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Comparison of the Enzyme-Linked Immunosorbant Assay III, Recombinant Immunoblot Third Generation Assay, and Polymerase Chain Reaction Method in the Detection of Hepatitis C Virus Infection in Haemodialysis Patients

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Hepatitis C virus (HCV) serotyping assays have evolved from simple antibody screening tests to complex RNA-based qualitative and quantitative methods. The objective of this study was to compare the HCV screening results from 161 patients in long-term maintenance haemodialysis (HD) as assessed by the recently developed Enzyme Linked Immunosorbant Assay III (ELISA III), confirmed by the Recombinant Immunoblot 3rd generation assay (RIBA 3rd) and determined by the qualitative HCV reverse transcription polymerase chain reaction (RT-PCR) method. One hundred sixty-one HD patients were tested for the presence of anti-HCV antibodies by the ELISA III and confirmed by the RIBA 3rd. HCV RNA was determined by an HCV RT-PCR method. All reported results that were designated as discrepant, anti-HCV (+) and/or HCV RNA (+) were further investigated by means of a quantitative HCV RT-PCR assay. Reported results obtained from ELISA III and qualitative RT-PCR assays were HCV positive for 16/161 patients (9,93%) and these were designated as anti-HCV (+)/HCV RNA (+). Subsequently, these 16 anti-HCV positive/161 HD patients were confirmed by the RIBA 3rd. Three individuals anti-HCV (–)/RIBA (+)/ HCV RNA (–)], the viral load that was reported from the quantitative RT-PCR was less than the assay detection level (< 2,000 viral copies/ml). In view of previous observations, our findings suggest that ELISA III remains still a highly reliable and valuable assay. However, despite the cost, the combination of both ELISA III and qualitative RT-PCR allows a definitive classification on HCV diagnosis. J. Clin. Lab. Anal. 13:122–125,1999.

Key Words: HCV; serotyping; HCV RNA; anti-HCV; haemodialysis

INTRODUCTION

Hepatitis C virus is responsible for 90% of non-A, non-B viral hepatitis cases (1). Diagnosis of HCV infection has serious implications, especially for high-risk patients such as those undergoing haemodialysis (2,3). The first commercially available HCV test was an Enzyme-Linked Immunosorbant Assay in which serum was tested for the c100-3 antigen derived from the nonstructural region of the virus. Since then, development of anti-HCV screening assays has made significant progress. Recent studies suggest that ELISA II and/or III screening assays are highly valuable in HCV diagnosis. However, interpretation of immunoserological tests (ELISA) is often difficult since 2–10% (depending on the risk group under evaluation and the immunoserological assay used) of samples that are repeatedly reactive in ELISA antibody screening assays are negative or indeterminate upon supplemental evaluation with RIBA testing (4–6). Therefore, detection of serum HCV antibodies does not imply unequivocally viremia and vice versa (7–9). Even more, in some cases of acute infection and/or immunodeficiency, individuals may fail to produce antibodies specific for HCV, thus it is of high risk to obtain a confused serological profile. HCV RNA determina-

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tion through qualitative RT-PCR method allows direct HCV detection prior to any serological alteration (10) including generation of antibodies or aminotransferase elevation.

Until recently, both quantitative and qualitative RT-PCR techniques had no practical application in hospital diagnostic laboratories because of sample handling, nonstandardized conditions, and contamination during the RNA amplification step. Despite the cost, several commercial diagnostic kits (11) have been developed, offering high reproducibility and reliability.

The objective of this study was to evaluate the frequency of HCV detection of two different screening assays in HCV diagnosis. Within this context, we determined the serologic and virologic profile of 161 HD patients by means of ELISA III and HCV-RNA qualitative measurement. Subsequently, all samples were subjected under confirmatory evaluation by RIBA 3rd. Furthermore, we measured the number of viral copies/ml (quantitative HCV-RNA measurement) in all HCV positive and "indeterminate" patients and we compared these data with the HCV screening results obtained from the two diagnostic tests (ELISA III and Qualitative RT-PCR).

MATERIALS AND METHODS

Patients and Controls

One hundred sixty-one haemodialysis patients (mean age: 43.3, range: 25.3–61.3, 83 males and 78 females) were examined three consecutive times over a 1.5 year follow-up period for the presence of anti-HCV antibodies and HCV-RNA by means of ELISA III and qualitative RT-PCR respectively. Subsequently all samples were confirmed by RIBA 3rd. Patients' sera were collected in EDTA-anticoagulant tubes, and stored in –20°C for up to one week or else in –70°C for up to one month.

