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Evaluation of ECLIA method for the early diagnosis of viral hepatitis B and C

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ABSTRACT

Infections with hepatitis viruses B and C occupy a special place in healthcare, regarding both their clinical and social importance. The objective of this study was to determine the efficiency of the ECLIA (electro-chemiluminiscence immunoassay) method for the qualitative assessment of the most important serological markers of infections with hepatitis B and C viruses. In this purpose, a total of 1825 patients admitted between 01.01.2011 - 28.02.2014 in the CF Witing University Hospital – Bucharest were selected for this study. The highest incidence for chronic hepatitis was found mainly in patients from the urban areas, specifically in the Bucharest region. The age group most affected by these infections both in women and men was that of 35-50 years. In the case of the HBV diagnosis of the total number of patients who had reactive result for the HBsAg, 220 men and 149 women, further determinations were made for the following infection markers: HBsAg, anti-HBs antibodies (Ab), HBeAg, anti-HbeAb, Hbc IgM antibodies and anti-HBc Ab. The HCV population consisted of 63 positive cases, from which 21 were men and 42 women. This study revealed the importance of ECLIA method for the determination of multiple serological markers early required for diagnosis and the initiation of therapy in the first stages of infection. The screening for HBVAg and anti-HCVAb must be used in the case of the risk population for developing infections with HBV or HCV. The advantages of ECLIA method consist in: a minimum amount of sample is required for analysis, short reaction time (18 minutes / test), specificity and high sensitivity (the positive results being confirmed by PCR). The early diagnosis and the initiation of therapy in the first stages of infection would reduce the number of incurable cases, prolongation of overall survival, and improving the patient's quality of life.

Keywords: *ECLIA, chronic viral hepatitis, HBsAg, anti-HBs antibodies, HBeAg, anti-Hbe antibodies, Hbc IgM, anti-HBc antibodies.*

1. INTRODUCTION

Clinical immunology contributes not only to the diagnosis of the pathogenic mechanisms of different diseases, but also to the assessment of the treatment efficacy [1]. Infections with hepatitis viruses B and C occupy a top place, regarding both their clinical and social importance [2,3]. The patients diagnosed with viral hepatitis B and C could develop forms of fatal complications, such as chronic hepatitis leading to cirrhosis or hepatocellular carcinoma [4,5]. A significant role in the pathogenesis of viral hepatitis B and C is held by the acute and chronic immunological processes [6,7]. Combined viral hepatitis cases with a worse prognosis are more common in intravenous drug users and represented by HBV and HCV, or HBV, HCV and HDV co-infections, sometimes associated with HIV [8]. In this context it is necessary to study the peculiarities of viral hepatitis with mixed etiology and to develop diagnostic criteria based on biochemical, serological and virological analyses [9,10]. Immunological analysis is an important step in the early diagnosis of viral hepatitis and correct diagnosis is very important for initiating immediate therapeutic management [11,12]. Of particular interest is the knowledge regarding changes that various viral hepatitis markers undergo during disease evolution and treatment [13,14]. In this context the objectives of this study were: i) to determine the efficiency of the ECLIA (electro-chemiluminiscence immunoassay) method used for the qualitative assessment of the most important serological markers of infections with hepatitis B and C viruses and ii) the evaluation and comparison of sensitivity and specificity results obtained by this method with those provided by confirmatory tests.

2. EXPERIMENTAL SECTION

In this study, a total of 1825 patients admitted between 01.01.2011 - 28.02.2014 in the CF Witing University Hospital - Bucharest, who had specific changes in biochemical parameters of liver were tested for hepatitis B and C using ECLIA method. The blood samples were collected in containers without anticoagulant and centrifuged for 10 minutes at 3500 rpm for serum separation. The patient's sample was mixed with biotin-coupled Ab and Ab complexes labeled with Ru (conjugate) using the ready to use reagent cassettes (compatible with the analyzer). After a 9 minute incubation period the paramagnetic microparticles coated with streptavidine were added to the reaction mixture (solid phase). Following a second 9 minutes incubation time the immune complexes attach to the paramagnetic particles due to the streptavidine's affinity for biotin. The reaction mixture was then introduced into the measuring cell, and the free conjugate was removed via a flushing solution containing TPA. The paramagnetic particles attached to immune complexes particles will be attracted by a magnet located inside the measuring cell. For infectious markers, the results of the cut-off value <1 are considered non-reactive, and the results with cut-off value> 1 are

thought to be reactive. The test lasts for 18 minutes. Before analysis of the patients' samples, the calibration curve was realized using two levels of calibrators, the calibration curve was validated on the basis of signals obtained and comparison with the reference signals and the quality control was performed on two levels: normal and pathological, using lyophilized control sera reconstituted with distilled water. The obtained values have been within the confidence interval [15-17].

