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Laboratory methods
in the screening of viral C hepatitis

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Abstract

Objective: The study aimed to manage and to analyse the results of the laboratory tests, available nowadays, used from routine clinical practice, for screening of hepatitis C.

Methods: comparison of ELISA method results (Enzyme-Linked Immunosorbent Assay) and chemiluminescence methods results. Beside previously mentioned, the study show the structural comparison of normal liver and pathologic liver with hepatic cirrhosis, using permanent samples colored after the technique protocol. Statistical analysis of this study results, was performed using the laboratory informatic system.

Results: The results of the study are substantial and intricate. For this purpose, the results of preliminary ECL screening method of patients at risk for HCV who took part in the study, are presented in tables and figures. Results of this study are various and are correlate from different perspectives. Also good to mention that the correlations of results were used in order to identify a possible relationships between indicators of ELISA method and ECL index. More than, correlations antibodies detected in ECL and ELISA are point out.

Conclusion: ECL and ELISA method results, are relevant for screening and for diagnostic confirmation in HCV risk patients. Unfotunately in the present study, were impossible to conclude about false-negative results. Good to know our opinion that RT-PCR technique, it is considered proper for the diagnosis of HCV.

Key words: Hepatitis C virus (HCV) , liver, samples, structure, electrochemiluminescence (ECL), ELISA method.(Enzyme-Linked Immunosorbent Assay), antigen-antibodies

Introduction

Viral hepatitis is a common patognomonic condition nowadays. (13)The causes can be varied, complex, insufficiently elucidated, in permanent scientific progress.(16) Potential determinants of the disease are implicated in HCV disease.(2,7,11) The susceptibility to infection of an individual person with the hepatitis C virus is determined by the patient's involvement in potentially environments, for infectiousness.(1) For HCV diagnostic purposes, various highly accurate laboratory methods and techniques are used.(4,6) Corroboration of the results of paraclinical examinations led to the establishment of a conclusive diagnosis of hepatitis C virus infection.(12) The signs and symptoms of patients infected with the hepatitis C virus, along with imaging results, also help to determine the exact diagnosis.(10,17) Knowing the diagnostic o the patients, infected with HCV, medical specialists could aplay the proper treatment. (14,15) HCV infection affect both genders, male and female in specially adults. (3,9)There are known patients diagnosed with HCV, also with comorbidities. (5) Medical specialists try to aplay good traetment for trat and for cure if possible HCV infection.(8)

Material and methods

For this study,investigations were in the OLYMP Clinical Diagnostic Laboratory, Karaganda, from October 2016 to December 2016.

6000 automatic modular analyzer (Roche Diagnostics model), were used for ECL analysis

A Bio RAD immunoassay analyzer, were used for ELISA method. (Enzyme-Linked Immunosorbent Assay). In this laboratory investigation, there are using a set of reagents Vector-BEST (Russia) for detecting total antibodies to each of the 4 antigens of the HCV, concretly the core (core) and non-structural proteins (NS3, NS4, NS5). Interpretation for conformation: tests with a positive result for the core antigen, or with two or three non-structural proteins (NS3, NS4 or NS5) were considered as positive.
Enzyme-Linked Immunosorbent Assay technique .results, were evaluated by optical density (OD) using a microplate spectrophotometer (BioRAD). Critical optical density (COD) is equal to the half-sum of optical density values of two negative control samples plus a correction factor.

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Results:

Statistical Analysis was performed using the laboratory information system LIS (Moscow, Russia) and the online calculator medstatistic.ru was used to calculate median (Me), lower (Q25) and upper (Q75) quartiles. A non-parametric Spearman correlation coefficient was used to determine data correlation.

Spearman correlation coefficient: Formula and Calculation with Example (18)

$$r_R = 1 - \frac{6\Sigma_i {d_i}^2}{n(n^2-1)}$$

Results

The first point and relevant in this study, is the morphologic analyse. In this context, are preparing microscopic preparates for samples with liver. So, following the steps of laboratory protocol, we can show the comparison of normal structure of the liver versus pathologic structure of the liver diagnosed with hepatic cirrhosis.

