Original article

Anti-HDV immunoglobulin M testing in hepatitis delta revisited: correlations with disease activity and response to pegylated interferon-α2a treatment

Ingmar Mederacke1, Cihan Yurdaydin2, George N Dalekos3, Birgit Bremer1, Andreas Erhardt4, Yilmaz Cakaloglu5, Kendal Yalcin6, Selim Gurel7, Stefan Zeuzem8, Kalliopi Zachou3, Hakan Bozkaya2, Hans Peter Dienes9, Michael P Manns1, Heiner Wedemeyer4*, Hep-Net/International Delta Hepatitis Study Group

1Department of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Germany
2Department of Gastroenterology, University of Ankara Medical School, Ankara, Turkey
3Department of Medicine and Research Lab of Internal Medicine, School of Medicine, University of Thessaly, Larissa, Greece
4Department of Gastroenterology, Hepatology and Infectiology, Heinrich-Heine University, Duesseldorf, Germany
5Department of Gastroenterology and Hepatology, Memorial Hospital, Istanbul, Turkey
6Department of Internal Medicine, Dicle University Medical School, Diyarbakır, Turkey
7Department of Gastroenterology, Uludag University Medical School, Bursa, Turkey
8Department of Medicine 1, Johann Wolfgang Goethe University Hospital, Frankfurt, Germany
9Institute of Pathology, University of Cologne, Cologne, Germany

*Corresponding author e-mail: wedemeyer.heiner@mh-hannover.de

Background: The role of anti-HDV immunoglobulin M (IgM) testing in patients receiving pegylated interferon-α therapy for hepatitis delta is unknown. We performed anti-HDV IgM testing in a well defined cohort of HDV-infected patients who were treated with pegylated interferon-α2a plus adefovir, or either drug alone.

Methods: Sera from 33 HDV-RNA-positive patients from the international HIDIT-1 trial were available for anti-HDV IgM testing (ETI-DELTA-IGMK-2 assay, DiaSorin, Saluggia, Italy) before therapy, at treatment weeks 24 and 48, and at 24 weeks after the end of treatment.

Results: Anti-HDV IgM tested positive in 31 out of the 33 patients (94%) prior to treatment. HDV IgM levels correlated with histological inflammatory activity ($r=0.51$, $P<0.01$) and were higher in patients with alanine aminotransferase and γ-glutamyl transpeptidase levels above the median ($P<0.05$). Quantitative anti-HDV IgM values declined in patients responding to antiviral therapy, however anti-HDV IgM remained positive after treatment in the majority of virological responders.

Conclusions: We suggest that anti-HDV IgM testing might give additional useful information to determine disease activity in hepatitis delta and to predict treatment response to antiviral therapy with type I interferons. However, determination of anti-HDV IgM can not substitute HDV RNA testing, which remains the primary virological marker for response to therapy.

Introduction

Hepatitis delta is an inflammatory liver disease caused by the hepatitis D virus (HDV) frequently leading to liver cirrhosis and hepatocellular carcinoma [1,2]. The diagnosis of HDV infection is usually based on anti-HDV immunoglobulin G (IgG) and HDV RNA testing. Recently, we established a protocol to quantify HDV RNA on a commercially available platform [3]; however, detection and quantification of HDV RNA is not well standardized yet between different laboratories. Moreover, various in-house PCR assays may have difficulties in appropriate quantification of HDV strains other than HDV genotype-1 [4]. Anti-HDV immunoglobulin M (IgM) testing was used to diagnose active HDV infection before PCR testing became widely available [5]. In addition, anti-HDV IgM levels correlated with disease activity [6] and treatment outcome [7] in few studies performed in the 1980s and 1990s.

Although the prevalence of HDV infections has declined within the last decades [8,9], hepatitis delta
is not a vanishing disease [10], as 8–12% of hepatitis B surface antigen (HBsAg)-positive patients still test positive for anti-HDV IgG in Central and Western Europe [1,11,12]. Subsequently, a re-emerging interest in hepatitis delta has been observed in recent years [13]. While therapeutic options significantly improved in the last decade for HBV, HCV and HIV infections, treatment options for hepatitis delta are still very limited with only 20–30% of patients responding to interferon (IFN)-α-based therapies [1]. We recently published the results from the so far largest study on the treatment of hepatitis delta, the Hep-Net/International Delta Hepatitis Intervention Trial 1 (HIDIT-1) study. This was an international randomized multicentre trial investigating pegylated IFN (PEG-IFN)-α2a with or without adefovir dipivoxil (ADV) versus ADV alone for the treatment of chronic hepatitis delta [14] showing that PEG-IFN-α2a but not ADV can lead to sustained HDV RNA clearance in approximately 25% of patients.

