INTRODUCTION

Hepatitis C virus (HCV) is a major health problem and chronic infection with this virus remains one of the most prevalent chronic diseases as well as an economic burden worldwide. It has been estimated that globally over 170 million people are infected with chronic HCV infection and about 20-25% of them are at risk of developing cirrhosis or even end stage liver Disease ***(Mazur et al., 2010).***

In Egypt, the problem is even more evident. HCV infection is the most important cause of liver disease in Egypt according to **Habib et al. (2001)** as the prevalence of antibodies to HCV is estimated to be 10 fold greater than in Europe and the US ***(Alter et al., 1999).***

It has been estimated that HCV is the leading cause of cirrhosis and hepatocellular carcinoma (HCC) in Egypt ***(AbdelAziz et al., 2000).***

Adipose tissue, previously thought of as a passive storage site for excess energy, is now recognized as a hormonally active system producing numerous molecules known as adipokines, which exert local, central and peripheral actions. During the last decade, interest has turned to the study of this group of molecules and their role in chronic liver diseases ***(ElRaziky et al., 2009).***

Resistin is one such adipokine, it is formed of 94 amino acids. It was cloned in 2001 and is thought to be mainly expressed

in adipose tissue. Resistin was shown to be involved in hepatic glucose and lipid metabolism and appears to play a pivotal role in hepatic insulin resistance ***(Murad et al., 2010).***

There have been a number of studies observing serum resistin levels in chronic liver diseases but they have mostly dealt with patients suffering from non-alcoholic fatty liver disease (NAFLD). In patients with NAFLD, abnormalities in the various adipokines (Resistin and leptin in particular) have been documented and possibly implicated in the progression of this disease towards cirrhosis ***(Wong et al., 2009).***

There are many data indicating, that steatosis, independently of being metabolic or viral origin, contributes to liver injury and fibrosis progression among the patients with chronic hepatitis C ***(Castera et al., 2003; Leandro et al., 2006).***

Early histopathological findings review performed by **Goodman and Ishak (1995)** revealed that steatosis is a common nonspecific feature observed in more than half of patients infected with HCV. Later studies revealed that the prevalence of steatosis among patients with chronic viral hepatitis C reached up to 86% in genotype-dependent groups ***(Hui et al., 2002).***

Due to relatively high prevalence of NAFLD in the general population, it might happen that both NAFLD as well as HCV infection are comrbidities affecting natural course of each one and promoting progression of liver damage and fibrosis.

The results of other studies suggest that adipose tissue can play active proinflammatory and profibrogenic role in the pathogenesis of NAFLD ***(Rajala and Scherer, 2003; Marchesini et al., 2008)*** as well as in infectious diseases including HCV and HIV infections ***(Desruisseeaux et al., 2007).***

AIM OF THE WORK

The aim of the present study is to study serum resistin level in cases of chronic hepatitis C and the effect of treatment on resistin level.

Chapter One

RESISTIN

Resistin (named after its ability to resist insulin) was originally described in 2000–2001 By three Different research groups ***(Steppan et al., 2001; Kim et al., 2001)*** as an adipocyte-secreted peptide involved in insulin resistance, type II diabetes and obesity. Resistin is also known as FIZZ3 (found in inflammatory zone) or ADSF (adipocyte-specific secretory factor), and belongs to the group of adipokines due to the protein being found originally in mouse adipose tissue ***(Kim et al., 2001).***

The initial studies in mice gave promising evidence for the role of resistin in the regulation of carbohydrate metabolism. In the transition to the study of human resistin, these properties of the protein were not evident and led to controversy about the role of resistin. Results regarding the possible metabolic role of resistin in humans are still inconclusive ***(Kim et al., 2001).***

Molecular structure of resistin

Resistin is a cysteine-rich peptide hormone of 12.5 kDa that belongs to the resistin-like family (RELM). The resistin gene, RETN, is conserved in chimpanzees, dogs, cows, mice, and rats. Resistin is 108 amino acids long in humans and 114 amino acids long in mice. In the two species, the genes are present on different chromosomes, as the human gene is located on chromosome 19 and the murine gene is on chromosome 8 ***(Steppan and Lazar,***

2004). The two proteins show similarities with respect to conserved cysteine residues. The first cysteine in the mouse protein (Cys22) is conserved, but is at position 26 (Cys26) in humans ***(Aruna et al., 2003).***

Human resistin can form a dimer through Cys26 ***(Banerjee and Lazar, 2001)*** and in line with this, murine resistin forms a dimer through Cys22 ***(Raghu et al., 2004).***

