# Value of serum Adiponectin level in Egyptian patients with chronic hepatitis B infection

Thesis

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by


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# Introduction

Hepatitis B virus (HBV) infection is a serious public health problem, with more than 350 million people worldwide suffering from chronic hepatitis B (CHB). Between 15% and 40% of HBV-infected patients will ultimately develop cirrhosis, liver failure, and hepatocellular carcinoma (HCC) **(Lok, 2002)**. Chronic viral hepatitis B is frequently associated with hepatic steatosis. The mechanism underlying HBV-mediated hepatic steatosis remains incompletely understood **(Gordon et al., 2005)**.

It was shown that over expression of the hepatitis B virus X (HBx) protein induces peroxisome proliferator-activated receptor γ (PPARγ) gene expression and transcriptional activation, leading to the upregulation of the fatty acid uptake-associated gene CD36 and of several adipogenic genes, including adipsin, aP2, and adiponectin **(Kim et al., 2007 and Na et al., 2009)**.

Adiponectin is an adipocytokine secreted by adipocytes, with antidiabetic, antilipogenic and antiatherogenic actions **(Weyer et al., 2001)**. Low plasma levels of adiponectin contribute to the pathogenesis of insulin resistance, type II diabetes mellitus and cardiovascular diseases **(Guerre-Millo, 2008)**. Adiponectin directly affects the inflammatory response by regulating both the production and activity of cytokines **(Ouchi et al., 2000)** and can also act as an antiapoptotic agent in a variety of cell types **(Kobayashi et al., 2004)**.

Adiponectin suppresses the proliferation and migration of vascular smooth muscle cells, whose characteristics are similar to those of hepatic stellate cells. Hepatic stellate cells play central roles in liver fibrosis. When they are activated, they undergo transformation to myofibroblast-like cells, so Adiponectin could have biological significances in liver fibrosis **(Yokota et al., 2000)**.

**Wong et al., 2010** found a positive correlation between serum HBV DNA and serum adiponectin level (but not with HOMA insulin resistance index). The positive correlation between HBV DNA and serum adiponectin may partly explain the purported protective effect of HBV infection against insulin resistance **(Kumar et al., 2008 & Wang et al., 2010)**

# Aim of the study

The aim of this study was to determine the value of serum Adiponectin level in Egyptian patients with chronic hepatitis B infection and to correlate these levels with the metabolic profile in these patients.

# Chronic hepatitis B Infection

## Introduction:

As a leading cause of cirrhosis and hepatocellular carcinoma, chronic hepatitis B virus (HBV) infection has become a massive economic burden world-wide. Hepatitis B has been recognized as a separate entity since the early 1940s, the first recorded cases of hepatitis B are thought to be those that followed the administration of smallpox vaccine containing human lymph to shipyard workers in Germany in l883. In the early and middle parts of the 20th century, HBV was repeatedly observed following the use of contaminated needles and syringes. The role of blood as a vehicle for virus transmission was further emphasized in 1943, when Beeson described jaundice that had occurred in seven recipients of blood transfusions. Australia antigen, later called hepatitis B surface antigen (HBsAg), was first described in 1965, and the Dane particle (complete hepatitis B virion) was identified in 1970 **(CDC, 2008).**

Since the 1970s, considerable progress has been made regarding the knowledge of the epidemiology, virology, natural history, and treatment of the hepatitis B virion; a hepatotropic virus particle. HBV is a hepadna virus, highly resistant to extremes of temperature and humidity.

The viral genome is a double-stranded, circular DNA linked to a DNA polymerase that is surrounded by an icosahedral nucleocapsid and then by a lipid envelope. Embedded within these layers are numerous antigens that are important in disease identification and progression. Within the nucleocapsid are the hepatitis B core antigen (HBcAg) and precore hepatitis B envelope antigen (HBeAg), and hepatitis B surface antigen (HBsAg) on the envelope **(Chang et al., 1997).**

# Epidemiology:

About 240 million people worldwide are chronically infected with HBV, which put them at great risk to develop liver cirrhosis and hepatocellular carcinoma (HCC) **(Trepo et al., 2014).**

HBV prevalence varies from 0.1% up to 20%. Low prevalence (<2%) areas represent 12% of the global population and include Western Europe, the United States and Canada, Australia and New Zealand. In these regions, the lifetime risk of infection is less than 20%. Intermediate prevalence areas is defined as 2% to 7%, with a lifetime risk of infection of 20-60% and includes the Mediterranean countries, Japan, Central Asia, the Middle East, and South America (about 43% of the global population).