The aim of the present study was to re-examine the role of anti-HDV IgM testing in samples obtained from the HIDIT-1 study and to investigate if anti-HDV IgM testing may give additional information to currently used virological and biochemical assays.

Methods

Patients and treatment

Patients were treated within the HIDIT-1 study. This was an investigator-initiated international, randomized controlled trial that enrolled patients in Germany, Turkey and Greece between March 2004 and September 2006. Subjects (age 18–70 years) with chronic hepatitis delta and compensated liver disease were eligible for inclusion. Detailed inclusion and exclusion criteria were published elsewhere [14].

Patients were randomized to one of three treatment arms, receiving PEG-IFN-α2a plus ADV versus either drug alone for 48 weeks. For this subanalysis, a total of 111 serum samples from 33 patients were available for baseline, week 24, week 48, and follow-up week 24. At each visit, blood was drawn for biochemistry, haematology and virology.

Liver biopsies were obtained before and at the end of treatment. Grading and staging of liver biopsy samples were performed according to the Ishak score [15] by an independent pathologist (HPD). Baseline characteristics of the 33 patients are shown in Table 1. Characteristics of the 33 patients included in this substudy did not differ significantly from the entire study cohort (P>0.05).

Determination of anti-HDV IgM

Anti-HDV IgM was determined using the ETI-DELTA-IGMK-2 assay (DiaSorin, Saluggia, Italy) according to the manufacturer’s instructions. The optical density (OD) 450/620 values were determined by photometry (Tecan Rainbow Thermo, Tecan, Crailsheim, Germany). This assay has been reported to have a sensitivity and specificity of 99.52% and 99%, respectively. As all of our patients were infected with HDV genotype 1 the HDV genotype was no concern for this study. Anti-IgM OD450/620 values were correlated with different histological, biochemical and virological parameters.

The cutoff value for anti-HDV IgM OD 450/620 is determined by addition of 0.1 to the mean extinction of the negative control serum according to manufacturer’s instruction. Patient samples 10% below or above the cutoff were retested as suggested by the manufacturer.

The samples were measured on two different plates. However, 12 samples were measured on both plates yielding slightly lower values on the second plate but well within the standard deviation.

Statistics

Statistical analyses were performed by using GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA) and SPSS version 15.0.1 (SPSS, Munich, Germany). P-values <0.05 were considered as an indicator for peculiarity.

Results

Qualitative analysis of anti-HDV IgM

Prior to treatment, anti-HDV IgM was positive in 31 out of 33 (94%) patients. The two anti-HDV IgM-negative patients had HDV RNA levels of 1.2×10^5 and 3.6×10^5 copies/ml and alanine aminotransferase (ALT) levels of 73 and 62 U/l, respectively. The distribution of anti-HDV IgM OD 450/620 values at baseline and during further follow-up is presented in Figure 1A, showing a considerable inter-individual variability of quantitative anti-HDV IgM levels.

At the end of treatment (week 48), seven additional patients became anti-HDV IgM-negative. At this time point, 4 of the 9 (44%) anti-HDV IgM-negative patients had HDV RNA levels of 1.2×10^5 and 3.6×10^5 copies/ml and alanine aminotransferase (ALT) levels of 73 and 62 U/l, respectively. The distribution of anti-HDV IgM OD 450/620 values at baseline and during further follow-up is presented in Figure 1A, showing a considerable inter-individual variability of quantitative anti-HDV IgM levels.

