Prevalence of Occult Hepatitis B Infection among Hemodialysis Patients and its Relation to Hepatitis C Virus Infection

Thesis

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List of Abbreviation

ALT	Alanine aminotransferase
Anti-HBc	Anti hepatitis B core antibody
Anti-HBe	Anti hepatitis B e antibody
Anti-HBs	Anti hepatitis B surface antibody
A nucleotide	Adenine nucleotide
A1762T	Adenine to Thymine substitution at position of the nucleotide 1762
APC	Antigen presenting cell
C nucleotide	Cytosine nucleotide
CD	Cluster of Differentiation
CD40	Cluster of Differentiation 40
CD40L	Cluster of Differentiation 40 ligand: CD154
CD80	Cluster of Differentiation 80: B7-1
CD86	Cluster of Differentiation 86: B7-2
cccDNA	covalently closed circular DNA
CDC	Centers for disease control and prevention
CRF	Chronic renal failure
CRP	C reactive protein
Ct	Threshold cycle
CTL	Cytotoxic T lymphocyte
CTLA-4	Cytotoxic T-lymphocyte associated antigen-4
DC cell	Dendritic cell
DR	Direct repeats
ER	Endoplasmic reticulum
ESRD	End stage renal disease
FasL	Fas Ligand
FoxP3	Forkhead/winged helix transcription factor on T_{reg} cells
G nucleotide	Guanine nucleotide
G145R	Glycine to Arginine substitution at codon 145
G1764A	Guanine to Adenine substitution at position of the nucleotide 1764

G1896A	Guanine to Adenine substitution at position of the nucleotide 1896
GM-CSF	Granulocyte monocyte colony stimulating factor
GN	Glomerulonephritis
HBcAg	Hepatitis B core antigen
HBeAg	Hepatitis B envelope antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HD	Hemodialysis
HIV	Human immune deficiency virus
HSPGs	Heparan sulphate proteoglycanes
Ig	Immunoglobulin
Ig-M	Immunoglobulin-M
IFN	Interferon
IFN-α/β	Interferon alpha/beta
IFN-γ	Interferon gamma
IL-2	Interleukin-2
IL-2R	Interleukin-2 receptor
IL-4	Interleukin-4
IL-12	Interleukin-12
IL-18	Interleukin-18
I.D.	Intradermal
I.M.	Intramuscular
IPC	Internal positive control
Kb	Kilo base
KDa	Kilo Dalton
LGL	Large granular lymphocyte
LN	Lymph node
L protein	Large protein
LPS	Lipopolysaccharide
LSEC	Liver sinusoidal endothelial cell

MHC	Major histocompatibility complex
M protein	Middle protein
MBL	Mannose binding lectin
mDC cell	Myeloid dendritic cell
moDC cell	Monocyte derived dendritic cell
MPL	Monophosphoryl lipid A
mRNA	messenger RNA
NAT	Nucleic acid testing
NF-κB	nuclear factor-κB
NK cell	Natural killer cell
NKT cell	Natural killer T cell
NS2 protein	Non-structural 2 protein
OLT	Orthotopic liver transplantation
ORF	Open reading frame
PAMPs	Pathogen associated molecular pattern
PCR	Polymerase chain reaction
pDC cell	Plasmacytoid dendritic cell
PD-1	Programmed death-1
PD-L1	Programmed death ligand-1
PEG-IFN	Pegylated interferon
P gene	Polymerase gene
PRR	Pattern recognition receptor
S protein	Small protein
sCD40	Soluble form of CD40
TCR	T cell receptor
TGF- ß	Transforming growth factor-ß
TLR	Toll like receptor
TNF- α	Tumour growth factor- α
T nucleotide	Thymine nucleotide
Th1 cell	T helper-1 cell
Th2 cell	T helper-2 cell
T_{reg} cells	Regulatory T cells
α-GalCer	α-galactosylceramide
ζ-chain	Zeta chain

INTRODUCTION

Hepatitis B virus (HBV) infection is one of the major health problems in the world with an estimation of 350 million people chronically infected. It is one of the major causes of chronic liver disease. It causes a broad spectrum of liver disease ranging from acute self limited hepatitis to fulminant hepatitis, chronic hepatitis including asymptomatic carrier state and chronic active hepatitis. It is also one of the main causes of liver cirrhosis and hepatocellular carcinoma (HCC) ***(Yalcin et al., 2003).***

The proportion of patients suffering from liver disease of unknown cause ranges from 5% in chronic hepatitis up to 40% in fulminant hepatitis cases. These patients may develop severe liver injury leading to an increased risk of cirrhosis. Several studies have called attention to HBV infection in the absence of serological markers or in the presence of anti hepatitis B core (anti-HBc) alone. It has been demonstrated that the serum of some patients without detectable hepatitis B surface Ag (HBsAg) may contain infectious virus. Accumulated data indicated that a low level of HBV DNA remains detectable in serum and liver tissue in some patients who cleared HBsAg from either acute self limited or chronic HBV infection ***(Honarkar et al., 2004).***

