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Molecular based identification and association of *Mycobacterium tuberculosis* and hepatitis C virus in patient samples of Muzaffargarh, Pakistan

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Abstract

Mycobacterium tuberculosis and Hepatitis C virus are the major diseases spreading more rapidly all over the Pakistan and its load increasing day by day. The main aim of the topic is to determine the correlation of Hepatitis C virus infection among the patient of *M. tuberculosis* and also to increase the awareness about co infection of HCV in *M. tuberculosis* patients. The study was cross-sectional and convenient sampling technique was used to approach the patients. In the study the basically patients were separated into different groups of male, female, age group (of <20 years, 20 to 40 years and > 40 years), married, unmarried, financially good, poor, literate and illiterate. The fresh EDTA samples of *M. tuberculosis* positive patients were taken to screen HCV infection. Further the HCV positive plasma was stored at -21^{0}C for later confirmation by PCR. The RNA was extracted first and converted into cDNA for amplification and detection. The standard curve was obtained by HCV reagent RT-PCR Kit for confirmation of qPCR assay. Finally CT value was used to get patients results. Among the 534 *M. tuberculosis* patients confirmed by genexpert in sputum, there were 94 patients of HCV infection. Overall the burden of HCV infection was 17.60%. The correlation of HCV co infection in male group was 49.46%, in female 50.54%, in literate was 31%, in illiterate 69%, age group < 20 years had 9.57% co-infection, age group 20-40 years had 39.36%, >40 years 51.01%, among married and unmarried the HCV co-infection was 17.03% and 82.97% respectively. There was very high correlation of HCV and *M. tuberculosis* infection in Muzaffargarh district. The *M. tuberculosis* patients were less immune to HCV infection as compared to non *M. tuberculosis* patients. The findings helps to give more awareness to physician to put more efforts towards *M. tuberculosis* patients who more prone to HCV infection and more work in future are needed to save the *M. tuberculosis* patients from getting HCV infection.

Keywords: *M. tuberculosis*, RNA extraction, qPCR, Identification.

Introduction

Mycobacterium tuberculosis infection is one of the most well-known health issues in developed and in poor countries. Globally 2 billion people were infected and risk of co infection was measured very high (Dye, Scheele, Dolin, Pathania, Raviglione, et al., 1999). There were about 8.6 million new cases were diagnosed all around the world in 2012 and about 1.3 million were death. The *M. tuberculosis* has got number of drug resistance reported by World Health Organization (WHO) and deadly effects in congestion with HCV and human immunodeficiency

virus (HIV). WHO also tried to develop public awareness about *M. tuberculosis* and its co-infection to HCV and HIV. The most important features of MTB are slow growth rate, complex cell structure, dormancy and complex genetic homogeneity (Cole et al., 1998; Zumla, George, Sharma, Herbert, & Baroness Masham of, 2013).

All over the world there were slow rate of incident of *M. tuberculosis* which were 2%. This 2% includes all age group, children and women in 2012. The number of women was 2.9 million and in children the numbers were 4.5 laces. And the undiagnosed numbers are also important which main cause of *M. tuberculosis* infection spread. In many countries the co infection of HIV was targeted in connection with HIV treatment and *M. tuberculosis* medication. As per a report there should be emergency in high infection risk countries to control the infection. To control the *M. tuberculosis* infection should on priority in high risk countries. *M. tuberculosis* need same care as the HIV, malaria and hepatitis (Zumla et al., 2013).

HCV is one of the leading causes of liver cancer worldwide. In the acute phase, HCV infection is usually asymptomatic, but more than 80% of patients progress to chronic HCV infection. About 1.3-1.7 million people worldwide are infected with HCV for a long time. (Kassa, Bane, & Kefene, 2016).

The aim of this study was to detect the *M. tuberculosis* by GeneXpert among the people of Muzaffargarh district and diagnosis the HCV in the patients with *M. tuberculosis* with RT-PCR to find out association of *M. tuberculosis* and HCV in patient samples of Muzaffargarh, Pakistan.