Correlation of quantitative anti-HDV IgM OD 450/620 values with disease parameters

Quantitative anti-HDV IgM OD 450/620 values correlated with histological inflammation (r=0.51,
Table 1. Baseline characteristics of all patients and according to treatment group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All patients</th>
<th>PEG-IFN-α2a+ADV</th>
<th>PEG-IFN-α2a+ placebo</th>
<th>ADV monotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, n</td>
<td>33</td>
<td>11</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>23 (70)</td>
<td>8 (73)</td>
<td>7 (64)</td>
<td>8 (73)</td>
</tr>
<tr>
<td>Median ALT, U/l (range)</td>
<td>84 (31–249)</td>
<td>88 (41–234)</td>
<td>67 (31–213)</td>
<td>134 (52–249)</td>
</tr>
<tr>
<td>Median AST, U/l (range)</td>
<td>62 (28–171)</td>
<td>64 (32–171)</td>
<td>41 (28–150)</td>
<td>74 (35–139)</td>
</tr>
<tr>
<td>Median GGT, U/l (range)</td>
<td>50 (16–297)</td>
<td>72 (19–288)</td>
<td>29 (16–178)</td>
<td>84 (18–297)</td>
</tr>
<tr>
<td>Median anti-HDV IgM OD 450/620, (range)</td>
<td>0.48 (0.08–2.77)</td>
<td>0.57 (0.08–2.77)</td>
<td>0.43 (0.10–1.05)</td>
<td>0.45 (0.13–1.43)</td>
</tr>
<tr>
<td>Anti-HDV IgM positive, n (%)</td>
<td>31 (94)</td>
<td>10 (91)</td>
<td>10 (91)</td>
<td>11 (100)</td>
</tr>
<tr>
<td>Median HDV RNA, log_{10} copies/ml</td>
<td>5.24</td>
<td>6.08</td>
<td>4.90</td>
<td>4.99</td>
</tr>
<tr>
<td>Median HBV DNA, log_{10} IU/ml</td>
<td>2.33</td>
<td>1.79</td>
<td>3.00</td>
<td>2.39</td>
</tr>
<tr>
<td>Cirrhosis, n (%)</td>
<td>7/30 (23)</td>
<td>1/9 (11)</td>
<td>2/11 (18)</td>
<td>4/10 (40)</td>
</tr>
<tr>
<td>HDV genotype 1, n (%)</td>
<td>33 (100)</td>
<td>11 (100)</td>
<td>11 (100)</td>
<td>11 (100)</td>
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<tr>
<td>Previous IFN treatment, n (%)</td>
<td>18 (55)</td>
<td>6 (55)</td>
<td>8 (73)</td>
<td>4 (36)</td>
</tr>
<tr>
<td>Response to treatment, n (%)</td>
<td>10/33 (30)</td>
<td>5/11 (45)</td>
<td>5/11 (45)</td>
<td>0/11 (0)</td>
</tr>
</tbody>
</table>

No significant difference between different treatment groups in terms of age, alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transpeptidase (GGT), anti-hepatitis delta virus (HDV) immunoglobulin M (IgM), log_{10} HDV RNA, log_{10} HBV DNA and log_{10} hepatitis B surface antigen (HBsAg; one-way ANOVA, P>0.05).

Response to treatment was defined as HDV RNA negativity at follow-up week 24. ADV, adefovir dipivoxil; OD, optical density; PEG-IFN, pegylated interferon.

Figure 1. Distribution of anti-HDV IgM values before treatment, at end of treatment and at end of follow-up

(A) The grey bar represents 10% below or above the cutoff value. (B) Correlation of anti-hepatitis delta virus (HDV) immunoglobulin M (IgM) values with histological inflammation score. Grouping anti-HDV IgM values in low (<0.3), medium (0.3–0.7) and high (>0.7) levels showed significantly higher (C) histological inflammation in patients with high optical density (OD) 450/620 levels, but no significant difference in (D) alanine aminotransferase (ALT) or (E) γ-glutamyl transpeptidase (GGT) between the three OD 450/620 groups. Significance was tested using Kruskal-Wallis test, Dunn’s multiple comparison test was used as post-test.
Patients with high anti-HDV IgM OD 450/620 values (>0.7) also showed a higher histological inflammation score compared to patients with low (<0.3) OD 450/620 values (P<0.05; Figure 1C). No direct correlation of anti-HDV IgM OD 450/620 values were evident with virological markers, although anti-HDV IgM levels tended to correlate with markers of biochemical disease activity (Additional file 1). These findings were confirmed by analysing anti-HDV IgM values after grouping of various biochemical, histological and virological parameters according to the median values. In this analysis, significantly higher anti-HDV IgM values were evident in patients with high ALT values (P=0.03), high γ-glytamyl transpeptidase values (P=0.01), and higher histological inflammation scores (P=0.03; Additional file 2).