The frequency of HBV DNA in patients with cryptogenic chronic liver disease (persistent alteration in liver biochemistry and the etiology could not be determined from clinical, biochemistry or serological data) varies depending on the baseline prevalence of HBV infection in certain geographical area, population studied and techniques used to detect HBV DNA. One study performed on patients with cryptogenic chronic hepatitis found that one of them had detectable HBV

DNA indicating an occult HBV infection. During follow up, repeated liver biopsy demonstrated that one fifth had progressed from chronic hepatitis to cirrhosis. These finding indicate that occult HBV infection is a common etiology of cryptogenic chronic hepatitis and a progressive disease at least in some patients ***(Chan et al., 2002).***

Occult HBV infection is defined as presence of HBV DNA without detectable HBsAg with or without anti-HBc or anti-HBs outside the pre-seroconversion window period. In most cases, occult HBV infection is related to low levels of HBV infection with sub-detectable levels of HBsAg and not infections with HBV variants that can not express S protein with aberrant epitopes which are not detected by conventional serological assay ***(Jafarzadeh et al., 2008).*** Another hypothesis is that the lack of detectable HBsAg in the blood may be due to rearrangement in HBV genome that interfere with gene expression or lead to production of antigenically modified surface (S) protein ***(Hui et al., 2006).***

The effect of virus interference of HBV by hepatitis C virus (HCV) in co-infected individuals has been well demonstrated in previous studies which showed that HCV core protein strongly inhibited HBV replication and gene expression ***(Lin, 2007).***

It is reported that there is a high prevalence of occult HBV infection in patients with chronic HCV, HCC, hemodialysis (HD) patients, in those with cryptogenic liver disease, drug injection users and HIV patients and those who underwent frequent blood transfusions e.g. hemophilic patients ***(Goral et al., 2006).***

HD patients are more vulnerable to HCV infection than others because of history of blood transfusion, frequent

injections, partial immunosuppression and history of kidney transplant. The duration of HD treatment and nosocomial HCV transmission have also been suggested as a contributing factor. The prevalence of HCV antibodies (HCV Ab) in dialysis patients has been reported to range from 20% to 81.6% in previous studies **(*Amiri et al., 2005*).**

Some kidney dialysis patients contract HBV during the course of their treatment, possibly from other members of the dialysis population with occult HBV which is detected through sensitive tests not typically performed on dialysis patients. A study found that the prevalence of occult HBV in adult HD patients is four to five times higher than standard HBsAg testing would suggest. If occult HBV status is known, transmission among HD patients might be limited by avoiding dialyzers reuse and dedicating dialysis rooms, machines and staff for infected patients. Vaccination may also protect HD patients from contracting HBV ***(Minuk et al., 2004).***

For these reasons, highly sensitive nucleic acid technology becomes essential. The introduction of polymerase chain reaction (PCR) based methods has resulted in a large increase in the sensitivity of HBV DNA detection. More recently, the development of real-time PCR methodology has further improved the ease with which HBV DNA levels can be monitored and has increased the range over which such level can be accurately quantified ***(Mendy et al., 2006).***

Aim of the Work

The aim of this study was to determine the prevalence of occult HBV in patients on maintenance HD in Ain Shams University Hospital and the relation of occult HBV infection to HCV infection and total anti-HBc among these patients.

I- HEPATITIS B VIRUS INFECTION

Hepatitis B virus infection (HBV) is a serious health problem worldwide. It is one of the most common infectious diseases globally. It is estimated that approximately 2 billion people have serological evidence of past or present infection with more than 50 million new infections occurring yearly. More than 350 million are chronic carriers of HBV and 500,000 to 1.2 million die of HBV infection annually ***(Wright, 2006).***

A) HBV Structure and Genome:

HBV is a prototype member of the Hepadnaviridae (hepatotropic DNA viruses) family which has a strong preference to infecting liver cells. The mature virion, also known as Dane particle, is 40-42 nm in diameter consisting of an outer lipoprotein layer that encodes the viral envelope proteins, the hepatitis B surface antigen (HBsAg), and surrounds a nucleocapsid core, the hepatitis B core antigen (HBcAg). The nucleocapsid contains the viral genome and viral polymerase **(Fig.1)** ***(Bergua et al., 2009).*** In addition to the mature virions, HBV infected serum contains two other distinct subviral particles that are either spherical or filamentous in shape and are approximately 20 nm in width. Subviral particles reach a 10,000 fold higher concentration than virions in the serum. They consist of an envelope glycoprotein and host derived lipids. The precise biological significance of this massive overproduction of empty envelopes is unknown; however, it has been speculated that they serve as decoys for host's immune system ***(Zekry and Mchutchison, 2007).***