High prevalence areas (≥8%) include Southeast Asia, China, and sub-Saharan Africa, where a lifetime likelihood of infection is greater than 60% **(Wasley et al., 2008).**

The diverse prevalence rates are probably related to differences in age at infection, which correlates with the risk of chronicity. The progression rate from acute to chronic HBV infection decreases with age. It is approximately 90% for an infection acquired perinatally and is 5% or lower for adults **(Wasley et al., 2008).**

## Taxonomic classification and genotypes

The Hepadnaviridae form their own taxonomic group as their biological characteristics differ from any other viral family. The family of Hepadnaviridae contains two genera: the orthohepadna viruses infecting mammals, and the avihepadna viruses that infect birds **(Funk et al., 2007).**

Orthohepadna viruses have been found in human are HBV, woodchuck (WHBV), ground squirrel (GSHBV), arctic squirrel (ASHBV) and woolly monkey (WMHBV) (Funk et al., 2007). Three unique hepadna virus species antigenically related to human HBV and capable of infecting human hepatocytes were also identified in bats. Thus, bats might constitute ancestral sources of primate hepadna viruses **(Drexel et al., 2013).**

Due to the lack of proofreading activity of the viral polymerase, disincorporation of nucleotide mutations occurs during viral replication. This has led to the emergence of eight HBV genotypes, A-H, which differ in more than 8% of the genome, as well as different subgenotypes, which differ by at least 4% **(Guirgis et al., 2010).**

The HBV genotypes have different geographic distribution **(Liaw et al., 2010)**, with predominance of genotype A in northwestern Europe, North and South America, genotype B and C in Asia and genotype D in eastern Europe and the Mediterranean area. The remaining genotypes are mostly found in West and South Africa (genotype E), in Central and South America (genotypes F and H), while genotype G is found in France and the USA **(Pujol et al., 2009)**.

In a study about the genotypes of HBV isolated from 100 serum samples of Egyptian carriers by sequencing, it was found that HBV genotype D was the most prevalent genotype in Egypt representing 87% of the cases, but did not detect mixed infection as sequence analysis provides information only on the majority strain, while LiPA method appears to overcome this limitation by its sensitive detection of mixed genotypes which showed that the other 13% showed mixed infections of genotypes D and F **(Osiowy & Giles, 2003).**

## HBV structure and genomic organization

**Genome:** the HBV virion genome is circular and approximately 3.2 kb in size and consists of double stranded DNA. It has compact organization, with four overlapping reading frames running in one direction and no noncoding regions **(Stares et al., 2002).**

HBV genome displays four major open reading frames (ORFs) that are organized in a highly condensed way **(Block et al., 2007).**

As shown in **(figure 1)**, all ORFs are in an identical orientation, partially overlap and are encoded by the negative strand. On the genome, 6 start codons, four promoters and two transcription-enhancing elements have been identified. The four major ORFs are:

I) The preS/S, encoding the three viral surface proteins.

II) The precore/core, encoding both the core protein, essential for the formation of the nucleocapsid, and the nonstructural pre-core protein, also known as the secreted e-antigen (HBeAg).

III) The pol ORF of the viral polymerase, which possesses reverse transcriptase, DNA polymerase and RNase H activities, and the terminal protein.

IV) The X ORF, coding for the small regulatory X protein, which has been shown to be essential *in vivo* for viral replication and is capable of transactivating numerous cellular and viral genes.

Characteristic of the 4 major HBV ORFs is that they utilize a single common polyadenylation signal motif. Thus, all RNA transcripts are polyadenylated and capped. **(Lucifora et al., 2011)**

