Diagnosis and Management of Paediatric Hepatitis C Virus Infection.

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SUMMARY

Background: Hepatitis C virus is a chronic life-long infection in the majority of patients who are infected with the virus. Without accurate diagnosis and follow up, these children cannot be offered optimal care, and are at risk of presenting in adult life with significant liver pathology and long-term sequelae.

Objective: To explore the possible diagnostic and management options available to those affected.

Materials and Method: Source of information was mainly from published works in and outside Nigeria. The information was extracted over a period of 12 months from January to December 2007.

Results: Treatment options available are use of immune response modifiers (interferons), antiviral agents (ribavirin), combination therapy with interferon and ribavirin, and liver transplantation.

Conclusion: Early identification and optimal treatment to those in which treatment are indicated**.**

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BACKGROUND AND STRUCTURE

Following the development of accurate diagnostic assays for hepatitis B virus infection, the existence of an additional form of transfusion transmitted infectious hepatitis was recognized. Because this diagnosis was one of exclusion, the new entity was then known as non-A, non-B hepatitis and is now known to be caused by hepatitis C virus (HCV) in most cases. HCV was discovered in 1988, using molecular techniques to identify the agent responsible for post- transfusion acquired non-A, non-B hepatitis[1]. The clinical importance of HCV infection is due to viral persistence in approximately 85% of those infected and the significant risk of subsequent development of chronic irreversible liver damage [2]. HCV is an RNA virus that belongs to the family of flaviviruses. The natural target of HCV are hepatocytes and possibly B-lymphocytes. The virus genome is a single strand of positive-sense RNA encoding three structural and seven non-structural proteins.[2] The genome is enclosed in a protein coat, which is wrapped in a lipid envelope derived from the host cell. The viral particle consists of an envelope derived from host membranes, into which are inserted the virally encoded glycoproteins (E1 and E2) surrounding a nucleocapsid. Sequence variation of the genome occurs mainly in the NS2, E1 regions whereas a high degree of conservation is observed in the non-coding, C, NS3, NS4 and NS5 regions. It also contains regions from which peptides have been produced for assays. The structure is shown in *fig.* 1.

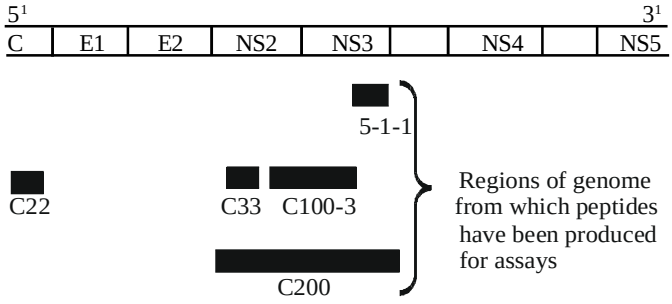

Fig 1: **Schematic diagram of hepatitis C Virus.** RNA Coding Regions [3] (C =Core Proteins; E= Envelope; NS = Non = structural Proteins)

Genotypic designations have produced epidemiological tools for studying geographical differences in HCV infection. The determination of HCV genotype is important since response to antiviral therapy with interferon correlates with HCV type. Laurer and walker,[4] have documented six distinct but related HCV genotypes and multiple sub-types which have been identified on the basis of molecular relatedness. Genotypes 1a and 1b are more common in the Untied States of American and Western Europe; followed by genotypes 2 and 4.[5] McOmish et al [6] have documented genotype 1, 2 and 3 as the predominant genotypes in Australia. Genotype 4 is present in Egypt, while genotypes 5 and 6 are found in South Africa and South East Asia respectively [5] But Akhtar et al [7] have documented genotype 3a and 3b in Pakistan. Genotypes 1 and 2 have been reported to be prevalent in Burkina Faso, Benin Republic and Guinea, all in the West African sub-region [8] However, in the Oni and Harrison study [9], the prevalent genotype in Nigeria were found to be 1 and 4. It is observed that genotype 2 and 3 respond better to the antiviral therapy than genotype 1 [5].

