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Validation of Statistical Quality Controls to Improve HCV Detection: An Efficient Approach for Accurate Diagnosis

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Abstract

HCV is the RNA enveloped virus belonging to the family of flavivirus, causing serious hepatic problems all over the world. While screening for HCV, many of the results false negative or false positive which reach chronic stages of the infection and eventually cancer. This study was conducted at molecular laboratory of University of Agriculture Faisalabad from June 2021 to September 2021. The aim of this study was to estimate various parameters like sensitivity, specificity, accuracy, likelihood ratios for HCV-RNA PCR and ELISA. All parameters for both diagnostic tools were compared. Sensitivity for HCV-RNA PPCR was 96% but for ELISA was 57%. Specificity for ELISA was 95% whereas 98% for HCV-RNA PPCR. The PPV of the HCV RNA-PCR test was calculated as 80% and 94% for PCR. Accuracy for both results varied as well showing among their efficiency and capacity. Results showed that HCV-RNA PCR were quite accurate and more reliable than the ELISA.

Introduction

HCV is the most prevalent deadly virus along the globe with various genotypes. HCV is an enveloped RNA virus i.e single stranded, and small in size belonging to the family of flavivirus with a high level of genetic diversity [1,2]. It is the only member in the genus of hepaci-virus which was identified in 1989 [3,4]. A huge nucleotide diversity was reported in the isolates after the discovery of HCV virus [5,6]. Currently, HCV has been classified into 11 genotypes (assigned as 1-11) that differ in their nucleotide sequences from 30% to 50%, six of which are major genotypes [7,8]. The distribution of the genotypes and subtype of HCV varies geographically [9,10]. Almost, 170 million people get infected with the HCV virus globally [11]. HCV is the third major reason of death due to the liver cancer worldwide [12]. Major cause of the prevalence is the undiagnosed individuals because mostly are asymptomatic at the early stages [13]. Without any therapy, acute HCV turns into chronic HCV, which increases the risk of liver diseases leading to the carcinoma and complete failure eventually [14]. The transfer rate of HCV is increasing rapidly and the reason of HCV prevalence in developing countries is the poor diagnosis and limited accessibility of the expensive quantitative assays. Therefore, the quantitation of HCV RNA load in serum or plasma tests can underrate the

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absolute circulating viral load [15]. The gaps in the diagnosis of HCV can be eliminated with efficient strategy like expanding the accessible and less expensive diagnosis and treatment [16]. HCV qPCR should be performed if screening is positive. The problem with the screening of the HCV is that many of the results declared as false positive and false negative [10] even if they are HCV positive which eventually leads to the chronic stages of the disease in the form of cancer. Hence, the betterment in the procedure to identify the HCV positive individuals effectively and directing them to proper treatment can decrease the risk of serious and severe issues thus helping in the elimination of deadly virus gradually.

Methodology

This study was conducted at molecular laboratory of University of Agriculture Faisalabad from June 2021 to September 2021. Four cities (Gojra, Samundri, Toba and Kamalia) peripheries of Faisalabad were targeted. Samples of the participants were obtained from the PINUM Cancer Hospital and Allied hospital Faisalabad. 1100 participants both male and female who had pre-diagnosis of HCV infection were selected for this comparative study. All the selected patients were informed and signed the consent form to use their information for the research analysis and Ethical Committee approved as well. All of the collected samples were centrifuged to separate serum and stored at -20c to -80C with the assigned codes until testing.

In this study, sensitivity and specificity of a modified third generation ELISA kit was compared with the SYSTAAQ HCV RT-PCR assay. HCV antibodies were assessed with the QIAGEN ELISA kit on the stored samples. As per manufacturer's instructions, a cut off value of <1.00 were considered as negative whereas >1.5 as positive and 1.0-1.5 as positive. According to the manufacturer's instructions, HCV viral load was measured on real-time PCR of $< 10^1$ IU/ml were considered as low levels of virus and $>10^1$ IU/ml showed positive results indicating the presence of virus.

For the statistical summary and data analysis, SPSS version 22.0 for windows was used. The results of specificity and sensitivity were obtained in the form of percentage. 2x2 cross tabulation of true disease status and test results were used for the calculations of the test performance measures. Sensitivity of both tests was calculated as the total number of true positive test results divided by the sum of false negative and true positive test results. Specificity was calculated as the number of true negative test results divided by the sum of true negative and false positive test results. Negative Predictive Values (NPV) were calculated as the number of true negative divided by the sum of false negative and true negative test results. Positive Predictive Values (PPV) were calculated as the number of true positive divided by the sum of true positive and false positive test results. NPV and PPV are affected by the prevalence of the disease whereas NPV shows a reverse relation with PPV. PPV tends to increase with the increase in prevalence and NPV decreases. Likewise, PPV decreases with the decreased prevalence and NPV increases.

