

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

**Thesis**

Submitted for Partial Fulfillment of Master Degree  
In Clinical Pathology

**By**

**Hoda Mohamed Hassan Elsoukkary**  
**M B, B Ch**

*Supervised by*

**Professor / Hala Ahmed Sherif Talkhan**  
Professor of Clinical Pathology  
Faculty of Medicine - Ain Shams University

**Professor / Salwa Ibrahim Bakr**  
Professor of Clinical Pathology  
Faculty of Medicine - Ain Shams University

**Doctor / Dina El-Sayed El-Shennawy**  
Lecturer of Clinical Pathology  
Faculty of Medicine - Ain Shams University

**Faculty of Medicine**  
**Ain Shams University**  
**2009**

## Acknowledgement

*Thanks are given to ALLAH the source of all knowledge for blessing this work till it has come to an end.*

*I would like to express my deepest thanks to Prof. Dr. Hala Ahmed Sherif Talkhan, Professor of Clinical Pathology, Faculty of Medicine, Ain Shams University, for her kind support guidance and valuable remarks. I am profoundly grateful for her continuous close supervision and constant help.*

*I would also like to express my deepest thanks and gratitude to Prof. Dr. Salwa Ibrahim Bakr, Professor of Clinical Pathology, Faculty of Medicine, Ain Shams University for her generous help, continuous encouragement and stimulating remarks throughout the study.*

*I would also like to express my great gratitude to Dr. Dina El-Sayed El-Shennawy, Lecturer of Clinical Pathology, Faculty of Medicine, Ain Shams University for her valuable comments, knowledge, experience and hand necessary for achieving this work.*

*Last but not least, I would like to thank my family especially my parents who encouraged and supported me all the time, to them I dedicate this work.*

# *List of Contents*

<b>Introduction .....</b>	<b>1</b>
<b>Aim of the work .....</b>	<b>4</b>
<b>Hepatitis B Virus .....</b>	<b>5</b>
HBV structure and genome .....	5
HBV lifecycle .....	9
HBV serotypes .....	13
HBV genotypes and its clinical significance .....	13
HBV mutants .....	15
Immunopathogenesis of HBV infection .....	21
Clinical picture of chronic HBV infection .....	38
Natural history of HBV infection .....	39
<b>Occult HBV Infection .....</b>	<b>45</b>
Virological aspects of persistent infection .....	45
Etiology .....	46
Types of occult HBV infection .....	49
Prevalence .....	50
Clinical relevance .....	52
Detection of occult HBV infection .....	58
<b>HBV Infection in Hemodialysis Patients .....</b>	<b>59</b>
Transmission of HBV to HD patients .....	59
Immune response of HD patients to HBV infection .....	60
Clinical outcome of occult HBV infection in HD .....	61

Prevention of HBV in HD patients .....	61
<b>Immune Response in HD Patients .....</b>	<b>68</b>
The innate immune response .....	69
The adaptive immune response .....	73
Alterations in the immune system in ESRD .....	73
<b>Subjects and Methods .....</b>	<b>85</b>
<b>Results .....</b>	<b>94</b>
<b>Discussion .....</b>	<b>100</b>
<b>Recommendations .....</b>	<b>112</b>
<b>Summary .....</b>	<b>113</b>
<b>References .....</b>	<b>117</b>
<b>Arabic Summary .....</b>	<b>1</b>

## *List of Figures*

<b>Figure 1:</b>	A simplified drawing of the HBV particle and surface antigen .....	6
<b>Figure 2:</b>	Domains of HBV surface proteins .....	7
<b>Figure 3:</b>	Representation of HBV genome Domains of HBV surface proteins .....	8
<b>Figure 4:</b>	The Replication cycle of HBV .....	12
<b>Figure 5:</b>	Immunoregulatory functions of hepatic NKT cells .....	24
<b>Figure 6:</b>	The cytokine/chemokine cascade through which NK cells recruit T cells .....	26
<b>Figure 7:</b>	DCs are the most potent APCs .....	29
<b>Figure 8:</b>	Cellular immune responses to HBV .....	32
<b>Figure 9:</b>	Schematic figure of how the B7-H1/PD-1 pathway may mediate the exhaustion of virus-specific T cells in chronic HBV infection .....	37
<b>Figure 10:</b>	HBV-specific T-cell tolerance causes .....	38
<b>Figure 11:</b>	Phases of chronic HBV infection .....	42
<b>Figure 12:</b>	Natural history of chronic HBV infection .....	43
<b>Figure 13:</b>	Model of HBsAg vaccination .....	66

<b>Figure 14:</b> Schematic presentation of secreted, endocytic and signalling pattern-recognition receptors .....	72
<b>Figure 15:</b> Mononuclear peripheral blood cells undergo a process of cyclic activation as a consequence of their interaction with HD membrane .....	76
<b>Figure 16:</b> Serum sCD40 levels in chronic HD patients in the course of HBV vaccination and correlation to their vaccine response status .....	83

## *List of Tables*

<b>Table 1:</b>	Geographical distribution of HBV genotypes .....	15
<b>Table 2:</b>	Stages of chronic HBV infection .....	41
<b>Table 3:</b>	Categories of occult HBV carriers known to be prone to viral reactivation .....	55
<b>Table 4:</b>	HBV vaccination protocols in uremia .....	64
<b>Table 5:</b>	Immune response in HD patients .....	84
<b>Table 6:</b>	Prevalence of total anti-HBc among HD patients .....	94
<b>Table 7:</b>	Prevalence of HCV Ab among HD patients .....	95
<b>Table 8:</b>	Comparative statistics of total anti-HBc with HCV Ab among HD patients .....	95
<b>Table 9:</b>	Levels of ALT and AST among HD patients with HCV Ab positive patients .....	96
<b>Table 10:</b>	Comparative statistics of kidney function tests with HCV Ab among HD patients .....	96
<b>Table 11:</b>	Prevalence of occult HBV infection among HD patients .....	97
<b>Table 12:</b>	Demographic and laboratory characteristics of HD patients with occult HBV infection .....	98
<b>Table 13:</b>	Comparative statistics of total anti-HBc with HBV DNA among HD patients .....	98
<b>Table 14:</b>	Comparative statistics of HCV Ab with HBV DNA among HD patients .....	99
<b>Table 15:</b>	Reported prevalence of occult HBV infection in HD patients .....	105

## *List of Abbreviation*

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



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

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