INTRODUCTION

Hepatitis B virus (HBV) infection is a major health problem leading to significant morbidity and mortality worldwide especially in the developing countries. The hepatitis D virus, also called delta virus (HDV) is dependent on the hepatitis B virus (HBV) and can cause co-infection in normal individuals with hepatitis B, or yet, superinfection in chronic HBV carriers. It has been estimated that 18 million people are infected with HDV virus amongst the 350 million carriers of the HBV around the world.

Liver cirrhosis develops in 60-70% of all patients with chronic HDV infection. This percentage is three times higher compared to the percentage that is observed in patients with chronic hepatitis B. Frequency of HDV associated fulminant hepatitis is 10 times higher than other viral hepatitis. HDV infection increases risk for HCC three-fold and mortality two-fold in patients with liver cirrhosis.

HBV infection, may be due to lack of proper health facilities, poor economic status and less public awareness about the transmission of major communicable diseases like HBV resulting in a rising prevalence of HDV in some parts of Pakistan.

Consensus on the treatment HDV infection is not outlined. However, the only approved treatment of chronic hepatitis D is interferon alpha (IFN-α). High dose (9 MU 3 times a week) IFN-α has higher rates of virological and biochemical response than IFN-α 3 MU 3 times a week or placebo. Patients have a viral relapse, however, an improvement in liver histology was seen 10 years post-treatment in patients who received high doses of IFN-α. Pegylated interferon has also been used for treatment of HDV infection, with higher costs compared to standard interferon. Hence, based on the available data high dose IFN-α or peg IFN-α for one year appears to have long-term beneficial effects in patients with chronic HDV infection (AASLD). A recent

ABSTRACT

Objective: To determine the response of one-year interferon-alpha therapy in hepatitis delta virus (HDV) infection in children and young adults at a tertiary care hospital, Karachi, Pakistan.

Study Design: An observational study.

Place and Duration of Study: Sarwar Zuberi Liver Centre (SZLC), Medical Unit IV, Civil Hospital, Karachi / Dow University of Health Sciences (DUHS), from June 2009 to July 2010.

Methodology: Paediatric patients (< 18 years age) and young adults (18-35 years) presenting were screened for hepatitis B virus (HBV) and HDV sero-markers. HDV anti-body positive by ELISA were further screened for hepatitis D ribonucleic acid (HDV-RNA) by real time PCR. HDV RNA PCR positive patients were treated with INF-α (children 6 MU/m²/day and adults 5 MU/day) for a period of one year. Patients were assessed monthly. Haematological parameters and ALT were monitored during treatment. Clinical progress (side effects) and negative HDV RNA were used as response criteria.

Results: Overall 49 patients were HDV RNA positive (children: n=15, mean age 15±2.92 years adults: n=34, mean age 27±4 years). Eighty percent were male. Treatment was given to 25 patients (children: n=11, adults: n=14). HBV genotype D was the predominant in all HDV RNA positive patients (73%). Eighty percent (20/25) were HDV-RNA negative after one year of treatment, and remaining patients are still under treatment. Side effects were tolerated well and children continued regular activity. Haematological parameters were unremarkable. Children maintained their pre-treatment centile for height and weight (growth parameters). ALT levels were significantly decreased post-treatment.

Conclusion: Conventional INF-α was safe in children with HDV infection in terms of side effects and growth parameters. Eighty percent were HDV-RNA negative one year after treatment. Further follow-up 2 years post-treatment will give conclusive results.

Key words: HDV. Viral hepatitis. Children. Young adults.
study suggests that interferon action against HDV replication is at the level of entry into primary human hepatocytes. Hence, in vivo, success of long-term interferon therapy for chronic HDV, may likewise involve blocking HDV spread by interfering with the initiation of productive infection of naïve hepatocytes.\(^9\) Interferon blocking HDV spread by interfering with the initiation of interferon therapy for chronic HDV, may likewise involve monitored closely.\(^7,8\)

In Pakistan, information about treatment of HDV infection is scarce; especially there is paucity of data on HDV infection among children and its treatment. Hence, the aim of this study was to determine the outcome of children and young adults treated with conventional interferon alpha 2a for HDV infection.

