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List of abbreviation

A	Early transmitral flow velocity
ACEI	Angiotesin converting enzyme inhibitor
AMPK	5AMP activated protein Kinase
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
CAD	Coronary Artery Diseases
CHF	Congestive heart failure
CKD	Chronic Kidney Disease
CRP	C- Reactive Protein
CVD	Cardiovascular Disease
CYP7A1	Cytochrome P7A1
DBP	Diastolic Blood Pressure
DM	Diabetes Mellitus
DN	Diabetic Nephropathy
E	Late transmitral flow velocity
$E^{/}$	Early peak diastolic annular velocity
ECGF	Extracellular growth factor
EF	Ejection Fraction
ERK	Extra cellular signal- Regulated Kinase
ESRD	End- stage renal disease
FBS	Fasting Blood Sugar
FFA	Free Fatty Acids
FGF	Fibroblast growth Factor
FGFR	Fibroblast growth Factor Receptor
FS	Fraction Shortening
GFR	Glomerular Filtration Rate
GH	Growth Hormone
GLUT	Glucose Transporter
HD	Hemodialysis
HDL	High Density Lipoprotein
HMW	High Molecular Weight
HOMA-IR	Homeostatic Model Assessment Insulin Resistance
HPSG	Heparan Sulphate Proteoglycan
ICAM	Intracellular Adhesion Molecule
Ig	Immunoglobulin
IL-2	Interleukin-2
IMT	Intima-Media Thickness
INF	Interferron
IVSd	Interventricular septal diameter
KDa	Killo dalton

KGFs	Keratinocytes Growth Factors
KO	Knockout
LADA	Latent Autoimmune Diabetes in Adult
LDL	Low Density Lipoprotein
LMW	Low Molecular Weight
LPWTd	Left Posterior Wall Thickness in diastole
LVEDd	Left Ventricular End Diastoli diameter
LVH	Left Ventricular Hypertrophy
LVMI	Left Ventricular Mass Index
MAPK	Mitogen Activated Protein Kinase
mRNA	Messenger RNA
NAFLD	Non Alcoholic Fatty Liver
ND	Non Detectable
NIDDKD	National Institute of Diabetes ,Digestive and Kidney Diseases
PCR	Polymerase Chain Reaction
PDGF	Platelet-Derived Growth Factor
PKA	Protein Kinase Activator
PPAR	Perxisome Proliferator Activated Receptor
PPC1 α	Perxisome Proliferator Co-activator protein-1 alpha
PTH	Parathyroid Hormone
RAAS	Renin- Angiotensin Aldosterone System
SBP	Systolic Blood Pressure
SCD	Sudden Cardiac Death
SREBP-1	Sterol regulatory element binding protein-1
SV	Stroke Volume
TDI	Tissue Doppler Imagine
TG	Triglycerides
TK	Tyrosine Kinase
Ucp-3	Uncoupling protein-3
VCAM	Vascular Intracellular Adhesion Molecule
WAT	White Adipose Tissue

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Introduction

Chronic kidney disease (CKD) is a growing public health disease that is associated with a markedly increased risk of cardiovascular disease and mortality. Although many traditional risk factors for atherosclerosis such as hypertension and diabetes mellitus promote the progression of CKD from early- to end-stage, these classic risk factors do not fully account for the burden of cardiovascular disease seen in patients with CKD. Left ventricular hypertrophy (LVH) is one of several common manifestations of cardiovascular disease that is a major independent risk factor for mortality in patients with CKD **(Sarnak et al., 2001)** . Exploring the early mechanism of LVH is necessary to develop therapies that block the progression of CKD and attenuate cardiovascular disease associated with CKD **(Sarnak et al., 2001)**.The fibroblast growth factor family is composed of 22 members with a wide range of biological functions, including cell growth, development, angiogenesis, and wound healing. FGF21 is a member of the endocrine FGF subfamily, which also includes FGF23, human FGF19, and its mouse homolog FGF15 **(Smallwood et al., 1996**; **Beenken** and **Mohammadi, 2009)**.

Human studies indicate that circulating levels of FGF21 increased in obese individuals **(Zhang et al., 2008)**, subjects with metabolic syndrome, type 2 diabetes mellitus**(Mraz et al., 2009**; **Stein et al., 2010)** and coronary heart disease**(Lin et al,2010)**. Furthermore, FGF21 was found to be closely associated with renal dysfunction in end-stage renal disease subjects **(Han et al, 2010; Stein et al., 2009)**. On the other hand, previous studies indicated that FGF receptors, particularly FGFR1, are expressed in adult myocardial cells, and their activation by locally secreted growth factors can stimulate myocardial hypertrophy and interstitial fibrosis**(Corda et al., 1997)**.

Aim of the study:

This study aims to assess FGF-21level and its relationship to cardiac dysfunction in the different stages of chronic kidney disease (CKD patients.

Fibroblast growth factors

Fibroblast growth factors, or **FGFs**, are a family of growth factors, with members involved in angiogenesis, wound healing, embryonic development and various endocrine signaling pathways. The FGFs are heparin-binding proteins and interactions with cell-surface-associated heparan sulfate proteoglycans have been shown to be essential for FGF signal transduction. FGFs are key players in the processes of proliferation and differentiation of wide variety of cells and tissues **(Finklestein and Plomaritoglou, 2001)**.

Historical background:

Fibroblast growth factors was found in pituitary extracts by Armelin in 1973**(Armelin, 1976)** later on, there were also found in a cow brain extract by Gospodarowicz, et al., and tested in a bioassay that cause fibroblasts to proliferate **(Gospodarowicz et al., 1974)**. They were further fractionated using acidic and basic PH and accordingly were named acidic (FGF-1) and basic (FGF-2) subtypes. Human FGF-2 found in low molecular weight (LMW) and high molecular weight (HMW) isoforms**(Vlodavsky et al., 1999)**. LMW FGF-2 is primarily cytoplasmic and functions in an autocrinemanner, whereas HMW FGF2is nuclear and exerts activities through an intracrinemechanism**(Böttcher and Niehrs, 2005).**

The bigger(third group) were isolated later(heparin sulphate growth factor) found to control proliferation of cells containing blood vessels(Endothelial cells) and classified into called ECGF-1 and ECGF-2, later on they found to be identical to acidic and basic FGF**(Pattoff et al., 2012)**.

FGFs Structures:

The crystal structures of heparin binding growth factors (HBGF) have been solved and found to be related to interleukin 1-beta. Both families have the same 12-stranded beta-sheet structure, and the beta-sheets are arranged in 3 similar lobes around a central axis with 6 strands forming an anti-parallel beta-barrel. In general, the beta-sheets are well-preserved and the crystal structures superimpose in these areas. The intervening loops are less well-conserved. The intervening loop between beta-strands 6 and 7 is slightly longer in interleukin-1 beta **(Murzin et al., 1992)**.

FGFs Members:

In humans, 22 members of the FGF family have been identified, all of which are structurally related signaling molecules **(Ornitz and Itoh, 2001)**:

1-Members FGF-1 through FGF-10: All bind fibroblast growth factor receptors (FGFRs).

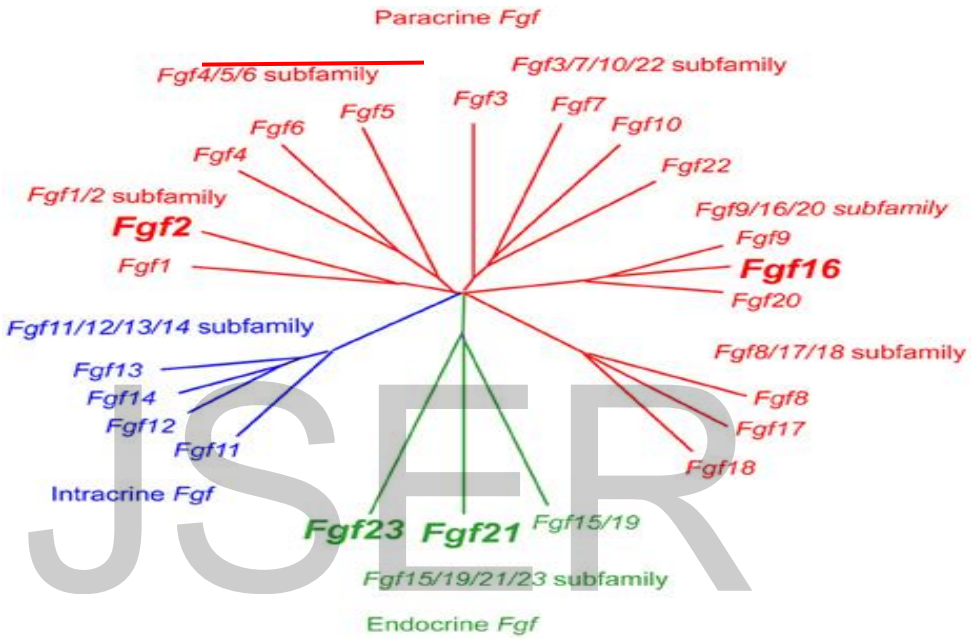
2-Members FGF-11 through 14: have similar sequence homology but they didn't bind to FGF receptors and are involved in intracellular processes unrelated to FGFs and known as intracellular growth factor "iFGF"**(Olsen et al., 2003; Itoh and Ornitz, 2008).**

3-Human FGF-18 is involved in cell development and morphogenesis in various tissues including cartilage **(Moore et al., 2005)**.

4-Human FGF-20 was identified based on its homology to Xenopus FGF-20(Xenopus: type of frog) **(Kirikoshi et al., 2000)**.

5-FGF-15 through 23:

- FGF-15 isn't present and known as FGF15/19.
- FGF 21-23 have systemic effects **(Fukumoto, 2008; Potthoff et al., 2012).**



- **Figure (1)**: FGFs Family classification **(Ornitz and Itoh, 2001)**.

FGFs receptors: The mammalian fibroblast growth factor receptors family has 4 members, FGFR1, FGFR2, FGFR3, and FGFR4.

The FGFRs consist of:

1- Three extracellular immunoglobulin- type domain (D1-D3).

2-A single-span trans-membrane domain.

3-An intracellular split tyrosine kinase domain.

-FGFs interact with the D2 and D3 domains, with the D3 interactions primarily responsible for ligand-binding specificity and heparan sulfate binding is mediated through the D3 domain.

A short stretch of acidic amino acids located between the D1 and D2 domains has auto-inhibitory functions. This 'acid box' motif interacts with the heparan sulfate binding site to preven receptor activation in the absence of FGFs**(Pattoff et al., 2012)**.

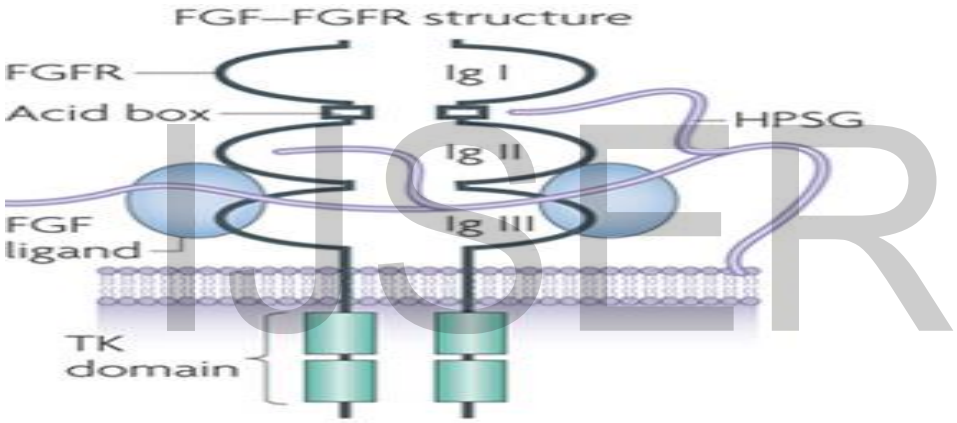


Figure (2): FGFR structure and signaling **(Turner and Grose, 2010).**

FGFs Functions:

Most of FGFs are secreted proteins that bind to heparansulphates therefore be caught in extracellular matrix.

1- FGF-1 and 2: promote endothelial cell proliferation and physical organization of the endothelial cells into tube like structure. Hence, they promote angiogenesis. They are more potent angiogenic factors than vascular endothelial growth factor (VEGF) or platelet-derived growth factor (PDGF) **(Zechel et al., 2010)**. In

addition, wound healing occurs through proliferation of fibroblasts under their control **(Zechel et al., 2010)**.

2- FGF-7 and FGF-10 (also known as Keratinocyte Growth Factors (KGFs): KGF1 and KGF2, respectively) stimulate the repair of injured skin and mucosal tissues by stimulating proliferation, migration and differentiation of epithelial cells, in addition they have direct chemotactic effects on tissue remodeling **(Jones,2012)**.

3- Members of the FGF-19 subfamily (FGF-15, FGF-19, FGF-21, and FGF-23) bind less tightly to heparan sulfates, and so can act in an endocrine fashion on far-away tissues, such as intestine, liver, kidney, adipose tissue, and bone**(Potthoff et al., 2012)**. For example:

- FGF-15 and FGF-19 (FGF15/19) are produced by intestinal cells but act on FGFR4-expressing liver cells to downregulate the key gene (CYP7A1) in the bile acid synthesis pathway **(Murzin et al., 1992)**.
- FGF-23 is produced by bone but acts on FGFR1-expressed in kidney cells to regulate the synthesis of vitamin D and phosphate homeostasis **(Eriksson et al., 1991)**.

Fibroblast Growth Factor 21

Since the discovery of the first fibroblast growth factor (FGF) almost 40 years ago, the FGF family has expanded over the years and currently consists of 22 members with a wide range of biological functions including cell growth, angiogenesis, wound healing and metabolism **(Itoh et al., 2004; Kharitonenkov, 2009)**.

The role of the FGFs in metabolism has been increasingly recognized in recent years. Unlike the classical FGFs which require heparin for efficient binding to the FGF receptors (FGFRs) and act in a paracrine or autocrine fashion **(Itoh et al.,**

2004), FGF-21 and the other endocrine FGFs lack the conventional heparin-binding domain and are secreted into the circulation, being able to escape the binding to the rich tissue depots of heparin sulphateproteoglycans**(Kharitonenkov, 2009)**.

Fibroblast growth factor 21 (FGF-21) was first cloned and identified from mouse embryos by homology-based PCR in 2000**(Nishimura et al., 2000).** Human FGF-21 is a polypeptide of 181 amino acids with 75% identity to mouse FGF-21. It is secreted predominantly by the liver **(Nishimura et al., 2000)**, but also by other tissues involved in glucose and lipid metabolism such as the adipose tissue, pancreas and skeletal muscle **(Fon-Tacer et al., 2010)**.

Studies in rodents have suggested FGF-21 to be a key physiological regulator of fasting response **(Inagaki et al., 2007)**, as well as a fed-state autocrine factor regulating the activity of Peroxisome proliferator-activated receptor gamma (PPAR-γ) in adipose tissues **(Dutchak et al., 2012)**. Administration of recombinant FGF21 in animal models **(Kharitonenkov et al., 2005);** including diabetic monkeys and findings in FGF-21 transgenic mice have revealed potent *in vivo* beneficial effects of FGF-21 on glucose and lipid metabolism, insulin sensitivity and body weight**(Kharitonenkov et al., 2007)** . Furthermore, unlike many of the other FGFs, FGF-21 does not have effects on cell proliferation and tumourigenesis **(Kharitonenkov et al., 2007)**. Instead, over-expression of hepatic FGF-21 delays the initiation of chemically induced hepatocarcinogenesis **(Huang et al., 2006)**. The favorable effects observed in animal studies would support the potential role of FGF-21 as a therapeutic agent for control of diabetes and obesity. However, high serum levels of FGF-21 are surprisingly found in obese subjects and patients with disorders related to obesity and insulin resistance **(Chen et al., 2011)**. The causes and underlying pathophysiology of elevated serum FGF-21 in

these pathological conditions warrant further clarification, although FGF-21 resistance has been demonstrated in a study done on obese mice **(Fisher et al., 2010).** Whereas these observations imply that supraphysiological doses of FGF-21 might be required for treatment of such disorders in humans, they also suggest the potential use of FGF-21 as a biomarker of obesity-related disorders **(Woo et al., 2013).**

Physiological roles of FGF-21

Production and regulation

The major site for FGF-21 production is the liver. In mice, hepatic expression and circulating levels of FGF-21 are raised by both fasting (for 12 h) and ketogenic diet and rapidly suppressed by refeeding **(Badman et al., 2007).** The nuclear receptor PPAR-α plays an indispensable role in fasting-induced hepatic expression of FGF-21**(Badman et al., 2007).** The mRNA expression of FGF-21 in mouse livers and human primary hepatocytes are strongly induced by fenofibrate, (a PPAR-α agonist). On the other hand, both fasting and fenofibrate-induced FGF-21 expression are abolished in the absence of PPAR-α action, as demonstrated by experiments in PPAR-α Knockout mice**(Badman et al., 2007)**. Apart from the liver, the adipocytes also express and secrete FGF-21. At times of thermogenic activation(cold exposure), adipocytes FGF-21 induce browning of white adipose tissue, in addition to being a FGF-21 target, also becomes a source of systemic FGF-21**(Itoh, 2014)**.

In addition, the degree of FGF-21 expression in several types of adipose tissue is markedly raised in obese mice and becomes comparable to its expression in the liver **(Zhang et al., 2008)**. On the other hand, it has recently been shown for FGF-

21 to act as an autocrine factor in the fed state, regulating the activity of PPAR-γ in adipose tissues through a feed-forward loop mechanism **(Dutchak et al., 2012)**. FGF-21 Knochout mice have defects in PPAR-γ signaling including decreased body fat and attenuation of PPAR-γ-dependent gene expression, and are refractory to the effects of the PPAR-γ agonist rosiglitazone, including both the beneficial insulin-sensitizing effects and the detrimental effects of weight gain and oedema. In summary, FGF21 can be secreted as an endocrine factor to co-ordinate the adaptive response to starvation or fasting; or as an autocrine factor induced in adipose tissue during the fed state to regulate adipocyte function **(Woo et al., 2013).**

Relatively little data are available on the regulation of FGF21 in humans as compared with mice. In a human study, ketosis induced by fasting for 2 days or feeding a ketogenic diet is not associated with increased serum FGF21 levels, and unlike reports in mice, a significant increase in serum FGF21 levels is seen only in the following conditions:

a- After fasting for 7 days **(Galman et al., 2008)**.

b- Treatment with fenofibrate, a PPAR-α ligand, in patients with hypertriglyceridaemia results in increased FGF-21 levels, suggesting that PPAR-α also regulates FGF-21 in humans**(Galman et al., 2008)**.

b- In line with such an observation, another group has also found that *in vitro* expression of human FGF-21 gene is increased by two fasting signals, namely PPAR-α and glucagon-PKA(glucagon protein kinase activity)**(Uebanso et al., 2011)**. On other hand, serum FGF-21 levels and FGF-21 mRNA expression in visceral fat are increased in subjects with obesity, a condition associated with over-nutrition **(Zhang et al., 2008)**. It is possible that FGF-21 is induced

in both extreme nutritional conditions, including prolonged fasting or over-feeding in human **(Uebanso et al., 2011)**.

c- Role of FGF-21 in glucose metabolism:

Glucose transporter (GLUT): are a wide group of membrane proteins that facilitate the transport of glucose over a plasma membrane.

Types:

GLUT 1: Is widely distributed in fetal tissues. In the adult, it is expressed at highest levels in erythrocytes membrane and also in the endothelial cells of barrier tissues such as the blood brain barrier. However, it is responsible for the low level of basal glucose uptake required to sustain respiration in all cells.

GLUT 2: Is a bidirectional transporter, allowing glucose to flow in 2 directions. It is expressed by renal tubular cells, small intestinal epithelial cells, liver cells and pancreatic beta cells. Bidirectionality is required in liver cells to uptake glucose for glycolysis, and release of glucose during gluconeogenesis. All three monosaccharides (glucose, galactose and fructose) are transported from the intestinal mucosal cell into the portal circulation by GLUT2.

GLUT 3: Expressed mostly in neurons (where it is believed to be the main glucose transporter isoform) and in the placenta.

GLUT4: Found in adipose tissues and striated muscle (skeletal muscles and cardiac muscle) **(Bell et al., 1990)**.

FGF-21 was found to activate glucose uptake in adipocytes, an effect independent of insulin and observed after at least 4 h of treatment, in contrast to the rapid action of insulin. FGF-21 is expressed in the pancreas **(Nishimura et al., 2000)**, as is the

single-pass transmembrane protein β-Klotho, (an important component of the FGF-21 receptor complex), which determines the tissue selectivity of FGF-21 action **(Kharitonenkov et al., 2008)**. In isolated rat pancreatic islets, FGF-21 inhibits glucagon secretion **(Kharitonenkov et al., 2005)** and increases insulin mRNA and proteins, but enhances glucose-induced insulin secretion only in islets from diabetic rodents **(Wente et al., 2006)**. Rat islets cells treated with FGF-21 are partially protected against glucolipotoxicity, probably through improved β-cell function and survival, via the activation of ERK 1/2 (extracellular signal-regulated kinase 1/2) and Akt signalling pathways (protein kinase B, involved in cellular survival pathway) **(Wente et al., 2006)**.

FGF-21 may also contribute to the glucose-lowering action of PPAR-γ agonists. Treatment of 3T3-L1 adipocytes (cell line derived from3T3 cell that is used in biological researches on adipose tissue) with FGF-21 and rosiglitazone (a PPAR-γ agonist), in combination leads to a synergistic increase in glucose transport **(Moyers et al., 2007)**. This suggests a profound functional synergy between the FGF-21 and PPAR-γ pathways. Whereas FGF-21 can enhance the transcription activity of PPAR-γ, the expression of β-Klotho is stimulated by rosiglitazone **(Kharitonenkov et al., 2008)**.

On the other hand, FGF-21 has also been implicated in the regulation of gluconeogenesis as fasting progresses to starvation **(Potthoff et al., 2009)** but, unlike glucagon, it does not stimulate glycogenolyis. Its effect is mediated by the induction of hepatic expression of peroxisome proliferator co-activator protein 1 α (PPC1α: a transcriptional co-activator controlling the expression of gluconeogenic genes). Mice lacking FGF-21 fail to fully induce PPC1α expression in response to fasting and have impaired gluconeogenesis **(Potthoff et al., 2009)**. However, FGF-21 may also act directly on the liver to stimulate the expression of gluconeogenic

genes, as suggested by the finding that FGF-21 can stimulate the same degree of gluconeogenic gene expression in a study of mice with liver-specific ablation of PPC1α**(Fisher et al., 2011)**. Thus, based on the aforementioned animal studies, FGF-21 has an impact on glucose metabolism via multiple mechanisms, acting through its receptor complex in the liver, adipose tissue and pancreas **(Woo et al., 2013)**.

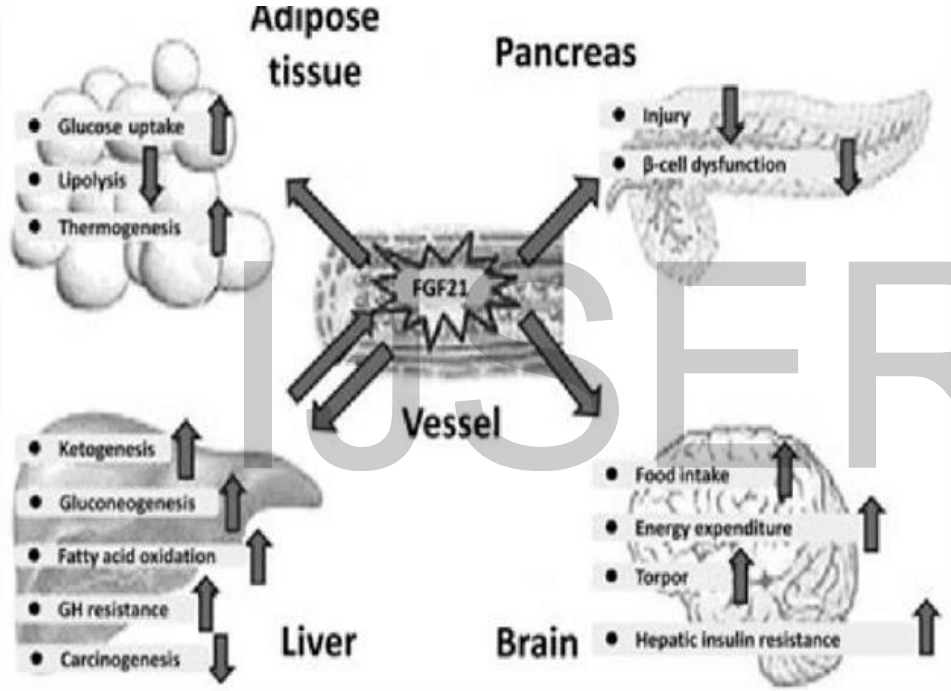


Figure (3).The pleiotropic metabolic actions of FGF21 on multiple organs **(Woo et al., 2013)**. (Torpor is a state of decreased physiological activity in an animal, usually by a reduced body temperature and metabolic rate. Torpor enables animals to survive periods of reduced food availability) **(Geiser, 1994)**.