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Epidemiology

The World Health Organization (WHO), [10] puts the average worldwide prevalence of HCV infection at 3.1%, with Africa recording the highest prevalence rate of 5.3%. Other regions of the world have lower rates – Americas 1.7%, Eastern Mediterranean 4.6%, Europe 1.03%, South East Asia 2.15% and Western Pacific 3.9%. [10] In Africa, [11] the following prevalence rates have been reported in the various countries – Gabon, 22%, Malawi, 16.5%, Madagascar, 3.3%, Ghana, 5. 4%, Burkina Faso, 4.9% and Guinea, 1.1%. A study in Zaire reported 6.4% [12] prevalence. However, a pilot study done in Nigerian adults and Children gave an average seroprevalence of 8% [9] While a study on adult blood donors in Nigeria reported a prevalence rate of 12.3% [13]. Another study in Ibadan, Nigeria conducted amongst doctors and dentist reported prevalence of 11% [14].

The Role of Risk Factors

Hepatitis C is transmitted parenterally. The most common risk factor for HCV infection in developing countries is transfusion of unscreened blood or blood derived products. [15] Thus, children at risk include those with sickle cell anaemia (SCA), those on haemodialysis, or who receive blood clotting factor concentrates as in hemophiliacs. Others include those who receive transplants or exposed to unsafe medical practices like re- usage of syringes and unsterile surgical procedures as can be found in alternative medical practices.

Needle –Stick Injury

Accidental needle stick injury in health care workers may lead to the transmission of the virus. The rate of transmission of HCV infection as a result of needle – stick injury is 3%. [2] This is less than that seen for HBV (30%) but greater than that which occurs with HIV (0.3%). [2]

Vertical Transmission

The rate of vertical transmission of HCV is been variable. More recent studies put the figure at 5 – 10% [2,16] in infants born to mothers who are positive for both HCV antibodies and HCV RNA, and not co- infected with HIV. The risk of transmission is increased when the mother is HIV positive or when there is a high HCV viral load. The transmission of infection via breastfeeding has not been documented, and therefore breastfeeding by an HCV- positive mother is not contraindicated.[16]

Sexual Transmission

Sexual exposure accounts for 10 – 20% of new cases, mostly among individuals who engage in high risk sexual practices such as non-use of condoms or those having multiple sexual partners.[16] The risk of infection from an HCV positive partner in a long term monogamous relationship is as low as 1.5% and the risk after single sexual exposure is negligible.[17]

Natural History of Hepatitis C Virus

The HCV is an RNA virus that has a high propensity to mutate.[16] The lack of vigorous T-lymphocyte response appears to promote a high rate of chronic infection. The incubation period from transmission to the initial rise in alanine aminotrasferease (ALT) is within 7 – 8 (range 2 – 26) weeks. Acute hepatitis C is symptomatic in a minority of cases and usually runs a mild clinical course, with only one-third or fewer of patients being jaundiced and many are completely asymptomatic.[16] The disease is rarely fulminant but this has been reported to occur.[18] Rather, indolence and chronicity are the hallmark of HCV infection.[9] Approximately 15 – 30% of patients exposed to HCV recover spontaneously while the remaining 70 – 85% develop chronic infection.[9] Chronic HCV infection is defined as the presence of HCV for longer than 6 months.[19] Most patients with HCV infection appear to have a mild to moderate histologic disease.[20,21,22] Cirrhosis may develop in as many as 15-20% of infected patients.[9] (see Fig 2 below). Factors that accelerate disease progression include ingestion of alcohol, coexisting human immuno- deficiency virus (HIV) or HBV, male gender and older age at infection [23,19] For example, the risk of cirrhosis in patients with both HIV and HCV infection is approximately 25% after 15 years.