3. RESULTS SECTION

In this study we have used the ECLIA method for the detection of serological markers used in the diagnosis of viral hepatitis with B and C viruses on a population of 1825 patients. ECLIA method uses a complex of ruthenium (II) tris (bipyridyl) [Ru (bpy) 3] 2+ with tripropylamine (TPA), which generates a light signal in an oxidation-reduction reaction cycle: Ru (bpy) 32+ has a reactive conjugation site with the analyte. Itt acts as an activating agent (N-hydroxysuccinimide - NHS), and it can be easily coupled with protein amino groups, haptens or nucleic acids, which makes it possible to apply this method to a wide range of analytes. The Ru complex will react with the TPA in the washing solution generating an oxidation-reduction reaction. The next step is applying an electric current to excite the ruthenium and generate a beacon that will enable the detection of Ag-Antibody complexes. The light produced is analyzed by a reader that will convert the signal. The substance concentration is directly proportional to the amount of Ag (Ac respectively for bridging principle) contained in the sample. The advantage of electric initiation of the chemiluminescence reaction is that the overall reaction can be precisely controlled. From the 1825 tested patients, 1186 came from urban areas and 639 from rural areas. Only 1003 were screened for the monitoring of hepatitis B viral infection status based on the known history, in order to analyze de possibility of reinfection or co-infection with other hepatitis viruses.

The diagnosis of hepatitis B based on the the HBs antigen (Ag) marker showed that from the total of 1003 patients, 369 presented reactivity and 634 were non-reactive (Fig. 1).

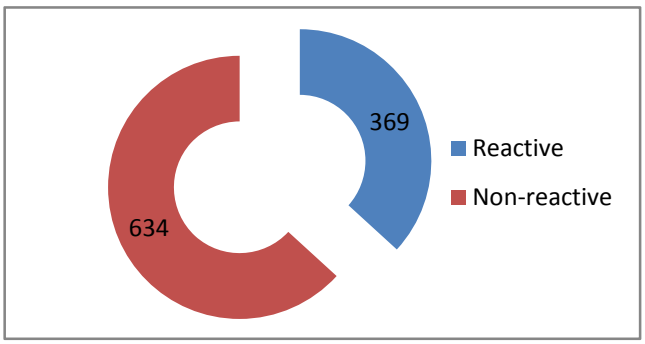

Figure 1. The distribution of the analyzed population depending on the HBsAg reactivity.

In the case of patients positive for HbsAg, further determinations were made for the following infection markers: HBsAg, anti-HBs antibodies (Ab), HBeAg, anti-HbeAb, Hbc IgM antibodies and anti-HBc Ab. The results are summarized in Table 1.

Table 1. Positive results regarding the HBV specific markers.

HBV specific markers												
HBsAg		HBeAg		Anti-HBs		Anti-HBc		Anti-HBc IgM		Anti-HBe	
F	B	F	B	F	B	F	B	F	B	F	B
149	220	17	21	198	182	244	166	8	21	25	17

Of the total number of patients who had reactive result for the HBsAg, 220 were men and 149 women. Both man and women were further grouped into five age groups (Fig. 2).

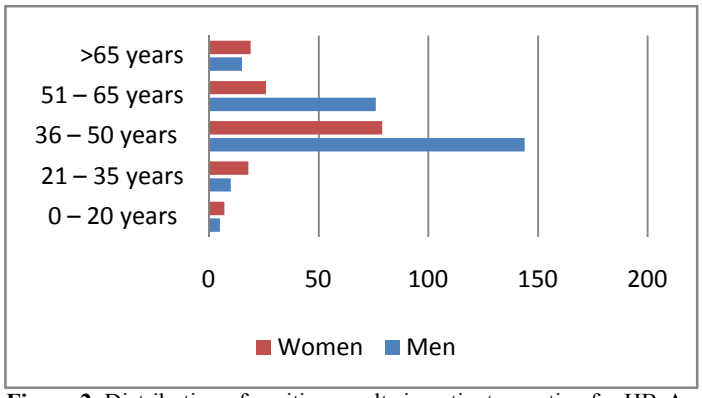

Figure 2. Distribution of positive results in patients reactive for HBsAg depending on age.

The results showed that both women and men within the 36-50 years age group had the highest HBV incidence. The men included in this age group revealed a significantly higher prevalence of HbsAg reactivity as compared to women from the same category.