Role of FGF-21 in lipid metabolism:

A-Effect of FGF-21 on ketogenesis

Different studies have demonstrated that FGF-21 is required for ketogenesis in mice in the fasting state. Transgenic mice with liver-specific overexpression of FGF-21 exhibit a significant increase in serum ketone bodies (β-hydroxybutyrate, acetoacetate and acetone) and a concurrent reduction in serum and hepatic triglyceride concentrations **(Inagaki et al., 2008)**. However, inconsistent results have been reported regarding the physiological role of endogenous FGF-21 in ketogenesis. FGF-21 KO(knockout) mice in one study demonstrate impaired adaptation to ketosis induced by a ketogenic diet**(Badman et al., 2009)**, whereas a Japanese group has reported increased ketogenesis in FGF-21 Kockout mice fasted for 24 hrs, as evidenced by a modest increase in serum β-hydroxybutyrate levels**(Hotta et al., 2009)**. On the other hand, a human study shows no correlation between plasma levels of FGF-21 and ketone bodies after a 2-day fast or feeding with a ketogenic diet **(Galman et al., 2008)**. In another human study, neither fasting up to 72 hrs nor a ketogenic diet for 12 days increases serum FGF-21 levels **(Dushay et al., 2010)**. With these conflicting findings, the physiological role of FGF-21 in regulating ketogenesis remains unclear **(Dushay et al., 2010)**.

B-Effect of FGF-21 on lipolysis

Despite early data suggesting that acute treatment with recombinant FGF-21 increases lipolysis **(Inagaki et al., 2005)**, more recent studies have shown an inhibitory effect. The observation of an increase in non-esterified fatty acids in adenovirus-mediated FGF-21 knockdown mice on a ketogenic diet is in line with the ability of FGF-21 to inhibit lipolysis **(Badman et al., 2007)**. More recently, it has been also shown that FGF-21 can suppress growth hormone (GH)-induced lipolysis in mice through a feedback regulatory loop **(Chen et al., 2011)**. GH is released from the pituitary in response to fasting and stimulates lipolysis in fat cells. The resulting increase in circulating free fatty acids (FFAs) induces hepatic

FGF-21 production via the action of PPAR-α. Raised FGF-21 in turn feedbacks negatively to terminate GH-induced lipolysis in adipocytes. The greater rise in serum glycerol and FFAs in response to GH in the FGF21 KO mice, compared with their wild-type littermates, also supports the inhibitory effect of endogenous FGF-21 on lipolysis **(Chen et al., 2011)**.

In human adipocytes, FGF-21 attenuates lipolysis stimulated by catecholamine and atrial natriuretic peptide after treatment for three days **(Arner et al., 2008)**. Human data have also shown that the 24-h profiles of FFAs correlated closely with that of FGF-21. A strong positive association is found between the peak levels of circulating FFAs and FGF-21 during both day and night, with the peak time of FFAs preceding that of FGF-21 by 3–4 h **(Yu et al., 2011)**. These findings also suggest the existence of feedback regulation between FFAs and FGF-21 and a role of endogenous FGF-21 in suppressing excessive lipolysis in humans **(Woo et al., 2013)**.

FGF-21 plays a part in the induction of hepatic fatty acid oxidation by PPAR-α **(Inagaki et al., 2008)**. In mice on a ketogenic diet, studies based on hepatic FGF-21 knockdown show that FGF-21 is required for the normal activation of hepatic lipid oxidation and triglyceride clearance **(Badman et al., 2007)**.Chronic treatment with recombinant FGF-21 reduces serum and hepatic triglyceride levels, and reverses fatty liver disease in diet-induced obese mice **(Xu et al, 2009)**, through the inhibition of sterol regulatory element binding protein-1 (SREBP-1: a transcription factor critical for lipogenesis). In diabetic monkeys treated with FGF-21, reductions in serum triglycerides, cholesterol and small dense LDL-cholesterol, together with increases in HDL-cholesterol, are observed **(Woo et al., 2013)**.

The reduction in circulating FFAs, consequent to the inhibition of excess lipolysis and enhanced hepatic fatty acid oxidation, may contribute to the reduction in systemic insulin resistance in the FGF-21 treated obese and diabetic animals. In this context, the reduction in hepatic steatosis by FGF-21 can also lead to an amelioration of hepatic insulin resistance **(Woo et al., 2013)**.

Clinical studies done on FGF-21

1-Role of FGF-21 in control of DM

Monoclonal Antibody trial:

As an alternative to native FGF-21, monoclonal antibody (mimAb1) has been developed that binds to β Klotho with high affinity and specifically activates signaling from the β Klotho/FGFR1c receptor complex (FGF receptor 1c). In obese cynomolgus monkeys (long-tailed macaque), injection of mimAb1 led to FGF-21 like metabolic effects, including decreases in body weight, plasma insulin, triglycerides, and glucose during tolerance testing. Mice with adipose-selective FGFR1 knockout were refractory to FGF21-induced improvements in glucose metabolism and body weight. These results in obese monkeys (with mimAb1) and in FGFR1 knockout mice (with FGF21) demonstrated the essential role of FGFR1c in FGF-21 function and suggest fat as a critical target tissue for the cytokine and antibody. Because mimAb1 depends on β Klotho to activate FGFR1c, it is not expected to induce side effects caused by activating FGFR1c alone. The unexpected finding of an antibody that can activate FGF21-like signaling through cell surface receptors provided preclinical validation for an innovative therapeutic approach to diabetes and obesity**(Foltz et al., 2012)**.

B-FGF-21 analog (LY2405319)

In a recent dose-finding study, an analog of FGF-21(LY2405319), was administered to 46 obese patients with type 2 diabetes for 28 days. Fasting plasma glucose and insulin concentrations declined significantly, total LDL cholesterol and triglycerides decreased by 10–20%, HDL cholesterol increased significantly**,** and plasma adiponectin(an insulin-sensitizing adipocytokine) increased, weight decreased by 1.5– 1.7 kg, although the decrease was not significant. Although the precise mechanisms via which FGF-21 exerts its beneficial effects on glucose and lipid metabolism in type 2 diabetes remain to be established, the drug appears to have a novel mechanism of action and may prove effective as a glucose- and lipid-lowering agent**(Gaich et al., 2013**).

C-Metformin trial

Metabolic status plays an important role in the regulation of FGF-21, and therefore metformin (AMPK-activator which is an enzyme that plays a role in cellular energy homeostasis, regulates FGF-21 expression in hepatocytes) effect on FGF-21regulation was examined. FGF-21 mRNA and protein expression were determined after incubation of primary cultured rat and human hepatocytes with metformin for 24 hours. To study the role of AMPK in the putative regulation of FGF-21, hepatocytes were incubated with Compound C (an AMPK inhibitor) in the presence of metformin. A strong dose-dependent increase in FGF-21 expression was observed in both rat and human hepatocytes treated with metformin. This effect was blocked by addition of the AMPK-inhibitor (Compound C). The study showed that metformin is a potent inducer of hepatic FGF-21 expression and that the effect of metformin seems to be mediated through AMPK activation. As FGF-21 therapy normalizes blood glucose in animal models of type 2 diabetes, the

induction of hepatic FGF-21 by metformin might play an important role in metformin's antidiabetic effect **(Eva et al., 2012)**.

Metabolic benefits of FGF-21 treatment in animal models:

Therapeutic administration of recombinant FGF-21 has led to various beneficial effects in animal models, including lowering of blood glucose and triglyceride levels, reversing hepatic steatosis and improving insulin resistance **(Kharitonenkov et al., 2005; Xu et al., 2009).**

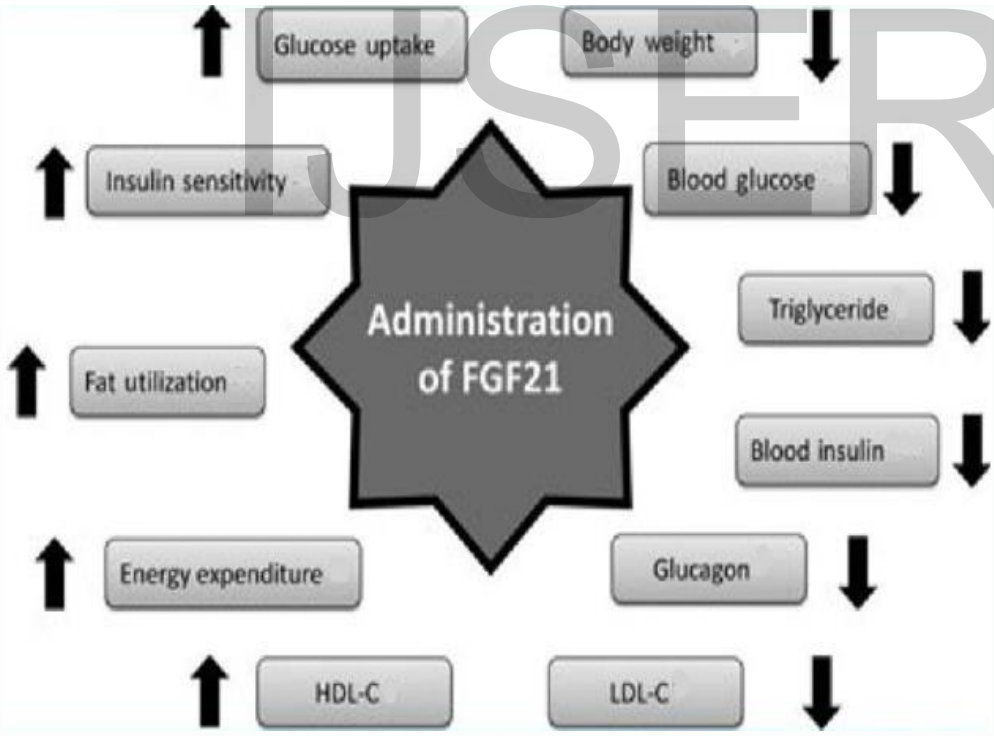


Figure (4). Metabolic benefits of FGF21 treatment in obese mice **(Coskun et al, 2008)** and diabetic monkeys **(Kharitonenkov et al, 2007).**

The effect of FGF-21 lasts at least 24 hours and with no weight gain, mitogenicity or hypoglycaemia being observed in the FGF-21 treated animals **(Kharitonenkov et al., 2007)**.

Chronic infusion of FGF-21 for 8 weeks in diabetic mice almost normalizes their glucose levels, in part contributed by the effects on β-cell survival and function in the diabetic animals **(Wente et al., 2006)**. This is of potential therapeutic importance as progressive β-cell failure is a major clinical challenge in the treatment of type 2 diabetes**(Woo et al., 2013)**.

2-Role of FGF-21 in lipids control:

Despite the presence of FGF-21 resistance in obese mice**(Fisher et al., 2010)** and possibly in humans, beneficial metabolic effects have been clearly observed in obese mice**(Coskun et al., 2008)** and diabetic monkeys**(Kharitonenkovet al., 2007)** after the administration of FGF-21. Systemic administration of FGF-21 for 2 weeks in mice with diet-induced or genetic obesity lowers their mean body weight by 20% predominantly via a reduction in adiposity, with no change in total caloric intake or physical activity. Improved hepatic steatosis is also observed. FGF21-treated obese mice also show increased energy expenditure **(Xu et al., 2009)** and a lower respiratory quotient, reflecting preferential utilization of fat as a fuel source**(Coskun et al., 2008)**.

The favorable effects observed in animal studies would support the potential role of FGF-21 as a therapeutic agent for control of diabetes and obesity. However, high serum levels of FGF-21 are found in obese subjects and patients with disorders related to obesity and insulin resistance **(Chen et al., 2011)**.

The causes and underlying pathophysiology of elevated serum FGF-21 in these pathological conditions warrant further clarification, FGF-21 resistance has been demonstrated in a study done on obese mice. These observations may imply that supraphysiological doses of FGF-21 might be required for the treatment of such disorders in humans. They also suggest the potential use of FGF-21 as a biomarker of obesity-related disorders **(Fisher et al., 2010)**.

3-Role of FGF-21 in skeletal homeostasis:

A recent study reported the role of FGF-21 in the regulation of skeletal homeostasis **(Wei et al., 2012)**. FGF-21 was shown to inhibit osteoblastogenesis and stimulate adipogenesis from bone marrow mesenchymal stem cells by potentiating the activity of PPAR-γ. Both genetic and pharmacological FGF-21 gain of function suggested a role of FGF-21 in inducing a remarkable decrease in bone mass. At this turning point, whether skeletal fragility and increased fracture risk may be an undesirable consequence of chronic FGF-21 administration remains to be confirmed by other investigators. Nonetheless, while the therapeutic efficacy and safety of FGF-21 in the treatment of obesity and its related disorders are being actively investigated, further research may reveal the possibility of developing tissue or pathway selective FGF-21 agonists as another strategy for FGF21-based therapy**(Wei et al., 2012).**

Relation between FGF-21 and Kidney Diseases

Circulating FGF-21 is increased in both acute and chronic kidney diseases. Renal excretion is a major route for FGF-21 elimination. Serum FGF-21 level have been shown to be increased in patients with impaired renal function. Patients undergoing chronic haemodialysis have elevated serum FGF-21 levels, more than 15-fold that of controls**(Stein et al., 2009)**. Serum FGF-21 concentration was associated with residual renal function and insulin resistance in end-stage CKD patients on long-term hemodialysis**(Han et al., 2010)**. These results suggested that elevated level of FGF-21 may be related to renal excretion functions in humans. Plasma FGF-21 concentration has been shown to be progressively increased with the development of early to end-stage CKD following the loss of renal functions in CKD patients. Furthermore, plasma FGF-21 level was 120 pg/ml in controls, 480pg/ml in patient with early stages CKD, 980pg/ml in patients with moderate stages CKD and much higher in hemodialysis patients(1800pg/ml). These results suggest that circulating FGF-21 concentration is associated with the CKD progression**(Lin et al., 2011)**.

The patho-physiological significance of other factors needs to be elucidated in more details **(Hindricks et al., 2014)**. Diabetes contributes to increased morbidity and mortality in patients with chronic kidney disease**(Whaley-Connell et al., 2009)**. Diabetes has additional effects on FGF-21 levels in CKD patients, which are supported by the fact that plasma FGF-21 levels in CKD individuals with DM were significantly higher than those CKD patients without this disease. Furthermore, stepwise logistic regression analysis revealed that plasma FGF-21 was

independently associated with diabetes. Take together; these results suggest that plasma FGF-21 levels in CKD subjects are affected by relevant co morbidities **(Woo et al., 2013).**

Relation between FGF-21 and cardiovascular risk factors in CKD

Patients with chronic kidney disease are at profoundly higher risk for cardiovascular (CV) morbidity and mortality. Multiple epidemiologic studies have demonstrated that both reduced renal function and proteinuria are significantly and independently associated with an increased risk of CV death **(Matsushita et al., 2010)**. In multivariate adjusted analyses, patients with end-stage renal disease (ESRD) had a 10 to 20-fold higher risk of CV mortality **(Rucker and Tonelli. 2009)**. Such increases in CV risk are not limited to patients with the most advanced renal disease on dialysis, those with mild-to-moderate chronic kidney disease (CKD) are also at significantly higher risk of CV disease **(Matsushita et al., 2010)**, and patients with both overt proteinuria as well as microalbuminuria without a reduction in estimated glomerular filtration rate (eGFR) are also at significantly higher risk **(Matsushita et al., 2010)**. The nature of CV disease in patients with renal failure differs from that of the general population. Whereas the most common manifestations of CV disease in the general population include coronary atherosclerotic disease, patients with renal failure are far more likely to suffer from chronic heart failure and sudden cardiac death **(Herzog et al., 2011)**. Sudden cardiac death is the leading cause of death in ESRD patients, and accounts for approximately 25% of all deaths in this population **(Green et al., 2011)**. In addition, the clinical presentation of CV disease is also different amongst those with renal disease when compared to the general population. For example, CKD

patients with acute myocardial infarction are more likely to present with atypical symptoms and shortness of breath than with more typical symptoms **(Sosnov et al., 2006)**. Thus, the pathophysiology of increased CV disease risk in CKD likely differs from that of the general population and may include factors that are specific to the diseased or ischemic kidneys. The mechanisms underlying CV risk in CKD are multifactorial and begin early in the course of renal disease. Not only the traditional CV risk factors such as hypertension and diabetes highly prevalent in the CKD population, but also nontraditional risk factors specific to CKD and ESRD patients are also highly prevalent and contribute to this risk **(Stenvinkel et al., 2008)**. These include oxidative stress, inflammation, endothelial dysfunction, anemia, extracellular volume overload, malnutrition, abnormal calcium and phosphorus metabolism, infection, uremic toxins, as well as sympathetic nervous system (SNS) overactivity **(Stenvinkel et al., 2008)**.

Mechanistic insights of traditional risk factors:

Hypertension

As a potentially modifiable risk factor, the impact of hypertension on cardiovascular disease in patients with CKD is of great interest. As kidney impairment progresses there is increasing activation of the RAAS in response to glomerular sclerosis and interstitial disease as well as fluid overload and increased arterial stiffness, all of which contribute to hypertension. Essential hypertension itself causes microvascular damage in the renal vascular bed causing kidney damage and hence, through Renin-Angiotensin-Aldosterone-System(RAAS) activation, exacerbating essential hypertension**(Wright and Hutchison, 2009)**. Hypertension is known to alter renal physiological function with increased

filtration fraction of sodium and increased renovascular resistance **(Fliseret al., 1997)**. Poor hypertension control clearly leads to increasing risk of cardiovascular morbidity and mortality and increasing risk of declining kidney function. A 'vicious circle' is created with worsening kidney function itself then contributing to hypertension. Hypertension is widespread among an otherwise healthy general population, especially in the elderly where approximately two thirds of people will be hypertensive. The majority of patients with CKD are hypertensive, with the prevalence increasing with increasing severity of CKD such that in the most advanced stages of CKD (eGFR< 30 mL/min) over 90% of patients are hypertensive**(Wanner et al., 2005)**. Sympathetic nervous system activation is incriminated. Plasma catecholamine concentrations are elevated and increased nerve sympathetic traffic has been demonstrated in renal failure**(Neumann et al., 2004)**. The participation of the sympathetic nervous system has become more complex with the recent discovery of renalase, a new regulator of cardiac function and blood pressure produced by the kidney **(Ernesto and Johannes, 2007)**. Renalase, a novel amine oxidase, is mainly expressed in the kidney, heart, and skeletal muscle. It has been known to degrade circulating catecholamines and plays a crucial role in human diseases**(Li et al., 2014)**.

Role of renalase in hypertensive cardiovascular disease:

Renalase is a 37.8-kDa oxidase, which contained flavin-adenine-dinucleotide, expressed mainly in glomeruli and proximal tubules of the kidney, also in cardiomyocytes and other tissues. Renalase metabolizes catecholamines in the following order: dopamine → epinephrine → norepinephrine. In contrast to other oxidases, renalase is secreted into plasma and urine of healthy persons **(Xu et al., 2005)**.

However, it is not detectable in uremic individuals. Recombinant renalase exerts a powerful and rapid hypotensive effect on rats. Mounting evidence from numerous studies demonstrates the capability of renalase recombinant proteins in lowering blood pressure as well as protecting myocardial cells from necrosis and apoptosis. The exact mechanism by which renalase regulates blood pressure and improves cardiac function is still unclear. However, renalase may be a potential drug or a novel therapeutic target for the prevention and treatment of hypertensive-ischemic cardiovascular diseases**(Li et al., 2014)**.

Studies have clearly shown a significant reduction in the rate of progression of CKD when hypertension is treated. Particularly this has been demonstrated in major studies investigating blockade of the RAAS with ACEi in both proteinuric and nonproteinuric CKD and with angiotensin II receptor blockers **(Wanner et al., 2005)**. Many studies have demonstrated a reduction in the rate of progression of CKD with drugs blocking the RAAS, compared to the same blood pressure reduction achieved with other antihypertensive regimens. This alludes to the importance of blocking angiotensin II which is a powerful endothelial growth factor in renal vascular bed, as well as other vascular beds in foot, it is the most powerful vasoconstrictor. Currently a full understanding of the pathophysiology of the effects of hypertension on the kidney in patients with CKD isn't fully understood; however the major effect is likely to be a progressive increase in intra-renal vascular resistance which may precede any changes in kidney structure **(Wright and Hutchison, 2009)**.

FGF-21 is a newcomer in field of hypertension

In humans, elevated circulating FGF-21 is associated with the metabolic syndrome**(Zhang et al., 2008)**, and dyslipidemia**(Lin et al.,2010)** which suggests that FGF-21 expression might be dysregulated due to abnormalities in FGF-21 secretion or circulating FGF-21 isoforms, or FGF-21 resistance in peripheral tissues. It is interesting to note that in an intervention study involving forty nondiabetic obese women, three months of supervised exercise training five times per week led to significant reductions in systolic and diastolic blood pressure, arterial stiffness, and circulating FGF-21 levels **(Yang et al., 2011)**. In 72 patients with end-stage renal disease, angiotensin receptor blocker treatment for six months reduced serum FGF-21 concentrations by 13 %**(Han et al., 2010)**. These findings suggest a possible link between the renin-angiotensin system and FGF-21. In a cross-sectional study serum FGF-21 levels has been shown to be independently associated with hypertension in community-dwelling adults after adjustment of multivariable analysis. Further studies are needed to address the biological mechanism(s) that could explain the association between FGF-21 and hypertension**(Richard et al., 2013)**.

Diabetes mellitus

DM is accompanied with various cardiovascular abnormalities including endothelial dysfunction, increased oxidative stress and micro- and macrovascular consequences leading to coronary artery disease, left ventricular dysfunction

(particularly diastolic dysfunction), hypertensive heart disease and reduced cardiac reserve**(Shange and Yip, 2011).**

CV risk in diabetic kidney disease

CV risk in diabetic kidney disease is about 10-40% of patients with diabetes mellitus (DM) developed nephropathy. Consequently with an increasing DM global prevalence and the aging of the population, DM has become the leading single cause of ESRD in many developed and developing countries **(Friedman et al., 2006)**.Moreover, it is now proven that even a mild reduction in kidney function among them is accompanied by an increased cardiovascular risk **(Ritz, 2006)**. In addition, cardiac and renal DM complications share many risk factors and markers including microalbuminuria (mA) and arteriosclerosis **(Zoccali, 2006)**. Therefore, evaluation and treatment of renal risk factors should not be only directed to prevent progression to End-Stage Renal Disease (ESRD), but also to reduce CV risk**(Vlachopoulos and O'Rourke, 2000)**. While the development and progression of renal damage in DM occurs very slowly, it often remains subclinical and undiagnosed for many years **(Caramori et al., 2000)** which inhibit effective prevention and intervention at a time when renal damage may be reversible. Therefore, early identification of diabetic nephropathy (DN) is a medical priority **(Taal and Brenner, 2006)**. Although microalbuminuria is currently regarded as an early marker of DN, it is now preferably considered as a marker of a generalized endotheliopathy and then a CV risk marker. However, irreversible damage has often occurred when microalbuminuria is detected **(MacIsaac et al., 2006)**. Furthermore, microalbuminuria may not accurately represent the severity of renal damage, it is absent in marked renal dysfunction and may regress or

fluctuate during the course of the disease**(Kramer et al., 2003)**. Consequently, other markers, preferably in their early stages, should also be investigated as a potential guide to the progression of ESRD **(Caramori et al., 2000)**. Arterial compliance changes occur early in DM and since arterial stiffness is an established independent predictor of mortality in the later stages of nephropathy, it should also correlate with renal function and BP profile in the earlier stages of DM**(London and Cohn, 2002)**.

Relation of FGF-21 to Diabetes Mellitus, Insulin and Microalbuminuria

Circulating FGF-21concentrations have been found elevated in insulin-resistant states, such as impaired glucose tolerance and type 2 diabetes (DM2) **(Mashili et al., 2011)**, but decreased in type 1 diabetes and latent autoimmune diabetes in adults (LADA). FGF-21 was also an independent predictor of DM2 in humans and its elevation is independent of the type 2 diabetes duration **(Cheng et al., 2011)**. Lastly, FGF-21 seems to be also independently associated with markers of insulin resistance and an adverse lipid profile in polycystic ovary syndrome **(Goraret al., 2010)** and gestational diabetes **(Stein et al., 2010)**.

The role of insulin on FGF-21 is currently not well understood. Some studies have shown that artificial hyperinsulinemia induced in healthy subjects is accompanied by an increase in FGF-21 levels **(Mai et al., 2009)**. However, it has also been reported that FGF-21 also increases in hypoinsulinemic states **(Mai et al., 2010)**. This discrepancy might be related to an elevation of stimulators of FGF-21 secretion such as FFAs, resulting from complete insulin deficiency. Therefore, FGF-21 responses to insulin might be affected by several confounders, such as obesity, endogenous circulating FFAs, insulin levels and insulin resistance. In

relation to therapy of diabetes, the addition of rosiglitazone **(Li et al., 2009)** or pioglitazone **(Samson et al., 2011)**, on ongoing metformin therapy in DM2 patients, as well as the use of mitiglinide **(Wang et al., 2012)** or short-term continuous subcutaneous insulin infusion **(Yang et al., 2011)** in patients with newly diagnosed DM2 was followed by a significantly reduction in circulating FGF-21 levels suggesting that FGF-21 decreases when insulin sensitivity improved. Based on all these observations, FGF-21 should be considered as new hormone with a significant role in insulin resistant states and complications associated with DM2 possibly promoting insulin sensitivity in human as a biological compensatory mechanism. Previous cross-sectional studies have demonstrated that circulating FGF-21 levels are associated with deterioration in renal function, both in patients with diabetic nephropathy **(Lin et al., 2011)**, and in community-dwelling adults with CKD **(Crasto et al., 2012)**.

