INTRODUCTION

Liver transplantation (LT) is considered the treatment of choice for patients with end stage liver disease (ESLD) who have failed standard therapy. The development of liver transplantation has revolutionized the field of hepatology and greatly improved the outlook of patients suffering from various liver diseases. This procedure is now applied worldwide as treatment for a large number of irreversible acute and chronic liver diseases for which there were previously no other treatment options ***(Zhu et al., 2009).***

The primary goals of liver transplantation are to prolong life and to improve the quality of life. Thus, it is essential to optimize patient selection and ideally time the transplant procedure so as to gain the maximum benefit ***(Kanwal et al., 2005).***

End-stage liver disease secondary to hepatitis C virus is the most common indication for liver LT ***(Burra, 2009).*** An association between hepatitis C virus (HCV) infection and insulin resistance (IR) has been recently reported, however the causality has not been established. This association is based on the finding of increased IR among HCV-infected subjects ***(Delgado-Borrego et al., 2008)***.

It was found also that liver transplant recipients with hepatitis C had significantly higher insulin resistance compared to those without hepatitis C, and this relationship apparently

could not be explained by differences in usual confounders such as body mass index, medications used, and degree of liver fibrosis ***(Alsatie et al., 2008).***

IR is a state in which a given concentration of insulin produces a less-than-expected biological effect. It has also been defined as the requirement of 200 or more units of insulin per day to attain glycemic control and to prevent ketosis ***(Olatunbosun and Dagogo-Jack, 2013).***

Insulin binds and acts mainly through the insulin receptor and also acts via the insulin like growth factor–1 (IGF-1) receptor; cellular actions of insulin involve a wide variety of effects on post-receptor signaling pathways within target cells. The **b** subunit of the insulin receptor is a tyrosine kinase, which is activated when insulin binds to the **a** subunit; the kinase activity autophosphorylates and mediates multiple actions of insulin ***(Olatunbosun and Dagogo-Jack, 2013)***.

The mechanisms responsible for insulin resistance syndromes include genetic or primary target cell defects, autoantibodies to insulin, and accelerated insulin degradation ***(Reaven, 1995).***

Obesity, the most common cause of insulin resistance, is associated with a decreased number of receptors and with post-receptor failure to activate tyrosine kinase. Insulin sensitivity and secretion are reciprocally related; consequently, insulin resistance results in increased insulin secretion to maintain normal glucose and lipid homeostasis ***(Olatunbosun and Dagogo-Jack, 2013).***

Insulin resistance plays a major pathogenic role in the development of the metabolic syndrome, which may include any or all of the following:

- Hyperinsulinemia.
- Type 2 diabetes or glucose intolerance.
- Central obesity.
- Hypertension.
- Dyslipidemia that includes high triglyceride levels, Low high density lipoprotein (HDL-C) level and high low-density lipoprotein (LDL) particles.
- Hypercoagulability characterized by an increased plasminogen activator inhibitor–1 (PAI-1) level

(Olatunbosun and Dagogo-Jack, 2013).

In metabolic syndrome, the major underlying mechanism is insulin resistance. However, this insulin resistance is not essentially caused by liver disease. It may lead to other conditions such as polycystic ovarian syndrome (PCOS), in which insulin resistance is the underlying mechanism as it causes hyperinsulinemia and so hyperandrogenism that leads to manifestations of PCOS ***(Olatunbosun and Dagogo-Jack, 2013).***

IR is a major component of nonalcoholic fatty liver disease. It is not only a precursor to diabetes, but is also itself associated with significant morbidity. It has been associated

with a 2-fold increase in the risk of hypertension, a 3-fold increased risk of coronary heart disease, and an 8-fold increased risk of type 2 diabetes. These undesirable health effects can develop in a period of less than 15 years ***(Zavaroni et al., 1999).***

Impaired glucose tolerance is frequently observed in patients with cirrhosis and 20% of these patients may develop overt diabetes mellitus during the natural history of their illness. The main cause of impaired glucose metabolism in chronic liver disease is a reduced insulin action. Despite high circulating insulin levels, the insulin sensitivity of peripheral tissues is significantly reduced in these patients. It has also been shown that the abnormality in insulin action results mainly in a reduced stimulation of non-oxidative glucose disposal (*i.e.*, glycogen synthesis) in muscle. Both B-cell hypersecretion and impaired hepatic insulin clearance, caused by a reduced first-pass hepatic uptake, may contribute in causing high plasma insulin concentrations in cirrhotic patients ***(Merli et al., 1999).***

The presence of IR in the setting of HCV infection is of particular importance because hyperinsulinemia appears to play a role in the progression of HCV-related liver disease ***(Delgado-Borrego et al., 2008)***. It has been reported that IR is an independent risk factor for advanced fibrosis and the rate of fibrosis progression ***(Hui et al., 2003).***

The role of insulin or insulin analogues in carcinogenesis is highly debated since insulin and IGF activate signaling pathways that stimulate cellular proliferation and cell survival.

