

Molecular Characterization, Expression and Purification of Hepatitis B Virus Core Antigen from Pakistani Population

by

Abdul Rauf
(M.Phil. Molecular Biology)



SUPERVISOR

Prof. Dr. Abdul Rauf Shakoori
Distinguished National Professor and Director
School of Biological Sciences
University of the Punjab
Lahore, Pakistan

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Dedicated to
My loving Ammi ji, caring Abba Huzoor
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Abdul Rauf
School of Biological Sciences,
University of the Punjab, Lahore. Pakistan

Summary

Viral hepatitis has become one of the greatest health problems across the globe. Almost half of the world population has been infected by one or the other type of hepatitis viruses. Hepatitis B virus (HBV) and hepatitis C virus (HCV) share the major load of all the viral hepatitis infections. Both HBV and HCV are generally transmitted through blood or blood products of the infected individuals. HBV is a DNA virus of hepadnaviridae family while HCV is the member of *Hepacivirus* genus in the family flaviviridae.

The present study encompasses the prevalence of HCV and HBV in the general (apparently healthy) population of Pakistan. Prevalence of HCV and HBV infection was determined among 1693 individuals of populations of three different areas. First population studied was that of Internally Displaced Persons (IDPs) of Swat during 2008-09. Second study was carried out in the village of Hill Surang district Bagh, Azad Kashmir, where general population of the village was screened. The third population studied was that of orphan children in Azad Kashmir and Rawalpindi district of Pakistan.

All subjects were screened for the presence of hepatitis B virus surface antigen (HBsAg) and antibodies against hepatitis C virus (anti HCV) in their blood. Subjects with positive screening results were confirmed for the presence of respective virus in the blood serum. Genotyping of HCV was done by using the blood sample of all those subjects who were positive for this virus. In all the HBV positive subjects HBV core gene was characterized and sequence comparison among symptomatic and asymptomatic isolates was done. Most commonly found isolate of the core gene was expressed in *E.coli*. This recombinant protein was purified and its immunogenicity was determined in the animal model.

Among the studied population 8.03% were found to have antigens and antibodies against the hepatitis B and C viruses, respectively, whereas 4.19% of the studied population were positive for viral DNA and RNA in the blood serum. HCV was the predominant infection (3.90%), while HBV was less prevalent (0.29%) in the studied population. HCV was found predominantly in females as

compared to males; of the positive individuals, 60.62% were females and 39.38% were males. About 62% of the subjects, confirmed with hepatitis C viral RNA were asymptomatic indicating a high percentage of chronic disease.

The age group 36-45 years exhibited the highest percentage (15.15%) of antibodies against HCV followed by 46-75 years (10.71%) >26-35 years (10.09%) >16-25 years (9.45%) > 5-15 years (3.93%). The highest percentage of HCV RNA was observed in age group 36-45 years (9.59%) followed by 26-35 years (5.77%) > 46-75 years (4.08%) >16-25 years (2.48%) > 5-15 years (2.47%). Genotype 3a was found to be the most predominant HCV genotype (43.93 %) followed by 2a (12.12%) > 3b (10.6%) > 1b (10.6%) > 2b (4.54%) >1a (1.51%). An unknown genotype was also found with the second highest percentage (16.66 %) which was even higher than that of 2a. The genotypes 4a, 5a and 6a were not found in any member of the studied population.

Hepatitis B virus DNA was confirmed in 5 out of 10 HBsAg positive individuals. The HCV was only studied in general and apparently healthy population, whereas HBV was studied in both healthy population as well as chronic patients of hepatitis B. HBV core gene was amplified using nested primers. A total of 25 samples of HBV were sequenced and characterized out of which 5 were from the healthy population while 20 samples were those of chronic patients. The sequencing results showed 99-92% homology with previously reported HBV core clone sequence (Accession No. GQ183476.1). Consensus sequence obtained after sequencing of the gene from different samples was expressed in *E. coli*. The immunogenic properties of recombinant core protein were studied by raising polyclonal antibodies in the rabbits. The antibodies against the core protein were determined by antigen-antibody precipitation and ELISA methods.

This study is helpful in determining the prevalence of hepatitis B and C in different areas of Pakistan. It has also helped in analyzing the Pakistani population of HBV regarding the core antigen, which can be a possible option of a potential vaccine against HBV. Different combinations may be tried to improve the efficiency of core-based vaccines in future.