**Anti-HDV IgM values during treatment**

We first evaluated anti-HDV IgM values according to different treatment arms. Median anti-HDV IgM levels did not change significantly during treatment in patients receiving PEG-IFN-α2a plus ADV or ADV alone. However, patients who received PEG-IFN-α2a plus placebo showed a significant decrease of anti-HDV IgM values after 48 weeks of treatment (P=0.04, paired t-test; Figure 2A). No significant differences in anti-HDV IgM levels were observed comparing the three treatment arms with each other at baseline, week 24, week 48 or follow-up week 24 (P>0.05, Kruskal-Wallis test; data not shown).

Analysing anti-HDV IgM values according to virological response to treatment, a significant decline of anti-HDV IgM levels after 24 and 48 weeks was observed in patients who had a sustained virological response as defined by a negative HDV RNA test at follow-up week 24. By contrast, patients with detectable HDV RNA at follow-up week 24 did not show a decrease of anti-HDV IgM levels over time (Figure 2B). Individual anti-HDV IgM values over time are shown in Figure 3A and 3B for the treatment responders of the two PEG-IFN-α2a-containing arms separately. In this analysis the decline of anti-HDV IgM values was only significant at follow-up week 24 for patients receiving PEG-IFN-α2a in combination with ADV (P=0.046, paired t-test; Figure 3A). Patients without treatment response to PEG-IFN-α2a-based treatment and all patients receiving ADV monotherapy did not show significant changes of anti-HDV IgM levels over time (Figure 3C and 3D). Of note, only three patients receiving IFN-based treatment had very high anti-HDV IgM levels before therapy (>1.1) and all of those did not only clear HDV RNA from serum but also showed a decline of HBsAg of >1 log10 IU/ml.

As histological inflammation correlated significantly with anti-HDV IgM at baseline we were interested in anti-HDV IgM responses in patients with or without an improvement in inflammatory activity in the post-treatment liver biopsy. Interestingly, only patients who showed a decrease in histological inflammation scores also experienced a decline of anti-HDV IgM levels (Figure 2C).

**Discussion**

In the current study we re-investigated the applicability of a commercially available anti-HDV IgM assay in a well-characterized multicentre cohort of patients with hepatitis delta. We show that anti-HDV IgM tested positive in almost all HDV-RNA-positive patients prior to treatment, that anti-HDV IgM levels correlated with histological inflammatory activity, and that anti-HDV IgM levels declined in patients responding to antiviral therapy; however, anti-HDV
IgM remained positive after treatment in the majority of virological responders. It has already been shown, two decades ago, that IgM antibodies to HDV may represent a marker of active HDV infection [6]. Indeed, also in our study, 94% of chronically HDV-infected patients were anti-HDV IgM-positive before treatment. However, anti-HDV IgM remained positive for ≥6 months after treatment in 8 out of 10 virological responders, suggesting that loss of anti-HDV IgM may be significantly delayed after viral clearance. These data are in line with earlier findings demonstrating that anti-HDV IgM disappeared in patients with sustained virological response to IFN-α-based treatment not before 3 to 15 months after ALT normalization [7]. Transient persistence of IgM antibodies after viral clearance is also not uncommon in patients with acute HAV, HBV or HEV infections, where loss of IgM antibodies has also been shown to occur as late as 28, 134, and 3 months after recovery, respectively [16–18]. Nevertheless, analysing anti-HDV IgM OD 450/620 levels over time clearly showed a decline during treatment with PEG-IFN-α2a, in particular in individuals responding to therapy. This finding further supports the relationship between the activity of infection and magnitude of HDV-specific IgM responses.