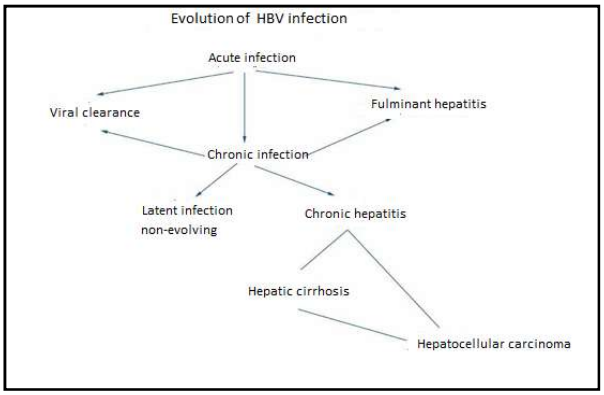

Figure 3. Evolution of the HBV infection.

For all HBsAg positive patients, additional tests were made to determine the clinical status of the hepatitis as well as for the monitoring of vaccinated patients. The stages of HBV infection are shown in Fig. 3.

Determinations were made for the assessment of the HBc-IgM antibodies and of the total anti-HBc antibodies in order to highlight the importance of the main serological markers for

hepatitis B (Fig. 4). The HBc-IgM antibodies are important for the diagnosis of a recurrent infection with HBV.

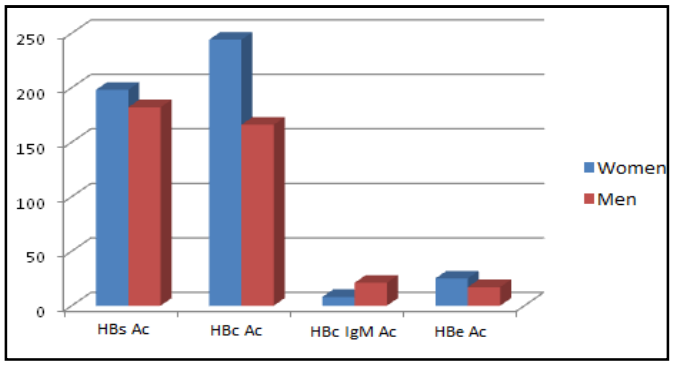

Figure 4. Distribution of HBV specific markers.

The presence of HBc IgM antibodies in the patient's serum is an early indicator of the type B viral hepatitis. A small part of the studied population presented HBc IgM antibodies. In these cases, highlighting the infectious markers present in the early stages of hepatitis B made possible the initiation of the antiviral therapy with a favorable prognosis. Patients who were anti-HBs reactive belonged to the group of patients immunized by vaccination or who have acquired immunity after antiviral treatment.

The presence of anti-HBc antibodies showed the existence of a previous infection and, when correlated with the anti-HBs antibodies present in serum titers greater than 10 IU / ml confirmed the eradication of hepatitis B infection. In the case of the 369 cases of moderate to severe HBV forms, the clinical status and therapeutic outcomes were analyzed by monitoring the transaminase levels. The chronic diseases associated with acute hepatitis B found in this study are shown in Fig. 5.

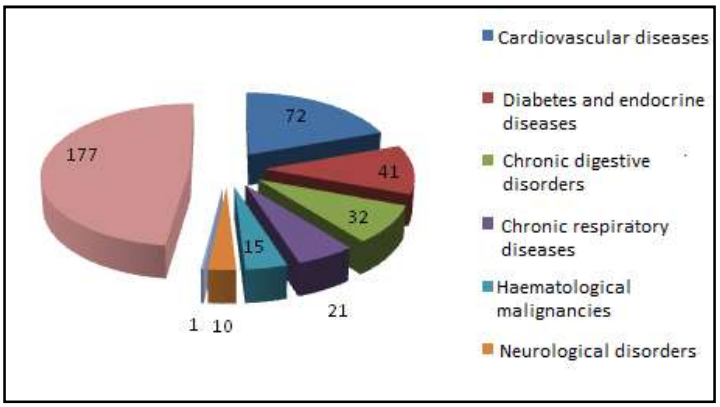

Figure 5. Chronic diseases associated with hepatitis B.

The acute illnesses associated with acute viral hepatitis B were: digestive parasitoses in 95 patients (24.3%), acute respiratory infections in 44 patients (11.2%), gastrointestinal infections in 30 patients (7.6%) and HDV co-infection in 15 patients (3.8%). From the total of the 369 patients with acute viral hepatitis B, 230 patients (59%) had normal transaminases levels, 105 patients (26.8%) had levels 20 times above the normal, while 57 patients (14.2%) had levels 10 times lower than normal. The hepatopriv syndrome was present in 330 patients with moderate to severe forms (84.1%), manifesting both at the level of the carbohydrate metabolism, as well as at the protein metabolism level. Acute liver failure, associated with severe hepatopriv syndrome, caused 12 (3%) cases of hepatic coma and 5 (1.2%) deaths (Fig. 6).