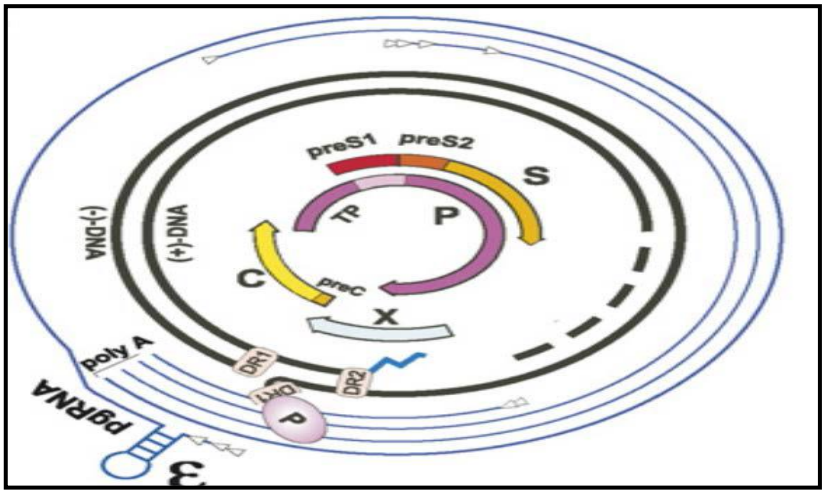


**Figure (1):** Genome organization and transcripts of the human hepatitis B virus. **(Lucifora et al., 2011)**

**HBV Particle Types:** The hepatitis B virion, also known as the Dane particle, is the infectious particle of HBV. This virion has a diameter of 42nm and its outer envelope contains a high quantity of hepatitis B surface proteins. The envelope surrounds the inner nucleocapsid which is made up of 180 hepatitis B core proteins arranged in an icosahedral arrangement. The nucleocapsid also contains at least one hepatitis B ploymerase protein (P) along with the HBV genome **(Garces & Robert, 2003)**, as shown in **(figure 2).**

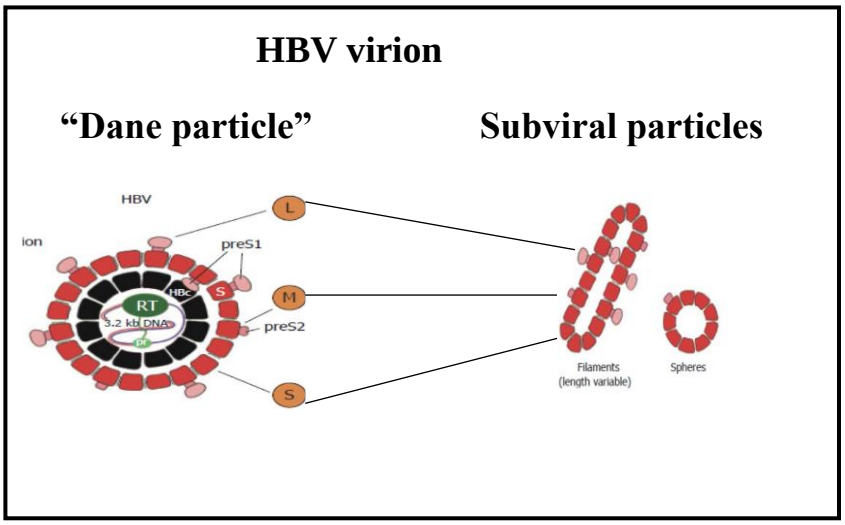


**Figure (2):** Schematic representation of the HBV virion and non-infectious empty subviral particles (filaments and spheres). **(Glebe & Urban, 2007)**

In infected people, virions actually compose a small minority of HBV-derived particles. Large numbers of smaller subviral particles are also present, that outnumber the virions by a ratio of 100:1. Two other subviral particles, the hepatitis B filament and the hepatitis B sphere, are often referred to as a group named surface antigen (HBsAg) particles. They are both 22nm in diameter and are totally composed of hepatitis B surface proteins **(Garces & Robert, 2003)**.

The sphere contains both middle and small hepatitis surface proteins whereas the filament also includes large hepatitis B surface protein. The absence of the hepatitis B core, polymerase, and genome causes these particles to have a non-infectious nature.

High levels of these non-infectious particles can be found during the acute phase of the infection. Since the non-infectious particles present the same sites as the virion, they induce a significant immune response and are thought to be non-advantagous for the virus. However, it is also believed that the presence of high levels of non-infectious particles may allow the infectious viral particles to travel undetected by antibodies through the blood stream **(Garces & Robert, 2003)**.

**Life Cycle:** In order to reproduce, the hepatitis B virus, must first attach onto a cell which is capable of supporting its replication. Although hepatocytes are known to be the most effective cell type for replicating HBV, other types of cells in the human body have be found to be able to support replication to a lesser degree. The initial steps following HBV entry are not clearly defined although it is known that the virion initially attaches to a susceptible hepatocyte through recognition of cell surface receptor **(Garces & Robert, 2003)**.

