The screening of the patients having risk for hepatitis c in low-income and middle-income countries: A comparison of elisa and chemiluminescence methods.

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Abstract

Objective: Hepatitis C virus (HCV) is one of the widespread causes of death and morbidity among viralhepatitis. The incidence rates of chronic viral hepatitis C in the Republic of Kazakhstan increased. The study aimed to compare two tests for screening hepatitis C available in low-income and middle-income countries for routine clinical practice.

Methods: Comparison of ELISA and chemiluminescence methods. Statistical analysis was performedusing the laboratory information system.

Results: The results of preliminary ECL screening of patients at risk for HCV who took part in the study, are presented in tables and figures. Results of the study are complex and relevant, for the purpose of the study,correlations were used to identify any possible relationships between ECL index and indicators of ELISA. Also correlations antibodies detected in ECL and ELISA are outlined.

Conclusion: The study showed that ECl and third generation of ELISA are good diagnostic tools for screening and conformation HCV risk patients. Antibodies defined in the ECL test correlated with core antibodies ELISA. We cannot conclude anything about false-negative results since our studies did not allow us to identify patients in the "gray zone". For the effective diagnosis of hepatitis C patients, it is necessary to confirm and also exclude by RT-PCR.

Keywords: Hepatitis c virus (HCV) infections, Electrochemiluminescence (ECL), Anti-HCV tests, ELISA.

Introduction

HCV is one of the widespread causes of death and morbidity among the different types of hepatic viruses. Globally, an estimated 71 million people have chronic hepatitis C virus infection. A significant number of those who are chronically infected will develop cirrhosis or liver cancer. (Figure 1a and 1b). WHO estimates that, as of 2016(the latest published data), approximately 399 000 people died from hepatitis C, mostly from cirrhosis and hepatocellular carcinoma (primary liver cancer).

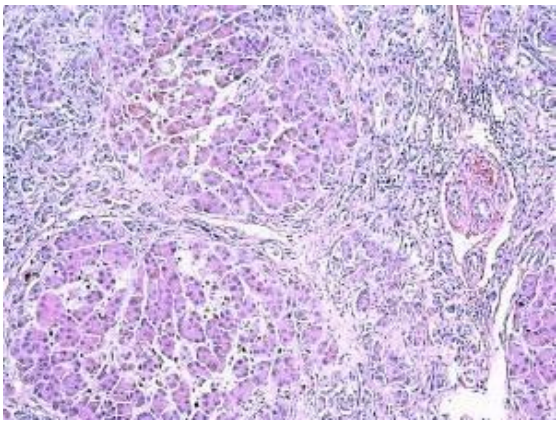


Figure 1a: *Cirrhosis Liver Hematoxylin and Eosin x20*.

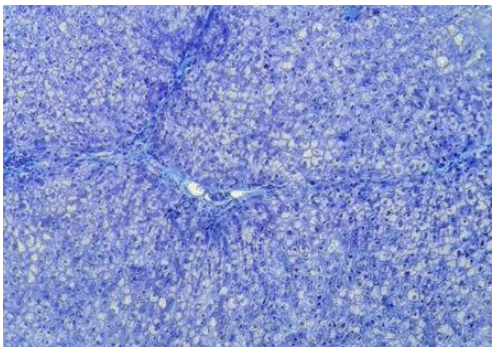
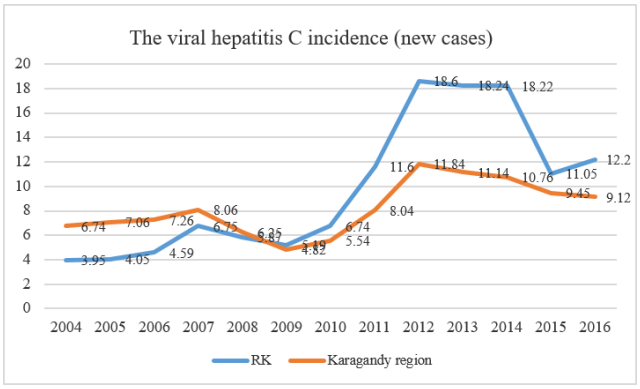


Figure 1b: *Normal Liver Masson stain x10.*

It has been estimated that while the incidence of HCV infection seems to decrease in the developed world, mortality secondarily related to HCV infection will continue to increase over the next 20 years. The 67 low-income and middle-income countries considered included 52 million people living with hepatitis C virus (90% of the world's total). The progress (by date) of HCV incidences in Kazakhstan and Karaganda are summarized in (Figure 2).

