM). Among 32 HBsAg negative non-survivors they found 3 cases with anti HAV, detectable by IEM and radio immune assay (RIA) but not by immune adherence hemagglutination. They classified latter cases as presumptively attributable to HAV. Thus, the overall incidence of HAV infection among HBsAg negative cases was 14% (6 cases). In 10 survivors among 30 HBsAg positive cases with fulminant disease they did not detect any instances of anti HAV seroconversion and in 20 HBsAg positive non-survivors, 1 case had anti HAV detectable by IEM and RIA but not by IAHA. This case was also considered to be presumptively caused by HAV. In another study on patients with chronic active hepatitis Rakela J et al found incidence of anti HAV in 13 HBsAg positive cases was 31%, which did not differ from 32% found in 22 HBsAg negative cases. They also looked for fecal shedding of HAV in 14 patients with HBsAg negative chronic active hepatitis without success.

Fulminant hepatitis is marked by clinical features of hepatic synthetic functions with associated bleeding diathesis and coma. HAV accounts for approximately 10 – 20% of all cases of fulminant viral hepatitis (Mathiesen et al 1980, Rakela et al 1978).

Fulminant hepatitis A is often fatal although survival rates are higher than with fulminant hepatitis B or non A, non B, non B, non C disease.

Mathiesen et al (1979) studied 115 patients hospitalized with acute viral hepatitis in Copenhagen for serological markers for hepatitis A and B virus. 39 patients had type B, 66 had type A. 3 had both type A and type B, and 7 had type non A, non B. 81% of the patients were between 15 and 40 years of age, and there was a
dominance of males due to an over representation of homosexual males (30%), in both the A and B groups. The main type of exposure to hepatitis type A was travel to foreign countries (53%), and for type B it was drug addiction (41%). In type A and type B the duration of jaundice was positively correlated to the age of the patients but did not vary with sex or type of exposure. There was no difference in maximum alanine aminotransferase levels, between the groups, but maximum bilirubin levels were lower for the type A group. Patients with type A had higher levels of IgM than those with type B and with type non A and non B. We conclude that both clinically acute hepatitis type A and type B occurs mainly in young adults and that foreign travel, drug addiction and homosexuality increase the risks of getting acute hepatitis.

A study was conducted by Khuroo et al in Kashmir from April 1979 to December 1981, where a total of 293 sporadic cases of acute viral hepatitis were identified. Out of these 44 (15%) were found serologically to be hepatitis A, 94 (32%) hepatitis B, and 155 (53%) non A, non B type. The non A, non B hepatitis observed was a disease of young adults (29.8 ± 15 years) with slight male predominance (1.4 : 1). Six of the 155 non A, non B cases had history of prior parenteral exposure, while 51 (35%) had a recent contact with another case of jaundice, suggesting that this form of hepatitis was spread by person to person contact. Fulminant hepatic failure occurred in 19 cases and six (31.5%) of the 19 cases occurred in pregnant women. None of 90 non-A, non-B cases followed up six months later had developed chronic hepatitis. The acute sporadic non-A, non-B hepatitis described in Kashmir resembles epidemic
non-A, non-B hepatitis epidemiologically and seems to be distinct from the non-A non-B hepatitis described in the west.

Tandon et al (1984) studied etiological spectrum of viral hepatitis and the prevalence of serological markers of hepatitis A and B virus infection in healthy persons in North India. Hepatitis A virus was found to be the most common cause of Acute hepatitis in children (67 %). It was a less frequent cause of this disease in adults (14 %). Hepatitis A virus was only rarely a cause of acute (12 %) and sub-acute (4 %) liver failure. It was recorded as the etiological agent in an epidemic among school children. Exposure to HAV occurs in early childhood and by the age of 10 years 90 % of healthy persons have serological evidence of Hepatitis A virus infection.