Materials and Methods

Sample collection

For the diagnosis of *M. tuberculosis* among the people of Muzaffargarh districts suspected people were screened and *M. tuberculosis* positive cases were screened for HCV. So, the lab work was done on approximately 6234 samples and stored them at -20C temperature in laboratory.

RNA isolation and cDNA conversion

RNA was isolated from all the collected samples and then cDNA was synthesized according to the manufacturer s' protocol (Qiagen, Germany).

PCR Amplification for HCV

The thermal protocol of the COBART HCV Quantitation Kit consists of a two-step initial denaturation for activation of HotStar Taq DNA polymerase, a two-step amplification cycle and final retention. The real-time data is collected in the second step of the amplification cycle. Reverse transcription was carried out at 50 ° C for 30:00 minutes. The initial denaturation process was carried out at 95 ° C for 14:30 minutes. The further denaturation was at 97 0C for 30 minutes. The annealing data Collection was at 55.0 ^{0}C in 01 hour and 20 minutes. The synthesis process lasts for 15 minutes at 72.0 ^{0}C. And hold the process at 22 ^{0}C for 05 minutes. There were total 50 cycles of denaturation, Annealing and synthesis. To start a real-time PCR reaction using the Bosphore® kit, complete the following steps on the instrument software: Select the filter pair (FAM and HEX) to use to identify unknown samples, standards, positive controls, negative controls, assign quantitative values and Select the correct hot protocol to automatically start the protocol.

Amplification curve

At the end of automated procedure the software had automatically calculated the baseline cycle and the threshold. A standard curve is drawn using data obtained from defined criteria, where the axis CT threshold cycle and logarithmic starting amount. The results are expressed in international units/mL (IU/mL). The molecular detection through PCR amplification is very specific and sensitive tool of diagnosis. It had given accurate results and specifies the presence or absence of species of HCV virus.

Results

In the figure 1 there was a detailed description of standard curve for HCV quantification to calibrate and validate the test results by using the kit HCV RG RT-PCR. Standard curve: CT = -2.593*log (conc.) + 39.185 Slope: -2.59285354869109 Y-intercept: 39.1849820981874 PCR efficiency calculated from slope: 1.43038718039951 r2 of standard curve: 0.9941268751749072.

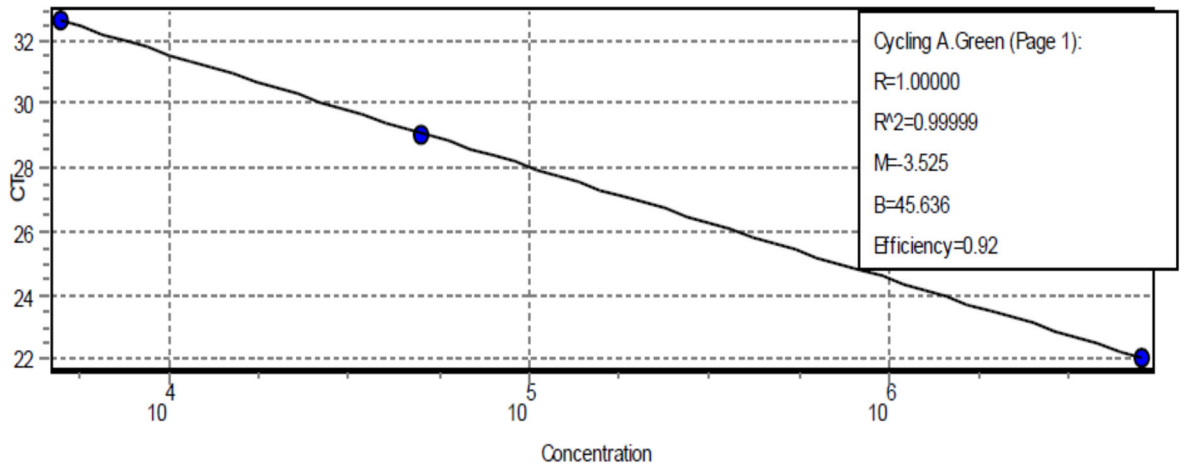

Figure.1. Amplification and development of the standard curve for HCV quantification at Muzaffargarh district obtained using HCV RG RT-PCR kit to validate the qPCR assay. The results are expressed in international units/mL (IU/mL).