Figure 2: *The dynamics of the incidence of viral hepatitis C per 100,000 population.*



The diagram shows that the incidence rate of hepatitis C in both Karaganda and the Republic stands high (at 12.22 and 9.12 respectively) in 2016 compared with the period2004-2009.

The increase in the incidence of HCV in KZ is associated with two trends: a real increase in the incidence and the consequence of improvements in diagnosis. According to the order of the Minister of Health of Kazakhstani N 451 of June 3, 2017viral hepatitis is included in the list of socially significant diseases that are dangerous to others. It should be noted that in recent years in RK, the detection of viral hepatitis has been associated with the expansion of the list of persons to be examined (medical workers, people before surgery, patients of centers and departments of hemodialysis, hematology, oncology, transplantation, cardiovascular and pulmonary surgery, drug users and others). Antiviral therapy of viral hepatitis is reflected in the order of the Minister of Health of the Republic of Kazakhstan dated May 4, 2019. We expect new prospects for reducing the incidence of viral hepatitis in the Republic of Kazakhstan, especially in the context of accurate and sensitive diagnosis of HCV infection being important.

For screening patients with a high risk of hepatitis C, we conducted two effective and possible in our conditions tests of ECL and ELISA of the third generation. ECL is a process of electrogenerated chemiluminescence that combines the advantages of both electrochemical and photoluminescence analysis. The ECL method possesses excellent characteristics such as, speed of response, economic, simple operation processes and high sensitivity, and has been widely used in the detection of antibodies. In contrast, ELISA is more sensitive and specific than Chemiluminescence for blood transfusion screening. Third-generation ELISA screening is recommended for confirmatory tests for HCV antibodies.

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. The characteristics for this method are shown in Table 1.

Table 1: *Statisical basis for Spearman correlation coefficient (Adapted from Dencey and Reidy 2011).*

Sperman (r)	Correlation
≥ 0,70	Strong relationship
4-6	Moderate relationship
1-3	Weak relationship

The statistical significance of the correlation coefficient was estimated by Student's criterion. Level of significance was at p <0.05.

Literature review

Data presented in this paper was drawn from a pilot project in the city of Karaganda, the Republic of Kazakhstan. Investigations were carried out in the OLYMP Clinical Diagnostic Laboratory from October to December 2016. 1369 patients at risk HCV were investigated (identified as those who have a history of numerous parenteral manipulations, including 24 patients with renal dialysis). From venipuncture, a blood sample (5 ml) was collected in a Vacutainer with a separation

gel. Blood sample was centrifuged. Part of the serum was used for initial testing for antibodies to HCV by ECL-test; positive sample sent for confirmation by ELISA.

ECL analysis was performed using a Cobas 6000 automatic modular analyzer (manufactured by Roche Diagnostics). Anti-HCV-total test system includes 3 recombinant antigens: c22-3, c200 and NS-5 to determine total antibodies (IgG + IgM).

Procedure steps:

1. Pipetting of the sample, reagent and microparticles.
2. The reaction mixture is supplied for measurement.
3. Each cycle is performed within 42 seconds.

4. The number of pipetting steps and preparation of the reaction mixture depend on the test.

Some tests require dilution with a diluent, which increases the number of pipetting steps. The incubation time at 37 °C ranges from 4.5 to 9 minutes depending on the test.

Calibration and quality control in ECL: Calculation of the calibration curve was carried out at the time of production of the reagent and it was encoded in a 2-dimensional bar code of the corresponding set with the reagent. This information is then read by the analyzer. Monitoring the operation of the analyzer was conducted with2 levels of controls (norm and pathology).Calibration and quality control were performed for each set of the reagent.

Results for the ECL assay were expressed as a signal to cut-off (s/co) ratio; a s/co ratio<0.9 was a negative and s/co ratio ≥1.0 indicated positive result. A s/co ratio within 0.9 and <1.0 was considered to fall into “a gray zone” and these serum samples were considered negative for calculations this study.