**METHODOLOGY**

This observational study was conducted at Sarwar Zuberi Liver Centre (SZLC), Medical Unit IV, Civil Hospital, Karachi / Dow University of Health Sciences (DUHS), from June 2009 to June 2010 for a period of one year. Patients of either genders aged up to 35 years were included. The patients were divided into two groups. Paediatric group included patients below 18 years of age and adult group included patients above the age of 18 not exceeding 35 years. Epidemiologic data including age, gender, height, weight and cultural background was recorded. All the patients who were HBsAg sero-positive were advised to check anti-HDV by ELISA. Of these, 49 had a positive HDV RNA by PCR and were included in this study. Majority of these patients were superinfected with HDV.

As recommended by American Association Society of Liver Disease (AASLD),\(^1\) all HDV RNA positive patients were advised for liver biopsy prior to their treatment. Of the 49 patients, only 25 received interferon alpha-2a treatment (11 children and 14 adults) and on liver biopsy had stage 2 grade 4 on histological activity index (HAI) scoring. The other 24 patients who were not treated either refused treatment or had advance fibrotic changes on liver biopsy, HAI stage of 5/6 or more and HAI grade of 9/18 or more.

Screening of all patients for HDV was done by HDV antibodies using ELISA technique (4th generation, Disorin, Italy). Those with positive HDV anti-body were further screened for hepatitis D ribonucleic acid (HDV RNA) by PCR. Silica column extraction of RNA from plasma was carried out with the instant virus RNA kit TH variante HDV Rev2 (AJ Roboscreen, Germany), according to manufacturer's instructions. Briefly, 0.2 ml plasma was mixed with 0.2 ml of Lysis Solution CLS in the given sample preparation tube coated with internal control (IC). The mixture was supplemented by adding 25 ul proteinase K provided with the kit and the mixture was incubated for 15 minutes at 70°C. After incubation, 400 ul binding solution was added to lysate and the whole mixture was applied to silica column. Centrifugation was done for 2 minutes at 12,000 rpm. Impurities were removed by spinning at 12,000 rpm by applying 500 ul washing solution (1X) and 650 ul washing solution LS (2X) consecutively. A final high speed centrifugation was done in order to remove any traces of washing solution LS. RNA was recovered by applying 60 ul of RNase free water and spinning the column at 8000 rpm. Negative and positive plasma of known normal and patient samples were included as extraction control in each batch. PCR amplification was carried out for HDV RNA quantification using RoboGene HDV RNA quantification kit tripleHyb version (AJ Roboscreen, Germany). Master mix was prepared according to scheme provided in the kit. Amplification was carried out in Rotor Gene 6000 with the profile as follows: RT 59°C for 45 minutes, Taq activation at 95°C for 2 minutes and 50 cycles of 95°C for 15 seconds, 45°C for 30 seconds and 57°C for 40 seconds. For quantification all 8 controls were amplified with sample in order to generate standard curve. Amplification negative controls were included in each run. Validation criteria was according to the protocol Slope (-3.09-3.22, CT value for IC 27-30, efficiency close to 1). The lower detection limit of this assay was 500 IU/ml.

All precautions were taken to avoid contamination during PCR as well as negative and positive control sera included in each run. The serum samples of the 49 patients who had positive HDV RNA were further analyzed for different genotypes of HBV by using genotype specific primers. The primers were designed in such a way that its specificity and sensitivity for HBV DNA detection was less than 2 pg/ml. Genotypes of HBV for each sample were determined by identifying the genotype-specific DNA bands. The sizes of PCR product were estimated according to the migration pattern of a 50-bp DNA ladder (Gibco BRL, Life Technologies). Mix-I allowed for the specific detection of PCR products for types A, B, and C, mix-II allowed for detection of types D, E, F, G, and H.

HDV RNA PCR positive patient were treated with INF-α (children 6 MIU/m²/day and adults 5 MIU/day), according to AASLD guidelines\(^1\) after an informed consent from the parents of children for a duration of one year. The parents were explained about the side effects and chances of relapse with interferon therapy. Patients were assessed before treatment and monthly by haematological and biochemical tests. The tests included complete blood picture, (CBC), haemoglobin (Hb) and platelet count, liver function tests (LFT’s) and blood sugar estimation. Children were monitored for their height and weight. For the initial 1-2 weeks the patients received injections in the hospital on an outpatient basis, under supervision. During the treatment period, the patient was counselled for cold chain
maintainence of interferon, its regular sub-cutaneous injection, methodology of injection and safe disposal of syringes after which they took the injections home for one month each time for a period of one year. Disposable needle cutters were provided to each patient or their parents. The process of counselling and injections was similar to our previous published work. Clinical progress (side effects), and negative HDV RNA were used as response criteria.