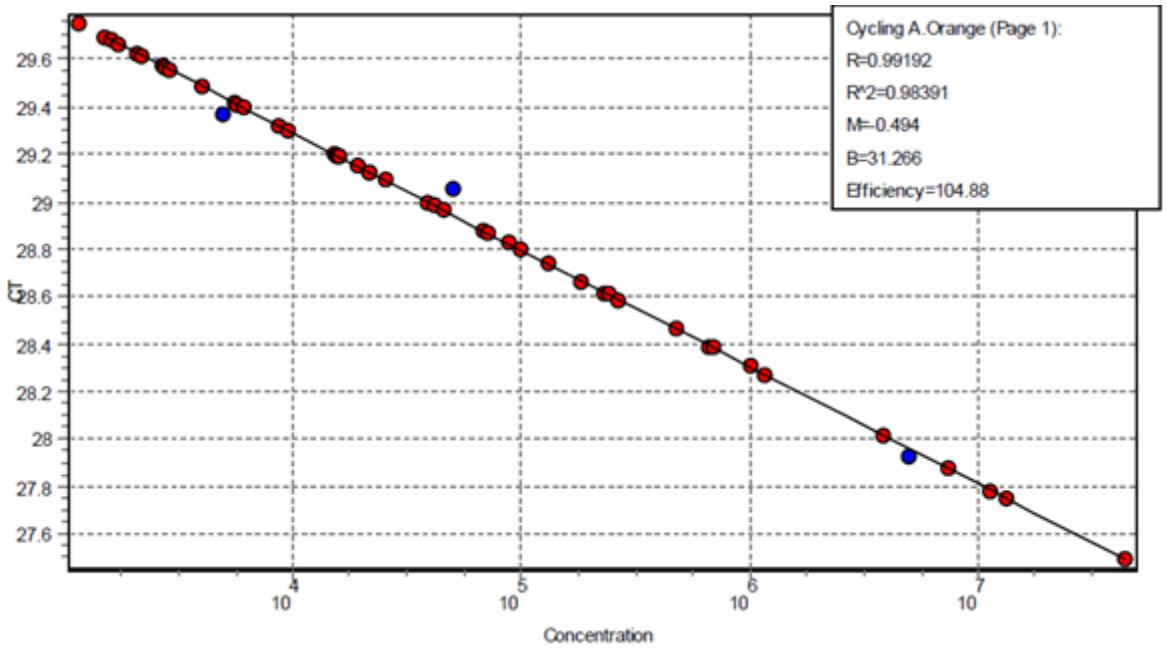

Figure. 2. Applications of the standard curve for HCV quantification in *M. tuberculosis* patients at Muzaffargarh district to validate the qPCR assay. The results were expressed in international units/mL (IU/mL).

In this figure.2 the results of unknown samples were compared with standard curve to get accurate results of *M. tuberculosis* patients. The standard curve validates the patients sample result and builds accurate concentration of viral load.

During the period of three (05) months sampling, total 534 *M. tuberculosis* samples positive were collected. All were screened for HCV by immunochromatic technique (ICT). All positive cases were verified by qPCR. Out of 534 *M. tuberculosis* patients 94 patients were HCV infection. The rate of HCV infection was 17.60% in *M. tuberculosis* infection (Figure.3).

