

# **Hepatitis B Virus Genotypes among Egyptian Patients**

**Protocol of thesis**

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*To My Mother,  
To my beloved, caring and  
understanding husband.*





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# List of Abbreviation

<b>AFP:</b>	Alpha-fetoprotein
<b>ALT:</b>	Alanine aminotransferase
<b>Anti-HBc:</b>	Anti hepatitis B core antibody
<b>Anti-HBs:</b>	Anti hepatitis B surface antibody
<b>APCs:</b>	Antigen-presenting cells
<b>BCR:</b>	B cell receptor
<b>ccc DNA:</b>	Covalently closed circular DNA
<b>CD:</b>	Cluster of differentiation
<b>CTL:</b>	Cytotoxic T lymphocytes
<b>DCs:</b>	Dendritic cells
<b>Fas L:</b>	Fas ligand
<b>GM-CSF:</b>	Granulocyte-macrophage colony-stimulating factor
<b>G145R:</b>	Glycine to Arginine substitution at codon 145
<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>ICU:</b>	Intensive care unit
<b>IFN-<math>\alpha/\beta/\gamma</math></b>	Interferons alpha/beta/gamma
<b>Ig:</b>	Immunoglobulin
<b>IHL:</b>	Intra-hepatic lymphocytes



<b>IL :</b>	Interleukin
<b>INNO-LIPA:</b>	Innogenetics line probe assay
<b>IRF3:</b>	Interferon regulatory factor 3
<b>ISGs:</b>	IFN-stimulated genes
<b>L protein:</b>	Large protein
<b>M protein:</b>	Medium protein
<b>MCA:</b>	Melting curve analysis
<b>M-CSF:</b>	Macrophage colony-stimulating factor
<b>MDA5:</b>	Melanoma differentiation associated gene 5
<b>mDCs:</b>	Myeloid dendritic cells
<b>MHC:</b>	Major histocompatibility complex
<b>MIP-1<math>\alpha</math>:</b>	Macrophage-inflammatory protein-1 $\alpha$
<b>mRNA:</b>	Messenger RNA
<b>NF-<math>\kappa</math>B:</b>	Nuclear factor- $\kappa$ B
<b>NK:</b>	Natural killer
<b>NKT:</b>	Natural killer T
<b>OBI:</b>	Occult HBV infection
<b>ORF:</b>	Open reading frame
<b>P:</b>	Polymerase
<b>PD:</b>	Programmed death
<b>pDC:</b>	Plasmacytoid dendritic cells
<b>PD-L1:</b>	Programmed death-ligand 1
<b>pg RNA:</b>	Pre-genomic RNA
<b>PRRs:</b>	Pattern recognition receptors
<b>RFLP:</b>	Restriction fragment length polymorphism

<b>RIG-I:</b>	Retinoic acid-inducible gene I
<b>S protein:</b>	Small protein
<b>SOI:</b>	Secondary occult infection
<b>SVP:</b>	Subviral particles
<b>Th1:</b>	T helper-1
<b>Th2:</b>	T helper-2
<b>TLRs:</b>	Toll-like receptors
<b>TRAIL:</b>	TNF-related apoptosis-inducing ligand

## INTRODUCTION

Hepatitis B virus (HBV) is very common worldwide. It is characterized by causing hepatitis B. However, it can also lead to cirrhosis and hepatocellular carcinoma (HCC). It has also been suggested that it may increase the risk of pancreatic cancer (**Hassan et al., 2008**). Chronic infection with HBV is a common cause of death associated with liver failure, cirrhosis, and liver cancer. Worldwide, approximately 350 million persons have chronic HBV infection, and an estimated 620,000 persons die annually from HBV-related liver disease (**Goldstein et al., 2005**).

A classification reflecting the phylogenetic origin of the virus isolates was proposed dividing HBV into eight genotypes designated A to H. These genotypes were differentiated by a sequence divergence in the entire genome exceeding 8%. While, subgenotypes have been described which differ by at least 4% (**Norder et al., 2004**). The genotypes show a distinct geographical distribution between, and even within, regions and are proving to be an invaluable tool in tracing the molecular evolution and patterns and modes of spread of HBV. Structural and functional differences between genotypes can influence the severity, course and likelihood of complications, hepatitis B e antigen (HBeAg) seroconversion as well as response to treatment of HBV infection and possibly vaccination against the virus (**Chu et al., 2002**).