The side effects monitored during therapy included fever, cough, blisters in mouth, fatigue, headache, musculoskeletal pain, nausea, anorexia, diarrhea, dyspepsia, vomiting, insomnia, depression, irritability, anxiety, impaired concentration, emotional liability, alopecia, rash, pruritis, dry skin and inflammation at injection site. Interferon treatment was continued, unless severe adverse effects occurred, such as very low platelet count, allergic reaction or severe uncontrollable depression requiring the therapy to be stopped completely. Adverse effects were more severe in the initial weeks of treatment. The patients were managed with analgesics such as paracetamol (acetaminophen; < 0.2 gm per kg per day) or NSAID. Anti-depressants such as serotonin re-uptake inhibitors (SSRI) were given when needed after psychiatric consultation.

The study was approved by ethical and research committee of DUHS. Informed consents were obtained from all the adults and parents of paediatric patients participating in the study.

Analysis of data was carried out with the aid of Statistical Package for Social Sciences (SPSS) package version 10.0 and measures of central tendency and dispersion were obtained.

RESULTS

The over all mean age was 23.5±11.9 years. There were 15 children with mean age of 15±2.92 years, ranging from 9-18 years. There were 34 adults with mean age of 27±4 years, ranging from 20-35 years. Males were 80%, and the rest were females. Demographic details show mean height and weight of the paediatric population, 140.1± 26.8 cm (ranging from 73-170 cm) and 35.4±15.3 kg (ranging from 08-60 kg) respectively. The corresponding values among adult population were 163.9±10 cm (ranging from 144-180 cm) and 59.6±13.5 kg (range 35-70 kg). Most of the children were between the 25-50th centile of the average weight and height for that age group according to the NCHS (National Centre for Health Statistics) growth charts. The children maintained their pre-treatment centiles between 25-50th centile during and after treatment.

None of the patients in the treatment group had acute infection; most of them had a history of HBV positivity for more than 6 months, so there was a probability of superinfection in all patients.

Fifty percent of the paediatric patients had fever, fatigue, and headache predominantly. While more than 50% of the adult patients had predominantly fever, fatigue, headache, musculoskeletal pain, malaise, anorexia, insomnia, anxiety and alopecia (Table II).

A total of 49 patients were positive for HDV RNA by PCR. Treatment was given to 25 patients, of whom 11 were children and 14 were young adults. Laboratory data analysis of the adult and the paediatric group patients for dual infection with HBV and HDV are shown (Table I). Among the serological markers of infection, HBeAg was present in 22.4% cases (n=11/49), while HBV DNA in about 40.8% patients (n=20/49). The range of quantitative load of HBV in these patients was 10x6 to 10x9 copies/ml. HBV genotype D was predominant in all HDV-RNA positive patients (77%).

Eighty percent (20/25) were HDV-RNA negative after one year of treatment; remaining patients are under treatment (Table I). Pre-treatment ALT levels of more than 30 I.U. was present in 77% of patients. Post-treatment levels of ALT in adult patients were 23±14 I.U and 22±12 I.U in the paediatric population (Table I). Pre-treatment the patients had normal white cell count (total and differential), platelet and red blood cell count. These were monitored during treatment and remained within normal limits.