Furthermore, its levels are also independently associated with urinary albumin excretion in type 2 diabetic patients, including those with microalbuminuria or subclinical diabetic nephropathy **(Jianet al., 2012)**. Elevated serum FGF21 levels may be a useful biomarker for predicting kidney disease progression especially in early stages of diabetic nephropathy with normoalbuminuria**(Lee et al., 2015)**.

Dyslipidemia

Dyslipidemia with high LDL and low high-density lipoprotein (HDL) cholesterol is associated with atherosclerotic vascular disease and an increased risk of CVD events, including acute myocardial infarction. Guidelines recommend aggressive lipid-lowering therapies in patients at high risk of CVD. Although patients with advanced CKD are at high risk of CV events, there is a reluctance to use statins for

several reasons. Unlike individuals in the general population, patients with CKD are at risk of malnutrition and inflammation that have a cholesterol-lowering effect. In this subset of patients, cholesterol levels and mortality risk are not lineary correlated. In contrast, a strong, graded, positive association of serum cholesterol with overall and CVD mortality has been observed in the absence of inflammation and malnutrition **(Liu et al., 2006)**. Fibrates are contraindicated in renal failure patients because of the risk of rhabdomyolysis, leading to reluctance to use this lipid-lowering agent in patients with CKD. Patients on hemodialysis (HD) predominantly have Frederickson type IV or III hyperlipidemias, characterized by hypertriglyceridemia (accumulation of very-low-density lipoprotein and intermediate-density lipoprotein, and lower levels of HDL). Several factors contribute to this condition**(Ardhanari et al**., **2014)**:

- Decreased levels of hepatic triglyceride lipase in uremia leads to an accumulation of triglycerides.

-A decreased apolipoprotein CII/CIII ratio impairs the function of lipoprotein lipase, leading to accumulation of very-low-density lipoprotein

- In dialysis patients, lower levels of apolipoprotein A-I (primarily because of increased catabolism) and lower levels of apolipoprotein A-II (primarily because of decreased production) lead to lowered HDL cholesterol and an abnormal HDL fraction.

- Dialyzer membrane type, dialysate, and use of heparin have all been shown to play a role in the phenotype of dyslipidemia in HD patients.

- Use of erythropoietin, calcium and disturbed parathyroid hormone homeostasis also seem to play a role in the genesis of dyslipidemia in patients with ESRD**(Ardhanari et al**., **2014)**.

Obesity

Visceral obesity is associated with CV events in CKD patients**(Mutsert et al., 2007)**. But, unlike the general population, dialysis patients have lower CV risk with higher body mass index. That paradox probably reflects the understanding that increased body mass index in dialysis patients is a general marker of better nutrition status. Metabolic syndrome imparts an increased risk for CV events in the general population that might or might not be extrapolated to patients with CKD. Adiponectin, an adipocyte hormone, has been implicated as a biomarker for metabolic syndrome; lower levels are associated with increased CV mortality in dialysis dependent patients with CKD **(Zoccali et al., 2002)**.

Role of FGF-21 in obesity

It has recently been reported that human FGF-21 gene expression is paradoxically and independently regulated by both fasting and feeding signals, suggesting that human FGF-21 is increased with nutritional crisis, including starvation and overfeeding **(Uebanso et al., 2011)**. Increased FGF-21 serum levels have been found to be associated with obesity in both children **(Reinehr et al., 2012)** and adults**(Dushay et al., 2010)** indicating a connection between FGF-21 and body fat mass. Obesity in childhood not only associated with increased FGF-21 serum levels compared with normal-weight children but also correlated with FFA **(Reinehr et al., 2012)**. This observation is important due to the fact that FFA have been defined as physiological stimulators of FGF-21 secretion **(Maiet al., 2009)**. In

addition, it has been reported that FGF-21 is significantly correlated to body mass index (BMI) and leptin as markers of white adipose tissue(WAT) in children **(Reinehr et al., 2012)**. This fact does not always occurs in adults, in whom several metabolic alterations such as high liver fat, TG, insulin and lower high-density lipoprotein cholesterol rather than overall adiposity is associated with high FGF-21 levels **(Tyynismaa et al., 2011)**. Therefore, FGF-21 seems to be an independent marker for the presence of metabolic stress in obesity**(Tyynismaa et al., 2011)**. Indeed, serum FGF-21 level has also been reported to be significantly increased and positively correlated with intrahepatic triglyceride content in Non-Alcoholic Fatty Liver Disease(NAFLD), representing an independent predictor of liver steatosis**(Yan et al., 2011)**. Studies that have analyzed the response of FGF-21 to weight loss in humans have shown controversial findings. On the one hand FGF-21 significantly increased after weight loss induced by short-term (3 weeks) very low calorie diet (VLCD) **(Mraz et al., 2009)**. These results are in concordance with those obtained in mice after fasting, indicating a possible response of FGF-21 to fasting after PPAR- activation, suggesting that FGF-21 might be related to the improvement in insulin sensitivity associated to weight loss induced by very low calorie diet (VLCD) in obese patients**(Mraz et al., 2009)**. However, on the other hand, FGF- 21 levels were not modified by moderate weight reduction (5 kg) after 6-month weight loss program based on hypocaloric diet and physical activity in a group of 30 obese subjects **(Mai et al., 2011)**. On the contrary, plasma FGF-21 concentrations have been found to be decreased in patients with anorexia nervosa (AN) compared to normal-weight healthy women. FGF-21 has also significantly correlated with serum levels of leptin, adiponectin, and insulin in both the normal-weight women and severely underweight patients with anorexia

nervosa and significantly reduced after 2 months of realimentation. Further clinical studies focused on alterations in circulating levels of FGF-21 linked to changes in body weight in both obesity and low weight are necessary**(Dostalova et al., 2008)**

Mechanistic insight on nontraditional risk factors

Studies have shown that traditional risk factors are not adequate to account for the excess CVD in patients with CKD. Further studies in this area led to the identification of multiple nontraditional risk factors that might play a direct causal role or considered as a marker for pre-existing CVD or other factors that increase the risk of CVD**(Ardhanari et al., 2014)**.

Uremic toxins

In 2012, the European Uremic Toxin Work Group (EUTOX) listed at least 88 uremic retention solutes **(Duranton et al., 2012)**.Uremic toxins are divided into three major groups: small-molecular-weight water-soluble compounds, protein-bound compounds, and large-molecular-weight compounds **(Vanholder et al., 2012)**. Protein-bound uremic toxins are poorly removed by current dialysis techniques because of their size, which is larger than the pore size of dialysis membranes **(Lekawanvijit et al., 2012)**. In particular, indoxyl sulfate and *p*-cresyl sulfate, which are considered representative protein-bound uremic toxins, are risk factors for CVD. Previous studies have suggested that these toxins are associated with the development of CVD and death in individuals with renal dysfunction **(Lekawanvijit et al., 2012)**.

Abnormal calcium phosphate metabolism: Hyperphosphatemia and hyperparathyroidism were found to be significantly associated with all-cause and CVD mortality in patients undergoing HD **(Blocket al., 2004)**. Possible mechanisms of this association include vascular calcification and stiffening, increased pulse pressure, decreased coronary perfusion pressure, and LVH**(Blocket al., 2004)**.

Relation of FGF-21 to phosphorus in CKD:

Elevated FGF-21 levels were associated with increase in both serum phosphate and CRP levels. The relative difference in mean serum phosphate concentration between normal subjects and those with early-stage CKD patients was significantly lower than that of plasma FGF-21 levels. These results suggest that FGF-21 is a better biomarker than blood phosphate to reflect the progression of CKD from the early-to middle stage, so further studies are needed to evaluate the possible role of FGF-21 as a cardiovascular risk factor in CKD. **(Woo et al., 2013)**.

Oxidative stress

Uremic patients have a high oxidative burden, and the resultant oxidative stress has been implicated in atherogenesis and CV morbidity and mortality. Activated phagocytes provide a link between oxidative stress and inflammation. Retained uremic solutes such as β2-microglobulin, Advanced Glycation End products (AGEs), cysteine, and homocysteine serve as substrates for oxidative injury **(Himmelfarb et al., 2002)**. Results from studies conducted to reduce oxidative stress are contradictory. The HOPE study showed no benefit for CVD outcomes with vitamin E; however, the SPACE trial demonstrated a benefit with antioxidants, reporting a lower incidence of a composite endpoint consisting of fatal and nonfatal acute myocardial infarction(AMI), CVD death, need for

coronary angioplasty or coronary artery bypass grafting, ischemic stroke, and peripheral vascular disease **(Mann et al., 2004)**. Acetylcysteine, an antioxidant, was shown to reduce events of CVD in patients with renal failure **(Tepel et al., 2003)**.

Oxidative stress burden on the heart

Oxidative stress mediated by reactive oxygen species (ROS) causes contractile failure and structural damage, and thus plays an important role in the pathogenesis of heart failure, especially after myocardial infarction**(Tsutsui et al.,2009)** Increased reactive oxygen species(ROS) production may contribute to the development of cardiac damage through different mechanisms, including cardiomyocyte loss via apoptosis or other cell death mechanisms in rabbits**(Heusch et al., 2010)**. Mitochondrial ROS production can be neutralized by uncoupling proteins (Ucps), which regulate proton leak across the inner mitochondrial membrane **(Brand et al., 2004)**. In fact, recent studies have shown the importance of uncoupling protein-3 (Ucp3) in the heart in controlling ROS production and thereby mediating its cardioprotection **(Ozcan et al., 2013)**.

Effect of FGF-21 on oxidative stress in the heart

A recent study done on mice, analyzed the role of FGF-21 in cardiac tissue in relation to pathological situations associated with oxidative stress. The study showed that FGF-21 induced expression of antioxidant genes such as Uncoupling proteins (*Ucp3, Ucp2)*, and superoxide dismutase-2(*Sod2),* in the heart, and showed that pro-oxidant signals induced Ucp3 expression in an FGF-21-dependent manner. Moreover, the study has shown that FGF-21 is expressed in and released by cardiomyocytes in response to lipopolysaccharides (a stimulus of pro-

inflammatory pathway), and its expression is under the control of the protein deacetylase Sirt-1 (sirtuin-1) pathway(Sirt1: is an important factor in cardiac protection, it protects the heart against hypertrophy, ischaemia-reperfusion injury, and oxidative stress in the heart**(Planavila et al., 2011)**.The study concluded that FGF-21regulates genes involved in antioxidant pathways in an autocrine manner, thus preventing Reacive Oxygen Species(ROS) production in cardiac cells. This is the mechanism by which FGF-21 acts as an antioxidant in heart, preventing induction of pro-oxidative pathways by inflammatory or hypertrophic conditions **(Planavila, et al., 2014)**.

Inflammation

An environment of chronic low-grade inflammation is common to both CKD and CVD and has therefore been postulated to contribute to the bidirectional relationship between these two systems **(Stehouwer and Smulder, 2006)**. Individuals with CKD have been shown to have elevated levels of CRP, IL-2, IL-6, INF-gamma, TNF-alpha, intracellular adhesion molecule 1(ICAM) and vascular adhesion molecule (VCAM-1) **(Costa et al., 2008)**.

Correlation between FGF-21 and inflammatory markers in CKD

FGF-21 was positively correlated with inflammatory markers (interleukin-6, fibrinogen, high selective C-reactive protein) and Homeostatic model assessment-insulin resistance(HOMA-IR; a method used to quantify insulin resistance and beta-cell function), and negatively correlated with residual renal function in a group of 72 nondiabetic peritoneal dialysis patients **(Hanet al., 2010)**.

Cardiovascular complications in CKD

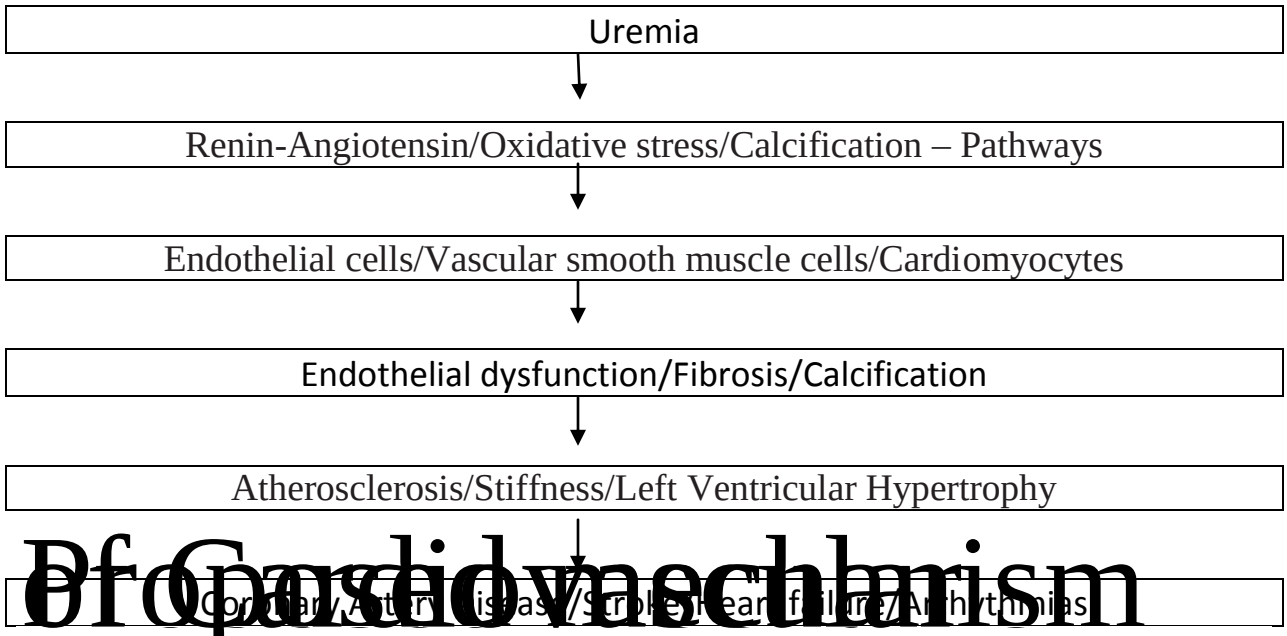


Figure (5): Proposed mechanism of cardiovascular complications in chronic kidney disease. The figure shows how uraemia activates different mechanistic pathways causing cell, tissue and organ injury leading on cardiovascular events **(Hajhosseiny et al., 2013)**.

Left Ventricular Hypertrophy

The pathogenetic factors involved in LV hypertrophy and fibrosis in CKD and ESRD have generally been divided into three categories: (1) afterload related, (2) preload related, and (3) not afterload or preload related. Afterload-related factors involve systemic arterial resistance, elevated systolic (and diastolic) arterial BP, and large-vessel compliance**(Ritz et al., 2009)**. The latter factor could be related in part to the common phenomenon of aortic "calcification" (more correctly, "ossification") seen in CKD and in ESRD. These afterload-related factors result in

myocardial cell hypertrophy and concentric LV remodeling. Activation of the intracardiac renin-angiotensin system (RAS) seems to be critically involved in this pathway, but angiotensin II as well as aldosterone can also be involved in myocardial cell hypertrophy and fibrosis, independent of afterload**(Steigerwalt et al., 2007)**. Recently, oxidative stress and xanthine oxidase activation have also been implicated in LVH caused by afterload induction. Preload-related factors involve expansion of intravascular volume (salt and fluid loading), anemia, and, in certain circumstances, large flow arterio-venous fistulas placed for vascular access. These latter factors result in myocardial cell lengthening and eccentric or asymmetric LV remodeling. Both afterload- and preload-related factors may operate simultaneously and probably have additive or even synergistic effects. Therefore, it is not easy to separate the effects of preload and afterload factors in the pathogenesis of LVH or even to establish a hierarchy of importance because they are intimately related to each other in ESRD patients. Nevertheless, evidence has accumulated to suggest that volume overload, related to inadequate salt restriction and ultrafiltration, plays a dominant role **(Charra, 2007)**.

Role of FGF-21 in cardiac remodeling

FGF-21 is regarede an endocrine FGF. FGF-21 mediates its biological responses in an FGFR-dependent manner. However, FGF-21 binds to FGFRs with heparin/heparan sulfate in very low affinity. FGF-21 efficiently binds to and activates FGFR1c, FGFR2c, and FGFR3c with β-Klotho, which is crucial for FGF-21 signaling as a cofactor **(Kharitonenkov et al., 2008; Suzuki et al., 2008)**.

Both FGFR1c and β-Klotho are predominantly expressed in cardiomyocytes. These findings indicate that FGF-21 acts on cardiomyocytes possibly in a paracrine

manner to prevent cardiac hypertrophy by activating MAPK signaling through the activation of FGFR1c with β-Klotho **(Planavila et al., 2013)**.

Relation between FGF-21 and LVH in CKD

Lin et al. 2011showed that Plasma FGF-21 levels in CKD patients with LVH were significantly higher than those in patients without LVH (P = 0.001). Furthermore, plasma FGF-21 level correlated positively with creatinine, blood urea nitrogen (BUN), β2 microglobulin, systolic pressure, adiponectin, phosphate, proteinuria, CRP and triglyceride, but negatively with creatinine clearance rate (CCR), estimated glomerular filtrate rate (eGFR), HDL-c, LDL-c, albumin and LVH after adjusting for BMI, gender, age and the presence of diabetes mellitus. Multiple stepwise regression analyses indicated that FGF-21 was independently associated with BUN, Phosphate, LVMI and β2 microglobulin (all P<0.05).Lin et al. concluded that Plasma FGF21 levels are significantly increased with the development of early- to end-stage CKD and are independently associated with renal function and adverse lipid profiles, however understanding whether increased FGF21 is associated with myocardial hypertrophy in CKD requires further studies**(Lin et al., 2011)**.

Atherosclerosis, Vascular calcification and Coronary Artery Disease in CKD

Atherosclerosis is a condition characterized with formation of plaques on the intimal layer of large vessels. According to the American Heart Association (AHA) guidelines, coronary atherosclerotic plaques constitute most of the CVD in general population **(Antman et al., 2004)**. However, the pathophysiology of vascular disease in CKD is quite different from that related to atherosclerosis, in the general population **(Kalantar-Zadeh et al., 2003)**. Beside traditional risk

factors including hypertension, diabetes, dyslipidemia, and advanced age, novel risk factors such as endothelial dysfunction (ED),chronic kidney disease-mineral and bone disorders (CKD-MBD), hyperphosphatemia, hyperparathyroidism associated vascular calcifications, increased oxidative stress, and inflammation are highly prevalent and seem to play a more important role for pathogenesis of vascular disease in CKD and ESRD patients compared to non uremic subjects **(Himmelfarb et al., 2002)**. Several studies demonstrated that systemic persistent inflammation in particular could be the main factor responsible for the increased risk in ESRD patients regardless of the renal replacement therapy. To prove this hypothesis, several biomarkers including C-reactive protein, interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α) received more attention in CVD and CKD populations**(Stenvinkel et al., 2008; Turkmen et al., 2011)**.

Apart from the factors mentioned above, why CKD patients are more prone to worse CV outcomes is still unclear. In the general population, many patients with CAD develop coronary collateral circulation to overcome obstruction of the atherosclerotic coronary arteries. **Charytan et al**.**2012** hypothesized that CKD patients might have less collateral blood supply to ischemic area of the myocardium and this hypothesis might partially explain why CKD patients have much worse CV outcomes and death. However, this study failed to prove this hypothesis because both CKD patients and patients without CKD had similar culprit artery collateral supply (25% versus 27.2%, resp.)**(Charytan et al., 2012)**.

Role of FGF-21 in atherosclerosis and CAD

The relationship between FGF-21 and atherosclerosis is not direct one. Thus, FGF-21 is significantly correlated with many atherosclerosis risk factors. High serum

FGF-21 levels were correlated positively with triglycerides, fasting blood glucose, apolipoprotein B100, insulin and HOMA test of insulin resistance (HOMA-IR) but negatively with HDL-C and apolipoprotein A1,indicating a positive association between FGF-21 and adverse lipid profile in CAD patients and a possible compensatory response or resistance to FGF-21**(Lin et al., 2010)**.In adults with type 2 diabetes, elevated FGF-21 is associated with the presence of carotid artery plaques. In turn these risk factors correlated well with some objective signs of atherosclerosis. The combination of dyslipidemia and hypertension is associated with an increased risk of atherosclerosis and cardiovascular disease **(An et al., 2012)**.Serum FGF-21 levels were positively correlated with carotid intima-media thickness (IMT, a marker of atherosclerosis) in women**(Chow et al., 2013)**.

Heart Failure

Heart failure is frequent and present in about one third of newly dialysis patients. Of patients with CKD, 20% experienced a worsening of HF in year 1,LVH, GFR, and hemoglobin were usually associated with the HF**(Levin et al., 2003)**. In dialysis patients with HF, the survival is very poor, the 2-year mortality rate being 49% in hemodialysis populations and 53% in peritoneal dialysis populations. The mortality rate currently remains the same as reported by the U.S. Renal Data System in 2013. Independent positive predictors for mortality in the patients were age, male sex, DM, HTN, history of CVD, and HF **(Banerjee et al., 2007)**.

Relation between FGF-21 and heart failure

Circulating FGF-21 was associated with decline in renal function from early to end stage renal disease. In turn, renal function deterioration was significantly correlated with LVMI, LVH and EF (all $P<0.001$). This may provide an indirect proof

about the role of FGF-21 in cardiac dysfunction **(Lin et al., 2011)**.**Crasto et al**. showed that serum FGF-21 was significantly correlated with hypertension (P<0.008) and heart failure(P<0.007)**(Crasto et al., 2013)**.

Sudden Cardiac death

SCD refers to an unexpected death from a cardiovascular cause with or without structural heart disease. In general, SCD events are defined as those that either arepreceded by a witnessed collapse, occur within 1 hour of an acute change in clinical condition, or occur not more than 24 hours since the deceased individual was known to be in his or her usual state of health**(Fishman et al., 2010)**. Despite major advances in cardiopulmonary resuscitation(**Field et al., 2010)** and post resuscitation care, survival to hospital discharge after cardiac arrest remains poor**(Hallstrom et al., 2004)**. The majority of SCDs occur at home, where the event is often unwitnessed **(Straus et al., 2004)**. The prognosis from cardiac arrests is even worse in patients with kidney dysfunction in which survival probability decreases with a declining GFR**(NIDDKD, 2006)**. Among patients with ESRD who have a witnessed cardiac arrest at an outpatient dialysis facility, more than three-quarters are not discharged alive from the hospital **(Davis et al., 2008)**. Coronary heart disease (CHD) or congestive heart failure (CHF) markedly increases the risk of SCD in this population**(Isaac et al., 2012)**. The majority of patients who suffer a cardiac arrest, however, will not have had a left ventricular failure (ejection fraction<35%) documented before SCD and thus would not have qualified for an intracardiac defibrillator (ICD)**(Zipes et al., 2006)**. In order to address effectively this public health dilemma, intermediate or other vulnerable subgroups of the population need to be identified so that preventive and management strategies can be evaluated. In addition, an understanding of the

mechanisms underlying SCD in well defined subgroupsmay help to provide additional insight into this condition across the entire population**(Isaac et al., 2012)**.

Patients and Methods

I. Patients

The study was conducted on eighty patients, sixty of them were outpatients with CKD and twenty patients with end stage renal disease on long-term hemodialysis. Sixty patients with CKD were recruited from outpatient nephrology clinics at Al-Housein University hospital. Patients recruited into this study had a sustained reduction (≥3 months) in estimated glomerular filtration rate (eGFR) ≤90 ml /min/1.73 m^2 based on the simplified Modification of Diet in Renal Disease formula**(MDRD)GFR =186 x SerumCr$^{-1.154}$ X age$^{-0.203}$ X1.212 (if patient is black) X 0.742 (if female) (Levey et al.,1999)** . Patients on long-term hemodialysis were recruited from hemodialysis unit at Al- Housein University hospital. Twenty apparently healthy control subjects with no history of medical disease, no proteinuria or hematuria, normal kidney in abdominal ultrasound and were not on regular medications were recruited. Subjects were divided into 5 groups as the following:

Group (1) (control): Twenty apparently healthy subjects matched for age, sex and BMI with patients groups.

Group (2): Twenty patients with stage 2 CKD (eGFR 60-90 ml / min /1.73 m^2).

Group (3): Twenty patients with stage 3 CKD (eGFR 30-59 ml / min / 1.73 m^2).

Group (4): Twenty patients with stage 4 CKD (eGFR 15-29 ml / min / 1.73 m^2).