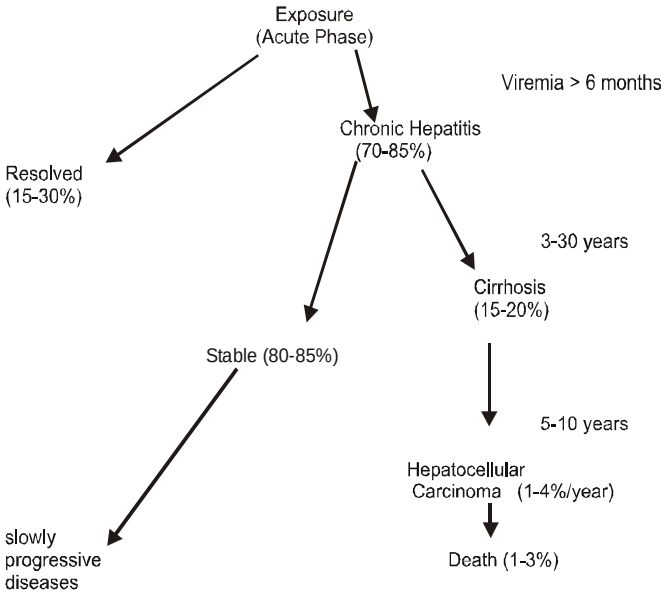

fig 2**. Natural History of Hepatitis C Virus Infection.[16]**

Clinical Presentation

Most patients with acute hepatitis C are asymptomatic, but when symptoms do occur the most common complaints are fatigue, abdominal pain, anorexia, jaundice, weight loss and pruritus. Symptoms usually subside after several weeks as alanine aminotransferase (ALT) level declines. Extra hepatic manifestations that are believed to be immunologic and vasculitic in origin may occur in HCV infected individuals.[9] These include, cryoglobulinaemia, membrane proliferative glomerulonephritis, sjorgren's syndrome, autoimmune thyroditis, lichen planus, idiopathic pulmonary fibrosis, polyarteritis nodosa, aplastic anaemia and B-cell lymphoma.[16]

DIAGNOSIS

Antibody Detection

The clinically available assays for detection of HCV are based on detection of antibodies to HCV antigens or testing directly for the virus RNA. The antibody assays are mainly used for the detection of chronic HCV infection, because antibodies remain negative for as long as 1-3 months after onset of infec-

tion.[24] Children born to HCV infected women should be tested for anti HCV after 12 months.[24] This is because babies lose the maternally acquired antibody at a median age of 6 months and the majority may have cleared it by 12 months.[25]

Enzyme lmmunosorbent Assay

Various tests are available for the diagnonsis and monitoring of HCV infection. Tests that detect antibody against the virus include the enzyme immunosorbent assays (EIAs) which contain HCV antigens from the core and non-structured genes, and the recombinant immunoblot assays (RIBAs).[26] The same HCV antigens are used in both EIAs and RIBAs. Target amplification techniques using either polymerase chain reation (PCR) or transcription mediated amplification (TMA) have been developed to detect HCV RNA.[27] Liver biopsy can provide direct histological assessment of liver injury due to HCV but cannot be used to diagnose HCV infection. After initial exposure, HCV RNA can be detected in blood in 1 to 3 weeks and is present at the onset of symptoms.[16]

EIA tests are reproducible, and approved for use in the diagnosis of HCV infection. They are suitable for screening at risk populations and are recommended as the initial test for patients with clinical liver disease.

Screening EIAs – EIAs can detect more than 95% of chronically infected patients but can detect only 50- 70% of acute infections.[10] Three generations of screening EIAs have been developed to detect antibodies against various epitopes of HCV genome proteins. The first generation EIAs (EIA -1) used the C 100-3 epitope of a non-structural (NS) protein (NS4). The sensitivity[28,29] of this EIA-1 was 80% which was low for a high prevalence population and has high false positive of 70% for a low prevalence population. Then the more sensitive and specific second generation EIAs (EIA-2) was developed and it incorporated additional antigens from NS (C33C) and structural (C22-3) proteins. Later, a third generation EIA (EIA-3) that added a fourth antigen (NS5) to those of EIA-2 was developed. These multiple antigens using recombinant protein and/ or synthetic peptides have been added in new serologic tests to avid non specific cross- reactivity and to increase the sensitivity of the HCV antibody tests.[30] Second and third generations HCV antibody assays have 99% sensitivity,[25] and become positive within 4 to 10 weeks of infection.[25] Fig 3 below shows HCV antigens used for serologic assays.

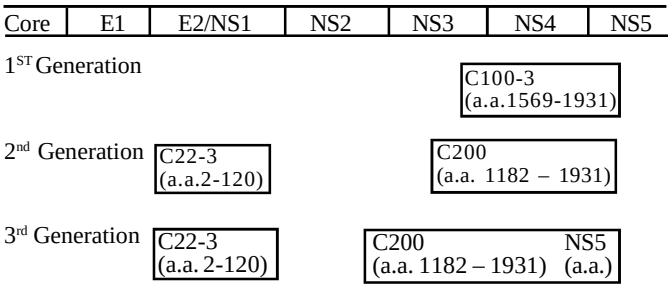

Fig3: HCV Antigens Used for Serologic Assays.[28]
C=Core; E=Envelop; NS=Non-Structural Protein; a.a.=Amino Acid Sequence of Recombinant Protein or synthetic Peptide Antigen

Negative Anti-HCV Test

A negative EIA test is sufficient to exclude a diagnosis of chronic HCV infection in immuno competent patients. False negative however can occur in some settings namely,

- Patients with immune deficiencies[27] and some patients on haemodialysis[19]
- Patients in whom exposure is quite recent and sufficient antibody response has not developed usually before 12 weeks post exposure.

