Acta Microbiologica et Immunologica Hungarica 63 (4), pp. 427–431 (2016)
DOI: 10.1556/030.63.2016.025

UNTYPEABLE HEPATITIS C VIRUS SUBTYPES IN PAKISTAN: A NEGLECTED SECTION

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(Received: 15 March 2016; accepted: 17 May 2016)

Diagnostically untypeable subtypes contribute a considerable percent of hepatitis C virus (HCV) subtypes in Pakistan. In the present study, chronically infected HCV patients with known viremia were subjected to HCV genotyping. Among the total retrieved samples, 92.7% (64/69) were found typeable while 7.24% (5/69) were diagnostically untypeable. In conclusion, the presence of large number of untypeable HCV subtypes emphasizes the need of an updated type-specific genotyping assay and consideration of primers for proportionally rare subtypes to minimize the number of untypeable HCV subtypes.

Keywords: hepatitis C, genotyping, untypeable subtypes, Pakistan

Introduction

Hepatitis C is a leading infectious disease caused by hepatitis C virus (HCV). HCV has been classified into genus *hepacivirus*, containing ssRNA of 9.6 kb. Globally, the anti-HCV prevalence is estimated to be greater than 180 million [1]. Pakistan carries the burden of about 10 million anti-HCV cases [2].

Currently, HCV has six major genotypes and multiple subtypes. HCV genotypes may vary in their acquisition route, therapy period, and response to therapy [3]. Genotypes 1–3 are prevalent worldwide, genotype 4 is found in Middle East, genotype 5 in South Africa while genotype 6 in Southeast Asia [2]. In Pakistan, genotype 3 occurs frequently while 3a is the most pervasive HCV subtype followed by 3b, 1a, and a considerable proportion of diagnostically untypeable subtypes [4]. To assess the latest proportion of diagnostically untypeable

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HCV subtypes, a pilot study was conducted at the Institute of Biotechnology and Genetic Engineering (IBGE), The University of Agriculture, Peshawar, Pakistan.

Methodology

The study design was duly approved from institutional ethical committee of IBGE before commencement of the study. The present study was conducted during April 2015–June 2015. Blood samples were collected from a total of 69 actively infected HCV patients. Collected blood samples were subjected to genotyping assay for determination of HCV subtypes proportion by type-specific primers using the previously described method [4].

Results and Discussion

It was identified that 92.75% (64/69) samples were HCV typeable subtypes while 7.24% (5/69) were diagnosed as untypeable. Among the typeable subtypes, 44.92% (31/69) were found to be 3a, 21.73% (15/69) were 2a, 10.14% (7/69) were 3b, 4.34% (3/69) were 1a, 2.89% (2/69) were 2b, 2.89% (2/69) were 1b, 1.44% (1/69) were genotype 4 while 4.34% (3/69) indicated mixed subtypes (Figure 1). The mixed subtypes included 2.89% (2/69) as 3a/1b while 1.44% (1/69) as 3a/3b. The untypeable subtypes have been previously reported from different parts of the country in various studies [4–8] for their considerable share in the overall HCV subtypes circulating in Pakistan (Table I). In general, the

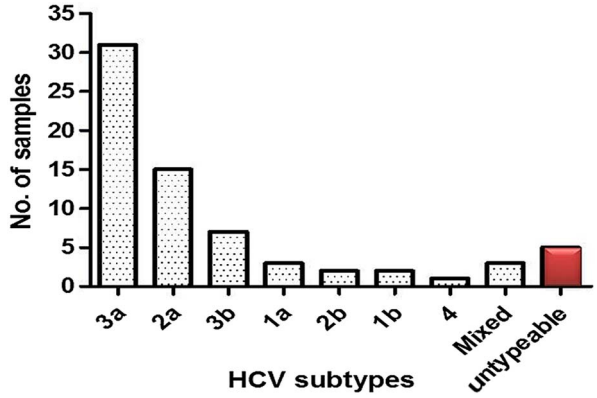

Figure 1. Proportion of typeable (3a, 2a, 3b, 1a, 2b, 1b, 4, and mixed) and untypeable HCV subtypes

Table I. Untypeable subtypes proportion in different studies

Study	Year	Typeable HCV subtypes, % (*n*)	Untypeable HCV subtypes, % (*n*)
Idrees	2008	93.84 (244)	6.15 (16)
Akhund et al.	2008	84.88 (292)	15.116 (52)
Ali et al.	2011	83 (166)	17 (34)
Inamullah et al.	2011	62.16 (115)	37.83 (70)
Waqar et al.	2014	87.82 (476)	12.17 (66)

polymerase chain reaction (PCR)-based HCV genotyping systems used in Pakistan relies on the method of Idrees [4] and Ohno et al. [9] developed in 2008 and 1997, respectively. Due to HCV high mutation rate of approximately 10^{-3} per nucleotide per replication [10], host immune pressure [11], antivirals' pressure [12], and viral/host immune escape mechanism [13, 14], substantial quasi-species population exists in HCV infected individuals. Some of these mutations may occur in diagnostically important regions of the genome that results in mistyping of the virus [15] which could lead to untypeable cases. Interestingly, in the present study, 1.44% (1/69) subtypes were identified as genotype 4. Although genotype 4 is rare in Pakistan, a previous study [4] confirmed its presence. However, in most commercial laboratories and diagnostic centers, genotyping is performed for subtypes 1a, 1b, 2a, 2b, 3a, and 3b, without including type-specific primers for genotype 4 and subtypes 1c, 3c, 5a, and 6a despite their existence [4], which could be a contributing factor to increased untypeable cases.

There is no definite treatment duration for untypeable subtypes as generally therapy duration is assessed in terms of qualitative or quantitative detection (viral load) [6]. In addition, the concept of "specialized therapies" for different HCV genotypes is not common in Pakistan, which leads to increased cases of non-responsiveness to treatment regimen [1]. Although the problem of untypeable subtypes is not solely an issue of Pakistan, however, in a developed world, sequencing practice is a routine strategy for HCV genotyping. Research and commercial laboratories that rely upon type-specific PCR as a tool for HCV genotyping have limitation over time in the case of HCV [3]. Therefore, a large number of patients with untypeable subtypes may face misdiagnosis and mistreatment.

Conclusion

Conclusively, substantial number of untypeable HCV subtypes was observed in the present study. PCR-based genotyping system must be constantly updated in light of local sequence data and type-specific primers for proportionally rare subtypes should be included in the genotyping assay to avoid chances of

mistyping and mistreatment. The practice of sequencing should be made mandatory in the case of untypeable HCV subtypes for precise genotype identification and determination of treatment duration.

Acknowledgement

The authors would like to thank the support of executive manager of Society of Awareness, Prevention and Control of Infectious Diseases (SAPCID) PCR Laboratory, Mr. Sajid Mohmand during the entire study period.

Conflict of Interest

The authors declare that they have no conflict of interest.

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