Detection of Anti-HCV Antibodies by ELISA III

Serum samples were tested three consecutive times during the period of study for the presence of anti-HCV antibodies by ORTHO ELISA III test system. This assay detects antibodies directed to core NS3, NS4, and NS5 antigens. The assay was performed according to the manufacturer's instructions and in each sample the optical density (OD) ratio was calculated by dividing the sample OD by the assay control OD. Sample with an OD ratio > 2.0 were considered positive, samples with an OD ratio between 1 and 2 were considered as weakly positive and samples with an OD ratio < 1.0 were considered negative. Low and high titer positive controls were included in each assay and these were always positive.

RIBA 3rd Generation Assay

Detection of anti-HCV antibodies (c33, NS5, C22p, c100p) by means of RIBA 3rd generation assay was performed three consecutive times according to the instructions provided by the manufacturer (Chiron Corporation, Emerville, CA). In brief, patients' sera were incubated with a nitrocellulose strip, which has been coated with HCV antigens and a number of positive and negative controls supplied by the diagnostic method.

HCV Qualitative Determination

HCV qualitative determination was performed in three well-isolated areas for contamination purposes named as: sample preparation area, amplification area, detection area. Specimen preparation was performed with an optimized isopropanol based method supplied by the kit. Sera were incubated with a lysis reagent followed by RNA extraction and ethanol precipitation. Both positive and negative controls were treated as unknown samples and were included in the initial step of sample preparation. PCR cycling conditions were set as instructed by the manufacturer (HCV Amplicor™, La Roche). Detection of HCV-RNA was performed by a non-isotopic method adapted to microwell format.

HCV Quantitative Measurement

Reverse transcription, amplification of HCV, and quantitation was performed simultaneously with the incorporation of an internal standardized target sequence control. In brief, RNA was isolated from viral particles by means of a chaotropic agent. Reverse transcription and PCR amplification were performed according to the instructions supplied by the manufacturer (HCV-Monitor™ Test, La Roche). Detection and colorimetric quantitation were based on a method adapted to microwell format. Results were expressed as viral copies/ml of human serum. The lower limit of the assay was estimated as 2,000 viral copies/ml. Below this cut-off value, the assay cannot confirm the presence or absence of viral particles and the provided protocol suggests to report the result as "no HCV RNA detected,'' less than 2,000 copies/ml, or less than 10 viral copies/PCR reaction.

RESULTS

Reported results obtained from ELISA III and qualitative RT-PCR assays were HCV positive for 16/161 patients (9,93%) and these were designated as anti-HCV (+)/ HCV RNA (+). Subsequently, 16 anti-HCV positive/161 HD patients were confirmed by the RIBA 3rd. In addition, three individuals anti-HCV (–)/HCV RNA (–) were reported as RIBA 3rd (+). Results did not vary during the three consecutive measurements for each assay. Discrepant results [ELISA (–)/ RIBA (+)/RT-PCR (–)] were obtained in cases 5, 6, and 10 (see Table 1). In order to minimize the possibility that the processed specimens were inhibitory for amplification or that RNA was not recovered the entire RT-PCR test procedure was repeated (including specimen preparation) for those samples. Furthermore, the three patients designated as ELISA (–)/RIBA (+)/RT-PCR (–), showed a viral load of less than the assay detection level (< 2,000 copies/ml). Three out of

TABLE 1. Immunologic and Virologic Profiles of HD Patients That Were Shown to be HCV Positive in at Least One of the Three Screening and/or Confirmatory Assays

Patient's no.	ELISA III	RIBA 3rd	RT-PCR (qualitative)	Viral load copies/ml[a]	Duration of haemodialysis (yrs)	HBV/HIV diagnosis
1	+	+	+	800,000 ± 50,000	8	–/–
2	+	+	+	100,000 ± 5,000	9	–/–
3	+	+	+	130,000 ± 10,000	18	–/–
4	+	+	+	150,000 ± 5,000	11	–/–
5	–	+	–	< 2,000[b]	10	–/–
6	–	+	–	< 2,000[b]	5	–/–
7	+	+	+	100,000 ± 5,000	9	+/–
8	+	+	+	50,000 ± 8,000	12	–/–
9	+	+	+	100,000 ± 8,000	11	–/+
10	–	+	–	< 2,000[b]	8	–/–
11	+	+	+	900,000 ± 60,000	7	–/–
12	+	+	+	150,000 ± 10,000	9	–/–
13	+	+	+	120,000 ± 10,000	10	–/–
14	+	+	+	1,000,000 ± 90,000	11	–/–
15	+	+	+	130,000 ± 10,000	8	–/–
16	+	+	+	100,000 ± 8,000	9	–/–
17	+	+	+	50,000 ± 5,000	7	–/–
18	+	+	+	150,000 ± 10,000	8	–/–
19	+	+	+	140,000 ± 9,000	10	–/–

[a]Average values measured three consecutive times during the period of study.
[b]No HCV RNA detected. The sensitivity limits of the assay is 2,000 copies/ml.

sixteen PCR positive samples had high levels of viral load (>500,000 copies/ml). All other patients had a viral load ranging from 50,000 to 150,000 copies/ml (see Table 1). Patients 7 and 9 were found to be HBV (+) and HIV (+) respectively. Patients' viral load remained relatively stable during three consecutive measurements.