The ELISA was performed on a Bio RAD immunoassay analyzer, using a set of reagents Vector-BEST (Russia) for detecting total antibodies to each of the 4 antigens of the HCV: the core (core) and non-structural proteins (NS3, NS4, NS5). ELISA results were evaluated by optical density (OD) using a BioRAD microplate spectrophotometer. An OD index (coefficient of positivity) was calculated by OD of the sample to OD of a cutoff provided in the kit or critical OD. Critical optical density (COD) is equal to the half-sum of optical density values of two negative control samples plus a correction factor. In this series, the correction factor was 0.25.COD = (OD-1 + OD-2) / 2 + 0.25. OD index values of ≥1 were considered positive.

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.

Results

The results of preliminary ECL screening of 1369 patients at risk for HCV are presented in Table 2.

Table 2:*Analysis of C results.*

s/co ratio < 1,0		s/co ratio ≥ 1,0		Total	
n	%	n	%	n	%
1164	85,06	205	14,94	1369	100

The table indicates that 205 (14, 94%) samples were positive and 1164 (85, 06%) negative, but S / CO ratio values ranged widely. The data of the positive S / CO ratiosare summarized in Table 3.

Table 3:*Values of S / C ratio in positive samples.*

S/CO ratio	1-4	5-10	11-20	21-30	31-40	41-50	51-60	61-100	100 >	Total
n	17	19	35	48	29	22	16	15	4	205
%	8,29	9,27	17,07	23,42	14,15	10,73	7,81	7,31	1,95	100

We noted the nonparametric distribution of the values of S / CO at Me30, 02 (Q75-Q25 49, 14-16, 89).205 positive blood samples were sent for confirmation in the ELISA test.

Discussion

The incidence rate of hepatitis C in both Karaganda and the Republic is high (12.22 and 9.12 respectively, based on data collated in 2016). These levels stand-out as specially high compared with the time period 2004-2009. This is drawn out from our data. Examination of the risk group for HCV showed that 205 (14.94%) samples had a positive result in the ECL test, S / CO ratio values ranged widely. From 205 positive samples in the ECL test 176 (85.85%) were confirmed in the ELISA test. Of the 176 samples confirmed in the ELISA, 174 (98, 86%) had S / CO ratio higher than 4.0. Our studies have shown that the diagnostically significant index of the ECL test is higher than 4.0. Unfortunately, we cannot say anything about the false negative results of the ECL test and “gray zone”, since we did not examine the ECL-negative samples with the ELISA. We also could not exclude a false negative result in the ELISA which may be during the HCV window period. Therefore, it is very important to screen blood using RT–PCR to avoid false positive and false negative results. Antibodies to core-antigen were detected in 172 (83.9%) samples. HCV core antigen has a relative strong role in a diagnostic algorithm for HCV infection. Anti-NS3 detected in 154 (75.12%) samples. Antibodies to NS3 are characteristic of the very early stages of hepatitis C and can be an independent diagnostic marker of the acute process. We believe that the majority of patients with hepatitis C were in the acute period. This is also evidenced by the number of positive samples with NS4 (43.9%) and NS5 (31.7%) antibodies significantly less in comparison with NS3 (75.12%).Anti-NS4 and anti-NS5 usually appear in a later period of the disease and also sign of chronization. The correlations between the ECL ratio and core antibody of were moderate (r=0, 54), and no relationships between ECL index and antibodies to nonstructural antigens NS3, NS4, NS5. The correlations between core and NS3, NS4, NS5 antibodies varied from moderate to weak r=0, 48; 0, 42; 0, 32 respectively. The largest correlations is between NS3 and NS4 (r=0, 67), also between NS5 and NS3, NS4 (r= 0,63).

Conclusion

Our study showed that ECl and third generation of ELISA are good diagnostic tools for screening and conformation HCV risk patients. Antibodies defined in the ECL test correlated with core antibodies ELISA. 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. Unfortunately, we cannot concludec anything about false-negative results since our studies did not allow us to identify patients in the "gray zone". For the effective diagnosis of hepatitis C patients, it is necessary to confirm and also exclude by RT-PCR.

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