Group (5): Twenty patients with end stage renal disease (eGFR<15ml/ min/1.73 m^2) on regular hemodialysis three times per week.

Inclusion criteria included: Sixty CKD patients with sustained reduction(≥3 months) in estimated glomerular filtration rate (eGFR) ≤90 ml /min/1.73 m^2 based on the simplified Modification of Diet in Renal Disease formula, in addition to twenty patients on regular hemodialysis three times per week.

Exclusion criteria included: overweight and obesity (BMI**>**25), uncontrolled hypertension (Blood pressure> 140/90), uncontrolled diabetes (HA1C% >7.5%), cardiac valve diseases (proved by echocardiography), ischaemic heart diseases, ischaemic cardiomyopathy (proved by electrocardiogram) and cirrhotic liver (proved by abdominal ultrasound, serum albumin, ALT, AST).

All the patients were subjected to:

Full history & detailed clinical examination stressing on blood pressure, body mass index (BMI = body weight in Kg/height in m^2), possibility of insulin resistance according to HOMA-IR equation: fasting insulin (iU/ml) X fasting glucose (mg/dl) / 405(Normal <3, moderate insulin resistance 3-5, severe insulin resistance>5) **(Matthews et al., 1985)**.

Laboratory tests included: Serum creatinine, blood urea, serum calcium, serum albumin, serum phosphorus, hemoglobin, cholesterol ,triglycerides, LDL, intact parathyroid hormone, fasting insulin level, HOMA-IR and serum fibroblast growth factor-21.

II. Analytical methods:

A. Sampling:

Blood samples were collected after overnight fasting for at least 10 hours under complete aseptic condition by venipuncture of 5 ml blood then were left for 20

minutes to be clotted then centrifuged at 4000xg for 10 minutes then aspiration with semi automated pipette of 2-3 ml serum for further analysis of serum fibroblast growth factor-21, intact parathyroid hormone, fasting insulin level, serum creatinine, blood urea, fasting blood sugar, serum calcium, serum albumin, serum phosphrous, serum cholesterol, triglycerides, LDL and hemoglobin. Blood collection tubes were quickly placed in a cooling box and transported to the laboratory where they were divided in aliquots and immediately used. Samples of patients on regular hemodialysis were collected before the session.

All samples were analyzed in Al-Housein University hospital main lab.

A-Assay of Fibroblast Growth Factor-21:

Principle of the test: The Quantikine Human FGF-21 Immunoassay is 4.5 hour solid-phase ELISA designed to measure human FGF-21 in cell culture supernates, serum and plasma. It contains E.coli expressed recombinant human FGF-21 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human FGF-21 showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that kit can be used to determine relative mass values for naturally occurring human FGF-21.

Principle of the assay: This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for FGF-21 has been pre-coated into microplate. Standards and samples are pipette into the wells and FGF-21 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the

amount of FGF-21 bound in the initial step. The color development is stopped and the intensity of the color is measured.

Sensitivity: Fifty-five assay were evaluated and the minimum detectable dose (MDD) of FGF-21 ranged from 1.61- 8.69 pg/ml. The mean MDD was 4.67 pg/ml. The MDD was determined by adding two standard deviations optical density value of twenty zero standard replicates and calculating corresponding concentration.

Calibration: This immunoassay is calibrated against a highly purified E.coli-expressed recombinant FGF-21 produced at R & D system.

Sample values: Serum/Plasma – Samples from apparently healthy volunteers evaluated for the presence of FGF-21 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable(pg/mL)	%Detectable	Range(pg/mL)
Serum	172	97	ND-914
EDTA	201	97	ND-1155
Heparine plasma	186	97	ND-1012

ND: non detectable.

B-Assay of Insulin level:

Principle of the Test: The biosource Human Insulin ELISA kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). The assay uses monoclonal antibodies (mAbs) directed against distinct epitopes of insulin. Samples including standards of known insulin content, control specimens, and unknowns are pipetted into these wells. A detector monoclonal antibody labeled

with horseradish peroxidase (HRP) is added. After an incubation period, the microtiter plate is washed to remove unbound enzyme-labeled antibody and a substrate solution [tetramethylbenzidine (TMB) – H202] is added and incubated. The reaction is stopped with HCl and the microtiter plate is read spectrophotometrically. The intensity of color is directly proportional to the concentration of insulin in the original specimen.

The range of insulin levels in subjects with normal oral glucose tolerance tests is 5 to 25 IU/ml however, it is recommended that each laboratory to establish its own normal concentration range.

C-Assay of Intact Parathyroid Hormone:

The Elecsys assay for determining intact PTH employs sandwich test principle in which a biotinylaled monoclonal antibody reacts with the N-terminal fragment (1-37) and a monoclonal antibody labeled with a ruthenium complex· reacts with the C-terminal fragment (38-84).

Principle of the Test:

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 50µL of sample, a biotinylaled monoclonal PTH-specifIc antibody, and monoclonal PTH-specific antibody Iabeled with a ruthenium complex form a sandwich complex.
- 2nd incubation: after addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin,
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to

the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

Expected values: 15-65 pg /ml (1.6-6.9 pmoI/L).

D-Assay of serum creatinine and urea, blood sugar, albumin,cholesterol, triglycerides, LDL, Hemoglobin, calcium and phosphorus were measured by using modular Eve autoanalyzer 2152- 70 through the following mechanisms:

- Creatinine: measured by Jafe method.
- Urea: measured by urease method.
- Cholesterol, triglycerides and LDL: Measured by enzymatic method.
- Fasting blood sugar: Measured by oxidase method.
- Albumin: Measured by BCG (BromoCresyle Green) method.
- Calcium: Measured by colorimetric method.
- Phosphorus: Measured by end point method with sample blanking.
- Hemoglobin: Measured by using Sysmex KX-21 N through colometric method.

Echocardiography:

All echocardiographic examinations were done with the use of PHILIPS sonos 7500 machine and transducer S8 (3.5 Hz).

All the patients were examined on the left lateral decubitus position. Left ventricular end-diastolic dimension (LVEDd), thickness of the interventricular septum in diastole (IVSd) and left ventricular posterior wall (LVPWTd) in diastole were determined by using standard echocardiographic 2-D and M-mode

measurements. Both fractional shortening and left ventricular mass were calculated from M-mode echocardiogram. LV Mass (g) and LVMI (g/m2) were calculated with the Devereux formulation: **LVM (g) = 0.8(1.04x ([LVEDd + IVSd +PWd]^3– LVEDd)^3) + 0.6** .

LWMI (g/m2) =LVM/BSA (normal range: 43-95 in female, 49-115 in male). Left ventricular ejection fraction (LVEF) was determined using biplane-modified Simpson's measurements **(Devereux et al., 1986)**.

Mitral inflow velocity was traced and the following variables were derived: early (E) peak and late (A) transmitral flow velocity, the ratio of early to late peak velocity (E/A) **(Labovitz and Pearson, 1987; Appleton et al., 1988)**.

Tissue Doppler Imagine (TDI)

A 5-mm sample volume at the lateral and septal corners of the mitral annulus was used for the apical –four chamber view. Annular velocities were displayed in spectral pulsed- wave TDI. The early peak diastolic annular velocity $E^{/}$ was determined from the TDI recordings and the mitral $E/E^{/}$ ratio was calculated **(Ommen and Nishimura, 2003).**

M-Mode Assessment of Left Ventricular Size and Function:

Parameter	Formula	Abbreviation	Values (range)
LV End Diastolic		LVEDd	3.6-5.6

dimension (cm)			
LV End dimension in systole (cm)		LVEDs	2.3-3.9
Fractional shortening (%)	(LVEDd-LVEDs)/LVEDd x100	FS	27-42

LV function assessment using Simpson's method:

Parameter	Formula	Value (range)
LV end-diastolic volume (LVEDV) ml/m^2		49-85
LV end-systolic volume ml/m^2		17-37
Stroke volume (SV) ml/m^2	LVEDV-LVESV	26-54
Ejection fraction (EF) (%)	SV/LVEDVx100	49-71

Echocardiography, ed. Feigenbaum, 6th Edition, Lippincotts, Williams & Wilkins.

The echocardiographies of the patients on regular hemodialysis were performed before dialysis session (in the day between the last and the next session to be nearly in euvolumic state).

Grading of diastolic dysfunction according to European Journal of Echocardiography:

In patients with **mild diastolic dysfunction (grade I)**, the mitral E/A ratio is < 0.8, deceleration time (DT) is >200 ms, (Isovolumetric relaxation time) IVRT is >100 ms, annular E´ is <8 cm/s, and the E/E´ ratio is <8 (septal and lateral). These patients have reduced diastolic reserve. However, reduced mitral E/A ratio in the presence of normal annular tissue Doppler velocities can be seen in volume-depleted normal subjects, so an E/A ratio < 0.8 should not be universally used to infer the presence of diastolic dysfunction. In most situations, when the E/A ratio is < 0.8, mean Left Atrial (LA)pressure is not elevated, except for some patients with severely impaired myocardial relaxation, as in long-standing hypertension or hypertrophic cardiomyopathy.

In patients with **moderate diastolic dysfunction (grade II)**, the mitral E/A ratio is 0.8 to 1.5 (pseudonormal) and decreases by >50% during the Valsalva maneuver, the E/E´ (average) ratio is 9 to 12, and E´ is <8 cm/s. Grade II diastolic dysfunction represents impaired myocardial relaxation with mild to moderate elevation of Left Ventricular(LV) filling pressures.

With **severe diastolic dysfunction (grade III)**, restrictive LV filling occurs with an E/A ratio ≥2, DT <160 ms, IVRT<60 ms, E´ is <8 cm/s and average E/E´ ratio >13 (or septal E/E´ >15 and lateral E/E´ >12) **(Sherif et al., 2009)**.

Statistical analysis:

Data were analyzed using Statistical Program for Social Science (SPSS) version 18.0. Quantitative data were expressed as mean± standard deviation (SD).

Qualitative data were expressed as frequency and percentage.

The following tests were done:

- **A one-way analysis of variance (ANOVA)** when comparing between more than two means.
- **Independent-samples t-test of significance** was used when comparing between two means.
- **Chi-square (X^2) test of significance** was used in order to compare proportions between two qualitative parameters.
- **Pearson's correlation coefficient (r)** test was used for correlating data.
- **Linear regression**: It is used to test and estimate the dependence of a quantitative variable based on its relationship to one or more independent variables.

- **Probability (P-value)**
  - **P-value <0.05** was considered significant.
  - **P-value <0.001** was considered as highly significant.
  - **P-value >0.05** was considered insignificant.

Table (1): **Descriptive data of group I(control):**

Group I	Min.	Max.	Mean	±SD
Age/ years	23.00	55.00	48.1	12
BMI(kg/m^2)	21.60	23.60	22.64	0.65
SBP (mm.Hg)	110.00	140.00	124.50	9.58
DBP (mm.Hg)	70.00	85.00	79.50	5.10
Creatinine(mg/dl)	0.70	1.10	0.88	0.12
Urea (mg/dl)	25.00	43.00	31.75	5.08
eGFR($ml/min/1.73m^2$)	91.40	118.00	101.94	8.07
C.Calcium (mg/dl)	8.50	9.50	9.03	0.38
Phosphorus (mg/dl)	3.10	4.50	3.69	0.35
iPTH(pg/ml)	23.00	50.00	35.05	7.78
Hemoglobin(g/dl)	12.00	15.10	13.29	0.90

Albumin (gm/dl)	3.50	5.30	4.41	0.55
Cholesterol (mg/dl)	160	191	175.6	8.5
Triglycerides (mg/dl)	76	114	90.6	12.1
LDL (mg/dl)	67	94	80.1	8.1
FGF-21 (pg/ml)	39.00	131.00	85.10	26.78
FBS (mg/dl)	75.00	105.00	86.9	9.50
Insulin (iu/ml)	5.00	9.00	6.75	1.41
HOMA-IR	0.9	2	1.4	0.4
LVEDd(cm)	3.30	5.10	4.24	0.34
IVSd(cm)	0.60	1.00	0.78	0.11
LPWTd(cm)	0.60	0.90	0.78	0.09
LVM (gram)	69.00	117.00	93.30	16.80
LVMI (gm/m^2)	36.50	89.00	53.00	12.52
EF %	65.00	71.42	69.34	2.2
FS %	30.50	40.10	38.42	3.40
E (cm/s)	48.00	96.30	72.24	11.18
A (cm/s)	40.30	61.40	53.37	5.14
E/A	1.00	1.80	1.35	0.41
$E^{/}$(cm/s)	9.80	12.50	10.20	0.77
$E/E^{/}$	3.70	8.00	7.07	1.20

Table (2): **Descriptive data group II:**

Group II	Min.	Max.	Mean	±SD
Age/ years	39.00	67.00	53.40	8.59
BMI(kg/m^2)	20.50	24.40	22.58	0.93
SBP mm.Hg	110.00	140.00	122.50	9.80
DBP mm.Hg	70.00	90.00	78.00	5.94
Creatinine mg/dl	0.70	1.40	1.07	0.18
Urea mg/dl	24.00	46.00	36.85	6.71
eGFR ml/min/1.73m^2	65.80	89.50	81.24	8.71
C.Calcium (mg/dl)	8.40	10.00	9.04	0.49
Phosphorus (mg/dl)	3.10	4.50	3.80	0.45
iPTH (pg/ml)	44.00	73.00	56.05	9.33

Hb (g/dl)	10.00	13.00	11.29	0.73
Albumin (gm/dl)	3.40	4.80	3.85	0.40
Cholesterol (mg/dl)	175.00	221.00	191.9	14.7
Triglycerides(mg/dl)	84.00	150.00	108.3	18.1
LDL (mg/dl)	76.00	112.00	91.9	9.9
FGF-21 (pg/ml)	70.00	324.00	184.56	78.13
FBS mg/dl	76.00	121.00	93.90	13.78
Insulin (iu/ml)	5.00	20.00	8.22	4.00
HOMA-IR	1.00	4.60	1.9	0.9
LVEDd (cm)	3.50	5.00	4.24	0.38
IVSd (cm)	0.60	1.10	0.85	0.13
LPWTd (cm)	0.60	0.90	0.79	0.09
LVM (gram)	72.00	127.00	100.15	14.88
LVMI (gm/m^2)	44.00	66.50	57.10	6.86
EF %	59.30	70.30	67.86	5.71
FS %	32.90	43.50	37.70	3.23
E (cm/s)	40.50	95.20	65.00	13.71
A (cm/s)	48.30	67.70	58.49	5.99
E/A	0.80	1.70	1.11	0.25
$E^{/}$ (cm/s)	7.20	10.30	8.33	1.20
$E/E^{/}$	5.40	11.10	7.80	2.19

Table (3): **Descriptive data group III:**

Group III	Min.	Max.	Mean	±SD
Age/ years	35.00	68.00	54.50	9.22
BMI(kg/m^2)	20.40	24.80	23.13	1.52
SBP (mm.Hg)	110.00	140.00	128.50	9.00
DBP (mm.Hg)	70.00	85.00	79.67	3.81
Creatinine(mg/dl)	1.50	3.40	2.64	0.49
Urea (mg/dl)	38.00	63.00	49.55	7.21
eGFR $ml/min/1.73/m^2$	30.00	58.50	40.72	8.24
C.Calcium (mg/dl)	8.40	10.10	8.89	0.45
Phosphorus (mg/dl)	3.30	5.50	4.47	0.74
iPTH(pg/ml)	66.00	148.00	107.05	27.03

Hemoglobin(g/dl)	9.70	13.00	11.14	0.91
Albumin gm/dl	3.30	4.00	3.67	0.23
Cholesterol (mg/dl)	178.00	249.00	210.30	23
Triglycerides(mg/dl)	83.00	178.00	118.90	25
LDL (mg/dl)	81.00	155.00	113.00	21.3
FGF-21 (pg/ml)	99.00	686.00	287.36	179.33
FBS (mg/dl)	76.00	125.00	96.20	14.77
Insulin (iu/ml)	5.50	17.00	10.2	4.00
HOMA-IR	1.10	4.80	2.50	1.20
LVEDd(cm)	3.30	5.50	4.41	0.55
IVSd(cm)	0.60	1.20	0.91	0.18
LPWTd(cm)	0.70	1.20	0.95	0.15
LVM (gram)	69.00	262.00	160.18	60.31
LVMI (gm/m^2)	36.50	134.00	85.38	31.09
EF %	54.60	69.00	65.76	5.10
FS %	30.30	41.12	36.50	2.83
E (cm/s)	31.90	70.60	55.14	12.03
A (cm/s)	47.90	78.50	64.64	10.64
E/A	0.62	1.30	0.85	0.23
$E^{/}$(cm/s)	5.50	7.50	6.51	0.64
$E/E^{/}$	5.00	11.40	8.5	1.89

Table (4): Descriptive data of group IV

Group IV	Min.	Max.	Mean	±SD
Age/ years	45.00	64.00	53.1	6.30
BMI(kg/m^2)	20.50	22.80	21.43	2.20
SBP(mm.Hg)	110.00	140.00	128.25	10.92
DBP (mm.Hg)	70.00	90.00	81.55	4.24
Creatinine mg/dl	3.10	5.00	4.01	0.50
Urea mg/dl	60.00	135.00	105.70	20.26
eGFR(ml/min/1.73/$m^{2)}$	15.80	26.40	20.09	9.73
C.Calcium(mg/dl)	8.20	9.50	8.61	0.32
Phosphorus (mg/dl)	3.40	6.90	5.10	1.03
iPTH (pg/ml)	70.00	407.00	201.40	82.10
Hemoglobin(g/dl)	8.60	11.50	10.08	0.86

Albumin (gm/dl)	3.30	4.50	3.80	0.35
Cholesterol (mg/dl)	194.00	235.00	225.1	23.2
Triglycerides (mg/dl)	100.00	242.00	146.5	46.8
LDL (mg/dl)	92.00	160.00	117.9	20.2
FGF-21(pg/ml)	203.00	2000.00	558.35	488.55
FBS (mg/dl)	76.00	124.00	99.80	15.17
Insulin (iu/ml)	7.00	23.00	14.03	5.69
HOMA-IR	1.40	6.20	3.40	1.50
LVEDd (cm)	3.50	5.50	4.54	0.64
IVSd (cm)	0.70	1.20	1.00	0.18
LPWTd (cm)	0.50	1.30	1.01	0.18
LVM (gram)	127.00	262.00	201.05	41.83
LVMI (gm/m^2)	69.50	176.00	108.94	25.82
EF %	50.22	67.50	65.04	9.47
FS %	27.70	39.20	36.11	5.32
E (cm/s)	39.50	88.30	62.07	12.29
A (cm/s)	24.00	71.60	48.93	9.99
E/A	0.80	1.80	1.30	0.31
$E^{/}$(cm/s)	4.30	7.50	6.00	0.9
$E/E^{/}$	5.90	15.10	10.3	2.78

Table (5): Descriptive data of group V:

Group V	Min.	Max.	Mean	±SD
Age/ years	37.00	70.00	56.50	8.75
BMI(kg/m^2)	19.20	24.50	22.32	1.60
SBP mm.Hg	90.00	140.00	126.00	15.40
DBP mm.Hg	60.00	90.00	77.0	6.40
Creatinine(mg/dl)	4.51	8.60	6.04	1.09
Urea (mg/dl)	125.00	258.00	168.30	33.71
eGFR(ml/min/1.73/$m^{2)}$	7.50	12.00	9.39	1.32
C.Calcium (mg/dl)	8.30	9.80	8.81	0.44
Phosphorus (mg/dl)	3.50	10.50	6.11	1.82
iPTH(pg/ml)	60.00	1163.00	419.50	297.50
Hemoglobin(g/dl)	8.20	12.50	10.31	1.16
Albumin gm/dl	3.30	4.00	3.63	0.19
Cholesterol (mg/dl)	200.00	270.00	233.00	23.60
Triglycerides (mg/dl)	101.00	245.00	161.50	49.40

LDL (mg/dl)	100.00	165.00	130.90	24.00
FGF-21 pg/ml	342.00	2000.00	1071.66	581.90
FBS mg/dl	78.00	105.00	88.90	9.72
Insulin iu/ml	6.00	24.00	11.15	5.94
HOMA-IR	1.00	5.90	2.40	1.30
LVEDd cm	3.40	5.50	4.51	0.62
IVSd cm	0.70	1.40	1.03	0.17
LPWTd cm	0.80	1.40	1.02	0.17
LVM gram	140.00	302.00	210.35	50.17
LVMI gm/m2	79.00	182.00	121.92	29.74
EF %	49.50	66.30	64.39	6.79
FS %	25.20	38.70	35.20	4.61
E (cm/s)	56.30	121.30	97.52	14.54
A (cm/s)	60.00	71.80	48.69	13.95
E/A	0.78	3.40	2.00	0.36
$E^{/}$(cm/s)	4.20	8.10	5.60	1.15
$E/E^{/}$	7.90	20.30	17.41	3.02

Table (6): **Demographic data of the study group:**

			Groups					Total	
			Group I	Group II	Group III	Group IV	Group V	ANOVA	
			Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	F	P-value
Age			48.1±12	53.4±8.6	54.5±9.2	53.1±6.3	56.5±8.7	2.18	**0.077**
BMI			22.6±0.6	22.6±0.9	23.1±1.5	21.4± 2.3	22.3±1.6	1.18	**0.325**
D.B.P.			79.5±5.1	78±5.9	79.7±3.8	81±4.2	77±6.4	1.83	**0.129**
S.B.P.			124.5±9.6	122.5±9.8	128.5±9	128.2±10.9	126±15.4	1.03	**0.397**
Sex	Female	No.	10	9	14	8	10	51	**P-value 0.385**
		% of Total	10.0%	9.0%	14.0%	8.0%	10.0%	51.0%	
	Male	No.	10	11	6	12	10	49	
		% of Total	10.0%	11.0%	6.0%	12.0%	10.0%	49.0%	
Total		No.	20	20	20	20	20	100	
		% of Total	20.0%	20.0%	20.0%	20.0%	20.0%	100.%	

This table shows no significant difference between groups as regard age, BMI, SBP, DBP and sex.

Table (7): Classification of patients groups according to their etiology of CKD:

Etiology		Patient groups				Total
		Group 2	Group 3	Group 4	Group 5	
Diabetes mellitus	Count	4	7	5	2	18
	% of Total	5.0%	8.8%	6.3%	2.5%	22.5%
Hypertension	Count	2	5	6	4	17
	% of Total	2.5%	6.3%	7.5%	5.0%	21.3
Polycystic Kidney disease	Count	1	1	0	3	5
	% of Total	1.3%	1.3%	.0%	3.8%	6.3%
Ch. Glomerulonephritis	Count	5	3	1	0	9
	% of Total	6.3%	3.8%	1.3%	.0%	11.3%
Ch. Interstitial nephritis	Count	2	0	4	1	7
	% of Total	2.5%	.0%	5.0%	1.3%	8.8%
Analgesic nephropathy	Count	1	1	0	3	5
	% of Total	1.3%	1.3%	.0%	3.8%	6.3%
SLE	Count	2	1	1	1	5
	% of Total	2.5%	1.3%	1.3%	1.3%	6.3%
Unknown	Count	1	2	1	4	8
	% of Total	1.3%	2.5%	1.3%	5.0%	10.0%
Obstructive nephropathy	Count	1	0	2	2	5
	% of Total	1.3%	.0%	2.5%	2.5%	6.3%
Amyloidosis	Count	1	0	0	0	1
	% of Total	1.3%	.0%	.0%	.0%	1.3%
Count	20		20	20	20	80
% of Total	25.0%		25.0%	25.0%	25.0%	100.0%

This table shows classification of patients groups as regard their etiologies of CKD.

Table (8): **Comparison between study groups as regard laboratory data:**

	Group I	Group II	Group III	Group IV	Group V	ANOVA	
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	F	P-value
eGFRml/ min/m^2)	101.9±8.1	81.2±8.7	40.7±8.2	20.1±9.7	9.4±1.3	497.5	**0.000**
Urea (mg/dl)	31.7±5.1	36.8±6.7	49.5±7.2	105.7±20.3	168.3±33.7	203.1	**0.000**
Creatinine (mg/dl)	0.9±011	1.1±0.2	2.6±0.5	4±0.5	6±1.1	270.9	**0.000**
Albumin (gm/dl)	4.4±0.5	3.8±0.4	3.7±0.23	3.8±0.4	3.6±0.2	14.8	**0.000**
Hb(g/dl)	13.3±09	11.3±0.7	11.1±0.9	10.1±0.9	10.3±1.2	37.5	**0.000**
Cholesterol (mg/dl)	175.6±8.5	191.9±14.7	210.3±23	225.1±23.2	233±23.6	29.03	**0.000**
TG (mg/dl)	90.6±12.1	108.3±18.1	118.9±25	146.5±46.8	161.5±49.4	14.3	**0.000**
LDL mg/dl	80.1±8.1	91.9±9.9	113±21.3	117.9±20.2	130.9±24	26.2	**0.000**
C.calcium (mg/dl)	9±0.4	9±0.5	8.9±0.4	8.6±0.3	8.8±0.4	3.5	**0.010**
Ph. (mg/dl)	3.7±0.3	3.8±0.4	4.5±0.7	5.1±1	6.1±1.8	19.1	**0.000**
iPTH(pg/ ml)	35±7.8	56±9.3	107±27	201.4±82.1	419.5±297.5	25.5	**0.000**

This table shows significant difference between groups as regard eGFR, creaitinine, urea, albumin, Hemoglobin, cholesterol, triglycerides, LDL, corrected calcium, Phosphorus, intact PTH, where dyslipidemia, hyperphosphatemia and hyperparathyroidism progress with the deterioration of renal functions.

