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Comparative analysis of HCV through ICT and ELISA, A contributing factor of HCV infection in district Killa-Saifullah, Balochistan

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Abstract

Hepatitis C virus (HCV) is a blood borne human pathogen infecting approximately 120-130 million worldwide and 17 million in Pakistan. An early diagnosis of HCV is the mainstay of an effective treatment providing a guide and/or to monitor the therapy of an HCV infection.

The most important marker for HCV diagnosis is the detection of anti HCV antibodies analyzed by immunochromatography test (ICT) and more reliable enzyme-linked immunosorbant assay (ELISA) and RNA test. ICT and ELISA are the primary quantitative screening techniques employed in the diagnostic centers, while ICT is a broadly utilized screening strategy in the Kila-Saifullah district (Balochistan).

A cross sectional study is carried out to compare the performance of ICT and ELISA for the detection of anti HCV antibody in blood donors from Kila-Saifulla to determine if the diagnostic technique employed is the cause of HCV spread when a false diagnosed blood is donated.

2000 blood samples collected from different diagnostic centers initially screed through ICT were rescreened through ELISA to

determine the viability of ICT to establish or/to exclude male diagnosis as a potential cause of HCV infection. The results showed 20 blood samples were HCV positive analyzed through ICT while 16 when analyzed through ELISA, which roughly translates to 1 % and 0.8% respectively. The ICT analysis showed results 20% false positive in comparison to gold standard ELISA technique.

Key words: ICT, HCV, ELISA.

INTRODUCTION:

Hepatitis C virus (HCV) is a blood borne human pathogen infecting approximately 120-130 million or 3% of the total world population. About 17 million people are HCV infected in Pakistan and is the country with the second highest infection rate in the world with 4.5–8% (Lavanchy D et al; 2011; Idrees M. et al. 2009). HCV causes acute hepatitis mostly subclinical but gradually evolves into chronic infection if left untreated and frequently progress to cirrhosis and hepatocellular carcinoma (HCC) (Alberti A et al., 2008; Forman MS et al,. 2011). Globally 130 million people are chronic carriers of the HCV at risk of developing liver cirrhosis and/or liver cancer with yet another 3-4 million people newly infected each year globally and about 70% will develop chronic hepatitis (WHO, 2009).

Hepatitis C virus is a hepatotropic RNA virus, transmitted primarily via blood however in developed countries the route of HCV transmission is intravenous through drug abuse, whereas in less developed countries invasive procedures or injection-based therapies with contaminated instruments used in dental procedures and infected objects like razors are the predominant source of new infections (Akhtar M. et al., 2013; Hauri AM. et al., 2004).

HCV virion is 55-65 nm in diameter with a single stranded positive-sense RNA of size 9.6 kb. Important feature of the HCV genome is its high degree of genetic variability with an estimation of mutational frequency 10^{12} per nucleotide per year (Del Campo et al., 2009). Approximately 10^{12} viral particles are generated every day in a chronically infected person. This remarkable replicative rate in

combination with the high rate of mutational frequency of the error prone polymerase activity of the virus results in tremendous genetic diversity expanding the number of recognized genotypes to 7 and subtypes 67 (Smith DB et al., 2014). The most prevalent infection causing genotypes of the HCV occurring globally are 1, 2, and 3 while others are limited to specific regions (Lemon SMet al. 2007; Liu J. et al., 2005). Almost 80% infection in Pakistani population is caused by HCV genotypes 3a followed by 3b and 1a respectively (Waqar M. et al., 2014). Correct genotypic identification of HCV is important for devising an appropriate treatment strategy, to assess the progression of disease and response to antiviral therapy (Aceijas C. et al., 2007; Cuypers L. et al., 2016).

HCV infection has declined in the past two decades in the developed countries such as United States (Armstrong GL. et al 2006; Williams IT. et al., 2010), Western and northern Europe (Duberg A. et al., 2008; Delarocque-Astagneau E. et al., 2010) Australia (Razali K. et al., 2007) and Japan (Chung H. et al., 2010) but the burden of this disease in the lesser developed and developing countries is continuously on the rise (Hajarizadeh B. et al., 2013).

Different diagnostic techniques are employed for screening of HCV antigen, anti HCV antibody or both by serological assay (Marwaha N. et al., 2014). Amongst the sensitive and reliable technique used for the HCV screening include kit, ELISA, chemiluminescence (CLIA) and PCR (Waqqar S. et al., 2015). In developing countries the diagnostic centers where facilities are limited such as electric supply, trained manpower and instruments, alternative screening methodologies are employed which does not require major equipment, electric supply, cost effective and the results can be read through visual aid following principles; agglutination, immunofiltration or immunochromatography (Batool A. et al., 2009). Advanced equipped diagnostic labs and blood banks in Pakistan are limited only to major cities of the country where as alternative cost effective and less reliable techniques are employed in remote areas. One such common method is immunoassay chromatography test (ICT) which is less sensitive rapid test with higher percentage of either false positive or false negative (unpublished reports).

