

RESEARCH ARTICLE

Frequency of active HCV infection among anti-HCV positive patients in selected districts of Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

Objective: The objective of this study was to determine frequency of active HCV infection among confirmed anti-HCV positive subjects of KPK in order to help the infected subjects decide about anti-viral treatment options.

Methods: Blood samples (3075 samples) were collected from patients in selected districts of KPK and were transported to Centre of Biotechnology and Microbiology, Diagnostic laboratory, University of Peshawar. These patients were already screened for anti-HCV by ICT (Immuno Chromatographic Technique) and ELISA (Enzyme Linked Immunosorbant Assay) in the local laboratories of the concern districts. Subsequently, viral RNA was isolated from serum sample and subjected to Real-time PCR. The frequency of the results was calculated for the HCV-RNA positive and negative samples.

Results: Out of 3075 confirmed anti-HCV samples, HCV-RNA positive and negative samples were 2055 (66.6%) and 1020 (33.3%) respectively. The frequency of male and female HCV-RNA positive samples was 57.6% and 42.4% respectively. Rate of false anti-HCV positivity was 33.3%. Moreover, rate of active HCV infection was found more in district Bunir followed by districts Dir and Mardan. Comparatively less positive percent frequency of active HCV infection was found in districts Swabi, Peshawar and Kohat, respectively.

Conclusion: It is concluded that viremia is present in more than 50% of confirmed anti-HCV positive patients. Anti-HCV positive, but HCV-RNA negative samples represent either false- positivity of anti-HCV or a spontaneous clearance of HCV. *J Microbiol Infect Dis 2013;3(4): 199-202*

Key words: HCV, Khyber Pakhtunkhwa, viremia, active infection, PCR

Pakistan Khyber Pakhtunkhwa eyaletinde seçilmiş bölgelerde anti-HCV pozitif hastalarda aktif HCV enfeksiyonu sıklığı

ÖZET

Amaç: Bu çalışmanın amacı, Khyber Pakhtunkhwa eyaletinde (KPK) antiviral tedavi seçeneklerini değerlendirmek amacıyla, anti-HCV pozitif olan kişiler arasında aktif HCV enfeksiyonu sıklığını belirlemektir

Yöntemler: Kan örnekleri (3075 adet) KPK'nın seçilmiş bölgelerindeki hastalardan toplandı ve Peşaver Üniversitesi Biyoteknoloji ve Mikrobiyoloji Merkezi Teşhis Laboratuvarına nakledildi. Bu hastalar zaten yaşadıkları bölgedeki yerel laboratuvarlarda immuno kromatografik teknik ve Enzim Linked Immunosorbant Assay yöntemleri ile anti-HCV için taranmış bulunuyorlardı. Daha sonra, viral RNA serum örneklerinden izole edildi ve gerçek zamanlı PCR ile araştırıldılar. HCV-RNA sıklığı negatif ve pozitif numuneler için hesaplandı.

Bulgular: Anti-HCV pozitif olan 3075 serum örneğinin 2055'inde (% 66,6) HCV-RNA pozitif bulunurken 1020'sinde (% 33,3) negatif bulundu. Erkekler ve kadınlar arasında HCV-RNA pozitiflik yüzdeleri sırasıyla % 57,6 ve % 42,4 idi. Yalancı anti-HCV pozitiflik oranı % 33,3 idi. Aktif HCV enfeksiyon sıklığı en çok Bunir bölgesinde idi ve onu Dir ve Mardan bölgeleri izledi. Aktif HCV enfeksiyonu sırasıyla Swabi, Peşaver ve Kohat bölgelerinde nispeten daha az sıklıkta bulundu.

Sonuç: Anti-HCV pozitif hastaların % 50'sinden fazlasında vireminin varlığı gösterildi. Anti-HCV pozitif olduğu halde HCV-RNA'nın negatif olması bu kişilerin anti-HCV pozitifliğinin yalancı olduğunu veya HCV'nin kendiliğinden temizlendiğini göstermektedir.

