# Study of Serum Retinol Binding Protein 4 and its Association with Insulin Resistance in HCV Patients

Thesis

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# دراسة البروتين 4 المرتبط بالرتينول وعلاقته بمقاومة الأنسولين في المرضى المصابين بالتهاب الكبدي ج

## رسالة

مقدمة توطئة لنيل درجة الماجستير
في الغدد الصماء

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# List of Abbreviations

**Adiponectin** : Adipocytokine
**AFABP** : Adipocyte fatty acid-binding protein
**AKT** : A family of a serine/threonine protein kinase that plays a key role in multiple cellular processes such as glucose metabolism, cell proliferation, apoptosis, transcription and cell migration.
**AMA** : Antimitochondrial antibody
**ANA** : Antinuclear antibody
**CHB** : Chronic hepatitis B ,CHC chronic hepatitis C
**CTGF** : Connective tissue growth factor
**CTL** : Cytotoxic lymphocyte
**CTL** : Cytotoxic lymphocyte
**ER** : Endoplsmic reticulum
**GLUT4** : Glucose transporter 4
**HCC** : Hepatocellular carcinoma
**HGV** : Hepatits G virus
**HOMA-IR** : Homeostasis model of assessment of insulin resistance
**HSV** : Herps Simplex virus
**IFG** : Impaired fasting glucose glucose
**IGT** : Impaired glucose tolerance
**IkkB** : Inhibitory Kapp beta kinase b
**IR** : Insulin resistance
**IRES** : Internal ribosomal entry site
**IRS** : Insulin receptor susbtrate
**JNK-C** : Jun N-terminal Kinase
**LDLR** : Low density lipoprotein receptor
**LKM** : Liver –Kidney microsomal antibody
**L-SIGN** : Liver /lymphnode –specific intracellular adhesion molecule 3-grabling integrin
**LT** : Liver transplantion
**MC** : Mixed cryoglobulinemia
**MMWR** : Morbidity and Mortality Weekly Report
**MTOR** : Mammlian target of rapomycin
**mTORC1** : mammalian target of rapamycin complex 1

**NAFLD** : Non alcoholic fatty acid liver disease
**NANHES-III** : Third Notional Health and Nutrition Examination survey
**NFKB** : Nuclear factor kappa –light chain enhancer of activated B cells
**ORF** : Open reading frame
**OSHA** : Occupational Safety and Health Adminstration
**PA28** : Proteasome Activator 28 gamma
**PEPCK** : Phosphoenol pyrovate carboxyl kinase
**PI3K** : Phosphatidylinositol-3-kinase
**PI3-Kinase** : Phosphatidylinositol 3 –kinase
**PNALT** : persistently normal alanine transaminase
**PP2A** : Protein phosphatase 2A
**PPAR** : Peroxisome proliferators activated receptor
**PPRE** : Peroxisome Proliferator response element
**RAR** : Retinoic acid receptor
**RBP** : Retinol binding protein
**Resistin** : Adipocytokine
**RT-PCR** : Real time polymerase chain reaction
**RXR** : Retinoid x receptor
**SLA** : Soluble liver antigen
**SMA** : Smooth muscle (actin) antibody
**SOCS3** : Supressor of cytokine signaling -3
**sTNFR2** : Soluble tumor necrosis factor receptor
**SVR** : Sustained virological response
**TH1** : T-cell helper 1
**TNT a** : Tumor necrosis factor alpha
**3′ UTR** : 3′ untranslated reigon
**5′ UTR** : 5′ untranslated reigon
**9 c R A** : 9 cis retinoic acid receptor a naturally occurring retinoid isomer

# Introduction

Hepatitis C virus a member of the Flaviviridae family of viruses is a positive single-stranded RNA virus which is one of several viruses that can cause hepatitis ***(Medicinenet, 2005).***

Several extrahepatic manifestations have been reported in the natural history of hepatitis C virus infection (HCV). Up to 40-74% of patients infected with HCV might develop at least one extrahepatic manifestation during the course of their disease ***(Galossi et al., 2007).***

In chronic hepatitis C, insulin resistance and type 2 diabetes mellitus are more often seen than in healthy controls or chronic hepatitis B patients. It has been reported that up to one-third of patients with chronic liver disease caused by HCV develop type 2 DM. Moreover, HCV seropositivty in patients with DM appears to be higher than general population ***(Taura et al, 2007).***

Mechanisms leading to HCV-induced insulin resistance and glucose intolerance are beginning to be elucidated. Insulin resistance in the setting of chronic HCV infection could be related etiologically to viral factors but is also often seen with concomitant nonalcoholic fatty liver disease. Insulin resistance decreases the likelihood of response to interferon-based therapies and may be an independent risk factor for the progression of HCV-related liver disease ***(Gholam et al., 2007).***

High levels of proinflammatory cytokines have been found in HCV-infected patients and, thereby they could be involved in the pathogenesis of insulin resistance associated with HCV.Yet the mechanisms by which HCV induces increased insulin resistance and the risk for development of diabetes has not been completely understood ***(Lecube et al., 2004).***

The adipose tissue is an important endocrine organ that secretes a variety of proteins termed adipocytokines which are biologically active polypeptides that are produced either execlusively or substantially by the adipocytes and act by the endocrine, paracrine, or autocrine mechanism.The adipokines appear to be involved in a wide range of physiological processes; these include haemostasis, lipid metabolism, blood pressure regulation, insulin sensitivity and angiogenesis ***(Perseghin et al., 2007).***