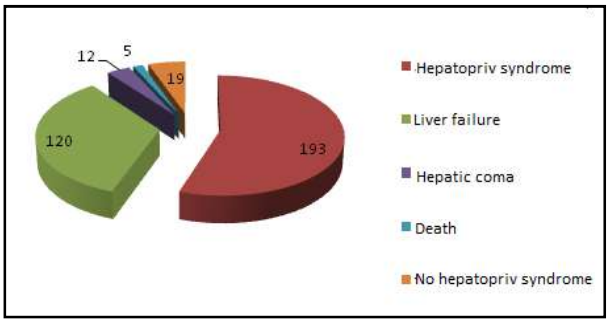

Figure 6. Hepatitis B complications.

Patients with severe forms were investigated by performing abdominal ultrasound imaging, radiographic and CT exams. Liver biopsy was performed in 2 cases with prolonged evolution, persistence of viral markers that indicated progression to chronicization.

In the present study, the population investigated for HCV infection consisted of 822 patients, of which 759 were negative for the presence of anti-HCV antibodies and 63 were positive, 21 being men and 42 women. The patients were assigned to the following age groups: 10 – 30 years; 31 – 55 years; > 55 years (Fig. 7).

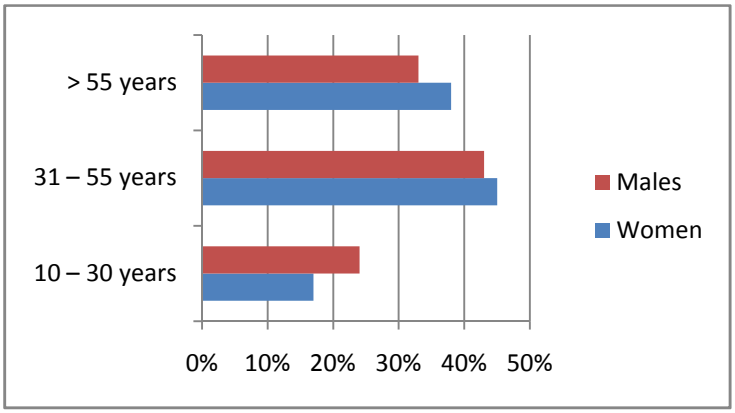

Figure 7. Distribution of the HCV positive studied population according to age.

The prevalence of HCV infection was similar in both sexes and higher in the age group of 31-55 years. In order to achieve a correct diagnosis, the patient's medical history should always be taken into account in collaboration with the clinical examinations and other assessments. Due to the long period of time between infection and sero-conversion, in the early stages of the infection anti-HCV antibodies test can be negative. The antibodies may be absent in the first 4 weeks after contracting the infection in approx. 30% of people. After 3 months post - infection, the antibody titer is detected in 90% of infected people. If acute infection with hepatitis C is suspected, the HCV ARN measurement by RT-PCR may indicate the presence of the HCV infection. To identify the stage of the infection, the liver biopsy is used. The biopsy provides information on the type and severity of liver damage (fibrosis) and is required to determine the specific type of treatment needed. If anti-HCV antibodies are found in the serum of the patient, a HCV infection is highly probable and further tests are needed. Detection of anti-HCV antibodies in the serum of the patient indicates the presence of an active infection with HCV or of a previous one, but it cannot differentiate between acute, chronic or treated infection. The scientific community has recognized that present methods for the detection of anti-HCV

antibodies are not sensitive enough to detect all possible cases of infection with HCV. The concentration of the antibodies may be below the detectable limit of the test or the antibodies do not react with the antigens because of cross-reactivity.

4. CONCLUSIONS

In the study group, the highest incidence was observed for chronic hepatitis, which was found mainly in patients from the urban areas, specifically in the Bucharest region. The age group most affected by these infections both in women and men was that of 35-50 years. Determination of serological markers is an important tool for diagnosis, treatment and prognosis. In order to identify the presence of absence of viral hepatitis, analyses of the correlation between the expressions of several markers is necessary. The screening for HBVAg and anti-HCV Ab must be used in the case of the risk population for developing infections with HBV or HCV. The present study confirms the benefits of immunological tests, the advantages consisting in: a minimum amount of sample is required for analysis, short reaction time (18 minutes / test), specificity and high sensitivity (positive results were confirmed by PCR). The study highlights the need for a combination of immunological and biochemical determinations in hospitals in order to support the correct diagnosis and the implementation of a targeted therapy. This study contributes to understanding the importance of early diagnosis and the initiation of therapy in the first stages of infection, with the purpose to reduce the number of incurable cases, prolongation of overall survival, and improving the patient's quality of life.

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