Table (9): **Comparison between study groups as regard glucoparameters:**

	Group I	Group II	Group III	Group IV	Group V	ANOVA	
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	F	P-value
FBS (mg/dl)	86.9±9.5	93.9±13.8	96.2±14.8	99.8±15.2	88.9±9.7	3.4	**0.013**
Insulin (Iu/ml)	6.7±1.4	8.2±4	10.2±4	14±5.7	11.1±5.9	7.6	**0.000**
HOMA-IR	1.4±0.4	1.9±0.9	2.5±1.2	3.4±1.5	2.4±1.3	8.7	**0.000**

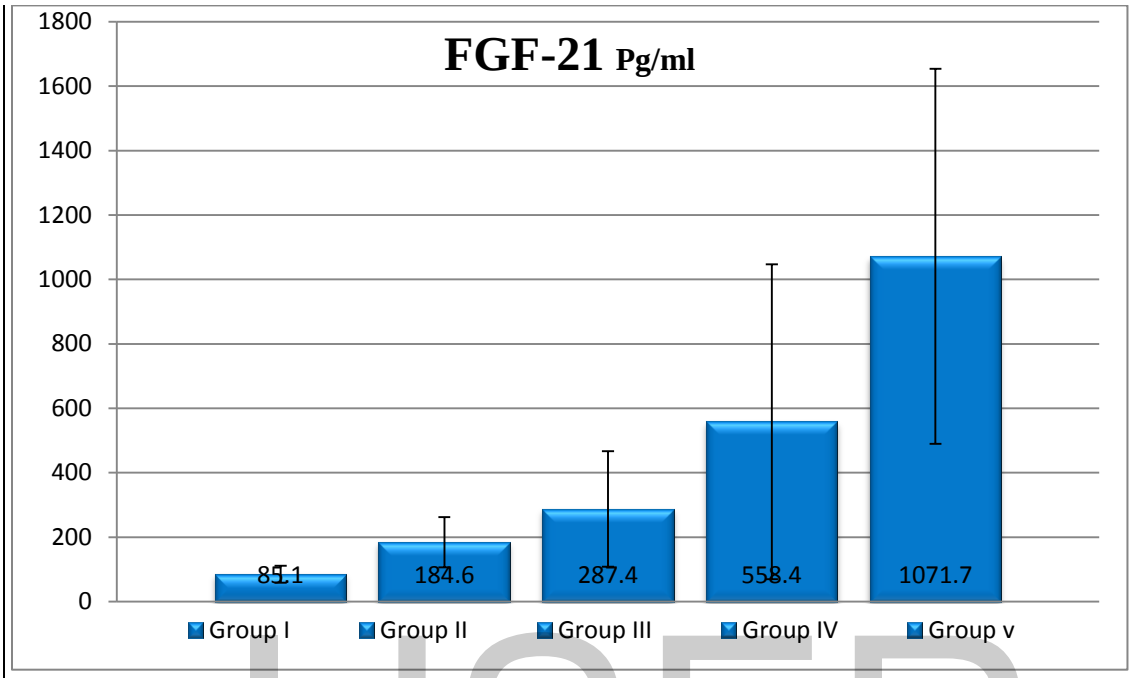
This table shows significant difference between groups as regard FBS, fasting insulin and HOMA-IR.

Table (10): Comparison between study groups as regard FGF-21 pg/ml level.

	FGF-21 pg/ml		**ANOVA**	
	Mean	**±SD**	**F**	**p-value**
Group I(Control)	85.1	26.8	25.455	**<0.001**
Group II	184.6	78.1		
Group III	287.4	179.3		
Group IV	558.4	488.5		
Group V	1071.7	581.9		
Mean total (FGF-21 pg/ml)	437.40	495.01		

This table show significant difference between groups as regard FGF-21 level**(P-value <0.001)**where FGF-21 level in group V is 12.6 fold higher as compared with group I, in group IV FGF-21 level is 5.6 fold higher as compared with group I, in group III FGF-21 level is 3.4 fold higher as compared with group I while FGF-21 level in group II is 2.2 fold higher as compared with group I. Mean FGF-21 between all subjects included in the study is 437.4± 495.01.

Figure (6): Barr chart shows Comparison between study groups as regard FGF-21 level.



This figure shows increased FGF-21 level progressively with the deterioration of renal functions.

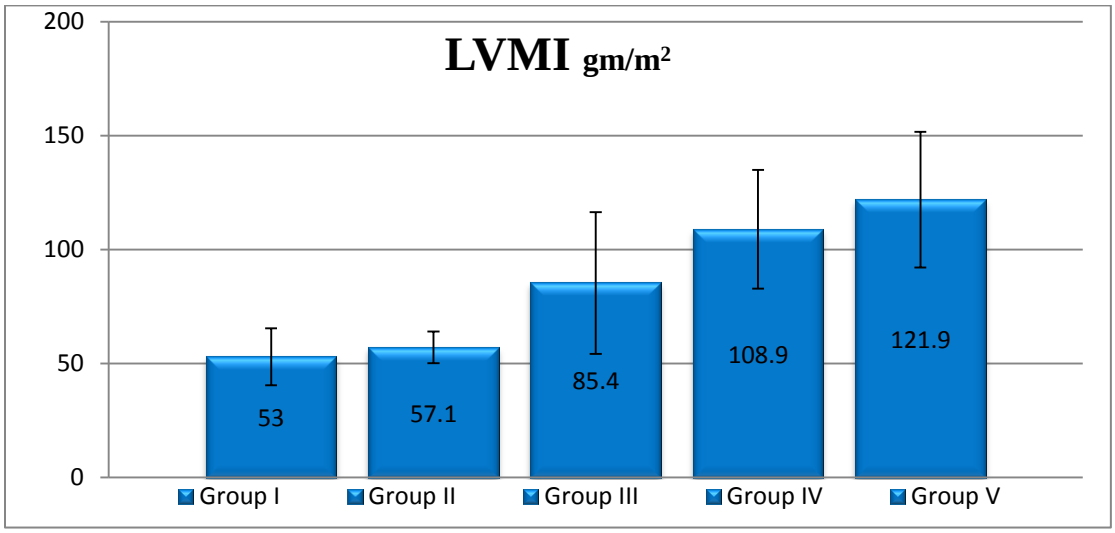
Table (11): **Comparison between study groups as regard echocardiographic parameters:**

	Group I	Group II	Group III	Group IV	Group V	ANOVA	
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	F	P-value
LVEDd cm	4.2±0.3	4.2±0.4	4.4±0.5	4.5±0.6	4.5±0.6	1.8	**0.139**
IVSd cm	0.8±0.1	0.8±0.1	0.9±0.2	1±0.2	1±0.2	7.617	**<0.001**
LPWTd cm	0.8±0.1	0.8±0.1	0.9±0.1	1±0.2	1±0.2	14.3	**<0.001**
LVM gram	93.3±16.8	100.1±14.9	160.2±60.3	201±41.8	210.3±50.2	35.7	**<0.001**
LVMI gm/m2	53±12.5	57.1±6.9	85.4±31.1	108.9±25.8	121.9±29.7	34.3	**<0.001**
EF%	69.3±2.2	67.9±5.7	65.8±5.1	65±9.5	64.4±6.8	2.4	**0.04**

FS%	38.4±3.4	37.7±3.2	36.5±2.8	36.1±5.3	35.2±4.6	2	**0.09**
E cm/s	72.2±11.2	65±13.7	55.1±12	62.1±12.3	97.5±13.5	16.6	**<0.001**
A cm/s	53.4±5.1	58.5±6	64.5±10.6	48.9±10	48.7±14	3.5	**0.010**
E/A	1.3±0.4	1.1±0.2	0.8±0.2	1.3±0.3	2.00±0.4	32.9	**<0.001**
$E^{/}$	10.2±0.8	8.3±1.2	6.5±0.6	6 ±0.9	5.6±1.1	79	**<0.001**
$E/E^{/}$	7.1±1	7.8±2.2	8.5±1.9	10.3±2.8	17.4±3	38.1	**<0.001**

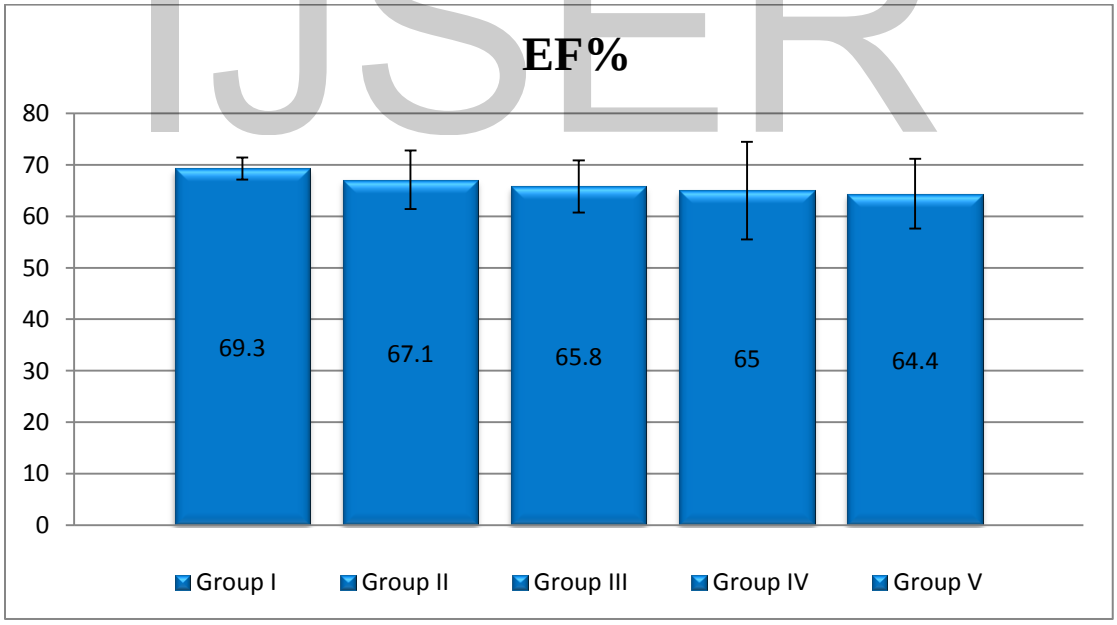
This table shows significant difference between study groups as regard: IVSd, LPWTd, LVM, LVMI, EF, E, A, E/A, $E^{/}$, $E/E^{/}$**,** while there is no significant difference between study groups as regard: LVEDd or FS **(P- value = 0.139, 0.09)** respectively.

Figure (7): **Barr chart shows comparison between study groups as regard LVMI.**



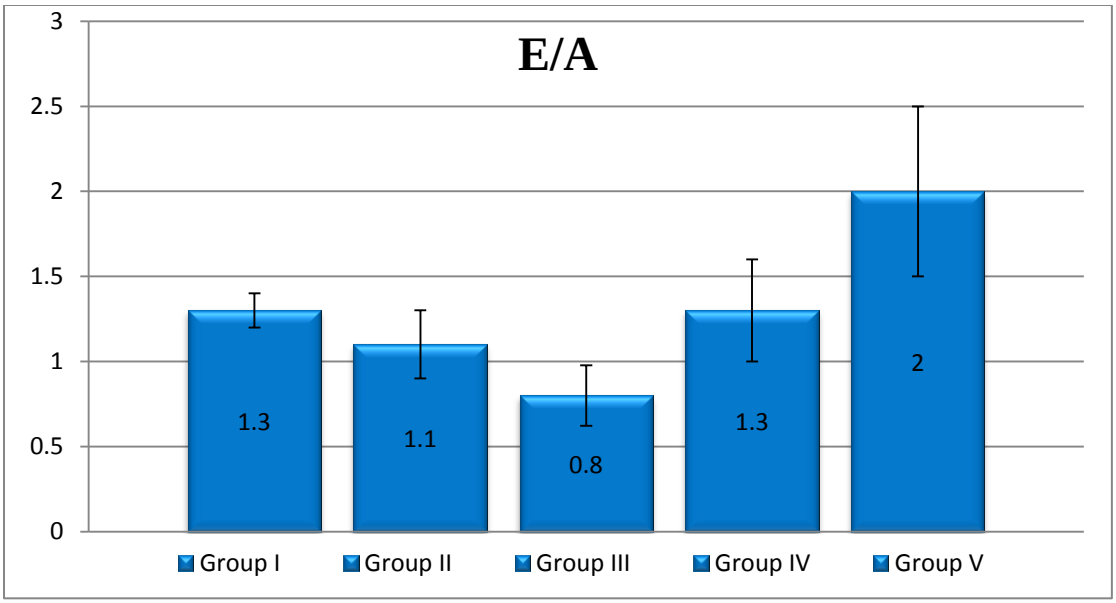
This figure shows that LVMI increases with the deterioration of renal functions.

Figure (8): **Barr chart shows comparison between study groups as regard EF:**



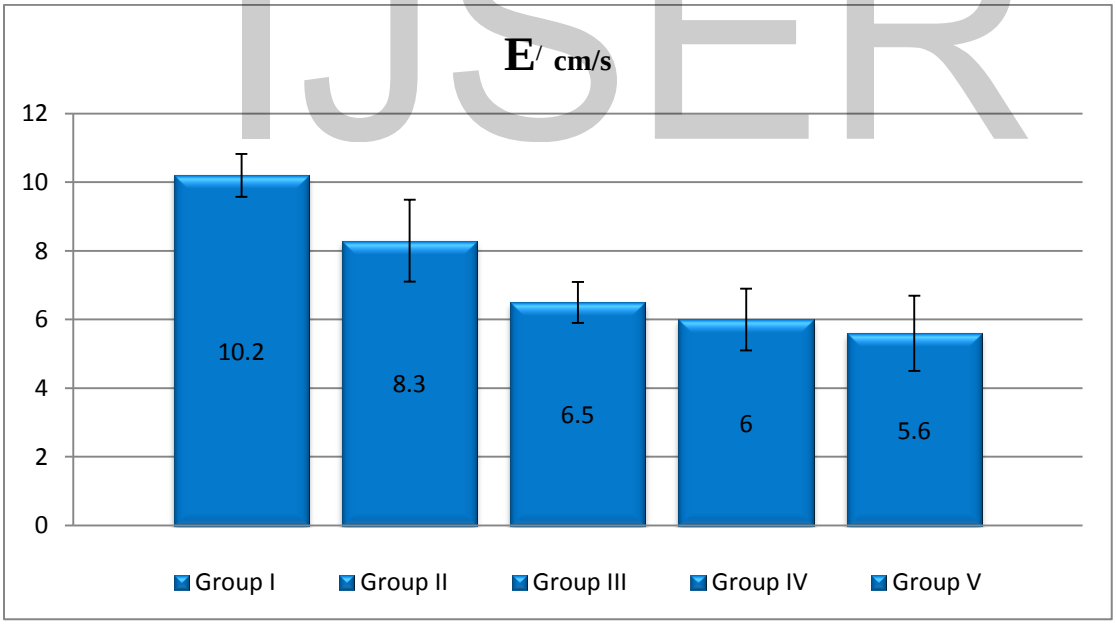
This figure shows that EF significantly decreases with the deterioration of renal functions **(P- value = 0.04)**.

Figure (9): **Barr chart shows comparison between study groups as regard E/A ratio:**



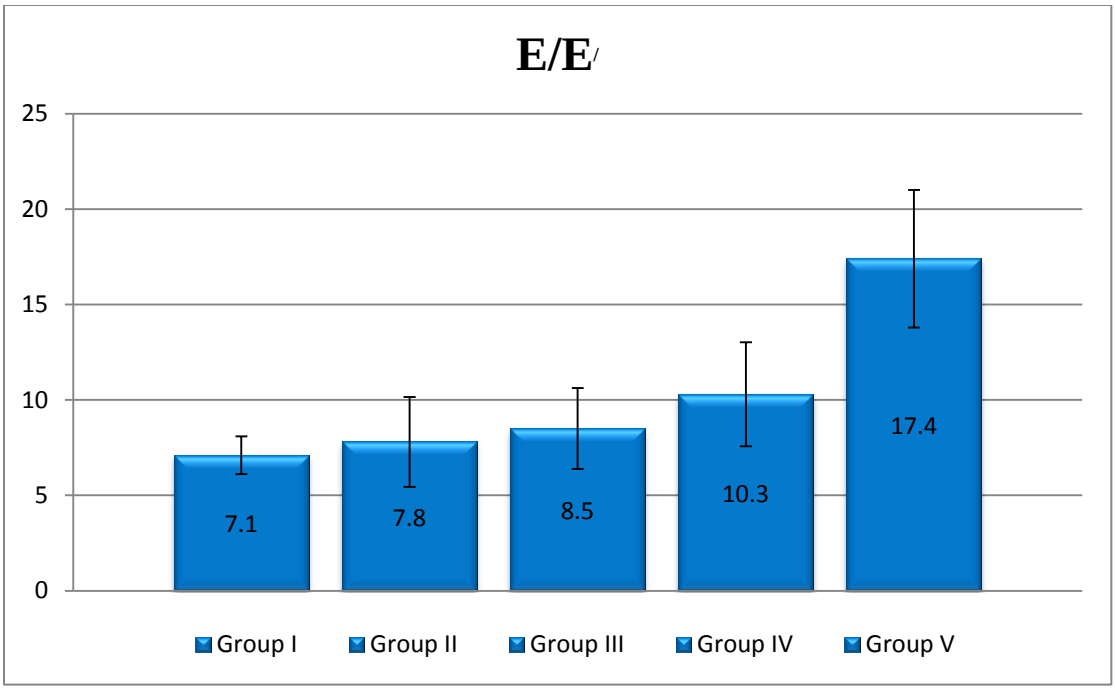
This figure shows that E/A ratio in group V is higher than other groups and E/A ratio in group III is reversed.

Figure (10): **Barr chart shows comparison between study groups as regard E/.**



This figure shows decreased E/ velocity with deterioration of renal functions.

Figure (11): Barr chart shows comparison between study groups as regard E/E/.



This figure shows that E/E/ ratio increases with the deterioration of renal functions.

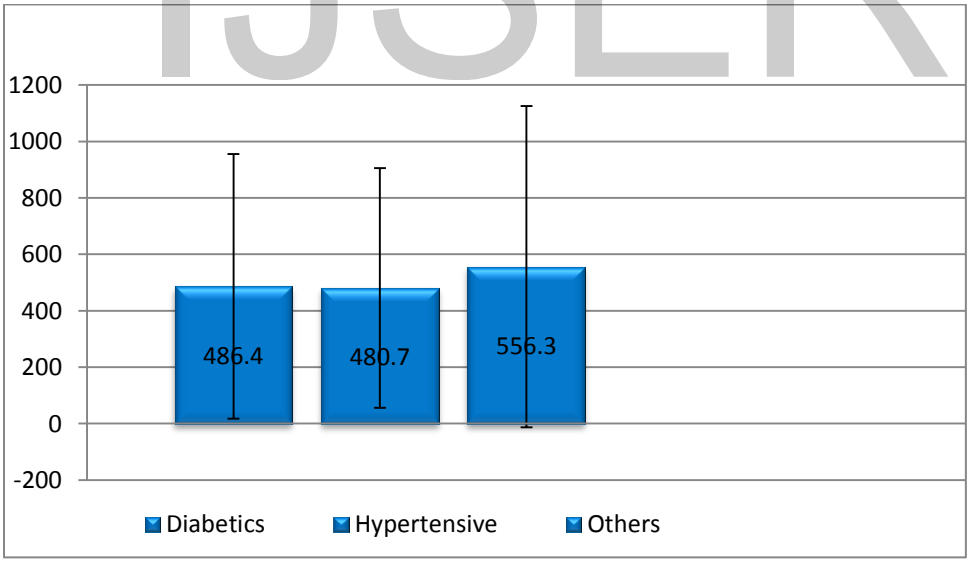
Table (12): Comparison between patients groups as regard mean FGF-21 level and mean echocardiographic parameters in relation to the etiology of CKD:

	N.	Diabetics Mean±SD	N	Hypertensive Mean±SD	N	Others Mean±SD	ANOVA F	ANOVA p-value
FGF-21	18	486.4±469.1	17	480.7±425.1	45	556.3±569.3	0.189	**0.828**
LVEDd	18	4.4±0.7	17	4.5±0.6	45	4.4±0.5	0.168	**0.846**
IVSd	18	1±0.2	17	0.9±0.2	45	0.9±0.2	0.480	**0.621**
LPWTd	18	0.9±0.1	17	1±0.1	45	0.9±0.2	0.258	**0.774**
LVM	18	167.7±67.7	17	177.5±57.2	45	164.7±62.5	0.247	**0.782**
LVMI gm/m2	18	90.6±34.6	17	95.7±31.6	45	93.5±37	0.090	**0.914**
EF%	18	66.1±8.3	17	63.4±7.4	45	66.4±6.2	1.135	**0.327**
FS%	18	36.2±5.4	17	34.9±4.2	45	36.9±3.4	1.439	**0.243**

E/A	18	1.1±04	17	1.3 ±0.4	45	1.2±0.4	1.044	**0.357**
E/	18	6.6±1.4	17	6.4 ±1.2	45	6.6±1.5	0.172	**0.842**
E/E/	18	9.3±2.9	17	10.3 ±4.5	45	10.2±3.9	0.425	**0.655**

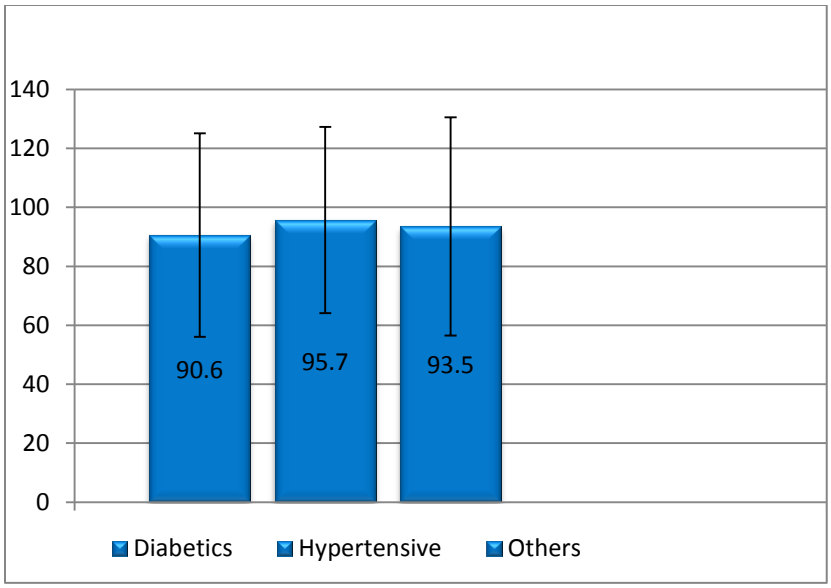
This table shows no significant difference between hypertensive, diabetic and other patients as regard mean FGF-21 level and echocardiographic parameters.

Figure (12): Barr chart shows comparison between diabetics, hypertensive and other patients as regards FGF-21 level:



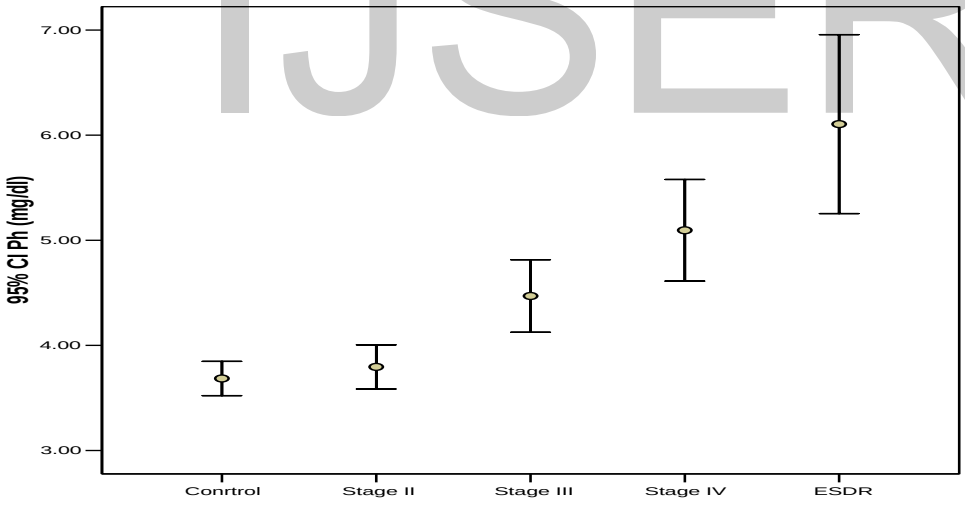
This figure shows no significant difference between diabetics, hypertensive and other patients as regard FGF-21 level **(P-value = 0.828)**.