Results

Out of the 1100 HCV suspects after the antibody testing by ELISA confirmed, 249 (23.8%) were infected and 799 (76.2%) un-infected. These results were divided into two groups i.e. infected and un-infected subjects. Antibody testing with the same procedure was performed again on 249 infected patients, of all 144 were positive and 105 were negative (un-infected). Other group consisting of 799 un-infected individuals showed positives results for 36 (whereas negatives for 763 declaring them un-infected. Overall the percentage ratio of positive results in both groups was 17.2% and negative was 82.8%.

For the detection of HCV RNA, the same samples of 1100 subjects were tested by RT-PCR diagnostic method. Of all 796 (76.2%) were sero-negative and 249 (23.8%) were sero-positives. There were some expected chances of the presence of sero-positives among the sero-negative results. Upon further testing of 796 sero-negatives, 14 plasma samples were sero-positive and 782 were declared as uninfected. Many of the individuals were still expected to be HCV RNA negatives even in the sero-positive results. Further testing of HCV RNA in 249 sero-positive, 9 were negative and 240 were positive.

However, the negative results obtained by the molecular diagnostic methods applied on positive results were considered as false-positive whereas positive results declared among the negative results were considered as false-negatives. Overall sensitivity of RT-PCR and ELISA was calculated as 96.386% and 57.831%, specificity as 98.241% and 95.494% respectively.

Figure 1: Various parameters calculated for ELISA and RT-PCR

Parameters	Estimate for ELISA	Interval (ELISA)	Estimate for RT-PCR	Interval (RT-PCR)
Sensitivity	57.831%	51.434 to 64.040 %	96.386%	93.250 to 98.334 %
Specificity	95.494%	93.817 to 96.825 %	98.241%	97.067 to 99.035 %
AUC	0.767	0.740 to 0.792	0.973	0.961 to 0.982
Positive Likelihood Ratio	12.835	9.169 to 17.968	54.802	32.589 to 92.157
Negative Likelihood Ratio	0.442	0.382 to 0.511	0.037	0.019 to 0.070
Disease prevalence	23.760%	21.212 to 26.456 %	23.828%	21.274 to 26.530 %
Positive Predictive Value	80.000%	74.076 to 84.847 %	94.488%	91.067 to 96.647 %
Negative Predictive Value	87.903%	86.260 to 89.374 %	98.862%	97.861 to 99.398 %
Accuracy	86.546%	84.329 to 88.555%	97.799%	96.716 to 98.600 %

The sensitivity and specificity of both theses molecular diagnostic tests were not similar. All the other parameters for both diagnostic methods such as positive likelihood ratios, negative likelihood ratios, PPV, NPV, accuracy and the prevalence of the disease showed different values shown in **Table 1**. The accuracy for both was different showing 97.799 % and 86.546 % respectively. Comparison of negative and positive ratios of testing for both ELISA in **Figure 1** and in PCR **Figure 2** are shown.

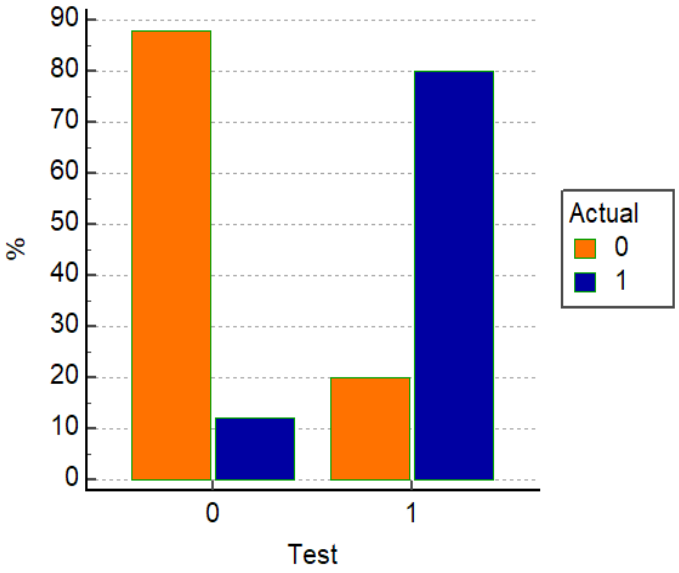
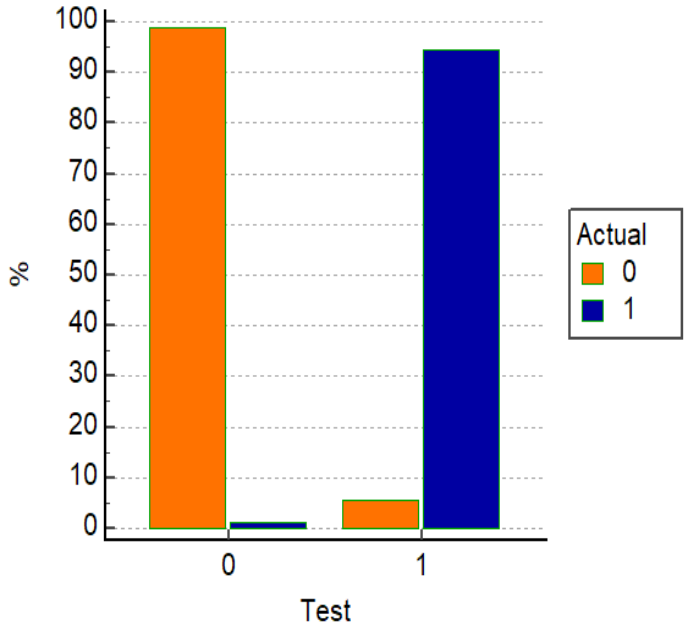


