

Comparison of Immunochromatography test (ICT) with real time polymerase chain reaction (RT – PCR) in detection of HCV.

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Abstract

Objectives: To compare ICT rapid test with RT- PCR.

Methodology: This study conducted at Pathological Laboratory Muhammad Medical College Hospital during July-August 2016 with collaboration of The Health Foundation District Sanghar at Goth Haji Khair Muhammad Junejo Kandiyari Dist: Sanghar Sindh. 400 subjects from whole the population approximately 674 persons of Goth Haji Khair Mohammad Junejo taulka kandiyari district Sanghar were selected in between the age of 10 years to 70 years. The purpose was to treat them if they are HCV positive. All the subjects were first screened for Anti – HCV on ICT method, and then all positive cases were confirmed on RT – PCR.

Results: This study conducted during the month of July and August 2016. Total 400 asymptomatic subjects from both male and female were screened by ICT method for Anti – HCV. Out of 400 subjects, 142 (35.5%) were positive. The serum of all 142 subjects positive on ICT, sent to Rawalpindi for HCV qualitative test on RT – PCR, HCV detected in 84 (59.16%) cases and 58 (40.84%) cases were negative. The ratio of variation (ICT: RT-PCR) was 1.44.

Conclusion: Rapid ICT method is less sensitive compare to RT – PCR. There is variation of results in between two methods. All positive cases on ICT must be confirmed on RT – PCR. There is false positivity in ICT method.

Key word: ICT (Immunochromatography test) RT – PCR (Real time polymerase chain reaction) RIA (Radio immunoassay) ELISA (Enzyme linked immunosorbent assay) EIA (Enzyme immunoassay).

Introduction:

Hepatitis C is single stranded enveloped flavivirus. It has identified in 1989 and formerly referred to as non-A non-B virus. HCV exists closely related genetic variants known as quasi species. Six genotypes are recognized. The virus infects liver cells, only small numbers of virus are excreted and circulate in the blood¹. Infection with HCV is often asymptomatic only about 10% of individuals become jaundiced. WHO however estimates that worldwide there are about 170 million chronic carriers of HCV at risk of developing liver cirrhosis and liver cancer². Chronic hepatitis C following acute infection develops in 70 – 80 % of individuals. Different methods are used for detection of hepatitis C virus, which are ICT, ELISA, EIA and PCR. The ICT kits are frequently used, ICT method is easy to perform and do not need higher equipment, manpower or infrastructure³. It is rapid and less expensive compare to other method which are expensive, time consuming and requiring specialized skills, equipment and manpower⁴. ICT kits are readily available and employed on minimal expenses but their sensitivity and specificity are less as compare to other methods.

Methodology:

This study had been conducted with collaboration of the health foundation district Sanghar at Goth Haji Khair Mohammad Junejo Taulka Kandiyari district Sanghar. From whole population approximately 674 persons of village, 400 subjects in between age of 10 years to 70 years were selected for the purpose to treat them if they are HCV positive. Selected subjects were screened for Anti – HCV by immunochromatography test at pathology laboratory Muhammad Medical College Hospital Mirpurkhas Sindh Pakistan. All cases which were positive on ICT, sent to Genetix Lab Rawalpindi for comparison of results on HCV