Anahtar kelimeler: HCV, Khyber Pakhtunkhwa, viremi, aktif enfeksiyon, PCR

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Received: 29.04.2013, Accepted: 06.10.2013

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INTRODUCTION

Hepatitis C virus (HCV) is a major worldwide cause of acute and chronic hepatitis which ultimately leads to cirrhosis and hepatocellular carcinoma.¹ It is estimated that 2.2% of the world population has been infected by HCV.² It has been studied that 50 to 80% of individuals having HCV infection lead to chronic hepatitis C.^{3,4} In 2004 it was estimated by the WHO that the annual deaths due to liver cancer caused by HCV and cirrhosis were 308,000 and 785,000 respectively.⁵

In Pakistan 170 million people are living with poor health and educational standards. It is ranked 134th out of 174 countries according to the human development index of the United Nations.⁶ About 10 million people in Pakistan are presumed to have HCV infection.⁷

Different laboratory tests are used for detection of circulating antibodies and HCV-RNA which are generally classified as screening tests or confirmatory tests. ELISA tests are mostly used for screening purposes while other screening tests which have been developed recently include agglutination, immunofiltration and immunochromatography (ICT).⁸ False positivity is a common problem associated with ICT devices.⁹⁻¹¹ Earlier studies have reported the prevalence of anti-HCV antibodies by using ICT tests among the blood donors or general population from KPK province.¹²⁻¹⁴ It has been showed by different studies that the percent prevalence of HCV in Pakistan in the general adult population was 5%.¹⁵

Hence, there is need to screen out HCV infection among confirmed anti-HCV positive patients; early detection enables the patients to take possible early curable and preventive measures, so as to minimize the complications associated with end-stage disease. Before this study, many attempts have been done to determine the general prevalence of HCV infection in different regions of KPK. Different districts have different rates of HCV infection. Still no study has been performed to screen out viremia among confirmed anti-HCV positive patients. The objective of this study was to determine frequency of active HCV infection among confirmed anti-HCV positive subjects of KPK in order to help out the infected subjects decide about anti-viral treatment options.

METHODS

KPK was formerly known as North West Frontier Province. It is one of the provinces of Pakistan, located in the north west of the country. Its total popu-

lation is 2,125,480. Its capital is Peshawar. Its main districts are Peshawar, Mardan, Swabi, Buner, Dir, Charsadda, Dera Ismail Khan, Karak, Lakki Marwat, Kohat, Abbotabad and Hangu.

In this study six districts were selected. These were districts Dir, Buner, Mardan, Swabi, Kohat and Peshawar. The map of KPK along with these districts is given in Figure 1.

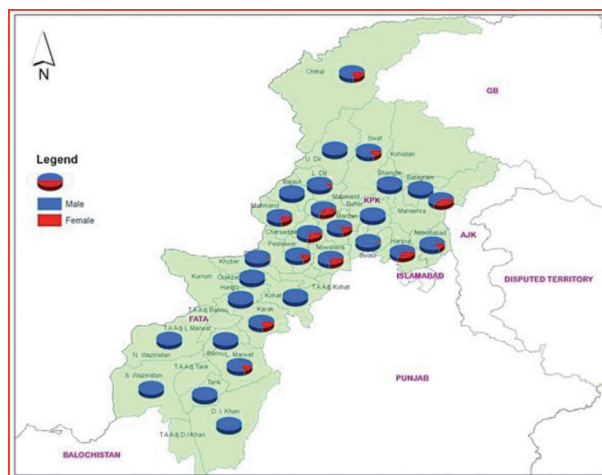


Figure 1. The distribution of study samples

In all these districts we made connections with health professionals that were already treating hepatitis patients. These health professionals were agreed and anti-HCV positive samples were then sent to us according to recommended transportation procedure. All these anti-HCV positive samples were then processed for active HCV infection by using Real Time PCR in the center of Biotechnology and Microbiology, diagnostic laboratory at the university of Peshawar/Pakistan. The total study population was 3075 patients having anti-HCV positive status. Serum was stored at -20°C and subsequently RNA was extracted from the sera by following instructions of column based extraction kit (Roboscreen Germany). PCR was performed for each sample by using real time PCR (Cepheid, USA) and Roboscreen amplification kit having internal control for each sample in order to rule out false negative results. We analyzed the results by calculating the Ct values for each sample. The Ct value less than 40 cycles were considered positive and more than 40 were considered as negative.