Positive Anti-Test[27]

A truly positive anti-HCV test indicates exposure to the virus and cannot differentiate between active (on going) infection, quiescent (inactive) infection or post/resolved infection. Conversely, falsely positive EIAs may occur.

- In patients with autoimmune disorders[27]
- When aged sera are used
- With persistence of antibody after vertical transmission.

In these patients assays for HCV RNA are necessary for diagnosis. RIBA remains a useful supplemental assay in the setting of large scale HCV screening of blood products.

Recombinant Immunoblot Assay (RIBA) – A Recombinant Immunoblot assay (RIBA) has been developed for confirmation of positive anti-HCV EIA result. Thus, they are supplemental assays to EIA testing. Both classes of antibody i.e., EIA and RIBA contain the same HCV antigens[31]. RIBA testing is currently in its third generation of development. Indeterminate RIBA test occurs when antibody is confined to only one of the HCV antigens. Among healthy blood donors, indeterminate RIBA results especially with RIBA-2 usually indicate non-specific reactivity. RIBA-3 is more specific and has greatly reduced the number of indeterminate test results[28,29,32].

Virus Detection by Molecular Assays – In view of the limitations of anti-HCV antibody tests, detection of HCV particles in blood is often necessary to confirm diagnosis. Since serum level of HCV RNA are usually low, the nucleic acid has to be amplified. Two principal methods used to detect hepatitis C viral RNA are target amplification and signal amplification[27,29,33] Target amplification involves qualitative HCV RNA detection by means of Polymerase Chain Reaction (PCR) in patient's serum,[27,29] while signal amplification involves quantification of HCV RNA by PCR.[27,29] The summary of stages involved in HCV diagnostic testing is shown in Fig. 4 below.

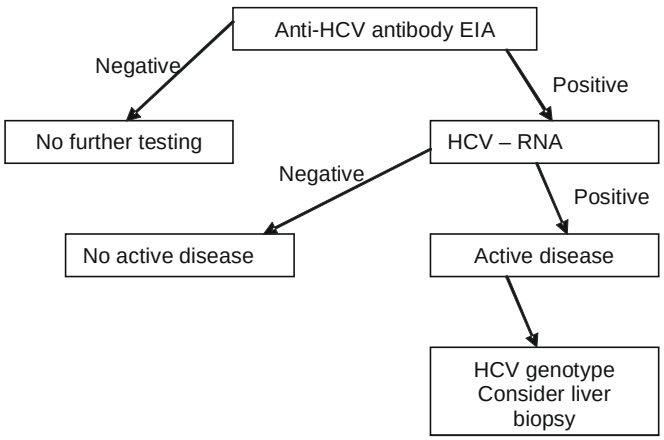

Fig 4: Algorithm for Hepatitis C Diagnostic Testing.

HCV Genotypes

HCV genotype is determined by sequence analysis with rapid PCR based hybridization techniques. The techniques are based on reverse hybridization analysis by means of genotype specific probes.[27,28] Another method of genotype determination is by means of serologic assays. Serotyping techniques are based on the detection of antibodies directed to genotype specific HCV epitopes.[27,28] compared to molecular assays, serologic genotyping is easier to perform and less expensive, but it has lower sensitivity and specificity.[28] Determination of HCV genotypes is important since response to antiviral therapy with interferon correlates with HCV type, although HCV genotype does not appear to affect the rate of disease progression. Infection with HCV genotype 1b has been noted to be assocated with more severe liver disease and may have a higher risk for the development of hepatocellular carcinoma.[31] Hassan and his coworkers[5] noted that patients with HCV genotype 1 respond poorly to interferon treatment when compared to patients with genotypes 2 or 3. Therefore patients infected with HCV genotypes 1 and 4 should be treated with combination of interferon and ribavirin therapy. Genotyping should be performed in all patients with HCV in whom treatment is being considered.