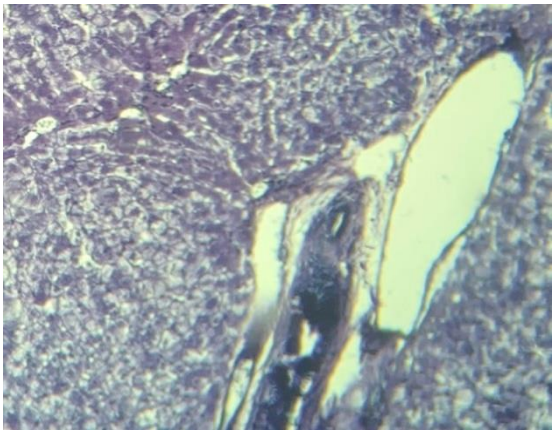

Figure 1. Liver x10. Iron Hematoxylin Heidenhain staining

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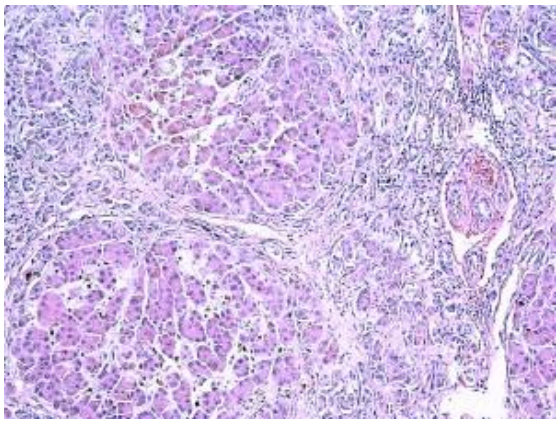

Figure 2. Cirrhosis Liver x10. Hematoxylin and Eosin staining

We noted the nonparametric distribution of the values of S / COat Me30,02 (Q75-Q25). Blood samples positive,were sent for confirmation in the ELISA test.The results of the ELISA and ECLare presented in Table 1.

A-body	N	Me	Q25	Q75
Core	205,00	14,17	10,98	14,50
NS3	205,00	7,18	1,11	11,32
NS4	205,00	1,14	0,18	5,89
NS5	205,00	0,30	0,15	9,65
ECL	205,00	30,02	16,89	49,14

Table 1. Data of ELISA and ECL test in ECL positive samples

Of the 205 samples with a positive result in the ECL test, 176 were confirmed in the ELISA. Of the 176 samples confirmed in the ELISA, 174 had an ECL index higher than 4.0 in only two samples S / CO ratio was 1,0 and 3,0. (figure 3)

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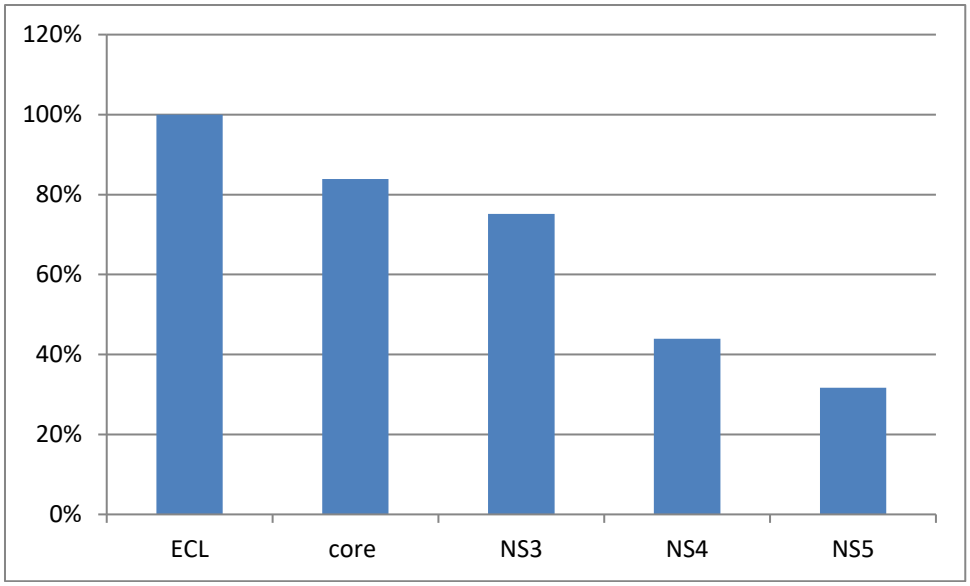

Figure 1. The percentage of positive samples in the ECL test and ELISA depending on antibody markers

As presented in figure 1, of the 205 samples with positive ECLtest, antibodies to core-antigen were detected in 172 samples. The detection rate of antibodies to non-structural antigens NS3, NS4, and NS5 ranged from lower to higher.The positive results of antibodies to NS3 were significantly higher compared to NS4 and NS5 (table 2). At the same time NS4 and NS5 did not differ significantly.