The DNA then enters into the nucleus where it is known to form a convalently closed circular form called ccc-DNA. The negative strand of such ccc-DNA is the template for transcription by cellular RNA polymerase II of a longer RNA than the genome length called the pregenome and another shorter subgenomic transcripts serve as mRNAs **(Flint et al., 2000).**

The shorter viral mRNAs are translated by ribosomes attached to the cell's endoplasmic reticulum and the proteins that are destined to become HBV surface antigens in the viral envelope are assembled. The pregenome RNA is translated to produce a polymerase protein P, which binds to a specific site at the 3' end of its own transcript, where viral DNA synthesis occurs. At the same time, the RNA-P protein complex is packaged and reverse transcription begins. At early times after the infection, the DNA is recirculated to the nucleus, where the process is repeated, resulting in the the accumulation of 10 to 30 molecules of ccc-DNA and an increase in viral mRNA concentrations **(Flint et al., 2000).**

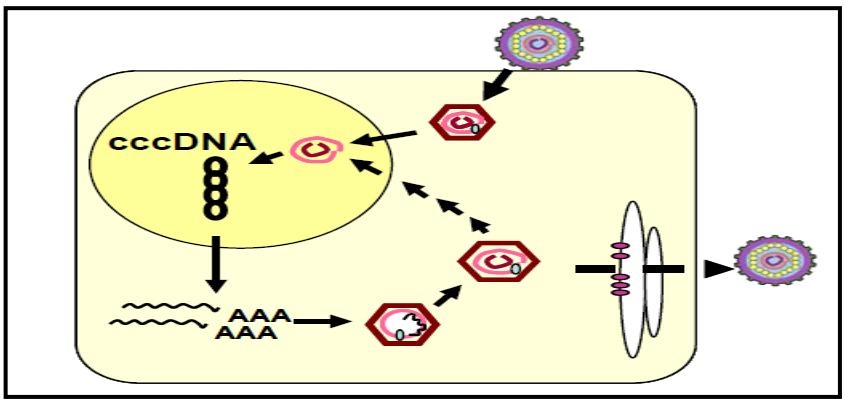


**Figure (3):** The HBV lifecycle. **(Bock et al., 2001).**

## Hepatitis B Antigens

There are three different types of hepatitis B antigens encoded by the HBV genome:

**Hepatitis B Surface antigen (HBsAg):** There are three different types of hepatitis B surface antigens; small hepatitis B surface antigen (HBsAg or SHBsAg), middle hepatitis B surface antigen (MHBsAg), and large hepatitis B surface Antigen (LHBsAg). HBsAg is the smallest protein of the hepatitis B surface proteins and has historically been known as the Australia antigen (Au antigen). It is very hydrophobic, containing four-transmembrane spanning regions. This protein is the prime constituent of all hepatitis B particle forms and appears to be manufactured by the virus in high quantities. It also contains a highly antigenic epitope which may be responsible for triggering immune response.

Reduced production of HBsAg leads to intracellular retention of the virus. MHBsAg contains an additional amino-acid domain and appears to reside extracellularly. MHBsAg is not required for HBV infectivity and therefore it is more likely that is contributes to viral attachment as a secondary mechanism. LHBsAg is the largest of the HBV surface proteins, containing three domains within the HBV encoding region. It is believed to be involved in liver attachment due to its variability among patients, and responsible for mediating viral attachment into host cells **(Yen & Benedict, 2002).**

**Hepatitis B core Antigen (HBcAg):** A 185 amino acid protein is expressed in the cytoplasm of infected cells, they are highly associated with nucleo-capsid assembly **(Stares et al., 2002).**

**Hepatitis B envelope Antigen (HBeAg):** This antigen which appears during an acute HBV infection and thought to be located in the core structure of the virus molecule, it is usually indicative of complete virus particles in circulation. **(Stares et al., 2002).**

It is not required for viral infection or replication, but appears to act as a decoy for the immune system, and hence, has tolerogenic functions in promoting viral persistence **(Visvanathan & Lewin, 2006),** and it is widely used as a marker for active viral replication **(Hadziyannis & Papatheodoridis, 2006).**

## Routes of HBV transmission:

There is considerable variation in the predominance of transmission modes in different geographic areas. For example, in low prevalence areas such as Western Europe, the routes are mainly unprotected sexual intercourse and intravenous drug use. In high prevalence areas like sub-Saharan Africa perinatal infection is the predominant mode of transmission. Horizontal transmission, particularly in early childhood, is regarded as the major route of transmission in intermediate prevalence areas **(Raphael Mohr et al., 2015).**