Murine resistin exists in two isoforms: the more abundant high-molecular-weight hexamer and the low-molecular-weight monomer form. The potency in impairing the action of insulin differs between the two forms. Human resistin is present in a mixture of oligomer and trimer, which can change into monomer and oligomer form ***(Aruna et al., 2008; Gerber et al., 2005).*** The oligomer shows more potent proinflammatory effects ***(Aruna et al., 2008)*** and the amino acid Sequence between residues 43 and 65 is suggested to carry the proinflammatory properties ***(Tarkowski et al., 2009).***

At the amino acid level, the two proteins exhibit 59% homology, at the mRNA level the nucleotide sequence identity is 64.4% whereas at the genomic DNA level this is reduced to 46.7%. This reduction in gene homology mainly reflects the differences within the introns, where the identity is as low as 28.7% ***(Ghosh et al., 2003).***

The expression and localization of resistin

The expression patterns of human and murine resistin are different. Murine resistin is almost exclusively expressed in white adipose tissue ***(Steppan et al., 2001)***, but it is also found in the pituitary gland the hypothalamus and in the circulation. In humans, only low levels of resistin are expressed in adipose tissue. In contrast, resistin expression in man is high in bone marrow, in the spleen, in lung tissue, and in the placenta; it is only scantily expressed in fat tissues of lean subjects ***(Adeghate, 2004).***

Resistin is upregulated during monocyte/macrophage differentiation and mononuclear cells serve as the main source of resistin in humans. Stimuli, such as cytokine stimuli by IL-1, IL-6, and TNF-α, by upregulation of resistin mRNA and resistin protein synthesis ***(Kaser et al., 2003).***

Microbial antigens (lipopolysaccharides) induce resistin synthesis ***(Lu et al., 2002)***, In addition, acute phase proteins (e.g. C-reactive protein) induce resistin mRNA and protein synthesis of resistin ***(Hu et al., 2007).*** Intriguingly, resistin itself has pro-inflammatory properties by upregulating the synthesis of IL-6, TNF-α, and IL-12 in mononuclear cells ***(Bokarewa et al., 2005; Silswal et al., 2005)*** and it has also been shown to trigger an inflammatory state in vivo ***(Bokarewa et al., 2005).***

In humans, non-myeloid cell types have been tested for the presence of resistin. Pre-adipocytes and adipocytes are devoid of

resistin mRNA expression ***(Nagaev et al., 2006)***, as Are Smooth-muscle cells ***(Nagaev and Smith, 2001)***, and in adipose tissue resistin is produced by infiltrating myeloid cells and not by adipocytes as shown in figure (1).

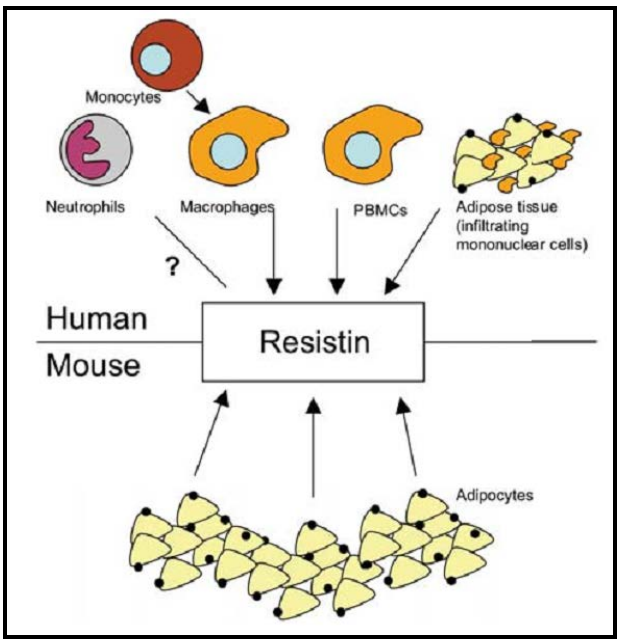

Fig. (1): Patterns of expression of resistin in humans and mice. Human resistin is mainly expressed in mononuclear cells and murine resistin is almost exclusively is almost exclusively expressed in adipocytes ***(Bokarewa et al., 2005).***

Human resistin has features similar to classical proinflammatory cytokines and has been shown to play a role in inflammation and immunity. The regulation it involves activation of the NFκB transcription pathway ***(Bokarewa et al., 2005).***

Receptor and signaling

Resistin was recently shown to mediate its proinflammatory effects via TLR-4.whether or not all the properties of resistin are mediated through activation of TLR-4 is yet unknown ***(Tarkowski et al., 2009).*** A search for additional receptor(s) mediating resistin activities is under way. Resistin induces NfκB activity dose-dependently in PBMC nuclear extracts stimulated ***(Tarkowski et al., 2009).***

In addition, NFκB inhibitor has been shown to abrogate the proinflammatory effects of resistin ***(Bokarewa et al., 2005)*** and blockade of IκBα, an inhibitor of NfkB, in monocytes leads to a reduction in the pro inflammatory properties of Resistin ***(Hu et al., 2007).***

Induction of pro inflammatory cytokines by resistin is mediated through complex formation by p65 and p50 subunits and translocation of these from the cytoplasm to the nucleus ***(Silswal et al., 2005).*** Moreover, resistin induces the phosphor-rylation of IκBα in hepatic stellate cells indicating NFκB activation ***(Bertolani et al., 2006).*** PI3k/AKT signaling is an important mediator of cell proliferation in response to growth factors and other mediators.

