Methods for Laboratory Diagnostic in Patients Presumptive Infected with Viral Hepatitis C

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Article

Methods for Laboratory Diagnostic in Patients Presumptive Infected with Viral Hepatitis C

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Abstract

One of common human disease nowadays is the well known the viral Hepatitis C (HCV). Untreated corectlly, viral hepatitis could be one of the most causes of morbidity and mortality. Actually, the incidence rates of chronic viral hepatitis C in the Republic of Kazakhstan have increased. Using in the laboratory, specific tests, the proposed aim is to identify the viral genome. We present data related to the screening of risk groups for hepatitis C and also the diagnostic significance and finally show their results, for disease diagnosis.

Keywords: patients; tests; Hepatitis C virus (HCV); techniques; diagnostic

1. Introduction

Hepatitis C virus (HCV) were identified in 1989. Actually are known seven types of genotypes. So from many different reasons is diagnosed in peoples from all of the world [1–3].

Globally, an estimated 71 million people have chronic hepatitis C virus infection. [4] A significant number of those who are chronically infected will develop cirrhosis or liver cancer. A proper treatment has a key point reffering to the usage of highly active direct-acting antivirals (DAAs) from years ago, concrely 2011. Actually are known three classes of antivirals (DAAs), used in different combinations for a proper treatment in patients diagnosed with Hepatitis C virus (HCV) [5–7].

The progress (by date) of HCV incidences in Kazakhstan and Karaganda are summarized in (Figure 3).

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 period 2004-2009.

For screening patients with a high risk of hepatitis C, we conducted two effective and possible in our conditions tests of ElectroChemiLuminescence (ECL) and ELISA of the third generation [8–10]. Third-generation ELISA screening is recommended for confirmatory tests for HCV antibodies [11].

There are known risk factors for a possible contamination with virus C which conduct to the liver illness installation in susceptible persons [12]. Risk factors are perinatal transmission, Injection or intranasal drug use, sexual transmission, exposure to contaminated blood, tattoos, and corelated nonsterile methods and list in relatively not to short [13–22].

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.

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Table 1. Statisical basis for Spearman correlation coefficient (Adapted from Dencey and Reidy 2011[15]).

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 $p < 0.05$.

2. Materials and Methods

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), following specific steps.

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 with 2 levels of controls.Calibration and quality control were performed for each set of the reagent.

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.

3. Results

We noted the nonparametric distribution of the values of S / CO at Me 30,02 (Q75-Q25 49,14-16,89). 205 positive blood samples were sent for confirmation in the ELISA test. The results of the ELISA and ECL are presented in Table 1.

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

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

We are also noted the nonparametric distribution of the antibody to **NS3-** Ag, at Me 7,18 (Q75-Q25 11,32-1,11); **NS4-** Ag at Me 1,14 (Q75-Q25 5,89-0,18) and **NS5-** Ag at Me 0,3 (Q75-Q25 9,65-0,15).

Of the 205 samples with positive ECL test, antibodies to core-antigen were detected in 172 samples (83.9%). The detection rate of antibodies to non-structural antigens NS3, NS4, and NS5 ranged from 31.7% to 75.12%. 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.

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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						

The correlations between core antibody 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). (Table 3).

Table 3. Correlation of indicators the ECL and ELISA.

Antibody	N	Spearman's Rank Correlation Coefficient	Student Criterion T (N-2)	p-level
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

4. Discussion

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 yo patients with different age and different social status. 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 ill liver. 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).

5. Conclusions

As a nowadays extended in all parts of the world, viral C hepatitis, may be well investigate and also vell diagnosed and treat. So modern methods are in daily usage. Also a proper treatment as we mentioned above play a great role in patient life. Management methods and future trends are also in attention.

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