As of today, HBV genotypes remain significant mostly from the research point of view. However, there is growing evidence from research being conducted around the globe that

the day when HBV genotypes will become as important clinically as hepatitis C virus genotypes may not be too far away (**Mahtab et al., 2008**).

## **Aim of The Work**

The aim of this work is to detect the predominant genotype(s) of HBV among the Egyptian chronic hepatitis B patients and its relation to the disease state.

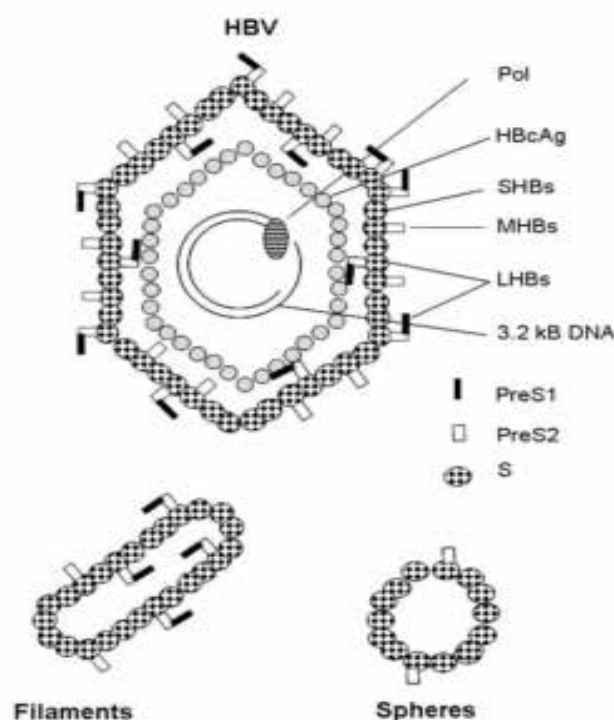
# HEPATITIS B VIRUS INFECTION

## **A) HBV Structure and Genome:**

The HBV is classified as the type species of the Orthohepadnavirus (**Hunt, 2007**). The genus is classified as part of the Hepadnaviridae (hepatotropic DNA viruses) family which have not been assigned to a viral order (**Mason et al., 2008**).

The infectious HBV virion (also known Dane particle) is a spherical particle, 42 nm in diameter. The virion contains the nucleocapsid which consists of the circular partially double-stranded genomic DNA covalently linked to the viral reverse-transcriptase. This nucleocapsid is surrounded by a lipid bilayer in which the three envelope proteins [small (S), medium (M) and large (L)] are anchored as transmembrane proteins playing a major role in HBV morphogenesis and infectivity (**Bruss, 2007**). The HBV surface proteins are not only incorporated into the virion envelope but also bud in an empty and non-infectious subviral particles (SVP). They consist of an envelope glycoprotein and host derived lipids. The SVP conformation is organized as an octahedral sphere about 20 nm in diameter or as a filament with the same diameter but with variable length (**Gilbert et al., 2005**) (**Fig.1**). The SVPs reach a 10.000 fold higher concentration than virions in the serum. The precise biological significance of this massive overproduction of such SVP is unknown; however, it has been speculated that they

serve as decoys for host's immune system (Zekry and Mchutchison, 2007).



**Fig. 1: Schematic Structure of the HBV Particle and Subviral Particles.** The envelope is formed by the three viral surface proteins LHBs, MHBs and SHBs that surround the viral nucleocapsid. The core protein (HBcAg) forms the nucleocapsid that harbors the partially double-stranded circular DNA genome that is covalently linked to the viral polymerase. In the serum of HBV-positive patients, large amounts of non-infectious subviral particles in the form of filaments or spheres are found; these are composed of the viral surface proteins, but lack the viral nucleic acid (Schädler and Hildt, 2009).

The genome of HBV is made of circular DNA, but it is unusual because the DNA is not fully double-stranded. The 5' end of the full length strand (minus strand) is linked to the viral DNA polymerase, while the 5' end of the short length strand (plus strand) is linked with short piece of capped RNA. The