Figure 1: (ELISA) The figure is showing the results of two testing groups used for the analysis. Group (0) is indicating the ratio of controlled or normal subjects (orange colored bar) out of which some positive subjects (who were infected) were detected upon further testing (shown with blue colored bar). Second group (1) is the set of infected patients (blue bar) out of which presence of some negative subjects (orange bar) with no infection were detected.



Graph 1: (RT-PCR): The figure is showing the results of two testing groups used for the analysis. Group (0) is indicating the ratio of controlled or normal subjects (orange colored bar) out of which some positive subjects (who were infected) were detected upon further testing (shown with blue colored bar). Second group **(1)** is the set of infected patients (blue bar) out of which presence of some negative subjects (orange bar) with no infection were detected.

Discussion

Infection with the Hepatitis C Virus (HCV) is problematic. It is the third main cause of cancer causing deaths around the world [12]. Many of those infected are not aware of their condition because mostly are asymptomatic in the early stages. Without any therapy, acute HCV turns into chronic HCV, which increases the risk of liver diseases leading to the carcinoma and complete failure eventually. Usually, severe complications or death may occur in the individuals with long term infection turned into cirrhosis which can be developed in to 15-20% patients approximately. HCV can be spread via some careless ways of blood transfusions, using contaminated instruments and unsterilized needles during the process of medical care etc globally. One less efficient route of infection is the blood exposure to the mucus membrane. Besides liver, the complications can be extra hepatic as diverse as lymphoma, diabetes, kidney diseases as well. The detection of HCV can be done in breast milk, saliva, semen and other fluids that are not considered efficient mode of transmissions. The HCV diagnostic assays available are expensive and out of reach so, the standardization of them is crucial. However, a small ratio of population has the accessibility for HCV RNA test leaving a huge ratio of undiagnosed individuals [10]. The problem with the screening of the HCV is that many of the results are resulted as false negative [10] even if they are HCV positive which eventually leads to the chronic stages of the disease in the form of cancer. The major issue of the HCV prevalence in populated areas is the unawareness about its disasters and types. It is possible that any unknown factor is involved in the prevalence of virus. Hence, the betterment in the procedure to identify the HCV positive individuals effectively and directing them to the treatment centers can decrease the risk of serious and severe issues thus helping in the elimination of deadly virus gradually.

For the estimation of the ratios and inspection of HCV's tests, this study was conducted for which samples from the live patients were collected and the procedure of RT-PCR and ELISA was applied on them. The percentage ratio for the positive testing was 24.3% and healthy was 75.5% in PCR testing. Whereas, ELISA showed 17.2% percentage ratio for the positive testing and healthy was 82.8%. This number determines whether the viral load is high or low. The gathered data and results were compared along with the healthy patients by using a statistical tool SPSS 22.

Diagnostic test sensitivity, specificity, positive predictive value, and negative predictive value are words used to characterize a test's capacity to discover a person with disease or exclude a person without disease. It is emphasized that while sensitivity and specificity are significant indicators of a test's diagnostic accuracy, they are useless in assisting clinicians in estimating the likelihood of illness in particular patients. Despite the fact, that predictive values may be used to estimate the risk of a disease, both prognostic and predictive values. This means that using predictive values generated for one group to another with a different disease prevalence would be inaccurate. Conclusion: Sensitivity and specificity are significant indicators of a test's diagnostic accuracy, but they can't be utilized to expect the likelihood of illness of an individual patient. Positive and negative predictive values offer estimates of illness likelihood, although both parameters change depending on the prevalence of the condition. The graphs were made for the feasible approach towards the understanding of the ratio among the people. Study showed that results of PCR were more accurate over ELISA. ELISA detects the antibody while HCV virus still can be inactive whereas PCR is able to detect the exact viral load in your blood stream in IU/ml. This number determines whether the viral load is high or low.

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