Liver Biopsy

Liver biopsy is done for the assessment of liver injury.[2] Liver biopsy results do not directly correlate to the virological stage of infection, but assist with prognosis and decisions about patient management. Biopsy is usually performed prior to starting antiviral therapy in order to exclude non-viral causes of liver injury. The same procedure is done after therapy to assess the response. Characteristic histopathological findings of chronic HCV infection include sinusoidal lymphocytosis, portal lymphoid aggregates/follicles and bile duct epithelial damage.[2] These changes seem to occur with the same frequency in children as in adults.[2] Necro inflammatory changes appear to be quite mild in children, however, there can be significant fibrosis, even after a short duration of infection.[2]

MANAGEMENT

The decision to treat a child with HCV infection is complex and should only be made in consultation with a pediatric gastroenterologist in the management of childhood infection. Hassan and his co-workers[5] noted indications for treatment of HCV infection which are

(a) Detectable level of HCV RNA
(b) Persistently elevated alanine aminotrasferase levels and/or
(c) Liver biopsy showing fibrosis or at least moderate necrosis and inflammation.

Therapy for HCV infection includes:-

(i) Immune response modifiers (i.e. interferons)
(ii) Antiviral agents (i.e., ribavirin)
(iii) Combination therapy with interferon and ribavirin
(iv) Liver transplantation

The primary goal of therapy in the patient with hepatitis C infection is to achieve a sustained virologic response (SVR) which is defined as undetectable serum or plasma HCV RNA level assessed by sensitive reverse transcription polymerase chain reaction (RTPCR) assay at least 6 months after the cessation of therapy.[34] Other goals of hepatitis C therapy include response to hepatitis C treatment, which can be.[34]

(a) biochemical (the normalization of serum liver enzyme levels) (b) histologic (improvement in necro inflammatory activity and fibrosis that is determined by serial liver biopsy) and (c) clinical outcomes (prevention of End Stage Liver Disease and improved survival rate). Interferon-alpha was the first drug shown to have low activity against HCV, and it remains the only approved monotherapy for chronic HCV infection. However, because of its poor sustained response rate (15 – 20%) among the general population with chronic HCV infection,[5] ribavirin was recently added to interferon in the treatment of HCV. Sustained virological response rates to this combination have shown a substantial improvement of approximately two fold over interferon mono therapy.[5]

Currently, the therapy for patients who qualify for treatment of chronic HCV infection is combination of the Interferon and Ribavirin. The recommended dosage of Interferon is a 48-week course of 3 million units given thrice weekly via subcutaneous route. Ribavirin for patients weighing = 75kg total oral daily dosage of 1,200mg in two divided doses. For patients weighing < 75kg the dosage is reduced to 1,000mg/day. Sustained virologic response (SVR) in this case is defined as non detection of the virus 24 weeks into treatment[5].

Recently, Pegylated (PEG)-interferon was introduced , which is a modification of interferon by the covalent attachment of polyethylene glycol (PEG) moiety which results in decreased proteolysis and prolonged serum half-life.[5] The advantages of this medication are once per week dosing and higher rates of response compared to conventional interferon monotherapy. Preliminary studies indicate a response rate of 80% with this combination.[5] Hadziyannis et al[35] presented the initial results of an ongoing study on the treatment of chronic HCV infection using PEG-interferon alpha-2A and ribavirin and recorded 76% overall response rate, with response rate of 68% in patients with genotype 1 virus and 87% in patients with genotype 2 or 3 at the end 24 weeks of treatment. Kugelmas et al[36] also suggested that the combination of PEG interferon and ribavirin seems to offer benefit to chronic HCV infected patients who did not respond to interferon and ribavirin. Contra indications to treatment with interferon include psychosis, severe depression, neutropenia, thromobocytopenia while ribavirin is not given to patients with anaema, end stage renal disease and haemoglobinapathies[5,2] However, in patients with end stage liver disease, liver transplaintation for HCV infection is indicated. The consequences for long term graft survival after liver transplantation are yet to be determined, but there is significant risk of recurrence.[2]

Future Therapies

Development of novel therapies to manage HCV infection are aimed at interfering with the replicative cycle of HCV. These include targeting the function of HCV proteases (helicase and polymerase), interfering with the internal ribosome entry site, interfering with the putative cell-surface receptor for the virus CD81, and interfering with replication using antisense oligonucleotides.[2]

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