Retinol-binding protein (RBP)-4 was reported in 2005 by Yang et al as a new fat-derived adipokine that specifically binds to retinol, that has been proposed as an adipokine involved in the regulation of systemic glucose metabolism and pathogenesis of insulin resistance, and that it may provide a link between obesity and insulin resistance. It has been demonstrated that RPB4 is secreted by human adipose tissue explants and that it is expressed almost exclusively in mature adipocytes. The expression of RBP4 has been demonstrated in subcutaneous and, in visceral adipose tissue in humans ***(Weiping et al., 2007).***

RBP4 was discovered while trying to identify the substance responsible for regulating insulin sensitivity in mice either lacking or over expressing GLUT4 in adipose tissues**.** Retinol binding protein 4 was found to be accompanied by decreased adipocyte GLUT4 which appears to be the signal for development of insulin resistance ***(Young et al., 2006).***

Plasma RBP4 concentrations were found to be elevated in patients with IGT or diabetes type 2 and are associated with various clinical parameters associated with insulin resistance**.** ***(Young et al., 2006)*** in addition plasma RBP4 concentration might be a biomarker of nephropathy and cardiovascular disease in type 2 diabetic subjects ***(Cabré et al., 2007).*** Also retinol-binding protein 4 levels were also found to be elevated in polycystic ovary syndrome in women with obesity and impaired glucose metabolism. ***(Hahn et al., 2007)***

Whereas RBP4 is also mainly expressed in hepatocytes as the principal transport protein for retinol (vitamin A) in the circulation, its pathophysiological role in liver remained unclear. It has been suggested that retinol binding protein 4 might contribute to the pathogenesis of nonalcoholic fatty liver disease; serum RBP4 levels were significantly associated with non alcoholic fatty liver disease (NAFLD) and high liver enzymes ***(Wu et al., 2008).***

Given the fact that inflammatory cytokines are an integral part of inflammation in chronic hepatitis C infection

and that there is a chronic subclinical inflammatory state associated with insulin resistance induced by various adipokines, Retinol binding protein 4 may be the link between HCV infection and diabetes, suggesting an additional mechanism of diabetes with important implications for prognosis and therapy ***(Wu et al., 2008).***

# Aim of the Work

The aim of the present study is to examine level of Retinol binding protein 4 and its association with insulin resistance in HCV infected patients.

# Hepatitis C

Viral hepatitis is a major health problem in developing and developed countries. Hepatotropic viruses are designated hepatitis A, B, C, D, E, and G viruses. Many other viruses can cause hepatitis as one component of a multisystem disease, including herpes simplex virus (HSV), cytomegalovirus (CMV), Epstein-Barr virus, varicella-zoster virus, human immunodeficiency virus (HIV), rubella, adenoviruses, enteroviruses, parvovirus B19, and arboviruses ***(Snyder and Pickering, 2004).***

The six hepatotropic viruses are a heterogeneous group that cause similar acute clinical illness, except for HGV, which appears to cause no or mild disease. HBV is a DNA virus, whereas HAV, HCV, HDV, HEV, and HGV are RNA viruses representing four different families. HAV and HEV are not known to cause chronic illness, whereas HBV, HCV, and HDV viruses can cause important morbidity and mortality through chronic infections. HGV can cause chronic infections but with little morbidity or mortality yet reported. In the United States, HAV appears to cause most cases of hepatitis. HBV probably accounts for about one third of symptomatic cases, whereas HCV is found in approximately 20%. HDV, which occurs only in the presence of HBV, occurs in only a small percentage.. HGV role is not yet completely known, but the virus appears to account for a small percentage of cases of non–HAV-HEV infections. ***(Snyder and Pickering, 2004)***

**Table (1):** Hepatotropic viruses

| Nucleic acid | HAV | HBV | HCV | HDV | HEV | HGV |
|---|---|---|---|---|---|---|
| | RNA | DNA | RNA | RNA | RNA | RNA |
| Incubation P | 30 days | 100-120 days | 7-9 wk | 2-4 mo | 40days | uknown |
| Transmision Per cutenous | Rare | Common | Common | Common | No | Common |
| Feco-oral | Common | No | No | No | Common | No |
| Sexual | Rare | Common | Rare | Rare | Rare | Rare |
| Transplacental | no | common | rare | no | no | rare |
| chronicity | no | yes | yes | yes | no | yes |
| Fulminant d | rare | yes | rare | yes | rare | no |

***(Snyder and Pickering, 2004)***

**Table (2):** Autoantibodies in autoimmune hepatitis

| Types | ANA | SMA | LKM | AMA | SLA |
|---|---|---|---|---|---|
| Type 1 | +++ | +++ | - | - | ++ |
| Type 2a | - | - | +++ | - | |
| Type 2b | - | - | + | - | |
| Type 3 | + | + | - | - | ++ |
| Autoimmune cholongopathy | +++ | + | - | - | |
| Primary biliary cirrhosis | - | + - | - | +++ | |

**ANA,** antinuclear antibody
**SMA,** smooth muscle (actin) antibody
**LKM,** liver-kidney microsomal antibody;
**AMA,** antimitochondrial antibody;
**SLA,** soluble liver antigen.

***(Sheila Sherlock and James Dooley, 2002)***