Serological studies of hepatitis viruses A and B were carried out on 362 patients with acute viral hepatitis, 130 with fulminant hepatitis, and 56 with subacute hepatitis, and on samples of serum from 230 subjects during 3 epidemics of viral hepatitis by B.N. Tandon et al(1985) in the state of Gujrat in north west of India. A diagnosis of non A non B viral hepatitis was made when serological tests showed that anti-HAV IgM and anti-HbcIgM were absent. Hepatitis virus non-A non-B was the causative agent responsible for 58% of cases with acute viral hepatitis, 58% with fulminant hepatitis, 87% with subacute hepatitis and 66% with epidemic hepatitis. A considerable proportion of patients (6-32%) were infected with both hepatitis virus non-A non-B and hepatitis virus B. Viral hepatitis non-A non-B is probably transmitted by infection of drinking water and is the principal cause of hepatitis in India.

Dhawan et al (1988) studied seroprevalence of Hepatitis A in Mumbai and immunogenicity and safety of Hepatitis A vaccine. 670
subjects (456 men age 6 mth – 60 yrs) answered questionnaires on social and medical history. Qualitative analysis of total anti HAV was performed in all subjects by ELISA. 107/147 anti HAV negative subjects received Hepatitis A vaccine at months 0,1 and 6. Subjects were followed up (month 1,2,6,7) to look side effects and seroconversion. They found seroprevalence of HAV was 523 / 670 (78 %); 38 % of children less than 5 years were anti HAV negative. Seroprevalence rates of 80 % were reached by 15 years. Prevalence was lower in higher socio-economic group (64.5 %) compared with lower socio-economic group (85%). One month after doses 1,2 and 3 of Hepatitis A vaccine seropositivity was 92%, 99% and 100% respectively. Minor self-limiting side effects occurred in 19.5% of subjects, there were, no major side effects. The overall conclusion of study was seroprevalence of anti HAV is high in Mumbai. Seroprevalence is lower in higher socio-economic groups. The hepatitis A vaccine is safe and immunogenic.

P. Kar et al over a period of 2 years (i.e., from May 1992-1994) evaluated one hundred and thirteen patients with acute viral hepatitis (AVH; n=70) or fulminant hepatic failure (FHF; N=43) for the presence of hepatitis A,B,C and E virus infection. Hepatitis C virus (HCV) testing was done using second generation anti-HCV ELISA test and reverse transcriptase polymerase chain reaction (PCR) for the detection of HCV RNA in the serum of patients with non-A non-B (NANB) hepatitis. Detection of IgM anti HEV antibody was done in patient found negative for the above viruses (n=53). Results; Hepatitis A and B viruses accounted for 3.5% and 42.5% of the 113 cases respectively. HCV infection accounted for 12% of the Non-A Non-B cases with AVH and 15.5% with FHF. PCR was more useful
than serological tests for the detection of HCV perfection. HEV infection accounted for 49% of the Non-A Non-B, Non-C cases with AVH and 25% WITH FHF, pregnant women with HEV infection had a fulminant course. No etiological agent could be established in 28.3% of cases. Conclusion: HEV is the most important cause of NANB hepatitis; hepatitis B virus is still a major concern, while HCV is not an important cause of acute viral liver disease in India.

Thapa et al 1995 studied pattern of viral markers in acute sporadic hepatitis in 329 children & those in 334 healthy school children from North West India. Hepatitis A is found to be the commonest infection in sporadic cases (78%) of these 86% were under 10 years & 50% less than 5 years of age. Hepatitis B was positive in 8%, non-A non-B in 13%. A as well as B both positive in 1% & none had Delta viral infection.

78% positively of anti HAV IgM in acute sporadic hepatitis is comparable to 81% reported from Hongkong but differ from 59% reported from Iraq. Studies from India have reported 67% & 56% incidence in sporadic hepatitis.

Viral marker in healthy school children showed anti HAV IgG positively in 96% & 85% in those belonging to low & high serio economic groups, respectively, indicating post infection HBsAg was positive in 1% of cases.

Hepatitis B is not a major problem in children as a cause of sporadic hepatitis. Similar observations are made by other authors from Hongkong (8%), Iraq (6%) & India (9-20%).