Over-expression of the IGF-I receptor and high levels of IGF-I or II ligand promote proliferation and tumor cell growth, while IGF-II receptor which is known to antagonize proliferation is frequently deleted in HCC ***(Schattenberg, 2009).***

In a recent study done to evaluate the long-term impact of glucose intolerance and to examine the expression of SHIP2 (Src Homology 2 domain-containing inositol phosphatase-which is negative regulator of intracellular insulin signaling and so suppress cell growth), it was found that glucose intolerance is an independent factor of poor prognosis in male HCC patients with HCV infection and expression of SHIP2 is significantly down-regulated in human HCC ***(Sumie et al., 2007).***

Although it was initially suggested that IR associated with chronic hepatitis C may be due to chronic inflammation, it is now known that HCV can induce IR directly, through specific viral effects. Much of the published literature in this area has focused on the HCV core protein, which has been proposed to cause IR in hepatocytes by reducing the level or activity of molecules involved in insulin signaling, particularly IRS (Insulin Receptor Substrate)-1 and IRS-2 ***(Douglas and George, 2009).***

Homeostatic model assessment of insulin resistance (HOMA-IR) has been reported in >500 publications as a method for assessing B-cell function and IR from basal (fasting) glucose and insulin or C-peptide concentrations.

HOMA analysis allows assessment of inherent B-cell function and insulin sensitivity and can characterize the pathophysiology in those with abnormal glucose tolerance ***(Wallace et al., 2004).***

In a recent prospective study done to evaluate the relation between the level of HCV RNA and the development of IR; a total of 34 adults [14 HCV(+) and 20 HCV(-)] who underwent liver transplantation were followed during the first year post-transplantation, it was found that patients with higher mean integrated HCV RNA levels (defined as mean HCV RNA level from month 1 post-LT to the time at which high HOMA - IR was attained) reached high HOMA levels significantly earlier than patients with lower HCV RNA levels ***(Delgado-Borrego et al., 2008)***.

Previous studies showed a controversy about the role of liver transplantation in normalization of glucose tolerance and insulin sensitivity in cirrhotic patients with IR. One study concluded that LT may reverse many alterations of glucose metabolism that are associated with cirrhosis. Fasting insulin levels, oral glucose tolerance, insulin sensitivity and non oxidative glucose disposal all were found to be normalized after LT ***(Merli et al., 1999).*** However, in a recent study, it was found that the disturbed glucose metabolism of patients with liver cirrhosis was improved by successful liver transplantation but was not corrected to normal ***(Tietge et al., 2004).***

Regarding the immunosuppressive medications following LT, calcineurin inhibitors were originally welcomed in the hope to reduce the risk of diabetes due to their glucocorticoid-sparing action, but soon it became evident that both cyclosporine and tacrolimus had a diabetogenic activity, impairing insulin sensitivity ***(Menegazzo et al., 1998).***

It was recently reported that calcineurin inhibitors also reduced insulin secretion and that tacrolimus was more diabetogenic than cyclosporine mainly in the early post-LT phase because of its multiple sites of action. However, after controlling for time from liver transplantation, the type of immunosuppression was not considered as prognostic factor for post-transplantation diabetes mellitus (DM). Once DM is established in the pretransplantation period, LT did not always produce a complete clinical regression ***(Bianchi et al., 2008).***

AIM OF THE WORK

- **Primary aim:** to study insulin resistance (IR) in Egyptian HCV cirrhotic patients before and after living donor liver transplantation (LDLT).
- **Secondary aims:**
  - To correlate the rate of development of IR with the level of HCV RNA pre and post LDLT.
  - To correlate the occurrence of IR with the status of HCV disease recurrence post LT.

Chapter 1

INSULIN RESISTANCE

Introduction:

Insulin is the most potent known anabolic hormone and is essential for appropriate tissue development, growth, and maintenance of whole-body glucose homeostasis. This hormone is secreted by the β cells of the pancreatic islets of Langerhans in response to increased circulating levels of glucose and amino acids after a meal. Insulin regulates glucose homeostasis at many sites, reducing hepatic glucose output (via decreased gluconeogenesis and glycogenolysis) and increasing the rate of glucose uptake, primarily into striated muscle and adipose tissue ***(Shulman, 2000).*** Insulin also profoundly affects lipid metabolism, increasing lipid synthesis in liver and fat cells, and attenuating fatty acid release from triglycerides in fat and muscle ***(Sesti, 2006).***

In addition to these classical insulin target tissues, there are many other important physiological targets of insulin, including the brain, pancreatic B-cells, heart, and vascular endothelium, that help to coordinate and couple metabolic and cardiovascular homeostasis under healthy conditions ***(Muniyappa et al., 2008).***