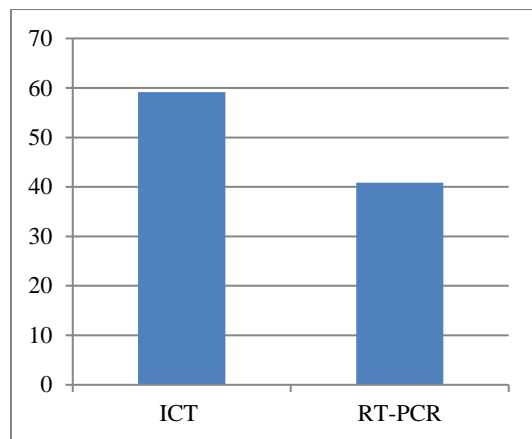
qualitative real time polymerase chain reaction (RT – PCR) by TaqMan probes. The ICT Kits used for detection of Anti – HCV were from various manufacturers available in market. Serum was used by collecting whole blood into the collection tube (not containing anticoagulant) by venipuncture, leaved to settle for 30 minutes for blood coagulation and then centrifugated to get serum specimen of supernatant. The results were interpreted in recommended time. The serum of all positive cases on ICT, extracted again and sent in cold chain to Rawalpindi for confirmation of results on RT – PCR. In molecular diagnosis, real time polymerase chain reaction is a laboratory technique used to monitor the progress of a PCR reaction in real time. Real time PCR is based on the detection of the fluorescence produced by reporter molecule which increases as reaction proceeds. This occurs due to the accumulation of the PCR product with each cycle of amplification these fluorescent reporter molecules include dyes that are sequence specific probes.

Results:

This study conducted during the months of July and August 2016. Total 400 asymptomatic subjects from both sexes. Among them 185 (46.25%) were male and 215 (53.75%) were female. The age of patient ranges from 10 years to 70 years, while male to female ratio was 1.16.

Age	Sex	
	10 Years to 70 Years	Male 185 (46.25 %)
n : 400	Male to Female Ratio 1.6	

400 subjects were screened by ICT method for Anti HCV out of 400 subjects, 142 (35.5%) were positive and 258 (64.5 %) were negative. The serum of all 142 subjects positive on ICT was sent to Rawalpindi for HCV qualitative test on RT-PCR, HCV detected in 84 cases (59.16%) and 58 cases (40.84%) were negative. The ratio of variation (ICT: RT-PCR) was 1.44.



Comparison of PCR and ICT ratio among the subjects.

ICT = 59.16%

RT-PCR = 40.84%

Discussion:

Goth Haji Khair Muhammad Junejo is a small village in district Sanghar Sindh Pakistan. It is at the one of gateways of the desert "THAR". A remote, faraway area without health facilities and low literacy rate. Where the healers are traditional "shamans" and dispensers. They use unsterilized syringes and dental instruments. The tools of barbers are spoiled. There is little data about prevalence of viruses causing hepatitis from remote areas of Pakistan. The prevalence of viruses causing hepatitis is more in remote areas than the proximities. There are many ICT kits available for screening of Anti - HCV, all are working on the same principle⁵. The RT - PCR diagnostic test is found to be more sensitive for detection of HCV as compared to ICT⁶. ICT method is less sensitive, and its specificity is low as compare to RT - PCR. The rapid ICT kits for HbsAg and Anti HCV were equally sensitive and specific when compared with ELISA⁷. The ICT is although economical and less expensive, may be equally sensitive as compare to other methods like ELISA, but its usage and effectiveness is not such as compare to RT - PCR. Quantitative tests that measure the actual levels of Hepatitis C viruses in blood may use the process of PCR. The viral load test computes the number of HCV RNA particles present and are expressed in either international units per liter (IU/L) or copies per milliliter (ml). The quantitative HCV RNA test is used to monitor individual who undergo antiviral treatment, prior to beginning therapy, during and upon completion⁸. The utility of HCV RNA quantification is well established. HCV

RNA assay is ideally should be sensitive, offer precise and reproducible quantification result⁹. In our study the positivity ratio (ICT: PCR) is 1.44 which shows remarkable variation in specificity.

Conclusion and Suggestion:

ICT tests are frequently used for detection of hepatitis viruses in Pakistan, which are less in sensitivity and specificity as compare to RT-PCR. False positivity is more by ICT method as compare to RT-PCR. ICT kits from various manufactures are available in our country, those are economical and rapid, but creating confusion among the users for their reliability. False positive results are producing havoc on one side and problems in blood banking on other side where healthy donors are rejected. The laboratories in hospitals should be well equipped with diagnostic facilities like RT - PCR for detection, genotyping of HCV and monitoring of the individuals who undergo anti-viral therapy.

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