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List of Abbreviations

ABBREVIATIONS	COMPLETE WORDS
X-gal	5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside
μL	Microlitre
FPLC	Fast Protein Liquid Chromatography
HPLC	High Performance Liquid Chromatography
M-MuLV	Moloney Murine Leukemia Virus
Taq	<i>Thermus aquaticus</i>
Da	Dalton
EDTA	Ethylenediamine tetra-acetic acid
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
pTZ-HBVc	pTZ57R/T ligated with HBV core gene
<i>E. coli</i>	<i>Escherichia coli</i>
O. D.	Optical Density
cDNA	Complementary DNA
RT	Reverse Transcriptase
PCR	Polymerase chain reaction
pTZ57R/T	pTZ57RT plasmid not ligated
IPTG	Isopropyl β-D-1-thiogalactopyranoside
IU	International Unit
ICT	Immuno Chromatographic Technique
V	Volts

1. INTRODUCTION

Hepatitis

Hepatitis is a term used to describe the disease or inflammation of liver usually caused by a viral infection (Booth *et al.*, 2001). Hepatitis and hepatocellular carcinoma are one of the serious health problems around the world. Hepatocellular carcinoma (HCC) is one of the major types of primary liver diseases. HCC is the fifth most frequently found type of carcinoma and the third among the most common causes of death from cancer worldwide. The major risk factors for this cancer are chronic infections of hepatitis B virus (HBV), hepatitis C virus (HCV), prolonged exposure to dietary mycotoxins and alcoholic cirrhosis. Hepatoblastomas encompass 1-2% of all malignant tumors of childhood, generally found in children less than 3 years of age. Hepatoblastomas are considered to be originated from undifferentiated hepatocytes (Taniguchi *et al.*, 2002).

Viral hepatitis

There are five main hepatitis viruses, namely A, B, C, D and E. Hepatitis A and E viruses are generally transmitted by intake of contaminated food or water (Table I).

Table I. Transmission routes of different hepatitis viruses.

Transmission route	Hepatitis A	Hepatitis B	Hepatitis C	Hepatitis D	Hepatitis E
Food-borne	Common	Never	Never	Never	Common
Fecal-oral	Common	Never	Never	Never	Common
Water-borne	Common	Never	Never	Never	Common
Raw shellfish	Common	Never	Never	Never	Suspected
Intra-Institutional	Common	Common	Common	Common	Common
I.V drug use	Infrequent	Common	Common	Common	Infrequent
Transfusion	Infrequent	Common	Common	Common	Infrequent
Hemodialysis	Never	Common	Common	Common	Never
Sexual	Infrequent	Common	Infrequent	Common	Infrequent
Anal-oral contact	Common	Never	Never	Never	Infrequent
Oral-oral contact	Common	Infrequent	Never	Never	Common
Household	Common	Infrequent	Infrequent	Infrequent	Common
Mother to newborn	Infrequent	Common	Infrequent	Common	Infrequent

Other three viruses Hepatitis B, C and D viruses are usually transmitted as a result of parenteral contact with contaminated body fluids (e.g. from blood transfusions or surgical and piercing procedures using infected equipment).

Prevalence of HBV and HCV

Hepatitis B virus (HBV) is a DNA virus having an icosahedral core covered by a lipoprotein envelop. The protein shell of HBV's core, the capsid, is a self-assembling complex of 120 core protein homodimers (Crowther *et al.*, 1994; Zlotnick *et al.*, 1996). The possibility of viral hepatitis transmission through blood and other body fluids are known since long (Mahoney, 1999). These viruses are transmitted through blood and blood products, although sexual transmission and intrafamilial transmission have also been reported (Fortuny *et al.*, 1991). It has been estimated that about two billion people have been infected with hepatitis B virus and 350 million have chronic life long infections including chronic hepatitis, liver cirrhosis, and HCC (Ganem and Schneider, 2001). Prevalence of HBV varies in different geographical regions of the world (Fig.1).



Fig.1. Global hepatitis B prevalence map, 2005. The map indicates a high prevalence of hepatitis B in Central and South-East Asia, South and Central Africa, central parts of South America and Northern parts of Canada. Intermediate prevalence is reported in Indian Sub-continent, Middle East (except Saudi Arabia), Northern Africa, Russia and Eastern South America. Low prevalence is found in Australia, South America, Western Europe and North America.