Current study is designed to employ sufficient number of blood donors from district Killa-Saifullah whose blood is screened either HCV negative or positive through ICT will be rescreened through an more sensitive and reliable Enzyme Linked Immunosorbant Assay (ELISA) at Bolan Medical Complex to determine if the ICT screening method is a contributing factor in HCV infection spread in the district Killah-Saifullah when a male diagnosed blood with HCV negative result ICT is donated.to patients.

Experimental Study design

Cross sectional evaluation study design was adopted to perform the study on "Immunoassay Chromatography Test efficacy for HCV as screening method for blood donors in district Killa-Saifullah. This cross sectional study design is used to obtain observations from a defined population in a specified time interval (Steiner MJ, 2011).

Blood samples and analyzed data was collected from different blood banks and clinical labs located in district Killa-Saifullah to be rescreened through ELISA at Bolan Medical College (BMC) Quetta.

Sample size

Sample size was 2000 ±10% assigned by supervisor. ±10% sample was used as margin of error to minimize error chance.

Sampling procedure

Current study is test based where collected blood samples from various blood banks and clinical labs located in district Killa-Saifullah and its surroundings. These samples were already screened for HCV infection by ICT techniques. We have taken these samples for rescreening by more sensitive and reliable screening technique (ELISA). The blood samples were centrifuged at 5000rpm for 8 minute and the separated serum was stored at -20^{o} C for further analysis.

Testing tools

Blood screening kits were used to screen the serum sampling through ICT and ELISA to compare if the results coincide with results of the diagnostic lab and then through ELISA for comparing the of efficiency of each test.

Data analysis

Descriptive statistics were applied to summarize the data.

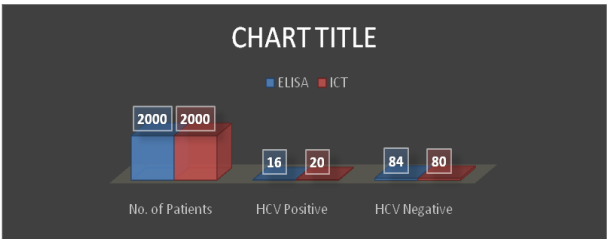
ELISA Vs ICT

During the study 2000 blood sample were screened for HCV using ELISA and ICT diagnostic techniques. The ICT method showed that only 20 patients were found to be HCV positive which roughly translates to be about 1 % of the total patients while the ELISA method showed that only 16 patients were with HCV positive which translates to 0.8 % (table.1, 2) The results of the current study reveal that the ELISA is potentially a better technique as compared to the ICT method. ICT HCV positive patients may be ELISA HCV negative if the patient is HCV carrier. The patients with HCV carrier is turned to be HCV positive in ELISA while HCV carrier patients are turned to be negative in ELISA technique which further reveals that the efficiency of ELISA is better than that of ICT.

Table 1: Blood samples analysed through ELISA and ICT

Method	No. of Patients	HCV Positive	HCV Negative
ELISA	2000	16	84
ICT	2000	20	80

Table 2: Comparison of results, obtained in ELISA and ICT



Results and Discussions

The current study was designed in district Kila-Saifullah of province Balochistan and patients whose ages were in between 18-54 years were included. During the study the blood samples of about 2000 were analyzed using ICT and ELISA. The patients were grouped based on their ages starting from 18-20, 21-23 and so forth till 52-54. The result of the current study revealed that the prevalence of HCV is about 1%

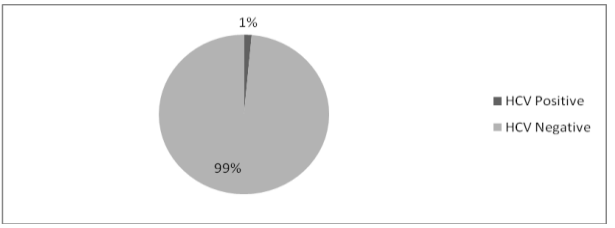


Figure-1 Percentage of HCV positive and negative samples

CONCLUSION

Finding of the study of HCV infection is huge in light of the fact that inconvenient treatment with PEG-IFN-α may provoke an upheld natural response (SVR) up to 90% and by maintain a strategic distance from the HCV corrupted cases from the development of liver cirrhosis and HCC. It is concluded with the study that HCV is growing here in Pakistan at a rapid rate in the entire region without any hurdle. In view of high inescapability of HCV illness in Pakistani people, the determination of HCV requires a highly sensitive screening test with central focuses like fast planning, clear in automation, high resolute quality and by and large straightforwardness, so the present examination was proposed to become such sort of test for the screening of neighborhood masses for HCV sullying using the helper antigenic recombinant antigens from the adjacent HCV strains. Study further concluded that the quantity of individuals who convey HCV contamination expanding quickly which is disturbing. It warrants the need to design greater, all around idea examines with a reasonable theory. In provincial territory like KillaSaifullah, HCV predominance studies may be done. In any case, control figuring for test size ought to be done that will illuminate about the measurable hugeness of our outcomes. An all-around spread out poll ought to likewise be utilized to record data on statistic, financial and clinical factors. The future examination could use increasingly delicate and exact systems, for example, quantitative PCR.

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