Table 2 The significance of differences* of the antibodies to non-structural antigens in ELISA in patients at risk for HCV

A-body	n	p%	m%	Significance of differences					
				NS3 and NS4		NS4 and NS5		NS3 and NS5	
				z1-2	p-level	z2-3	p-level	z1-3	p-level
NS3	154	75,12	3,48	4,97	0,0000*	1,57	0,119	6,44	0,0000*
NS4	90	43,9	5,23						
NS5	65	31,7	5,77						

Table 2 The significance of differences* of the antibodies to non-structural antigens in ELISA in patients at risk for HCV

As table 2 displays, correlations antibodies detected in ECL and ELISA are outlined.Correlations were used to identify any possible relationships between ECL index and indicators of ELISA, as set out in Table 3.The correlations between the ECL ratio and core antibody were moderate and no relationships between ECL ratio antibodies to nonstructural NS3, NS4, NS5 antigens. The correlations between core antibody and NS3, NS4, NS5 antibodies varied from moderate to weak respectively.The largest correlations is between NS3 and NS4 , also between NS5 and NS3, NS4.

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Antibody	N	Spearman's Rank Correlation Coefficient	Student Criterion T (N-2)	p-level
ECL& core	205	0,54	2,04	0,0425
ECL& NS3	205	0,09	1,28	0,2013
ECL& NS4	205	0,00	0,02	0,9840
ECL& NS5	205	-0,06	-0,92	0,3587
core & NS3	205	0,48	7,81	0,0000
core & NS4	205	0,42	6,66	0,0000
core & NS5	205	0,32	4,86	0,0000
NS3 & NS4	205	0,67	12,72	0,0000
NS3 & NS5	205	0,63	11,54	0,0000
NS4 & NS5	205	0,63	11,66	0,0000

Table 3. Correlation of indicators the ECL and ELISA

Discussion

Knowing the data collated in 2016, we can conclude after results of the study, that the incidence of the HVC, is higher than in the one of the previous period studied, respectively, 2004-2009. Electrochemiluminescence method and Enzyme-Linked Immunosorbent Assay method, is not able to show unclear results as false negative or false positive, in HCV diagnostic. It is important to test vein blood using RT–PCR technique, in order to exclude false results.Antibodies detected in samples and HCV core antigen play a signifiant role in a diagnostic for HCV infection[16]. In this context antibodies to NS3 are specific for diagnosis the early stages of hepatitis C. Also is considered as an independent diagnostic marker of the HCV acute process. The number of positive samples with NS4 and NS5 antibodies significantly less in comparison with NS3, show us in this study that a higher number of patients were in the acute form of HCV. The cronicisation process of the liver distruction could be relevant studing in the laboratory,anti-NS4 and anti-NS5.The study show also differents correlative results between the ECL ratio and antigens as NS3, NS4, NS5.

Conclusion

The study showed that ECL and third generation of ELISA are important for screening and for conformation HCV risk patients.

Antibodies defined in the ECL test correlated with core antibodies ELISA are also good to know for a proper diagnostic.

The highest correlation were among antibodies to non-structural antigens. NS3, NS4, NS5 antibodies had an independent value for the differentiation of an acute or chronic process.

The study cannot conclude about false negative and false positive in this study. So unclear to find the presumptive enlarged “gray zone”, including false results.

RT-PCR technique is consider one of the modern and with high potential for the diagnosis of hepatitis C patients, in order to confirm or to exclude this disease.

ABBREVIATIONS

HVC (VIRAL C HEPATITIS)

ECL (Electrochemiluminescence)

ELISA (Enzyme-Linked Immunosorbent Assay)

RT – PCR (Reverse transcription polymerase chain reaction)

NS3, NS4, NS5 (antigens)

Author Contributions:

Conceptualization, A.M. and G.A.; methodology, G.A.; software, B.K.; validation, S.A.; formal analysis, A.C.; investigation, A.M; resources, A.M.; data curation, G.A.; writing—original draft preparation, A.C., T.S; writing—review and editing, A.C. T.S; visualization, G.A.;

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