DISCUSSION

Our data demonstrated that only a small proportion of haemodialysis patients in our unit were anti-HCV positive and/or HCV RNA (+) upon ELISA III and RT-PCR evaluation respectively. Reported results obtained from ELISA III were further subjected under RIBA 3rd confirmatory assay. As shown in Table 1 patients 5, 6, and 10 were reported as ELISA (–)/RIBA (+) / RT-PCR (–). HCV-RNA negativity in anti-HCV positive patients is in this study within the reported range in the literature (12). However, a patient designated with the above screening profile cannot be reported as an anti-HCV (+) patient, since RIBA 3rd is not an HCV screening assay and does not allow a definite HCV classification. In addition, discordant results in these three cases between ELISA III and RIBA 3rd assays and the parallel agreement of ELISA III and qualitative RT-PCR reported results might imply a higher sensitivity of RIBA 3rd in anti-HCV immunoreactivity. In fact, we are not in a position to confirm the higher sensitivity of RIBA 3rd since this cannot be deduced from the reported results of any of the screening assays used in this study. Even more, quantitative HCV RNA evaluation of these samples cannot confirm the presence or absence of HCV RNA since due to sensitivity limits of the assay, it is impossible to measure viral loads of less than 2,000 viral copies/ml. Quantitative assays are intended for use in conjunction with clinical presentation and other laboratory markers as an aid in assessing viral response to antiviral treatment as measured by changes in serum or plasma HCV RNA levels. These assays are not intended as screening HCV tests and/or as diagnostic tests to confirm HCV infection.

Several studies suggest ELISA III to be a specific, reliable, and necessary screening assay in routine HCV determination. However, this assay may not be sufficient in all cases of HCV infection (13,14). On the other hand, some patients who demonstrate HCV RNA only in the liver may be designated HCV RNA (–)/anti-HCV (+) due to the limited sensitivity of HCV-RNA in the blood. In addition, this discrepancy could be attributed to post-viral clearance. A positive antibody result indicates that the patient has been exposed to HCV but the test cannot reliably distinguish between acute, chronic, or resolved infection. When normal liver enzyme levels are present in anti-HCV positive patients, or a RIBA 3rd (–)/ELISA III(+) profile is obtained then the patient could be considered either as false positive, or positive but with minimal lesions (15). In such cases, a combination of ELISA III and qualitative HCV-RNA evaluation allows a definitive classification in HCV diagnosis since such a strategy seems unlikely to be fallible though not impossible (16,17). In cases where an anti-HCV negative result is obtained but disease resembles viral hepatitis, it should be taken in serious consideration whether the patient is immunocompromised (18). This is of particular importance especially for patients in renal failure and those infected with Human Immunodeficiency Virus (HIV). In this

case, it seems that PCR diagnosis should be considered and that similar diseases should be excluded. In acute infections ELISA III and subsequent confirmation by RIBA 3rd are often negative (9) since antibodies have not yet been produced. In that case, HCV-RNA-based technique appears to be a valuable tool since it offers the advantage of detecting current HCV viremia (10). Three to nine weeks later, an anti-HCV ELISA III immunoassay may confirm if any seroconversion has occurred (19,20). Hence, because virologic and immunologic profiles often differentiate, we suggest that in certain cases, despite the cost, both ELISA III and RT-PCR assays be considered, and if not, assay selection should be based on a case-sensitive manner and on a cost-effective strategy scheme (21–27). Particularly for high-risk groups such as haemodialysis patients, a combination of the ELISA III screening assay and a standardized RT-PCR method should be highly encouraged.

In view of the above observations, our findings may suggest that ELISA III remains a highly valuable assay in routine HCV diagnosis since it combines a direct evaluation of immune anti-HCV response, simplicity in both handling and performance, and a significantly lower cost. Nevertheless, a combination of ELISA III immunosorbant assay with a standardized RT-PCR assay is the only effective screening strategy to date that could allow a definitive classification in HCV diagnosis.

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