Insulin has concentration-dependent saturable actions to increase whole body glucose disposal. The maximal effect of insulin defines “insulin responsiveness,” whereas the insulin concentration required for a half-maximal response defines "insulin sensitivity" ***(Muniyappa et al., 2008).***

Insulin resistance occurs when normal circulating concentrations of the hormone are insufficient to regulate these processes appropriately. Thus, by definition, insulin resistance is a defect in signal transduction ***(Pessin and Saltiel, 2000).***

The Insulin Receptor and Mechanism of Action:

The insulin receptor is a heterotetrameric bifunctional complex, consisting of two extracellular **a** subunits that bind insulin and two transmembrane b subunits with tyrosine kinase activity. insulin binding to the **a** subunits activates the intrinsic kinase activity located in the **b** subunits and subsequently initiates a cascade of phosphorylation events that leads to different biological functions ***(White, 2003).***

Unlike other receptor tyrosine kinases, most functions of the insulin receptor require accessory molecules known as insulin receptor substrates (IRSs) to engage multiple downstream signaling pathways ***(Withers, 2001)***.

Insulin binding results in autophosphorylation of the receptor and tyrosine phosphorylation of intracellular IRS proteins, mainly IRS-1 and IRS-2. These actions are manifested via insulin's action on a complex network of intracellular pathways in hepatocytes, adipocytes and muscle cells upon binding to its cellular receptor. Two major cellular signaling pathways, phosphoinositide-3 kinase (PI3K)/Akt and the Ras/mitogen-activated protein kinase (MAPK) pathways, can be activated. Fig. (1) ***(Sesti, 2006).***

Mammalian target of rapamycin (mTOR) is another signaling pathway that is present in at least two different complexes and playes multiple roles in insulin signaling as reviewed recently ***(Wang and Proud, 2006)***. The mTOR-receptor complex 1 (mTORC1) appears to mediate the downstream effects of insulin on cell growth and proliferation and also provides negative feedback of insulin signaling by phosphorylating IRS-1 at inhibitory serine residues 636 and 639 ***(Ozes et al., 2001)*.**

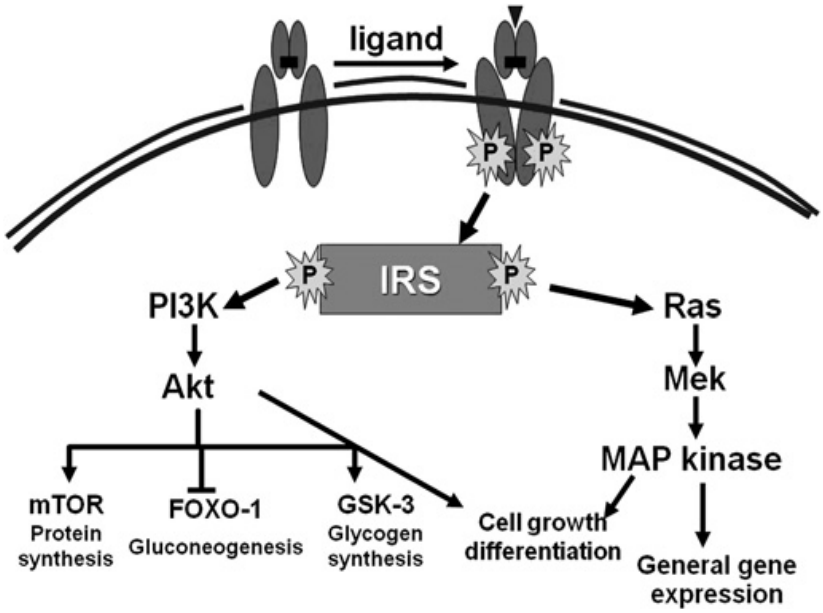

Fig. (1): Insulin signalling pathways ***(Saltiel and Kahn, 2001)***.

These different cascades regulate diverse cellular processes, such as gene expression, protein synthesis and vesicle trafficking, which result in the regulation of glucose, lipid and protein metabolism, cell growth and differentiation.

One of the main results of these processes is the final translocation of glucose transporter 4 (GLUT-4) from its intracellular pool to the cell membrane, facilitating glucose transport along the concentration gradient into the cytoplasm ***(Saltiel and Kahn, 2001)***.