Resistin induces proliferation of smooth muscle cells through activation of PI3k/Akt where its effect is inhibited by PI3k inhibitor ***(Calabro et al., 2004).***

Resistin has also been shown to activate the mitogen-activated protein (MAP) kinase pathway ***(Kushiyama et al., 2005; Bertolani et al., 2006).***

There are three MAPK subfamilies, namely ERK, JNK, and p38 MAP. ERKs are activated by mitogens and growth factors, and JNK and p38 are activated by proinflammatory cytokines and cellular stress.

Resistin phosphorylates ERK in hepatic stellate cells ***(Bertolani et al., 2006).*** The MAPK subfamily p38 is related to proinflammatory cytokines and it has been shown to be phosphorylated by resistin ***(Kushiyama et al., 2005).***

This indicates that resistin has a role in both inflammation and growth. Resistin has several features in common with proinflammatory cytokines. Like TNF-α and IL-1, resistin promotes inflammation through induction of a cytokine cascade of other cytokines ***(Bokarewa et al., 2005).***

This maintains an inflammatory state where more inflammatory cells are recruited and activated upon stimulation of chemokines and cytokines. In contrast to TNF-α, however, resistin does not induce the suppression of adipose-specific markers suggesting that resistin's intracellular signaling pathway is distinct from that of TNF-α ***(Nagaev et al., 2006).*** The proinflammatory effects of resistin are mediated via TLR-4. Anti-

TLR-4 antibodies abolish resistin-induced cytokine production by PBMCs ***(Ghosh et al., 2003).***

Epithelial cells stably transfected with TLR-4 were found to respond to stimulation by resistin, which was not evident in untransfected cells or cells transfected with TLR-2 ***(Ghosh et al., 2003).*** The stably transfected TLR4-epithelial cells that were transfected with siRNA targeting TLR-4 lost their ability to respond to resistin stimulation ***(Ghosh et al., 2003).*** SiRNA targeting myeloid differentiation factor 88 (Myd88), which is downstream of TLR-4, lead to similar results.

The part of resistin molecule from amino acid 43 to amino acid 64 has properties similar to that of the whole resistin lomecule, suggesting that this part is active in binding to TLR-4 ***(Ghosh et al., 2003).*** Peroxisome proliferator-activated receptor Ý, (PPARÝ) is a ligand-activated nuclear receptor that regulates gene transcription and is the molecular target of anti-diabetic drugs (e.g. Thiazolidinedione, (TZD). PPARÝ negatively Regulates resistin expression as shown in figure 2 ***(Ghosh et al., 2003).***

Treatment with the PPAR agonist Rosiglitazone decreased resistin mRNA expression as much as 80%. The decrease was also observed at the protein level ***(Patel et al., 2003).*** Moreover, statins reduce resistin expression in diabetic patients and downregulate its expression in PBMCs ***(Hu et al., 2007; Koufany et al., 2008).*** Resistin expression is significantly reduced following TNFα

blockade in RA and IBD Patients ***(Karmiris et al., 2007; Gonzalez-Gay et al., 2008).***

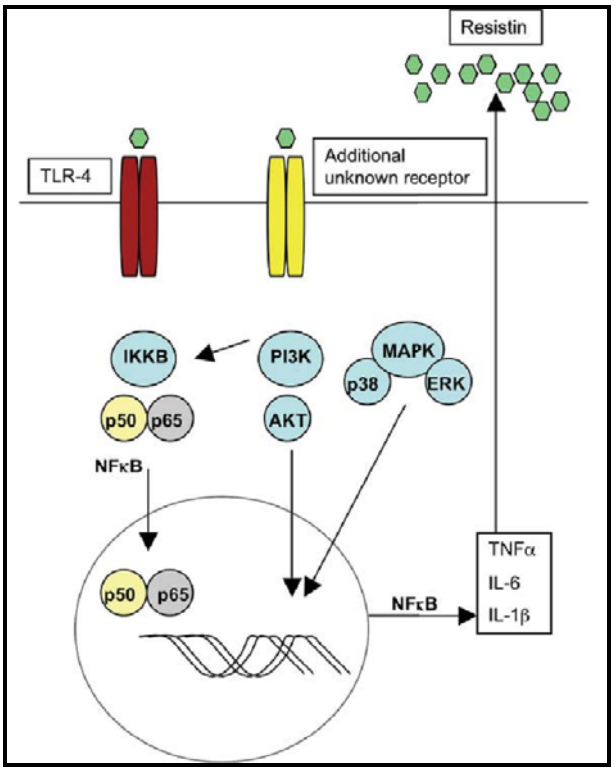

Fig. (2): Resistin signaling in humans. The proinflammatory effects of resistin are mediated via TLR-4. Resistin activates the NFκB Signaling pathway and leads to movement of the P65 subunit to the nucleus. No additional receptor(s) have been identified. Resistin has also been shown to activate PI3k/AKT signaling and MAPK signaling ***(Ghosh et al., 2003)***.