The two patients with negative anti-HDV IgM tests at baseline despite detectable HDV viraemia remained anti-HDV IgM-negative until week 48. Surprisingly, both patients became anti-HDV IgM-positive at

Figure 2. Anti-HDV IgM according to treatment arm, treatment response and inflammation

Anti-hepatitis delta virus (HDV) immunoglobulin M (IgM) optical density (OD) 450/620 values according to (A) treatment arm, (B) in patients responding to treatment (HDV-RNA-negative at follow-up week 24 [FU24]) versus treatment non-responders and (C) in patients with a decrease of inflammation versus increase or no change of inflammation when comparing pretreatment and post-treatment histological inflammation. Significance was tested using the paired t-test. ADV, adefovir dipivoxil; NS, non-significant; PEG-IFN, pegylated interferon; W0, week 0 (baseline); W24, week 24; W48, week 48 (end of treatment).
follow-up week 24, although one of them even cleared HDV RNA. It is tempting to speculate that IFN-α may have activated plasma cells, which led to the production of HDV-specific IgM antibodies. Indeed, IFN-α may exert a direct stimulatory effect on plasma cells [19,20]. This would be in line with our previous observation that anti-HCV antibody responses can transiently increase during IFN-α based treatment of acute hepatitis C [21].

However, in the majority of patients, anti-HDV IgM antibody titres clearly declined during treatment but increased after treatment was stopped. As this decline was only observed in patients clearing HDV RNA, a direct correlation between the presence of hepatitis delta antigen and antibody production is suggested. Moreover, although IFN-α may stimulate B-cells it may also confer antiproliferative effects on plasma cells, and thereby reduce IgM antibody levels during, but not after, treatment [22]. Overall, anti-HDV antibody production during IFN treatment is regulated by different mechanisms leading to distinct outcomes in different individuals depending on responsiveness to IFN and antigen load.

Another interesting finding of this study was that anti-HDV IgM OD 450/620 values correlated with histological inflammation scores and biochemical activity before treatment. Thus, these data clearly support earlier findings that anti-HDV IgM levels may indicate disease activity [6]. We therefore suggest that, if anti-HDV IgM testing is performed, quantitative OD 450/620 values should be reported as this may represent clinically valuable information for the treating physician. Compared to liver biopsies, anti-HDV IgM testing can easily be repeated, thus providing potentially useful additional information if a liver biopsy cannot be performed for other reasons. However, anti-HDV IgM testing was not helpful in determining the stage of liver disease, as no correlation with fibrosis scores was evident.

Finally, patients with very high OD 450/620 values may respond better to PEG-IFN-α-based treatment as all three patients with anti-HDV IgM levels >1.1 cleared...

![Figure 3. Individual anti-HDV IgM response](image-url)
HDV RNA. Interestingly, these patients also showed a decline of HBsAg of >1 log10. High anti-HDV IgM levels may therefore indicate a particular strong anti-HDV immunity, which could contribute to the control of HDV infection and also to response to therapy [23]. However, this hypothesis seems to be in contrast to an earlier study showing HDV-specific CD4+ T-cell responses, in particular in anti-HDV IgM-negative but not -positive patients [24]. Thus, more studies are needed to investigate the role of humoral and cellular immune responses against HDV in the response to antiviral therapy [25].

Several limitations of this study, which was a retrospective analysis of sera obtained from a prospective treatment trial, need to be considered. Importantly, serum samples were available for only one-third of the overall study cohort and, thus, the findings need to be confirmed in an independent cohort. Comparisons with adaptive anti-HDV immune responses would also be of interest. Finally, a follow-up longer than 24 weeks should be performed to determine the duration of anti-HDV IgM persistence in sustained virological responders.

In summary, we here suggest that anti-HDV IgM testing might provide additional useful information to determine disease activity in hepatitis D and to predict treatment response to antiviral therapy with type I IFNs. However, determination of anti-HDV IgM cannot substitute HDV RNA testing which remains the primary virological marker for response to therapy.

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Disclosure statement

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Additional files

Additional file 1: Supplementary Table 1 displaying Spearman correlations of histological, biochemical and virological parameters at baseline with anti-HDV IgM OD 450/620 values can be accessed via http://www.intmedpress.com/uploads/documents/AVT-11-OA-2011_Mederacke_Add_file1.pdf

Additional file 2: Supplementary Table 2 displaying mean anti-HDV IgM OD 450/620 of histological, biochemical and virological parameters according to median values can be accessed via http://www.intmedpress.com/uploads/documents/AVT-11-OA-2011_Mederacke_Add_file2.pdf

References