Insulin not only specifically activates its receptor, but it can also transactivate the IGF (insulin like growth factor)-I receptor, which is similar to the insulin receptor, a member of the receptor tyrosine kinase family of growth factor receptors ***(Pessin and Frattali, 1993).***

When insulin levels increase (as in the postprandial surge in insulin-resistant subjects or after insulin injection), insulin binds and activates the related IGF-I receptor which has a more potent mitogenic and transforming activity. Moreover, insulin decreases IGF-I-binding proteins (IGF-BP1). This results in increased free IGF-I, the biologically active form of the growth factor, the mechanism of which has been implicated in the pathogenesis of several malignancies ***(Su et al., 2010).***

Insulin resistance:

The concept of insulin resistance was proposed as early as 1936 to describe diabetic patients requiring high doses of insulin ***(Muniyappa et al., 2008)***. Insulin resistance is classically defined as a state of decreased responsiveness of target tissues to normal circulating levels of insulin ***(Sesti, 2006)***. It has also been arbitrarily defined as the requirement of **200** or more units of insulin per day to attain glycemic control and to prevent ketosis ***(Olatunbosun and Dagogo-Jack, 2010).***

Internationally, early studies indicated a more significant association between insulin resistance and the various components of the metabolic syndrome in white persons than in members of other ethnic groups. Prevalence rates of insulin resistance syndrome reported for white populations ranged from 3-16%; a rate of less than 2% was reported among Japanese populations ***(Moadab et al., 2009).***

IR plays a major role in the development of type 2 diabetes mellitus. Insulin resistance is also a feature of a number of other health disorders, including obesity, glucose intolerance, dyslipidaemia and hypertension clustering in the so-called metabolic syndrome (also commonly referred to as syndrome X) ***(Sesti, 2006 and Bernsmeier and Heim, 2009).***

Pathophysiology of IR:

The mechanisms responsible for insulin resistance syndromes include genetic or primary target cell defects, autoantibodies to insulin, and accelerated insulin degradation ***(Reaven, 1995).*** Obesity, the most common cause of insulin resistance, is associated with a decreased number of receptors and with post-receptor failure to activate tyrosine kinase. While adiposity and insulin resistance are related, they are not necessarily synonymous, and each may make independent and different contributions to increasing the risk of cardiovascular disease ***(Olatunbosun and Dagogo-Jack, 2010).***

Insulin sensitivity and secretion are reciprocally related; consequently, insulin resistance results in increased insulin secretion to maintain normal glucose and lipid homeostasis. Several potential mediators are thought to signal the pancreatic B cells to respond to insulin resistance; these potential mediators include glucose, free fatty acids, autonomic nerves, fat-derived hormones (e.g. adiponectin), and the gut hormone glucagon-like peptide 1 (GLP-1). GLP-1 is an incretin hormone that stimulates insulin secretion, causes B-cell mitosis while inhibiting apoptosis, inhibits glucagon secretion, and delays gastric emptying with overall antidiabetic effects. Failure of the signals or of the pancreatic B cells to adapt adequately in relation to insulin sensitivity results in inappropriate insulin levels, impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and type 2 diabetes ***(Olatunbosun and Dagogo-Jack, 2010)*.**

Mechanisms of insulin resistance:

1- In-vivo insulin resistance: contributions of glucose transport and muscle glycogen synthesis to whole-body insulin-stimulated glucose metabolism:

The primary targets for insulin are skeletal and cardiac muscle, adipose tissue and liver. In-vivo studies have shown that in humans the skeletal muscle is the principal site of glucose uptake under insulin-stimulated conditions, accounting for approximately 75% of glucose disposal in the postprandial state, whereas less glucose is metabolized by adipose tissue ***(DeFronzo, 1988)*.**

Glucose uptake is the rate-limiting step in glucose utilization and storage. After entering the cell, glucose is phosphorylated by hexokinase and either stored as glycogen via the activation of glycogen synthase, or oxidized to generate ATP via activation of enzymes such as pyruvate kinase. In adipocytes, glucose is stored primarily as lipids via increased activation of lipid synthetic enzymes. Insulin also inhibits lipolysis in adipocytes through inhibition of the enzyme hormone-sensitive lipase. Most of these insulin-mediated changes in enzyme activities are mediated by attenuation of their phosphorylation state due to a combination of protein kinase inhibition and phosphatase activation. In the liver, insulin inhibits the production and release of glucose by blocking gluconeogenesis and glycogenolysis through a mechanism involving regulation of the expression of a number of genes encoding hepatic enzymes such as phosphoenol-pyruvate carboxylase (PEPCK), the rate-limiting step in gluconeogenesis ***(Schulman, 2000)*.**

Some recent advances in our understanding of human insulin resistance have been obtained using nuclear magnetic resonance (NMR) spectroscopy. Employing this technique, it has been shown that under hyperglycaemic, hyperinsulinaemic conditions, muscle glycogen synthesis is the major pathway for glucose metabolism in both normal and type 2 diabetic subjects, and that defective muscle glycogen synthesis (due to defects in glucose transport or in hexokinase) plays a major role in causing insulin resistance in patients with type 2 diabetes ***(Olatunbosun and Dagogo-Jack, 2010)*.**