Figure (13): Barr chart shows comparison between diabetics, hypertensive and other patients as regards LVMI:



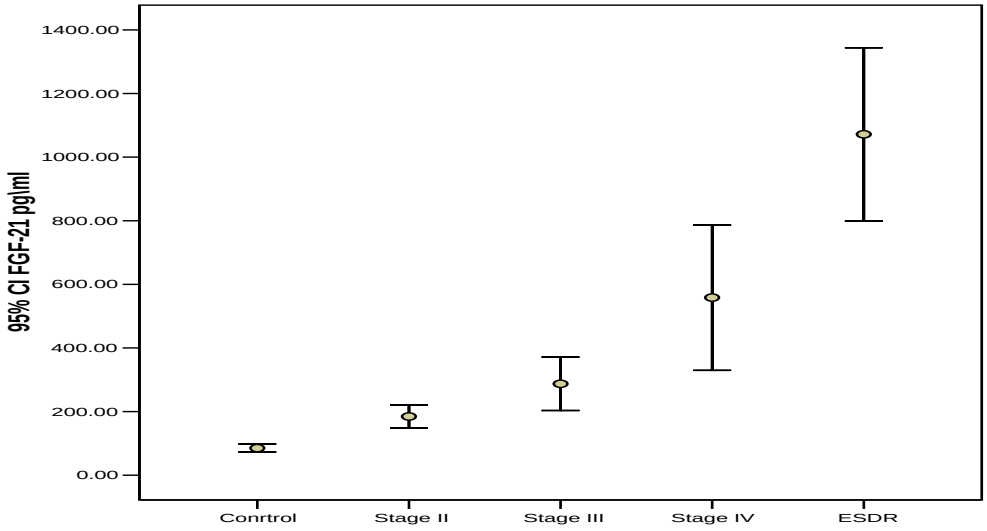
This figure shows no significant difference between diabetics, hypertensive and other patients as regard LVMI **(P-value = 0.914)**.

Figure: (14): Error bar between groups as regard Ph. (mg/dl).



This figure shows that absolute difference in mean serum phosphorus is 0.1mg/dl (relative difference (2.7%) between stage II group and control group.

Figure (15): Error bar between groups as regard FGF-21 (pg/ml).



This figure shows that absolute difference in mean serum FGF-21 is 99.5pg/ml(relative difference (117%) between stage II group and control group.

Table (13): **Pearson's correlation coefficient test shows relation between FGF-21 level and other studied parameters:**

	r	Sig.
Age/ Years	0.115	**0.253**
BMI	-0.018	**0.862**
DBP (mm.Hg)	0.149	**0.138**
SBP (mm.Hg)	0.191	**0.060**
eGFR(ml/min/ml)	-0.631	**<0.001**
Urea mg/dl	0.774	**<0.001**
Creatinine (mg/dl)	0.707	**<0.001**
Albumin (gm/dl)	-0.272	**<0.001**
Hemoglobin (g/dl)	-0.398	**<0.001**
iPTH(pg/ml)	0.444	**<0.001**
Phosphorus (mg/dl)	0.569	**<0.001**
C.Calcium (mg/dl)	-0.097	**0.335**
Cholesterol mg/dl	0.673	**<0.001**
Triglycerides(mg/dl)	0.606	**<0.001**
LDL (mg/dl)	0.598	**<0.001**

r: correlation. sig.: significance.

This table shows that FGF-21 has significant positive correlation with creatinine, urea, PTH, phosphorus, cholesterol and triglycerides while it has significant negative correlation with eGFR, albumin and Hemoglobin but it has no significant correlation with age, BMI, SBP, DBP or corrected calcium.

Table (14): **Pearson's correlation coefficient test shows relation between FGF-21 level and glucoparameters:**

	r	**Sig.**
FBS (mg/dl)	-0.094	**0.351**
Insulin (Iu/ml)	0.225	**0.025**
HOMA-IR	0.159	**0.115**

This table shows that FGF-21 has significant positive correlation with fasting insulin level but it has no significant correlation with fasting blood sugar or HOMA-IR.

Table (15): **Pearson's correlation coefficient test shows relation between FGF-21 level and echocardiographic parameters:**

	r	Sig.
LVEDd(cm)	0.045	**0.660**
IVSd(cm)	0.344	**0.000**
LPWTd(cm)	0.412	**<0.001**
LVM (gram)	0.563	**<0.001**
LVMI (gram/$m^{2)}$	0.558	**<0.001**
EF%	-0.186	**0.064**
FS%	-0.168	**0.095**
E (cm/s)	0.179	**0.075**
A (cm/s)	-0.112	**0.268**
E/A	0.168	**0.096**
$E^{/}$**(**cm/s)	-0.565	**<0.001**
E/ $E^{/}$	0.595	**<0.001**

This table shows that FGF-21 has significant positive correlation with IVSd, LPWTd, LVM, LVMI and E/E/**,** while it has significant negative correlation with E/ but it has no significant correlation with LVEDd, EF, FS,E,A or E/A.

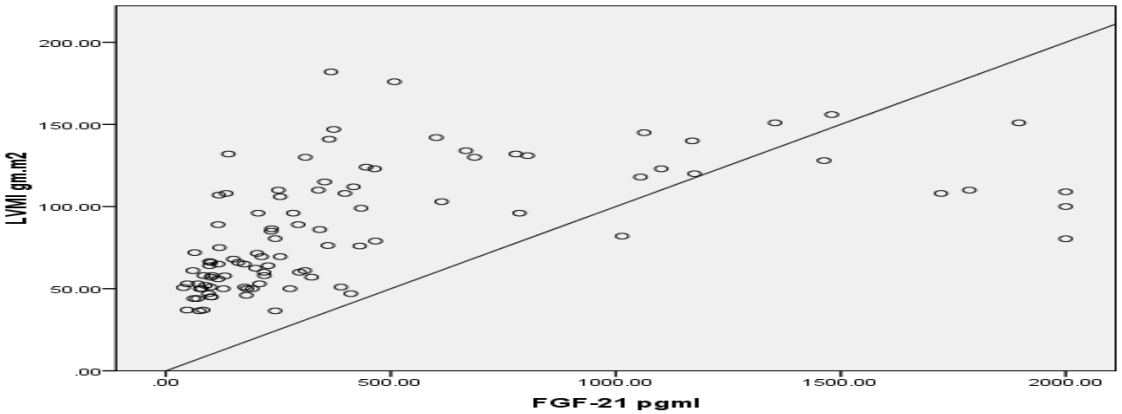


Figure (16): Shows positive correlation between FGF-21 and LVM.

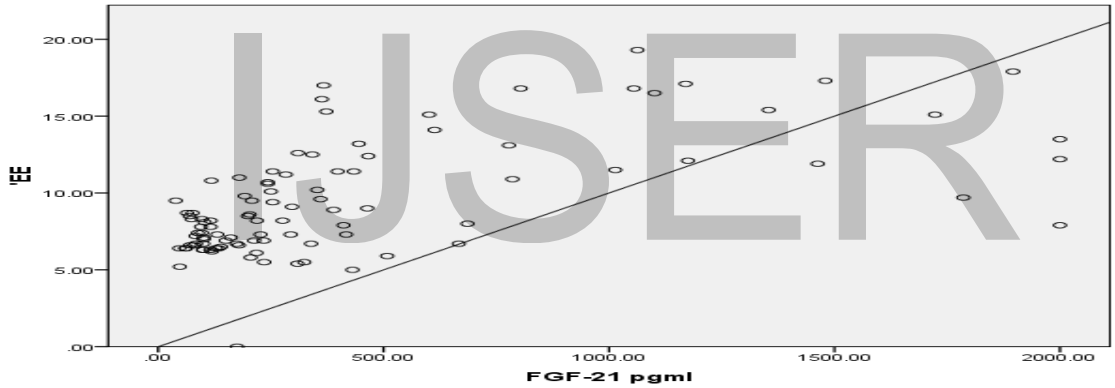


Figure (17): Shows positive correlation between FGF-21 and E/E/.

Table (16): **Pearson's correlation coefficient test shows relation between HOMA-IR level and other studied parameters:**

	r	Sig.
Age	0.141	0.160
BMI	-0.059	0.557
DBP	0.060	0.551
SBP	0.055	0.585
eGFRml.min.	-0.424	<0.001
Urea mg/dl	0.298	0.001
Creatinine (mg/dl)	0.364	<0.001
Cholesterol mg/dl	0.358	<0.001

Triglycerides(mg/dl)	0.376	<0.001
LDL (mg/dl)	0.341	<0.001
FBS(mg/dl)	0.411	<0.001
Insulin iu/ml	0.952	<0.001

This table shows that HOMA-IR has significant positive correlation with urea, creatinine, phosphorus, cholesterol, triglycerides, LDL, insulin and FBS, while it has significant negative correlation with eGFR but it has no significant correlation with age, BMI, SBP or DBP.

Table (17): **Stepwise linear regression analysis by using FGF 21 as dependent variables and all tested variables were entered**:

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	ANOVA	
					F	P-value
1	0.774[a]	0.598	0.594	315.3	146	**<0.001[a]**
2	0.809[b]	0.654	0.647	293.9	91.8	**<0.001[b]**
3	0.821[c]	0.674	0.664	287.1	66.1	**<0.001[c]**
4	0.832[d]	0.692	0.679	280.5	53.3	**<0.001[d]**
5	0.842[e]	0.708	0.693	274.4	45.6	**<0.001[e]**
6	0.851[f]	0.723	0.706	268.6	40.5	**<0.001[f]**
7	0.858[g]	0.736	0.716	263.8	36.65	**<0.001[g]**

a. Predictors: (Constant), Urea mg/dl.

b. Predictors: (Constant), Urea mg/dl, Cholesterol.

c. Predictors: (Constant), Urea mg/dl, Cholesterol, Creat. (mg/dl).

d. Predictors: (Constant), Urea mg/dl, Cholesterol, Creat.(mg/dl), Calcium (mg/dl).

e. Predictors: (Constant), Urea mg/dl, Cholesterol, Creatinine (mg/dl), Calcium (mg/dl), LVEDd cm.

f. Predictors: (Constant), Urea mg/dl, Cholesterol, Creatinine(mg/dl), Calcium (mg/dl), LVEDd cm, DBP.

g. Predictors: (Constant), Urea mg/dl, Cholesterol, Creatinine(mg/dl), Calcium (mg/dl), LVEDd cm, DBP, Phosphorus (mg/dl).

Dependent Variable: FGF-21 pg/ml.

This table shows stepwise linear regression analysis by using FGF 21 as dependent variable and all tested variables were entered showed 7 models of predictions. The first dependent predictor for FGF- 21 was blood urea with regression coefficient (r = 0.774) then the prediction went higher with blood urea and serum cholesterol (r = 0.809), blood urea, serum cholesterol and serum creatinine (r= 0.821),blood urea, serum cholesterol, serum creatinine and serum calcium (r = 0.832),),blood urea, serum cholesterol, serum creatinine, serum calcium and LVEDd (r = 0.842), blood urea, serum cholesterol, serum creatinine, serum calcium, LVEDd and diastolic blood pressure (r = 0.851), the highest prediction with regression coefficient (r = 0.858) associated with blood urea, serum cholesterol, serum creatinine, serum calcium, LVEDd, diastolic blood pressure and serum phosphorus.

Table (18): **Correlation between FGF-21 and LVM, LVMI and Insulin level after adjustment for age, sex, BMI ,DM and hypertension:**

Adjusted Variables		**FGF-21**	
		r	Sig.
Age, Sex, BMI, DM and Hypertension.	LVM gram	0.541	**<0.001**
	LVMI gm/m^2	0.544	**<0.001**
	Insulin iu/ml	0.191	**0.063**

This table shows that FGF-21 still has significant positive correlation with LVM and LVMI while became insignificantly correlated with insulin (sig. = 0.063) after adjustment for age, sex, BMI, DM and hypertension.

Table (19): Correlation between FGF-21 and LVM, LVMI and insulin level after adjustment for corrected calcium, phosphorus, iPTH, cholesterol, triglycerides and LDL:

Adjusted variables		r	Sig.
C.calcium, phosphorus, iPTH,cholesterol,TG and LDL.	LVM gram	-0.030	**0.774**
	LVMI gm/m^2	0.000	**0.997**
	Insulin iu/ml	-0.192	**0.064**

This table shows that FGF-21 became insignificantly correlated with LVM, LVMI and insulin (sig. = 0.774, 0.997, 0.064 respectively) after adjustment for corrected calcium, phosphorus, i PTH, cholesterol, triglycerides and LDL.

Discussion

Human FGF-21 is a polypeptide of 181 amino acids with 75% identity to mouse FGF-21. It is secreted predominantly by the liver **(Nishimura et al., 2000)**, but also by other tissues involved in glucose and lipid metabolism such as the adipose tissue, pancreas and skeletal muscle**(FonTacer et al., 2010)**.Human studies indicate that circulating levels of FGF-21 increased in obese individuals **(Zhang et al., 2008)**, subjects with metabolic syndrome, type 2 diabetes mellitus**(Mraz et al., 2009**;**Stein et al., 2010)** and coronary heart disease**(Lin et al.,2010)**. Furthermore, FGF-21 was found to be closely associated with renal dysfunction in end-stage renal disease subjects **(Han et al., 2010)**.

Although several clinical studies focusing on FGF-21 and its relevant human diseases have been reported in recent years, the definitive mechanism of FGF-21 is not fully understood **(Zhang et al., 2008)**.

The current study aims to assess FGF-21level and its relationship to cardiac dysfunction in the different stages of chronic kidney disease patients.

In the present study, mean FGF-21 level between all subjects included in the study was 437.4± 495.01and its level increased with the development of early to end-stage CKD patients (Figure 6).Our data showed that serum FGF-21 in ESRD was 12.6 fold higher compared with normal subjects. Furthermore, serum FGF-21 level in patients with CKD stage IV was 5.6 fold higher compared with normal subjects; in patients with CKD stag III, serum FGF-21 level was 3.4 fold higher compared with normal subjects and in patients with CKD stage II it was2.2 fold higher compared with normal subjects (Table 10).The present study showed that serum FGF-21 level is significantly correlated with eGFR, urea and serum creatinine in CKD subjects (Table 13). These results suggest that FGF-21 concentration is

closely related to the change of renal function in CKD patients. Similar results were reported by **Stein et al**. who found that serum FGF21 concentration was 15-fold higher in chronic hemodialysis patients (CKD end-stage) than normal subjects and was associated significantly with the loss of renal function **(Stein et al., 2009)**. Also, **Han et al**. reported that serum FGF-21 level was significantly associated with the residual of renal function and insulin resistance in ESRD patients with long term hemodialysis **(Han et al., 2010)**. **Lin et al**. found that plasma FGF-21 was 10 fold higher in ESRD patients compared with normal subjects **(Lin et al., 2011)**. In the current study, stepwise linear regression analysis by using FGF-21 as dependent variable and all tested variables were entered revealed that FGF-21 was associated significantly with blood urea, serum cholesterol, serum creatinine, serum calcium, LVEDd, diastolic blood pressure and serum phosphorus (Table: 17).

Hypertension and diabetes contribute to increased morbidity and mortality in patients with chronic kidney disease **(Whaley-Connellet al., 2009)**.In the current study, we found that hypertension and diabetes have no additional effects on serum FGF-21 level in CKD patients where there was no difference between mean FGF-21 level in diabetics, hypertensive and other patients with different comorbidities (486.4±469.1), (480.7±425.1), (556.3±569.3) respectively, (P-value 0.828) (table: 12, figure: 12). On contrary to our results, **Lin et al**. reported that plasma FGF-21 level was higher in all CKD patients with DM, HTN and coronary heart disease than those without corresponding comorbidities; also, plasma FGF-21 level was higher in CKD patients with diabetes than other CKD patients without diabetes but **Lin et al.** didn't find significant difference in plasma FGF-21 level between all CKD patients with and without hypertension **(Lin et al., 2011)**. The possible explanation of these differences may lie in that the current study excluded patients

with obesity, uncontrolled hypertension and uncontrolled diabetes; in addition, small numbers of patients were recruited.

Previous clinical studies indicated that the plasma lipid profile frequently evolves during the course of progression of CKD, and dyslipidemia is a strong predictor of myocardial infarction in subjects with CKD. Patients with mild to moderate CKD, especially those with significant proteinuria, commonly exhibit hypercholesterolemia and elevated LDL levels **(Vaziri, 2006)**. Serum triglycerides and very low-density lipoprotein (VLDL) levels are elevated, and clearance of VLDL and chylomicrons and their atherogenic remnants is impaired in patients with advanced CKD or end stage renal disease **(Vaziri, 2010)**.The present study showed that dyslipideamia progress with the deterioration of renal functions from early to end stage renal disease and serum FGF-21 level was significantly correlated with adverse lipid profiles including elevating cholesterol, LDL and triglycerides(Table: 13),suggesting that the elevation of circulating FGF-21 level may be directly or indirectly linked to hyperlipidemia of CKD. The same results were reported by **Lin et al., 2011**. Also, **Jin et al**. showed that serum FGF-21 level was independently and significantly associated with triglyceride level and systolic BP. Serum FGF-21 level was significantly higher in subjects with high triglycerides level and high systolic BP compared with those who had normal triglycerides level and normal systolic BP respectively, **Jin et al.** concluded that FGF-21 level might be a biomarker for some metabolic disorders associated with metabolic syndrome **(Jin et al., 2014)**.

Increased serum FGF-21 concentrations have been recently associated with abnormal glucose metabolism and insulin resistance in adults **(Semba et al., 2012)**. In this regard, circulating FGF-21concentrations have been found to be elevated in insulin-resistant states, such as impaired glucose tolerance and type 2 diabetes.

FGF-21 seems to be also independently associated with markers of insulin resistance and an adverse lipid profile in polycystic ovary syndrome and gestational diabetes. In the current study, serum FGF-21 level was significantly correlated with fasting serum insulin level, but it wasn't correlated with fasting blood sugar or HOMA-IR (Table: 14) and after adjustment for age, sex, BMI,DM, hypertension, calcium, phosphorus, iPTH and adverse lipid profiles FGF-21 level wasn't correlated with insulin level(Table: 18,19). The current study also showed that HOMA-IR has significant positive correlation with fasting blood sugar, fasting insulin level and adverse lipid profiles (Table: 16). These results are consistent with a previous study done by **Li et al.** who didn't find significant association between FGF-21 level and HOMA-IR**(Li et al., 2009)** also, the results of the present study are consistent with the results of **Galman et al.** who didn't find significant association between FGF-21 level and fasting plasma glucose level**(Galman et al., 2008)**. On contrary to results of the current study, **Chavez et al**. showed that elevated serum FGF-21 concentrations are significantly associated with abnormal glucose metabolism and insulin resistance in humans**(Chavez et al., 2009)**. This contrast lies in the patients selection where **Chavez** did his research on patients with glucose intolerance and type II diabetes without stress on presence of CKD or its absence while in the current study the research was more specific and take into account both diabetes and degree of CKD.

Hyperphosphatemia is a common manifestation of CKD and has been significantly associated with FGF-23**(Gutierrez et al., 2009)**. In the present study FGF-21was positively correlated with serum phosphorus($r = 0.569$, sig.< 0.001),(Table:13) and the relative difference in mean serum phosphorus concentration between normal subjects and early stage CKD patients (stage II) was significantly lower than that of serum FGF-21 level(relative difference,2.7% versus 117%)(Fig.14,15). These

results suggest that FGF-21 may be used as a better biomarker than phosphorus to reflect the progression of CKD in early stages of CKD. Similar results and conclusion were reported by **Lin et al.** who found that relative difference in phosphorus and plasma FGF-21 level between normal subjects and early CKD patients were (0.8% versus 150%) **(Lin et al., 2011)**.

Left ventricular mass index (LVMI gm/m^2) in all CKD subjects in this study ranged from 36.5 to 182 gm/$m^2$and 27.5% of patients experienced left ventricular hypertrophy. These results as regard LVMI and LVH among CKD patients were lower than that in the previous studies and that's because patients recruited for this study were apparently free of traditional cardiac diseases (uncontrolled hypertension, uncontrolled DM, ischeamic heart diseases and obesity) consistent with the study design. LVMI ranged from 49.02 to 247.9gm/m^2, LVH was found in 31.7% in **Lin et al**. study **(Lin et al., 2011)**. The current study showed that LVMI increased with the progression of renal function (Table 11, figure 7) also, there was no significant difference between mean LVMI in diabetics, hypertensive and other CKD patients with other comorbidities (P- value = 0.914, Table: 12, figure: 13). In the present study, we found that FGF-21 level was significantly correlated with LVMI(r: 0.558, P-value <0.001), (Table 15, Figure16). After adjustment for age, sex, BMI, DM and hypertension, serum FGF-21 level still significantly correlated with LVMI(Table:18) but after adjustment for calcium, phosphorus, iPTH and adverse lipid profiles, serum FGF-21 didn't correlate with LVMI(Table:19). These results show that neither hypertension nor diabetes affect FGF-21 level nor LVMI but effect of FGF-21 level on LVMI is enhanced by other cofounders as calcium, phosphorus, PTH and lipids. Similar to these results, **Lin et al**. study showed significant correlation between plasma FGF-21 level and LVMI but after adjustment for age, sex, BMI and diabetes there was no significant correlation. On

contrary to results of the current study, **Lin et al**. showed that mean LVMI in hypertensive CKD patients was more than that in patients without hypertensive although there was no difference in plasma FGF-21 level between patients with and without hypertension, **Lin et al**. concluded that hypertension affects LVMI but doesn't affect plasma FGF-21level in CKD patients**(Lin et al., 2011)**.These difference between **Lin et al**. and the current study as regard effect of hypertension on LVMI may be due to small numbers of hypertensive patients included in the current study, also uncontrolled hypertensive patients had been excluded from the current study in addition, antihypertensive given, ethnic difference and targeted blood pressure difference in both studies.

As regard left ventricular systolic function in patients recruited in this study, ejection fraction (EF) was mildly decreased with the progression of CKD (P-value =0.04), (Table:11, figure: 8). Serum FGF-21 was not significantly correlated with EF or FS (sig. =0.064, 0.095 respectively), (Table: 15).

Diastolic dysfunction is an abnormality of relaxation, filling, or distensibility of the left ventricle that is associated with augmented cardiovascular mortality. Transmitral pulsed Doppler is a non-invasive method of evaluation of diastolic dysfunction, but is influenced by factors such as loading condition of the left atria and heart rate. In contrast, Tissue Doppler Imagine (TDI) can be used to measure mechanical wall function directly by calculating the velocity of myocardial longitudinal movement and to monitor diastolic function of the myocardium more effectively. In contrast to standard mitral flow patterns, $E^{/}$ velocities tend to remain consistently reduced through all phases of diastolic dysfunction **(Gulel et al., 2008)**.

The current study showed normal diastolic functions in patients with stage II CKD as E/A ratio was 1.1,$E^{/}$ was 8.3cm/s, E/E´ ratio was 7.8,in stage III CKD patients, E/A ratio was reversed(0.8),$E^{/}$: 6.5cm/s, $E/E^{/}$:8.5 indicating that this patients group had mild impairment in left ventricular relaxation, while in stage IV CKD patients, E/A ratio was 1.3 it looks normal but with correlation with parameters of Tissue Doppler Imagine in patients of the same group $E^{/}$ and $E/E^{/}$it was appeared to be pseudonormal where $E^{/}$:6cm/s, $E/E^{/}$:10.3 indicating that this group of patients had moderate diastolic dysfunction. In patients with ESRD, E/A ratio was 2, $E^{/}$: 5.6cm/s and $E/E^{/}$:17.4 indicating that this group of patients had severe diastolic dysfunction (Table: 11 figure 9, 10, 11). These results show a progressive decline in left ventricular diastolic function with the progression from moderate to advanced CKD stages. Similar findings had been shown by **Otsuka et al**. who used conventional and Tissue Doppler Imagine (TDI) echocardiograms to detect diastolic dysfunction in CKD patients **(Otsuka et al., 2009)**, also, **Franczyk-Skora et al.** found that diastolic functions declined with the progression of CKD using conventional and Tissue Doppler Imagine (TDI) echocardiograms **(Franczyk- Skora et al. 2014)** .

The current study showed that serum FGF-21 was significantly correlated with $E^{/}$and $E/E^{/}$while it wasn't correlated with E/A (Table: 15). As these results showed that FGF-21 was correlated with LVMI, $E^{/}$ and $E/E^{/}$ so, we can conclude that serum FGF-21 is associated with increased LVMI and left ventricular diastolic dysfunction in CKD patients but it is not associated with systolic dysfunction in CKD patients. Also, the current study is consistent with the previous studies which concluded that Tissue Doppler Imagine is more reliable than transmitral pulsed Doppler in assessment of left ventricular diastolic dysfunction in CKD patients. To

our knowledge, there were no previous studies in the literature that evaluated the relationship between serum FGF-21 level and cardiac dysfunction in CKD patients.