RESULTS

As screening tests may be associated with false positivity and could not confirm viremia, therefore

we carried out our study by using real time PCR technique for confirmation and screening out of viremia. Out of 3075 anti-HCV positive samples, HCV-RNA positive and negative samples were 2055 and 1020, while male and female positive samples were 1200 and 855 respectively. Rate of both anti-HCV and HCV-RNA positive was 66.6% and the rate of anti-HCV positive but HCV-RNA negative was 33.30%. While male and female percent positivity was 57.7% and 42.4% respectively. The highest percent positivity was found in district Bunir followed by in districts Dir and Mardan (Table 1).

Table 1. District wise distribution of Chronic HCV RNA positive and negative samples

Districts	Total Samples	Positive Samples n (%)	Male Positive n (%)
Peshawar	1587	1016 (64.0)	571 (56.2)
Mardan	514	366 (71.2)	221 (60.4)
Bunir	378	275 (72.8)	165 (60)
Kohat	157	93 (59)	51 (54.8)
Swabi	126	84 (66.6)	49 (58.3)
Dir	313	221 (70.6)	116 (52.4)
Total	3075	2055 (66.8)	1173 (57)

DISCUSSION

Hepatitis C infection is spreading rapidly due to poor and unsatisfactory health care conditions and its prevalence is very high in general population of Pakistan.¹⁶ In KPK, where the health care facilities are poorly equipped with essentials for screening and sterilization, HCV has become an economic burden over a population with considerable number of people living below the poverty line.

Determination of viremia is important for early decision about whether a patient should take the interferon-based therapy or not. Untimely diagnoses and treatment in the case of chronic HCV have serious limitations and the infection leads to cirrhosis, hepatocellular carcinoma and finally death. Therefore, it is necessary to investigate viremia among confirmed anti HCV patients by using PCR techniques as anti-HCV test is not informative about active infection.

In KPK, internationally approved treatment and diagnostic procedures are rarely followed. As anti-HCV positivity and elevated Liver function tests are least informative about whether a person is actively infected, so according to the accepted norms, the patients should not be subjected to antiviral treat-

ment before confirmation of the active infection. Lack of facilities and knowledge about the proper diagnosis and treatment of HCV infection, both on the part of the general population and physicians is doing havoc with the health of the masses in the form of irrational treatments in KPK.

This study was not designed to reveal the rate of HCV infection. We noted that around 2/3 of the cases with anti-HCV positivity had HCV-RNA positivity. This situation can be explained by either false positivity of anti-HCV positivity or spontaneous clearance of HCV-RNA. However considering that spontaneous clearance is around 15-20%, there should be some false anti-HCV positives. Nevertheless some districts have relatively higher numbers of the patients (anti-HCV and HCV-RNA positives) (Table 1).

The previous hospital based study about the general prevalence of anti- HCV positivity in district Bunir and Mardan, showed high HCV infection in these two districts.^{12,17} The high number of patients in Bunir may be attributed to high rate of dental surgery.¹⁶ Comparatively lower number of cases with HCV infection was found in districts Swabi and Kohat respectively. Previously no study was conducted on the general prevalence rates in the above-mentioned districts. The low infection rate may be attributed to more education and awareness about the transmission of HCV infection in these districts. It was also noted that chronic HCV infection was high in male as compared to female, which has also been shown by different studies. Thus it is clear that anti-HCV tests are associated with false positivity and the reliable tool for screening viremia is PCR technique.

In conclusion, about two thirds of the patients who have anti HCV positivity had chronic HCV infection. Whether this is a false positivity of anti-HCV or spontaneous clearance of HCV-RNA remains to be clarified.

Conflict of interest: Authors declare that they have no conflict of interest.

Acknowledgment: The authors are thankful to the University of Peshawar (UOP), Pakistan for funding the research work.

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