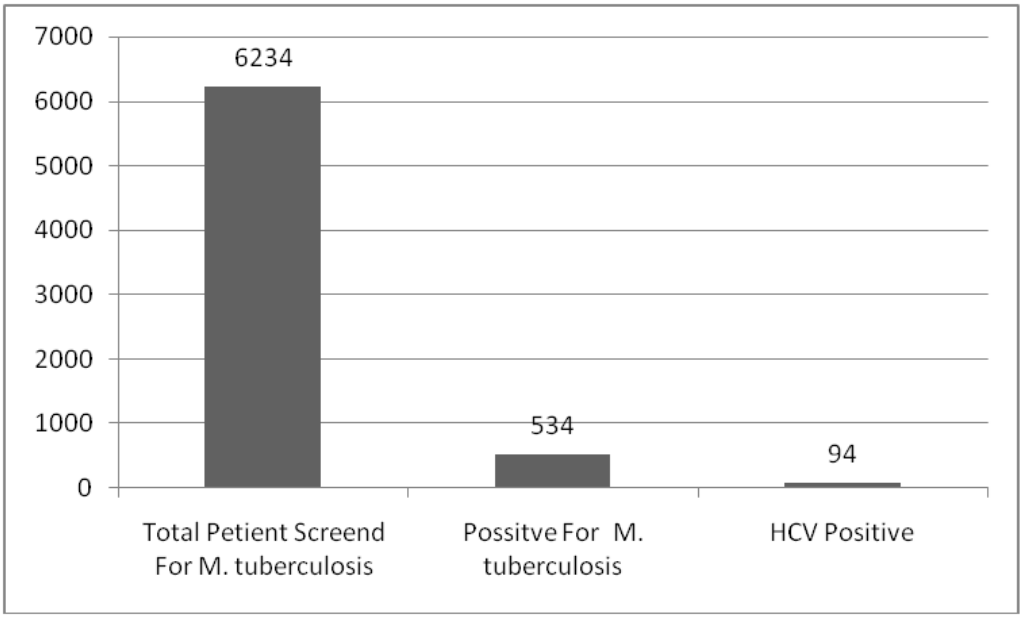

Figure 3. Total patients tested for *M. tuberculosis* and HCV positive.

Discussion

This study was the first study of the epidemiology of HCV infection in patients with *M. tuberculosis* in Muzaffargarh, Punjab, Pakistan. The load of HCV infection exceeds the normal population. Based on these findings, the risk of HCV infection increased during the management of anti-tuberculosis drugs. Due to *M. tuberculosis* infection patients became less immune and HCV infection risk increased many times. The drug management of *M. tuberculosis* infection is very long. It may need 9 months continually medication to treat the *M. tuberculosis* infection. During the medication, the patients get lot of complications and due to heavy doses and complication; the patients lost their immunity and become more prone to HCV infection. This study showed that the infection increases up to 17.60 % among *M. tuberculosis* patients. Similarly the HCV infection can cause *M. tuberculosis* infection. It may cause reactivation of latent *M. tuberculosis* infection. The infection of HCV in *M. tuberculosis* patients increased in high incidence of areas of the world. Similar were found in another research of HCV co-infection in *M. tuberculosis* patients. That study showed 21% co-infection among *M. tuberculosis* patients of Georgia. The common serotype was 1b (45%).

The HCV co-infection was independent and importantly associated to *M. tuberculosis* infection. The drug may induce the HCV infection and hepatotoxicity. The incident of hepatotoxicity was common during the drug management of *M. tuberculosis*. Poor medical facilities, misuse of needles and tattoo centers were the major cause of HCV among the *M. tuberculosis* patients. Data from the patients of HCV co-infection with *M. tuberculosis* shows increase in hepatotoxicity and genotype was also similar to other studies. There was limitation in our study (Lomtadze et al., 2013).

We had searched only for HCV co-infection in *M. tuberculosis* patients. There was no plan to measure the hepatotoxicity among these patients. At least the correlation HCV infection was 17.60 % in *M. tuberculosis* patients. That was similar to different other studies. We had also some new cases of *M. tuberculosis* in this study that was screened for HCV infection confirmed by RT PCR. Majority of the *M. tuberculosis* infected patients getting the treatment for last 6 months.

A similar study was done in Brazil. Where the co-infection of HCV was 5 times more than normal population in *M. tuberculosis* infected patients. In other countries this load was 11.8% (in Argentina) and in Georgia it was 12 %. But this load of co-infection in Thailand was 31.5 % among *M. tuberculosis* patients. In Brazil it was reported 23.5 %. The risk of infection was commonly related to misuse of syringes, blades and mainly parental way. In Brazil two third of HCV patients had developed the HIV infection. The study of polygenetic analysis showed some relation of HCV and *M. tuberculosis* (Reis et al., 2011).

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