Summary

Since the discovery of the first fibroblast growth factor (FGF) almost 40 years ago, the FGF family has expanded over the years and currently consists of 22 members with a wide range of biological functions including cell growth, angiogenesis, wound healing and metabolism. The role of the FGFs in metabolism has been increasingly recognized in recent years **(Itoh et al., 2004; Kharitonenkov, 2009)**.

Fibroblast growth factor 21 (FGF-21) was first cloned and identified from mouse embryos by homology-based PCR in 2000**(Nishimura et al., 2000).** Human FGF-21 is a polypeptide of 181 amino acids with 75% identity to mouse FGF-21. It is secreted predominantly by the liver **(Nishimura et al., 2000)**, but also by other tissues involved in glucose and lipid metabolism such as the adipose tissue, pancreas and skeletal muscle **(FonTacer et al., 2010)**. Studies in rodents have suggested FGF-21 to be a key physiological regulator of fasting response **(Inagaki et al., 2007).**

Administration of recombinant FGF-21 in animal models **(Kharitonenkov et al., 2005);** including diabetic monkeys have revealed potent *in vivo* beneficial effects of FGF-21 on glucose and lipid metabolism, insulin sensitivity and body weight. Furthermore, unlike many of the other FGFs, FGF-21 does not have effects on cell proliferation and tumourigenesis **(Kharitonenkov et al., 2007)**. Instead, over-expression of hepatic FGF-21 delays the initiation of chemically induced hepatocarcinogenesis **(Huang et al., 2006)**.The favorable effects observed in animal studies would support the potential role of FGF-21 as a therapeutic agent for control of diabetes and obesity.

Renal excretion is a major route for FGF-21 elimination. Serum FGF-21 levels have been shown to be increased in patients with impaired renal function.

Circulating FGF-21 is increased in both acute and chronic kidney diseases. **(Stein et al., 2009)**.

Serum FGF-21 concentration was associated with residual renal function and insulin resistance in end-stage CKD patients with long-term hemodialysis **(Han et al., 2010)**. These results suggested that FGF-21 may be related to renal excretion functions in humans. Plasma FGF-21 concentration has been shown to be increased with the development of early- to end-stage CKD following the loss of renal functions in CKD patients. These results suggest that circulating FGF-21 concentration is associated with the CKD progression **(Lin et al., 2011)**.

Patients with chronic kidney disease are at profoundly higher risk for cardiovascular (CV) morbidity and mortality. The nature of CV disease in patients with renal failure differs from that of the general population. Whereas the most common manifestations of CV disease in the general population include coronary atherosclerotic disease, patients with renal failure are far more likely to suffer from chronic heart failure and sudden cardiac death **(Herzog et al., 2011)**.Sudden cardiac death is the leading cause of death in ESRD patients, and accounts for approximately 25% of all deaths in this population. In addition, the clinical presentation of CV disease is also different amongst those with renal disease when compared to the general population **(Green et al., 2011)**.

The mechanisms underlying CV risk in CKD are multifactorial and begin early in the course of renal disease. Not only are traditional CV risk factors such as hypertension and diabetes highly prevalent in the CKD population, but nontraditional risk factors specific to CKD and ESRD patients are also highly prevalent and contribute to risk **(Stenvinkel et al., 2008)**. These include oxidative stress, inflammation, endothelial dysfunction, anemia, extracellular volume

overload, malnutrition, abnormal calcium and phosphorus metabolism, infection, uremic toxins, as well as sympathetic nervous system (SNS) overactivity **(Stenvinkel et al., 2008)**.

The current study aims to assess FGF-21level and its relationship to cardiac dysfunction in the different stages of chronic kidney disease patients.

This work is a cross- sectional study, conducted on 100 subjects divided into 5 groups according to eGFR, each group included 20 patients where group I included 20 apparently healthy control subjects with matched age, sex and BMI, group II included 20 patients with CKD stage II, group III included 20 patients with CKD stage III, group IV included 20 patients with CKD stage IV, group V included 20 patients with ESRD on regular hemodialysis three times per week. Inclusion criteria included: Sixty CKD patients with sustained reduction(≥3 months) in estimated glomerular filtration rate (eGFR) ≤90 ml /min/1.73 m^2 based on the simplified Modification of Diet in Renal Disease formula, in addition to twenty patients on regular hemodialysis three times per week. Exclusion criteria included: overweight and obesity, uncontrolled hypertension, uncontrolled diabetes, cardiac valve diseases, ischemic heart diseases and liver cirrhosis. CKD patients included in this study were recruited from nephrology outpatient clinic at AL-Housein University hospital while hemodialysis patients included in this study were recruited from hemodialysis unit at AL-Housein University hospital. All the patients were subjected to full history & detailed clinical examination at the start of the study. Levels of serum FGF-21 and serum fasting insulin were measured by ELISA technique while serum iPTH was measured by an immunoradiometric assay. Insulin resistance according to HOMA-IR = ([fasting insulin (iU/ml) **x** fasting glucose (gm/dl)]/405). All blood tests were done at Al-Housein University hospital main lab. Assessment of data was described as mean ± standard deviation

(SD) for parametric numerical variables. Chi-square test: comparison of distribution of qualitative variables among different group. One-way ANOVA test: comparison of quantitative variables among more than two independent groups. Pearson's correlation coefficient: testing association between different parametric variables. Linear regression analysis is used to test and estimate the dependence of a quantitative variable based on its relationship to one or more independent variables.

In the present study, serum FGF-21 level increased with the development of early to end- stage CKD patients. FGF-21 concentration is closely related to the change of renal function in CKD patients.

In the current study, stepwise linear regression analysis by using FGF-21 as dependent variable and all tested variables were entered revealed that FGF-21 was associated significantly with blood urea, serum cholesterol, serum creatinine, serum calcium, LVEDd, diastolic blood pressure and serum phosphorus. The current study showed that hypertension and diabetes have no additional effects on serum FGF-21 level in CKD patients where there was no difference between mean FGF-21 level in diabetics, hypertensive and other patients with different comorbidities(486.4±469.1), (480.7±425.1), (556.3±569.3) respectively, (P-value = 0.828).

In the present study, our data showed that elevation of serum FGF-21 level was significantly correlated with adverse lipid profiles including elevating cholesterol, LDL and triglycerides(Table: 13),suggesting that the elevation of circulating FGF-21 level may be directly or indirectly linked to the progression of pathophysiology of cardiac and CKD. In the current study, serum FGF-21 level was significantly correlated with fasting serum insulin level, but it wasn't correlated with fasting

blood sugar or HOMA-IR and after adjustment for age, sex BMI, hypertension, DM, calcium, phosphorus, iPTH and adverse lipid profiles, FGF-21 level didn't correlate with fasting insulin level. The current study also showed that HOMA-IR was correlated significantly with fasting blood sugar, fasting insulin level and adverse lipid profiles.

The current study showed that FGF-21 level was positively correlated with serum phosphorus(r:0.569, sig.<0.001),(Table:13) and the relative difference in mean serum phosphorus concentration between normal subjects and early stage CKD patients (stage II) was significantly lower than that of serum FGF-21 level(relative difference,2.7% versus 117%). These results suggest that FGF-21 may be used as a better biomarker than phosphorus to reflect the progression of CKD in early stages of CKD.

The current study showed that LVMI increased with the progression of renal function (Table 11, figure 7) also, there was no significant difference between mean LVMI in diabetics, hypertensive and other CKD patients with other comorbidities (P- value: 0.914) The present study showed that FGF-21 level was significantly correlated with LVMI(r: 0.558, P-value <0.001). After adjustment for age, sex, BMI, DM and hypertension, serum FGF-21 level still significantly correlated with LVMI (Table: 18) but after adjustment for calcium, phosphorus, iPTH and adverse lipid profiles, serum FGF-21didn't correlate with LVMI (Table: 19). These results show that neither hypertension nor DM affect FGF-21 level or LVMI and effect of FGF-21 level on LVMI is enhanced by other cofounders as calcium, phosphorus, PTH and lipids.

The current study showed that ejection fraction (EF) was mildly declined with the progression of CKD (P-value = 0.04), (Table: 11, figure: 8). Serum FGF-21 was

not significantly correlated with EF or FS (sig. = 0.064, 0.095 respectively), (Table: 15).

The present study showed that diastolic functions deteriorated with the progression of CKD (Table: 11) and serum FGF-21 level was significantly correlated with left ventricular diastolic dysfunction (Table:15).

The present study agreed with the previous studies which recommended using TDI echocardiogram as a more accurate and reliable method for assessment of diastolic functions in CKD patients.

Recommendations:

1. FGF-21 has multiple metabolic actions so; it can be used in treatment of dyslipidemia, obesity and diabetes.

2. FGF-21 can be used to monitor the deterioration of kidney functions in CKD patients.

3. Assessment of the relationship between FGF-21 and cardiac dysfunction need more studies on large number of patients.

Conclusion:

It may be concluded that serum FGF-21 level is increased from early to end stage renal disease and its increase is associated with the deterioration of renal function. FGF-21 is associated with increased left ventricular mass index and diastolic dysfunction but not associated with systolic dysfunction in CKD patients.

Relation between FGF-21 and insulin resistance needs further studies to be more elucidated.

Referrences:

An S., Lee S., Yi A., et al., 2012: Serum fibroblast growth factor 21 was elevated in subjects with type 2 diabetes mellitus and was associated with the presence of carotid artery plaques. **Diabetes Res Clin Pract. 2012. Pb. ahead of print**.

Antman E., Anbe D., Armstrong P., et al., 2004: "ACC/AHA guidelines for the management of patients with ST-elevation myocardial infarction. **Journal of the American College of Cardiology, vol. 44, no. 3, pp. 1–211**.

Appleton C., Hantle L. and Popp R., 1988: Relation of transmitral flow velocity patterns to left ventricular diastolic function. **J Am Coll Crdiol 12:426- 40**.

Ardhanari S., Martin A. and Aggarwal K. 2014: Cardiovascular Disease in Chronic Kidney Disease: Risk Factors, Pathogenesis, and Prevention. **Advances in Peritoneal Dialysis, Vol. 30**.

Armelin H., September 1973: "Pituitary extracts and steroid hormones in the control of 3T3 cell growth".**Proc. Nat. Acad. Sci. U.S.A. 70 (9): 2702–6**.

Arner P., Pettersson A., Mitchell J., et al., 2008: FGF21 attenuates lipolysis in human adipocytes - a possible link to improved insulin sensitivity. **FEBS Letters, 582, 1725–1730**.

Badman K., Pissios P., Kennedy R., et al., 2007: Hepatic fibroblast growth factor 21 is regulated by PPAR alpha and is a key mediator of hepatic lipid metabolism in ketotic states. **Cell Metabolism, 5, 426–437**.

Badman K., Koester A., Flier S., et al., 2009: Fibroblast growth factor 21-deficient mice demonstrate impaired adaptation to ketosis. **Endocrinology, 150, 4931–4940.**

Banerjee D., Ma J, Collins A., et al., 2007: Long-term survival of incident hemodialysis patients who are hospitalized for congestive heart failure, pulmonary edema, or fluid overload. **Clin J Am Soc Nephrol; 2:1186–90**.

Beenken A. and Mohammadi M., 2009: The FGF family: biology, pathophysiology and therapy. **Nat Rev Drug Discov. 8:235–253.**

Bell G., Kayano T., Buse J., et al.,1990: Molecular biology of mammalian glucose transporters". **Diabetes Care 13 (3): 198–208**.

Block A., Klassen S., Lazarus M., et al., 2004: Mineral metabolism, mortality, and morbidity in maintenance hemodialysis. **J Am Soc Nephrol;15:2208–18**.

Böttcher T. and Niehrs C., 2005:"Fibroblast growth factor signaling during early vertebrate development". **Endocr. Rev. 26 (1): 63–77**.

Brand D., Affourtit C., Esteves C., et al., 2004: Mitochondrial superoxide: production, biological effects, and activation of uncoupling proteins. Free Radic **Biol Med; 37:755-767**.

Brand R. and Kreuzer J., 2005: "Vascular NADPH oxidases: molecular mechanisms of activation,"**Cardiovascular Research, vol. 65, no. 1, pp. 16–27**.

Brandes R. and Kreuzer J., 2005: "Vascular NADPH oxidases: molecular mechanisms of activation,"**Cardiovascular Research, vol. 65, no. 1, pp. 16–27**.

Caramori M., Fioretto A., and Mauer M., 2000: The need for early predictors of diabetic nephropathy risk: is albumin excretion rate sufficient? **Diabetes, 49(9): p. 1399-408**.

Cerasola G., Nardi E., Mulè G., et al., 2010: Left ventricular mass in hypertensive patients with mild-to-moderate reduction of renal function. **Nephrology (Carlton) 15: 203–210**.

Chang S., Chen C., Chen C., et al., 2004:"Enhancement of quality of life with adjustment of dry weight by echocardiographic measurement of inferior vena cava diameter in patients undergoing chronic hemodialysis," **Nephron—Clinical Practice, vol. 97, no. 3, pp. 90–97**.

Charra B., 2007: "Fluid balance, dry weight, and blood pressure in dialysis," **Hemodialysis International, vol. 11, no. 1, pp. 21–31**.

Charytan M, Stern M, Mauri L, et al., 2012: "CKD and coronary collateral supply in individuals undergoing coronary angiography after myocardial infarction," **Clinical Journal of the American Society of Nephrology, vol. 7, no. 7, pp. 1079–1086**.

Chavez A., Molina-Carrion M ., Abdul Ghani M. , et al. 2009: Circulating fibroblast growth factor-21 is elevated in impaired glucose tolerance and type 2 diabetes and correlates with muscle and hepatic insulin resistance. ***Diabetes Care*** **32:1542–1546**.

Chen C., Cheung M., Tso W., et al. 2011: High plasma level of fibroblast growth factor 21 is an Independent predictor of type 2 diabetes: a 5_4-year population-based prospective study in Chinese subjects. **Diabetes Care, 34, 2113–2115**.

Chen S., Law C., Grigsby C., et al. 2011: Cardiomyocyte-specific deletion of the vitamin D receptor gene results in cardiac hypertrophy. ***Circulation*** **;124:1838–1847**.

Chen W., Hoo L., Konishi M., et al. 2011: Growth hormone induces hepatic production of fibroblast growth factor 21 through a mechanism dependent on lipolysis in adipocytes. **Journal of Biological Chemistry, 286, 34559–34566**.

Cheng X., Zhu B., Jiang F., et al., 2011: Serum FGF-21 levels in type 2 diabetic patients. **Endocrine Research. 36 142-148**.

Cheung A., Sarnak M., Yan G., et al., 2011: "Atherosclerotic cardiovascular disease risks in chronic hemodialysis patients," **Kidney International, vol. 58, no. 1, pp. 353–362**.

Chow W., Xu A., Woo Y., et al., 2013: Serum fibroblast growth factor-21 levels are associated with carotid atherosclerosis independent of established cardiovascular risk factors. **ArterioscIerThrombVascBiol (2013) 33:2454–9**.

Chue C., Edwards N., Moody W., et al, 2012:"Serum phosphate is associated with left ventricular mass in patients with chronic kidney disease: a cardiac magnetic resonance study," **Heart, vol. 98, no. 3, pp. 219–224**.

Corda S., Mebazaa A., Gandolfini P., et al., 1997: Trophic effect of human pericardial fluid on adult cardiac myocytes. Differential role of fibroblast growth factor-2 and factors related to ventricular hypertrophy. **Circ Res. 81:679–687.**

Coskun T., Bina H., Schneider A., et al., 2008: Fibroblast growth factor 21 corrects obesity in mice.**Endocrinology; 149: 6018–6027**.

Costa E., Lima M., Alves J., et al.,:2008.Inflammation, T-cell phenotype, and inflammatory cytokines in chronic kidney disease patients under hemodialysis and its relationship to resistance to recombinant human erythropoietin therapy.**J Clin Immunol; 28: 268–75**.

Crasto C., Semba D., Sun K., et al., 2012 : Serum fibroblast growth factor 21 is associated with renal function and chronic kidney disease in community-dwelling adults.**J Am Geriatr Soc. 2012;60:792– 793**.

Crasto C., Semba R., Strait J., et al., 2013: Elevated FGF-21is associated with hypertension in community –dwelling adults .**Journal of human hypertension; 37:397-399.**

Davis T, Young B, Eisenberg M, et al., 2008: Outcome of cardiac arrests attended by emergency medical services staff at community outpatient dialysis centers. **Kidney Int 73: 933–939**.

Devereux R., Alonso D., Lutas E., et al., 1986: Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. **Am J Cardiol 57: 450–458**.

Devereux R., Casale P., Kligfield P., et al., 1986: Performance of primary and derived M-mode echocardiographic measurements for detection of left ventricular hypertrophy in necropsied subjects and in patients with systemic hypertension, mitral regurgitation and dilated cardiomyopathy. **Am J Cardiol 57: 1388–1393**.

Dostalova I., Kavalkova P., Haluzikova D., et al. 2008: Plasma concentrations of fibroblast growth factors 19 and 21 in patients with anorexia nervosa. **Journal of Clinical Endocrinology and Metabolism, 93, 3627–3632**.

Duranton F., Cohen G, de Smet R, et al., 2012:European Uremic Toxin Work Group Normal and pathologic concentrations of uremic toxins. **J. Am. Soc. Nephrol.23:1258–1270**.

Dushay J., Chui C., Gopalakrishnan S. et al. 2010: Increased fibroblast growth factor 21 in obesity and nonalcoholic fatty liver disease. **Gastroenterology, 139, 456–463**.

Dutchak A., Katafuchi T., Bookout L., et al., 2012: Fibroblast growth factor-21 regulates PPARgamma activity and the antidiabetic actions of thiazolidinediones. **Cell, 148, 556–567**.

Echocardiography, ed. Feigenbaum, 6th Edition, Lippincotts, Williams & Wilkins.

Eriksson E., Cousens S., Weaver H., et al., 1991:"Three-dimensional structure of human basic fibroblast growth factor". **Proc. Natl. Acad. Sci. U.S.A. 88 (8): 3441–5**.

Ernesto L. and Johannes F. 2007: Cardiovascular Involvement in General Medical Conditions.Circulation.116: 85-97.

Eva B., Sara G., Cathrine O, et al., 2012: Metformine stimulates FGF-21 expression in primary hepatocytes. **Experimental Diabetes Research;vol. 2012,article ID 465282,8 pages**.

Field J, Hazinski M, Sayre M, et al., 2010: American Heart Association Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. **Circulation 122[Suppl 3]: S640–S656**.

Finklestein P. and Plomaritoglou A. 2001: "Growth factors".In Miller L.P., Hayes R.L., eds. Co-edited by Newcomb J.K. Head Trauma: Basic, Preclinical, and Clinical Directions. **New York: Wiley. pp. 165–187**.

Fisher M., Chui C., Antonellis J. et al., 2010: Obesity is a fibroblast growth factor 21 (FGF21)-resistant state. **Diabetes, 59, 2781–2789**.

Fisher M., Estall L., Adams C. et al., 2011: Integrated regulation of hepatic metabolism by fibroblast growth factor 21 (FGF21) in vivo. **Endocrinology, 152, 2996–3004**.

Fishman G, Chugh S, Dimarco P, et al., 2010: Sudden cardiac death prediction and prevention: Report from a National Heart, Lung, and Blood Institute and Heart Rhythm Society Workshop. **Circulation 122: 2335–2348**.

Fliser D, Franek E, Joest M, et al., 1997: Renal function in the elderly: impact of hypertension and cardiac function. **Kidney Int; 51(4):1196–1204**.

Foltz N., Hu S., King C., et al., 2012: FGF-21 mimetic shows therapeutic promise.**Sci. Tromsl. Volume (4):p.153**.

Fon-Tacer K., Bookout L., Ding X., et al., 2010: Research resource: comprehensive expression atlas of the fibroblast growth factor system in adult mouse. **Mol. Endocrinol. 24, 2050–2064**.

Franczyk- Skora B., Gluba A., Olsze –Wski R., et al., 2014: Heart function disturbances in chronic kidney disease echocardiogram indices. **Arch Med sci; Dec. 22; 10(6): 1109- 1116**.

Friedman E, 2006:ESRD in diabetic persons. **Kidney International, 70: p. S51-54**.

Fukumoto S., 2008:"Actions and mode of actions of FGF19 subfamily members". **Endocr. J. 55 (1): 23–31**.

Gaich G., Chien J., Fu H., et al., 2013: The effects of LY2405319, an FGF21 analog, in obese human subjects with type 2 diabetes. **Cell Metab. 3;18(3):333-40**.

Galman C., Lundasen T., Kharitonenkov A. et al., 2008: The circulating metabolic regulator FGF21 is induced by prolonged fasting and PPARalpha activation in man. **Cell Metabolism, 8, 169–174**.

Geiser, F., 1994: Hibernation and Daily Torpor in Marsupials – a Review. **Australian Journal of Zoology 42 (1): 1–16**.

Gilbert F., 2014: Developmental Biology.10th edition. Sunderland (MA): Sinauer Associates. **Early Development in Birds**.

Gorar S., Culha C., Uc Z., et al., 2010: Serum fibroblast growth factor 21 levels in polycystic ovary syndrome. **Gynecological Endocrinology: The Official Journal of the International Society of Gynecological Endocrinology. 26 819-826** .

Gospodarowicz D., 1974: "Localisation of a fibroblast growth factor and its effect alone and with hydrocortisone on 3T3 cell growth". **Nature 249 (453): 123–7**.

Green D, Roberts R, New D, et al., 2011:"Sudden cardiac death in hemodialysis patients: an in-depth review," **American Journal of Kidney Diseases, vol. 57, no. 6**.

Gulel O., Soylu K., Yuksel S., et al., 2008: Evidence of left ventricular systolic and diastolic dysfunction by colour tissue imaging despite normal ejection fraction in patients on chronic hemodialysis program. **Echocardiography, 25, pp. 569–574.**

Gutierrez O., Januzzi J., Isakova T., et al. 2009: Fibroblast growth factor 23 and left ventricular hypertrophy in chronic kidney disease. **Circulation 119: 2545–2552.**

Hajhosseiny R., Khavandi K., Goldsmith D., 2013: Cardiovascular complications in CKD. **Int J Clin Pract. 67(1):14-31**.

Hallstrom A, Ornato J, Weisfeldt M, 2004: Public Access Defibrillation Trial Investigators: Public-access defibrillation and survival after out-of-hospital cardiac arrest. **N Engl J Med 351: 637–646**.

Han H., Choi H., Cho J., et al., 2010: Serum fibroblast growth factor-21 concentration is associated with residual renal function and insulin resistance in

end-stage renal disease patients receiving long-term peritoneal dialysis. **Metabolism: Clinical and Experimental; 59 1656-1662** .

Herzog C, Asinger R, Berger A, et al., 2011: "Cardiovascular disease in chronic kidney disease. A clinical update from Kidney Disease: improving Global Outcomes (KDIGO)," **Kidney International, vol. 80, no. 6, pp. 572–586**.

Heusch P., Canton M., Aker S., et al., 2010: The contribution of reactive oxygen species and p38 mitogen-activated protein kinase to myofilament oxidation and progression of heart failure in rabbits. Br **J Pharmacol; 160:1408-1416**.

Hillege H, Fidler V, Diercks G, et al. 2002: Prevention of Renal and Vascular End Stage Disease (PREVEND) Study Group: Urinary albumin excretion predicts cardiovascular and noncardiovascular mortality in the general population. ***Circulation*** **106: 1777–1782**.

Himmelfarb J., Stenvinkel P., Ikizler T., et al., 2002:"Perspectives in renal medicine: the elephant in uremia: oxidant stress as a unifying concept of cardiovascular disease in uremia,"**Kidney International, vol. 62, no. 5, pp. 1524–1538**.

Hindricks J., Ebert T., Bachmann A., et al., 2014: Serum levels of fibroblast growth factor-21 are increased in chronic and acute renal dysfunction.**Clinical Endocrinology. 80, 918–924**.

Hotta Y., Nakamura H., Konishi M. et al., 2009: Fibroblast growth factor 21 regulates lipolysis in white adipose tissue but is not required for ketogenesis and triglyceride clearance in liver. **Endocrinology, 150, 4625–4633**.

Housch L., House E., Glascock B., et al., 2010: Fibroblast growth factor 2 mediates isoproterenol-induced cardiac hypertrophy through activation of the extracellular regulated kinase. Mol. Cell. **Pharmacol. 2, 143–154**.

Hu C., Shiizaki K., Kuro-o M., et al., 2013: Fibroblast growth factor 23 and Klotho: physiology and pathophysiology of an endocrine network of mineral metabolism. **Annu. Rev. Physiol. 75, 503–533**.

Huang X., Yu C., Jin C., et al., 2006: Forced expression of hepatocyte-specific fibroblast growth factor 21 delays initiation of chemically induced hepatocarcinogenesis. **Molecular Carcinogenesis, 45, 934–942**.

Inagaki T., Dutchak P., Zhao G., et al., 2007: Endocrine regulation of the fasting response by PPARalpha-mediated induction of fibroblast growth factor 21. **Cell Metabolism, 5, 415–425**.

Inagaki T., Lin Y., Goetz R. et al., 2008: Inhibition of growth hormone signaling by the fasting-induced hormone FGF21. **Cell Metabolism, 8, 77–83**.