Resistin induces chemotaxis of CD4+ T cells .The chemotactic effects of resistin on T cells have been suggested to be mediated by a pertussis toxin-sensitive G-protein-coupled receptor ***(Walcher et al., 2009).***

Downstream activation of Src kinase by resistin was observed, leading to activation of PI3k ***(Walcher et al., 2009).*** In inhibiting Src kinase, resistin-induced migration was also inhibited ***(Walcher et al., 2009).*** Apart from this, little is known about the interaction between resistin and T cells. The NFκB pathway is used in mononuclear cells, but also in adipocytes.

Activation of TLR-4 in adipocytes generates insulin-resistant adipocytes and TLR-4 has been suggested as a link between inflammation and insulin resistance ***(Chung et al., 2006).***

Mice deficient in TLR-4 are protected from fatty acid-induced insulin resistance, suggesting that the innate immune system is involved in the regulation of insulin sensitivity ***(Shi et al., 2006).***

Resistin in inflammatory disease

Elevated levels of resistin in man are frequently found in association with inflammation and autoimmune disease ***(Senolt et al., 2007).***

Rheumatoid arthritis

Several research groups ***(Schaffler et al., 2003; Bokarewa et al., 2005; Migita et al., 2006; Senolt et al., 2007)*** have implicated resistin in RA. Rheumatoid arthritis is a progressive autoimmune disease characterized by a massive infiltration of

leukocytes into synovial tissue, resulting in synoviocyte proliferation, cartilage injury, and bone erosion.

Resistin is present in the synovium in both RA and osteoarthritis (OA); however, its presence is more pronounced in the sublining layer of the RA synovia. Resistin is expressed in synovial fibroblasts, macrophages, B cells, and plasma cells but not in T cells of the synovial tissue ***(Senolt et al., 2007).***

patients compared to controls with non-inflammatory joint diseases ***(Bokarewa et al., 2005 ; Senolt et al., 2007)*** and they are associated with elevated TNF-α, IL-6, disease activity, acute-phase reactants (CRP, IL-1Rα), and erythrocyte sedimentation rate ***(Schaffler et al., 2003; Migita et al., 2006).***

Expression of the resistin gene is upregulated in PBMCs ***(Kaser et al., 2003).*** Moreover, circulating resistin has correlations with levels of CRP, IL6 and TNF receptor 2 ***(Reilly et al., 2005).***

In traumatic joint injury, resistin has a direct effect on cartilage matrix turnover ***(Lee et al., 2009).*** Taken together, the data available support the hypothesis that resistin is a modulator of inflammation with potent regulatory functions.

Diseases of the liver and gastrointestinal tract

Resistin is expressed in hepatocytes and hepatic stellate cells. In addition, hepatic stellate cells respond to resistin stimuli

by increased expression of proinflammatory cytokines and NFkB activation ***(Bertolani et al., 2006).***

In cirrhosis of the liver, there is a correlation between resistin and both TNFα and free fatty acids ***(Bahr et al., 2006).*** Cirrhotic patients who have undergone liver transplantation have a reduced degree of insulin resistance that is not associated with a change in resistin levels ***(Bahr et al., 2006).***

Resistin in metabolic conditions

Resistin expression is upregulated in rodent models of diabetes ***(McTernan et al., 2003)*** and obesity ***(Steppan et al., 2001),*** indicating its role in these diseases. Resistin inhibits insulin-mediated reduction of gluconeogenese. Moreover, glucose and fatty acid uptake in skeletal muscle is inhibited.

Resistin knockout mice have low blood glucose levels whereas high resistin disturbs glucose homeostasis. When treated with monoclonal anti-resistin antibodies, the condition in these animals becomes stable. Thiazolidinedione treatment suppresses resistin production in patients with diabetes, and suppresses resistin secretion from monocytes and macrophages in vitro ***(Bajaj et al., 2004; Lehrke et al., 2004).***

Chronic inflammation has been proposed to be important in obesity-related insulin resistance ***(Wellen and Hotamisligil, 2005)***, and there is a correlation between resistin and inflammatory markers in patients with metabolic diseases.