Table I: Demographic, laboratory data and treatment outcome of HDV infected patients.11

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total (n=49)</th>
<th>Paediatrics (&lt; 18 years)</th>
<th>Adults (&gt; 18 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>9 - 35</td>
<td>23.5±11.9</td>
<td>22±12</td>
</tr>
<tr>
<td>Mean ± S.D</td>
<td>9-18</td>
<td>15±2.92</td>
<td>27±4.0</td>
</tr>
<tr>
<td>Gender</td>
<td>40 (81.6)</td>
<td>09 (18.3)</td>
<td>03 (20.0)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>151.9±23.7</td>
<td>35.9±15.3</td>
<td>59.6±13.5</td>
</tr>
<tr>
<td>Mean ± S.D</td>
<td>9 - 90</td>
<td>73±180</td>
<td>144 - 180</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>49±12±18.5</td>
<td>140.1±26.8</td>
<td>163.9±10</td>
</tr>
<tr>
<td>Growth parameters</td>
<td></td>
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<tr>
<td>Pre-treatment centile</td>
<td>25-50 centile</td>
<td>25-50 centile</td>
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<tr>
<td>Post-treatment centile</td>
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<td></td>
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<tr>
<td>HBeAg (+)</td>
<td>11 (22.4)</td>
<td>4 (26.6)</td>
<td>7 (20.5)</td>
</tr>
<tr>
<td>HBV DNA (+)</td>
<td>20 (40.8)</td>
<td>6 (40.0)</td>
<td>14 (41.1)</td>
</tr>
<tr>
<td>ALT (I.U) Means±S.D #</td>
<td>107.3±130</td>
<td>113±119</td>
<td>107.3±130</td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>25±15</td>
<td>22±12</td>
<td>23±14</td>
</tr>
<tr>
<td>Post-treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment given</td>
<td>11/15 (73.3%)</td>
<td>9/11 (81.8%)</td>
<td>11/14 (78.5%)</td>
</tr>
<tr>
<td>ETR (–VE) at 12 months*</td>
<td>2 (18.1%)</td>
<td>3 (21.4%)</td>
<td></td>
</tr>
<tr>
<td>Lost to follow-up</td>
<td>10 (20.4%)</td>
<td>7 (46.7%)</td>
<td>4 (11.8%)</td>
</tr>
</tbody>
</table>

* End treatment response at 12 months.
# NCHS standards / (CDC charts) for height and weight in children -ref:
around 42.3%. Various local studies have documented a virus also.12 Another study conducted during 2003-2005 patients suffering from hepatitis B are infected with delta conducted from 1994-2001, showed that 16.6% of geographical distribution of HDV is variable. A study was done in this study. The geographical distribution of HDV is variable. A study conducted from 1994-2001, showed that 80% of the children suffering from HBV and HDV in this study were malnourished, their weight falling below the 50th centile (NCHS) for their age, probably due to low dietary intake due to poverty in confirmation with the previous studies.11 Monitoring of growth in children is essential and more so when receiving drugs such as interferon alpha with known adverse effects; fatigue, anorexia, nausea, diarrhea, abdominal pain and vomiting. These side effects may result in weight loss and fall in the growth centiles. Hence, height, weight, and diet taken by the child should be monitored strictly, as was done in this study.

The geographical distribution of HDV is variable. A study conducted from 1994-2001, showed that 16.6% of patients suffering from hepatitis B are infected with delta virus also.12 Another study conducted during 2003-2005 revealed a much higher proportion of 26.8%.13 The study results showed a further rise in this frequency of around 42.3%. Various local studies have documented a higher seroprevalence of HDV in younger male subjects who are positive for HBsAg;12-14 but not enough data on the treatment of HDV in paediatric population is available. Most of the patients (41.3%) belonged to the province of Sindh, majority from the rural areas, followed by Khyber Pakhtunkhwa (25.6%), Urdu speaking population (15.2%), Balochistan (9.2%) and Punjab (4.9%). This pattern of presentation is also observed in previous studies.14

Even countries with high prevalence rates like Bangladesh, 25% HBV infected children are simultaneously infected with HDV.15 Likewise results from Peru showed 13% prevalence, Uzbekistan 14% and Spain 13%.16-18 Comparison of these results with this study showed a much higher frequency of delta infected children of around 45%. However, this 45% is a crude estimate of the rising prevalence of hepatitis D in our country, since it was not a randomized sample including mostly patients from a single province. Moreover, the sample size is very small to apply the results to the entire population of Pakistan or even the province of Sindh. Furthermore, there is no data regarding the treatment of HDV in the paediatric population, though prevalence data for HDV is available in the adult population.6 However, long-term outcome in terms of treatment for HDV infection is meager.