The diagnosis of non-A non-B hepatitis is still by way of exclusion in developing countries due to high cost of C & E markers.
S. Malathi et al studied 127 children of age ranging from 2 months to 12 years with features of acute hepatitis during the period February 1995 to January 1996 in Madras (attending either Department of Gastroenterology in the Institute of Child Health & Hospital for children, Egmore or the Department of Digestive Health & Diseases, Govt. Peripheral Hospital Anna Nagar, Madras). The diagnosis of acute hepatitis was made on the basis of history & clinical findings. Biochemical investigations such as serum bilirubin, ALT, AST were performed sera were analyzed for anti HAV IgM, HBsAg, anti HBcIgM & anti delta using ELISA kits from Murex Diagnostic, U.K., anti HCV was measured using the Recombinant Immuno Blot Assay manufactured by Chiron Corporation Ltd., U.S.A. Anti HEV IgM was detected using ELISA kits from Gene Labs Ltd. U.S.A. Specific etiological agents were identified in 89 percent. Of these 67.7 % were due to single virus, whereas 21.3% were due to two or more hepatitis viruses. HAV was the sole infecting agent in 38.6% of cases. Mixed infections were due to HAV & HBV co-infection (7.1%), HAV & HEV (13.4%), & the combination of HAV, HBV & HEV (0.8%). In 11% none of the markers (HAV to HEV) were identified. Acute sporadic hepatitis in children can occur due to single virus type or at times due to infection with a combination of two enterally transmitted viruses or enteral & parenterally transmitted viruses. Improving personal hygiene & active immunization are essential in prevention of these viral illness. Out of 127 children, 110 (87%) recovered without any complication, 17 (13%) developed hepatic failure & 16 children (13%) died.

Singh et al (1997) undertook study to estimate the incidence of acute sporadic viral hepatitis and described its epidemiology in an
urban population. A retrospective community survey for jaundice was done in Bastar, Madhya Pradesh, India. Trained paramedics surveyed about 51,643 population to detect cases of jaundice which occurred in past one year. Cases were examined to collect clinical and epidemiological data. Blood samples were drawn from all cases who had jaundice in the past three months for testing them for markers of viral hepatitis. Study estimated that annual incidence of jaundice cases as 244/lac population. Almost 95% jaundice cases occurred in summer and monsoon months. People from all socio-economic strata were affected. The incidence of jaundice was found to be highest in children below 15 years of age, which declined significantly with the increase in age. The overall incidence in two sexes was not different statistically. Hepatitis A and E combined together contributed 68% of acute sporadic cases of viral hepatitis, whereas B, C and D accounted for the remaining 32% of the cases. The study found the annual incidence of laboratory supported cases of viral hepatitis to be 81/lac population, which suggested that it is an important public health problem in India. Hepatitis A was much more prevalent than hepatitis E.

Aggarwal et al (1999) undertook a study to estimate the seroprevalence of antibodies to HAV among children in North India. Sera were obtained from 73 children (6 Months to 18 years) who had attended this institution for minor non-infective illnesses. These sera were tested for the presence of anti-HAV antibodies using commercially available immunoassay. Of the 73 sera, 61(84%) tested positive for anti-HAV. Prevalence rates were similar among boys and girls. Anti-HAV positivity rates in different age group
showed a progressive rise with age; 68% (0-5 yrs.); 91% (6-10 yrs.) and 96% (11-18 yrs.) respectively.

A prospective study was done by Dutta et al (2000) to determine the age specific prevalence of hepatitis A antibodies (antiHAV Abs) among children in Delhi. 420 children aged 0-12 years. Attending OPD for vaccination or any minor illness were studied. Sera was tested by ELISA for antiHAV Abs using a commercial kit. 30 samples of cord blood were similarly analyzed. The study revealed that all samples of cord blood were positive for antiHAV Abs. Prevalence of antiHAV Abs was 80% by 5 years of age. The positivity was minimum (16.6%) between 0.5-1.5 years of age and is the most vulnerable group. Only 3% children had history of jaundice signifying that majority of infection is asymptomatic and anicteric in younger age group. The study also revealed that socioeconomic status of the family significantly affect prevalence of antiHAV Abs, but sex, water supply, history of jaundice in self or family did not have significant effect on antiHAV Abs positivity.