HBV isolates have been classified into eight genotypes, A-H, which exhibit distinct geographical distributions.19 Studies have shown that in Asia, genotypes B and C are the most prevalent. One study result showed that in Pakistan, all the four common genotypes of HBV found worldwide (A, B, C and D) were isolated. Genotypes B and C are predominant in Punjab and Pakhtunkhwa, whereas genotype A is predominant in Sindh. While another study showed that hepatitis B genotypes A, D and A/V combination were present in all categories of patients with predominance of D genotype, which is also our observation in this study.20-21 It has been shown that compared with children with genotype A, children with genotype D showed a significantly higher viral load. Hence, it is important to determine the genotype in HBV and HDV infections.22 Also strict secondary T-cell immunodeficiency, more expressed in children with chronic viral hepatitis D,23 poses them to be at risk of acquiring other infections.

Analysis of hepatitis delta virus (HDV) isolates from around the world has indicated that there are at least three phylogenetically distinct genotypes with different geographic distributions.1,24 A previous local study concluded that all the HDV strains belonged to genotype-I.6 The limitation of this study is that HDV genotype could not be done in all patients due to cost constraints.

HBV and HDV share similar routes of transmission. In Italy and other developed countries the disease seems
to be declining due to the high overall rate of vaccination against HBV. The most common risk factor responsible for acquiring HBV and HDV was the lack of immunization against HBV in around 95% patients. Lack of knowledge and poverty were found to be the main issues regarding low vaccination coverage in Pakistan. Based on these facts vaccination for HBV as a part of Expanded Programme of Immunization (EPI) was launched in a nationwide vaccination campaign in 2004.

Therapeutic response of interferon-alpha against HDV infection in children is limited. In the study population, 80% (n=20/25) of the adults and paediatric patients treated with regular interferon alpha 2a were HDV RNA negative after one year of treatment. This requires a further follow-up, of one year more as HDV infection appears to have long-term beneficial effects and there is improvement in liver histology with treatment. None of the patients in our treatment group had acute infection; most of them had a history of HBV positivity for more than 6 months, so the probability of super infection in all our patients’ exists.

Predominant side effects of interferon were fever, fatigue, headache, musculo-skeletal pain, malaise, anorexia, insomnia, anxiety and alopecia seen in this cohort of population (Table II) and were similar to previous study conducted in adult population. In children side effects were similar to previously published data. In this study, the side effects in children were not particularly disturbing and children remained active on treatment, felt well and attended school regularly. All thrived well during treatment and maintained their 25-50 centile indicating height velocity and body mass increase. This is similar to previous published study in children treated with interferon alpha in HDV infection.

Fulminant disease occurs more commonly in HBV and HDV than in other forms of acute viral hepatitis. Chronic HDV infection is usually associated with severe histological changes in the liver and with a rapidly progressive course that can lead to liver cirrhosis, liver failure and death. Treatment of chronic HDV is currently unsatisfactory and interferon alpha is the only agent found to have some effect on the course of chronic hepatitis. Cost of treatment for chronic viral hepatitis is very high and efforts have to continue to extend HBV vaccination to the general population nationwide to reduce its transmission and hence prevent HDV infection also.

The limitations of this data included small sample size, non-randomized, un-controlled study, majority of patients were from a single community or single province of the country, based on the patients' data that visited hospital or were found to be clinically affected, and the inability to do HDV genotyping. Majority of the patients could not afford treatment, for HDV infection, for a period of one year or more. Hence, we were unable to use pegylated interferon? which though more effective and convenient is expensive. Therefore, conventional interferon was used.

Thus further data is needed to support the results of this study. Also there is an urgent need of the time to develop public health care policies with special emphasis towards the control of HBV and hence HDV infection, through vaccination for HBV, public awareness and proper health care facilities in a cost effective manner. However, young children with HDV infection should be treated with interferon alpha, with very careful monitoring of the side effects their nutrition and height and weight centiles so as to try and avoid the long-term sequela of morbidity and mortality associated with HBV and HDV co-infection.

CONCLUSION

Conventional INF-α in children with HDV infection had minimal side effects but required very close monitoring. Eighty percent were HDV-RNA negative one year after treatment. Further follow-up 2 years post-treatment will give conclusive results.

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REFERENCES