Isaac R., Harold I. and Deo R., 2012: CKD and Sudden Cardiac Death: Epidemiology, Mechanisms, and Therapeutic Approaches**. J Am Soc Nephrol. (12): 1929–1939**.

Itoh N. and Ohta H., 2013: Pathophysiological roles of FGF signaling in the heart. **Front. Physiol., 06 September 2013.**

Itoh N. 2010: Hormone-like (endocrine) Fgfs: their evolutionary history and roles in development, metabolism, and disease. **Cell Tissue Res. 342, 1–11**.

Itoh N. 2014: FGF21 as a hepatokine, adipokine, and myokine in metabolism and diseases. **Endocrinol., 07 July 2014.**

Itoh N. and Ornitz M., 2008: Functional evolutionary history of the mouse Fgf gene family". **Dev. Dyn. 237 (1): 18–27**.

Itoh N., and Ornitz M. 2011: Fibroblast growth factors: from molecular evolution to roles in development, metabolism and disease. **J. Biochem. 149, 121–130**.

Jian X., Peng H., Jin J., et al., 2012: Association between serum fibroblast growth factor 21 and diabetic nephropathy. **Metabolism, 61, 853–859**.

Jin Q., Bando Y., Miyawaki K., 2014: Correlation of fibroblast growth factor 21 serum levels with metabolic parameters in Japanese subjects. J. Med. Invest. 61 : 28-34.

Jones A., 2012: Physiology of FGF15/19: **Advances in Experimental Medicine and Biology 728: 171–82**.

Kalantar-Zadeh K, Block G, Humphreys M, et al. 2003: Reverse epidemiology of cardiovascular risk factors in maintenance dialysis patients. **Kidney Int. 63:793–808**.

Kamimura M., Carrero J., Canziani M., et al., 2013: Visceral obesity assessed by computed tomography predicts cardiovascular events in chronic kidney disease patients. **Nutr Metab Cardiovasc Dis; 23:891–7**.

Kanauchi M., Akai Y., Hashimoto T., 2002: Transferrinuria in type 2 diabetic patients with early nephropathy and tubulointerstitial injury. **Eur J Intern Med.13**: **190-193**.

Kharitonenkov A., Shiyanova L., Koester A., et al., 2005: FGF-21 as a novel metabolic regulator. **Journal of Clinical Investigation, 115, 1627–1635**.

Kharitonenkov A., Wroblewski J., Koester A., et al., 2007: The metabolic state of diabetic monkeys is regulated by fibroblast growth factor-21. **Endocrinology, 148, 774–781**.

Kharitonenkov A., Dunbar D., Bina A. et al., 2008: FGF-21/FGF-21 receptor interaction and activation is determined by beta Klotho. **Journal of Cellular Physiology, 215, 1–7**.

Kharitonenkov A., 2009: FGFs and metabolism. **Current Opinion in Pharmacology, 9, 805–810**.

Kirikoshi H., Sagara N., Saitoh T., et al., 2000: Molecular cloning and characterization of human FGF-20 on chromosome 8p21.3-p22". **Biochemical and Biophysical Research Communications 274 (2): 337–43**.

Kramer H. Nguyen D, Curhan G, et al., 2003: Renal insufficiency in the absence of albuminuria and retinopathy among adults with type 2 diabetes mellitus. **Jama, 289(24): p. 3273-7**.

Lapovitz A and Pearson A, 1987: Evaluation of left ventricular diastolic function. Clinical relevance and recent Doppler echocardiographic insight. **Am Heart J 114: 836- 51**.

Le N, 2009:"Oxidized lipids and lipoproteins: indices of risk or targets for management," **Future Lipidology, vol. 4, no. 1, pp. 41–45**.

Lee C., Hui E., Woo Y., et al., 2015: Circulating fibroblast growth factor 21 levels predict progressive kidney disease in subjects with type 2 diabetes and normoalbuminuria. **J Clin Endocrinol Metab.**

Lekawanvijit S, Kompa R, Wang H, et al., 2012: Cardiorenal syndrome: The emerging role of protein-bound uremic toxins. **Circ. Res; 111:1470–1483**.

Levey A, Bosch J, Lewis J, et al., 1999: A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation.

Modification of Diet in Renal Disease Study Group. **Ann Intern Med. Mar 16;130(6):461-70**.

Levin A, 2002: Anemia and left ventricular hypertrophy in chronic kidney disease populations: A review of the current state of knowledge. ***Kidney Int Suppl* 80 : 35 –38**.

Levin A. 2003: Clinical epidemiology of cardiovascular disease in chronic kidney disease prior to dialysis. **Semin Dial; 16:101–5.**

Levin A, Thompson C, Ethier J, et al., 2003: Left ventricular mass index increase in early renal disease: Impact of decline in hemoglobin. **Am J Kidney Dis 34: 125–134**.

Li X, Yang HY, Giachelli CM, 2006: Role of the sodiumdependent phosphate cotransporter, Pit-1, in vascular smooth muscle cell calcification.**Circ Res; 98: 905–12**.

Li K., Li L., Yang M., et al., 2009: Effects of rosiglitazone on fasting plasma fibroblast growth factor-21 levels in patients with type 2 diabetes mellitus. **European Journal of Endocrinology, 161, 391–395**.

Li H., Bao Y., Xu A., Pan X., et al. 2009: Serum fibroblast growth factor 21 is associated with adverse lipid profiles and γ-glutamyl transferase but not insulin sensitivity in Chinese subjects. ***J Clin Endocrinol Metab* 94:2151–2156.**

Li H., Fang Q., Gao F., et al., 2010: Fibroblast growth factor 21 levels are increased in nonalcoholic fatty liver disease patients and are correlated with hepatic triglyceride. **Journal of Hepatology, 53, 934–940**.

Li X, Speer M, Yang H, et al. 2010: Vitamin D receptor activators induce an anticalcific paracrine program in macrophages: requirement of osteopontin. ***Arterioscler Thromb Vasc Biol*; 30:321–326**.

Li X., Huang Z., Lin M., et al., 2014: Renalase, a new secretory enzyme: Its role in hypertensive-ischemic cardiovascular diseases. **Med Sci Monit; 20: 688–692**.

Lin Z, Wu Z, Yin X, et al., 2010: Serum levels of FGF-21 are increased in coronary heart disease patients and are independently associated with adverse lipid profile. ***PLoS One* (2010) 5:e15534**.

Lin Z., Zhou Z., Liu Y., et al., 2011: Circulating FGF21 levels are progressively increased from the early to end stages of chronic kidney diseases and are associated with renal function in Chinese. **PLoS ONE, 6, e18398**.

Lin Z., Gong Q., Wu C., et al., 2012: Dynamic change of serum FGF21 levels in response to glucose **challenge in human. Journal** of Clinical Endocrinology and **Metabolism, 97, E1224–1228**.

Liu Y, Coresh J, Eustace J, et al. 2006: Association between cholesterol level and mortality in dialysis patients: role of inflammation and malnutrition. **JAMA. 291:451–9**.

London G. and. Cohn J, 2002: Prognostic application of arterial stiffness: task forces. **Am J Hypertens, 15(8): p. 754-8**.

MacIsaac R, Panagiotopoulos S, McNeil K, et al., 2006: Is nonalbuminuric renal insufficiency in type 2 diabetes related to an increase in intrarenal vascular disease? **Diabetes Care, 29(7): 1560-6**.

Mai K., Andres J., Biedasek K., et al., 2009: Free fatty acids link metabolism and regulation of the insulin-sensitizing fibroblast growth factor-21. **Diabetes, 58, 1532–1538**.

Mai K., Andres J., Biedasek K., et al., 2009: Free fatty acids link metabolism and regulation of the insulin-sensitizing fibroblast growth factor-21. **Diabetes. 58 1532- 1538**.

Mai K., Bobbert T., Groth C., et al., 2010: Physiological modulation of circulating FGF21: relevance of free fatty acids and insulin. **Am J Physiol Endocrinol Metab; 299:E126-130**.

Mai K., Schwarz F., Bobbert T., et al., 2011: Relation between fibroblast growth factor-21, adiposity, metabolism, and weight reduction. **Metabolism; 60:306-311**.

Mann F., Lonn M., Yi Q., et al., 2004: on behalf of the HOPE Investigators. Effects of vitamin E on cardiovascular outcomes in people with mild-to-moderate renal insufficiency: results of the HOPE study. **Kidney Int; 65:1375–80**.

Mashili F., Austin R., Deshmukh A., et al., 2011: Direct effects of FGF21 on glucose uptake in human skeletal muscle: Implications for type 2 diabetes and obesity. **Diabetes/metabolism Research and Reviews; 27 286-297**.

Matsumoto E., Sasaki S., Kinoshita H., et al., 2013. Angiotensin II-induced cardiac hypertrophy and fibrosis are promoted in mice lacking Fgf16. **Genes Cells 18, 544–553**.

Matsushita K., van der Velde M., Astor B., et al., 2010: "Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis," **Lancet, vol. 375, no. 9731, pp. 2073–2081**.

Matthews D., Hosker J., Rudenski A., et al., 1985: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. **Diabetologia 28(7):412—9**.

Meyer Tand Hostetter T, 2007: Uremia. **N Engl J Med 357: 1316–1325.**

Moe M and Chen X, 2008: "Mechanisms of vascular calcification in chronic kidney disease**. Journal of the American Society of Nephrology, vol. 19, no. 2, pp. 213–216**.

Moore E., Bendele A., Thompson D., et al., 2005: Fibroblast growth factor-18 stimulates chondrogenesis and cartilage repair in rat. Model of injury induced osteoarthritis. **Osteoarthritis Reaserch Society 13(70):623-631**.

Moyers S., Shiyanova L., Mehrbod F., et al., 2007: Molecular determinants of FGF-21 activity-synergy and cross-talk with PPARgamma signaling. **Journal of Cellular Physiology; 210, 1–6**.

Mraz M., Bartlova M., Lacinova Z. et al., 2009: Serum concentrations and tissue expression of a novel endocrine regulator fibroblast growth factor-21 in patients with type 2 diabetes and obesity. **Clinical endocrinology (Oxford); 71, 369–375**.

Murzin G., Lesk M. and Chothia C., 1992: Patterns of structure and sequence in the Kunitz inhibitors interleukins-1 beta and 1 alpha and fibroblast growth factors". **J. Mol. Biol. 223 (2): 531–43**.

Mutsert R., Snijder M., van der Sman F., et al., 2007: Association between body mass index and mortality is similar in the hemodialysis population and the general population at high age and equal duration of follow-up. **J Am Soc Nephrol; 18:967–74**.

Myerberg R. andCastellanos A., 1997: Cardiac arrest and sudden death. In: *Heart Disease: **A Textbook of Cardiovascular Medicine*, edited by Braunwald E, Philadelphia, Saunders, pb. 742–779**.

National Institute of Clinical Excellence Chronic Heart Failure (NICE), National Clinical Guideline for Diagnosis and Management in Primary and Secondary Care, Royal College of Physicians, London, UK, **2003**.

National Institute of Diabetes and Digestive and Kidney Diseases(NIDDKD): 2006: Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States, Cardiovascular Special Studies.

Neumann J., Ligtenberg G., Klein I., et al., 2004: Sympathetic hyperactivity in chronic kidney disease: pathogenesis, clinical relevance, and treatment. ***Kidney Int*.65: 1568–1576**.

Nishimura T., Nakatake Y., Konishi M., et al. 2000: Identification of a novel FGF, FGF-21, preferentially expressed in the liver. **Biochimica Biophysica Acta, 1492, 203–206**.

Oguzhan A., Arinc H., Abaci A., et al., 2005: Preload dependence of Doppler tissue imaging derived index of left ventricular diastolic function **Echocardiography, 22, pb. 320–325.**

Olsen K. and Garbi M., et al., 2003:Fibroblast growth factor (FGF) homologous factors share structural but not functional homology with FGFs". **J. Biol. Chem. 278(36): 34226–36**.

Ommen S. and Nishimura R., 2003: A clinical approach to the assessment of left ventricular diastolic function by the Doppler echocardiography: update 2003. **Heart 89(Suppl. 3): 18-23**.

Ornitz M. and Itoh N., 2001:Fibroblast growth factors". **Genome Biol. 2 (3): reviews3005.1–reviews3005.12**.

Otsuka T., Suzuki M., Yoshikawa H., et al., 2009: Left ventricular diastolic dysfunction in the early stage of chronic kidney disease. **Journal of Cardiology; 54, 199—204.**

Ozcan C., Palmeri M., Horvath L, et al., 2013: Role of uncoupling protein 3 in ischemia-reperfusion injury, arrhythmias, and preconditioning. **Am J Physiol Heart; 304: 1192-1200**.

Planavila A., Iglesias R., Giralt M., et al., 2011:Sirt1 acts in association with PPAR{alpha} to protect the heart from hypertrophy, metabolic dysregulation, and inflammation. **Cardiovasc Res ; 90:276-284**.

Planavila A., Iglesias R., Giralt M., et al., 2011:Sirt1 acts in association with PPAR{alpha} to protect the heart from hypertrophy, metabolic dysregulation, and inflammation. **Cardiovasc Res ; 90:276-284**.

Planavila A., Redondo I., Hondares E., et al., 2013: Fibroblast growth factor 21 protects against cardiac hypertrophy in mice. **Nat. Commun;4:2019**.

Planavila A., Redondo-Angulo I., Ribas F., et al., 2014: Fibroblast growth factor 21 protects the heart from oxidative stress. **Cardiovasc Res.106(1):19-31.**

Potthoff J., Inagaki T., Satapati S., et al., 2009: FGF21 induces PGC-1alpha and regulates carbohydrate and fatty acid metabolism during the adaptive starvation response. **The Proceedings of the National Academy of Sciences USA, 106, 10853–10858**.

Potthoff J., Kliewer A.and Mangelsdorf J, 2012: Endocrine fibroblast growth factors 15/19 and 21: from feast to famine". **Genes & Development 26 (4): 312–324**.

Reinehr T., Woelfle J., Wunschb R., et al., 2012: Fibroblast growth factor 21 (FGF-21) and its relation to obesity, metabolic syndrome, and nonalcoholic fatty liver in children: a longitudinal analysis. **Journal of Clinical Endocrinology and Metabolism, 97, 2143–50**.

Richard D., Crasto C., Strait J., et al., 2013: FGF-21 is a newcomer in field of hypertension. **J Hum Hypertens; 2013 Jun; 27(6): 397–399**.

Ritz E., 2006: Heart and kidney: fatal twins? **Am J Med, 119(5 Suppl 1): p. S31-9**.

Ritz E., 2009: "Left ventricular hypertrophy in renal disease: beyond preload and afterload," **Kidney International, vol. 75, no. 8, pp. 771–773**.

Rivas-Gotz C., Manolios M., Thohan V., et al., 2003: Impact of left ventricular ejection fraction on estimation of left ventricular filling pressures using tissue Doppler and flow propagation velocity. **Am J Cardiol; 91:780–4**.

Rucker D and Tonelli M, 2009: "Cardiovascular risk and management in chronic kidney disease," **Nature Reviews Nephrology, vol. 5, no. 5, pp. 287–296**.

Ruth E. and David E., 2014: FGF21-based pharmacotherapy – potential utility for metabolic disorders. **Trends E ndocrinology and Metabolism, volume 25; pages 303- 311**.

Samson L, Sathyanarayana P, Jogi M, et al., 2011: Exenatide decreases hepatic fibroblast growth factor 21 resistance in non-alcoholic fatty liver disease in a mouse model of obesity and in a randomised controlled trial. **Diabetologia; 54 3093-3100**.

Sarnak J., Parfrey S and Foley N., 2001: Left ventricular hypertrophy in the renal patient. **J Am Soc Nephrol. 12:1079–1084.**

Semba D., Sun K., Egan J., et al. 2012: Relationship of serum fibroblast growth factor-21 with abnormal glucose metabolism and insulin resistance: The balitmore longitudinal study of aging. **Journal of clinical endocrinology and metabolism; 97: 1375- 1382**.

Shange, Q. and Yip G., 2011: Diabetic heart disease: the story continues. **Journal of Human Hypertension, 25: p. 141-143**.

Sherif F., Christopher P., Thierry C., 2009: Recommendations for the Evaluation of Left Ventricular Diastolic Function by Echocardiography. **European Journal of Echocardiography; 10, 165–193**.

Shiizaki K., Kuro-o M., et al., 2013: Fibroblast growth factor 23 and Klotho: physiology and pathophysiology of an endocrine network of mineral metabolism. **Annu. Rev. Physiol. 75, 503–533**.

Simpson R., Hershey S. and Nibbelink K., 2007. Characterization of heart size and blood pressure in the vitamin D receptor knockout mouse. J Steroid **Biochem Mol Biol; 103: 521–4**.

Smallwood P., Munoz-Sanjuan I., Tong P., et al., 1996: Fibroblast growth factor (FGF) homologous factors: new members of the FGF family implicated in nervous system development. **Proc Natl Acad Sci U S A.93:9850–9857**.

Sosnov J., Lessard D., Goldberg R., et al., 2006: Differential symptoms of acute myocardial infarction in patients with kidney disease: a community-wide perspective. **American Journal of Kidney Diseases, vol. 47, no. 3, pp. 378–384**.

Souberbielle J, Roth H, Fouque D. 2010: Parathyroid hormone measurement in CKD. **Kidney Int ;77:93–100**.

Stehouwer C. and Smulders Y., 2006: Microalbuminuria and risk for cardiovascular disease: analysis of potential mechanisms. **J Am Soc Nephrol; 17: 2106–11.**

Steigerwalt S., Zafar A., Mesiha N., et al., 2007: "Role of aldosterone in left ventricular hypertrophy among African-American patients with end-stage renal disease on hemodialysis," **The American Journal of Nephrology, vol. 27, no. 2, pp. 159–163**.

Stein S., Bachmann A., Lossner U., et al., 2009: Serum levels of the adipokine FGF21 depend on renal function**. Diabetes Care. 32:126–128.**

Stein S., Stepan H., Kratzsch J., et al., 2010: Serum fibroblast growth factor 21 levels in gestational diabetes mellitus in relation to insulin resistance and dyslipidemia. **Metabolism: Clinical and Experimental. 2010 59 33-37**.

Stein S; Stepan H; Kratzsch J; et al., 2010: Serum fibroblast growth factor 21 levels in gestational diabetes mellitus in relation to insulin resistance and dyslipidemia. **Metabolism. 59:33–37.**

Stenvinkel P., Carrero j., Axelsson J., et al., 2008: Emerging biomarkers for evaluating cardiovascular risk in the chronic kidney disease patient: how do new pieces fit into the uremic puzzle? **Clinical Journal of the American Society of Nephrology, vol. 3, no. 2, pp. 505–521**.

Straus S., Bleumink G., Dieleman J., et al., 2004: The incidence of sudden cardiac death in the general population. **J Clin Epidemiol 57: 98–102**.

Suzuki M., Uehara Y., Motomura-Matsuzaka, K., et al., 2008: betaKlotho is required for fibroblast growth factor (FGF) 21 signaling through FGF receptor (FGFR) 1c and FGFR3c. Mol. **Endocrinol. 22, 1006–1014**.

Taal M. and Brenner M., 2006: Predicting initiation and progression of chronic kidney disease: Developing renal risk scores. **Kidney Int, 70(10): p. 1694-705**.

Tepel M., van der Giet M., Statz M., 2003: The antioxidant acetylcysteine reduces cardiovascular events in patients with end-stage renal failure: a randomized, controlled trial. **Circulation; 107:992–5.**

Tsutsui H., Kinugawa S., Matsushima S., et al., 2009: Mitochondrial oxidative stress and dysfunction in myocardial remodelling. Cardiovasc Res 2009;81:449-456.

Turkmen K., Gorgulu N., Uysal M., et al., 2011: "Fetuin-A, inflammation, and coronary artery calcification in hemodialysis patients," **Indian Journal of Nephrology, vol. 21, no. 2, pp. 90–94**.

Turner N., and Grose R., 2010: Fibroblast growth factor signaling. **Nature Reviews Cancer; 10: 116-129**.

Tyynismaa H., Raivio T., Hakkarainen A, et al., 2011: Liver fat but not other adiposity measures influence circulating FGF21 levels in healthy young adult twins. **Journal of Clinical Endocrinology and Metabolism; 96 E351-5** .

Uebanso T., Taketani Y., Yamamoto H., et al. 2011: Paradoxical regulation of human FGF21 by both fasting and feeding signals: is FGF21 a nutritional adaptation factor? **PLoS ONE, 6, e22976**.

Vanholder R., Schepers E., Pletinck A., et al., 2012: Neirynck N., Glorieux G. An update on protein-bound uremic retention solutes. **J. Ren Nutr; 22:90–94**.

Vaziri N., Ni Z., Oveisi F., et al., 2002: Enhanced nitric oxide inactivation and protein nitration by reactive oxygen species in renal insufficiency. ***Hypertension*; 39: 135–141**.

Vaziri N. 2006: Dyslipidemia of chronic renal failure: the nature, mechanisms, and potential consequences. **Am J Physiol Renal Physiol ; 290: 262–272**.

Vaziri N. 2010: Lipotoxicity and impaired high density lipoprotein mediated. **J Ren Nutr.(5 suppl):35-43**.

Verma A., Anaverkar N., Meris A., et al., 2007: The relationship between renal function and cardiac structure, function and prognosis after myocardial infarction: The VALIANT Echo Study**. J Am Coll; 50: 1238- 1245.**

Vlachopoulos C. and O'Rourke M., 2000: Genesis of the normal and abnormal arterial pulse. **Curr Probl Cardiol; 25(5): p. 303-67**.

Vlodavsky I, Korner G, Ishai-Michaeli R, et al., 1999: "Extracellular matrix-resident growth factors and enzymes: possible involvement in tumor metastasis and angiogenesis".**Cancer Metastasis Rev 9 (3): 203–26**.

Wang A., 2012: "Clinical utility of natriuretic peptides in dialysis patients," **Seminars in Dialysis, vol. 25, no. 3, pp. 326–333**.

Wang A., Wang M., Lam C., et al., 2011: Sanderson, "Heart failure in long-term peritoneal dialysis patients: a 4-year prospective analysis," **Clinical Journal of the American Society of Nephrology, vol. 6, no. 4, pp. 805–812**.

Wang B., Yang G., Yang M., et al., 2012: Mitiglinide treatment may decreases plasma fibroblast growth factor-21 levels in individuals with new-onset T2DM. **Cytokine. 2012 57 300- 303**.

Wanner C., Krane V., Marz W., et al., 2005: Atorvastatin in patients with type 2 diabetes mellitus undergoing hemodialysis. **N Engl J Med.; 353(3):238–248**.

Wei W., Dutchak A., Wang X. et al., 2012: Fibroblast growth factor 21 promotes bone loss by potentiating the effects of peroxisome proliferator-activated receptor gamma. **The Proceedings of the National Academy of Sciences USA, 109, 3143–3148**.

Wente W., Efanov M., Brenner M., et al., 2006: Fibroblast growth factor-21 improves pancreatic beta-cell function and survival by activation of extracellular signal-regulated kinase 1/2 and Akt signaling pathways.**Diabetes, 55, 2470–2478**.

Whaley-Connell A., Sowers J., McCullough P., et al., 2009: Diabetes mellitus and CKD awareness: the Kidney Early Evaluation Program (KEEP) and National Health and Nutrition Examination Survey (NHANES). **Am J Kidney Dis 53: S11–S21**.

Whaley-Connell A., Sowers J., McCullough P., et al.2009: Diabetes mellitus and CKD awareness: the Kidney Early Evaluation Program (KEEP) and National Health and Nutrition Examination Survey (NHANES). **Am J Kidney Dis 53: S11–S21.**

Woo Y, Aimin X, Wang Y, et al., 2013: Fibroblast Growth Factor 21 as an Emerging Metabolic Regulator.**ClinEndocrinol. 78(4):489-496**.

Wright j and Hutchison A., 2009: Cardiovascular disease in patients with chronic kidney disease.**Vascular health and risk management; 713-722.**

Xiao Y., Xu A., Law S, et al., 2012: Distinct changes in serum fibroblast growth factor 21 levels in different subtypes of diabetes. **Journal of Clinical Endocrinology and Metabolism, 97, E54–58**.

Xu J., Li G., Wang P., et al., 2005: Renalase in a novel soluble monoamine oxidase that regulate cardiac function and blood pressure. **J Clin Invest; 115(5): 1275-80**.

Xu J., Lloyd J., Hale C. et al., 2009: Fibroblast growth factor 21 reverses hepatic steatosis, increases energy expenditure, and improves insulin sensitivity in diet-induced obese mice. **Diabetes, 58, 250–259**.

Yan H., Xia M., Chang X, et al., 2011: Circulating fibroblast growth factor 21 levels are closely associated with hepatic fat content: A cross-sectional study. **PloS One; 6: e24895** .

Yang M., Dong J., Liu H., et al., 2011: Effects of short-term continuous subcutaneous insulin infusion on fasting plasma fibroblast growth factor-21 levels in patients with newly diagnosed type 2 diabetes mellitus. **PLoS ONE, 6, e26359**.

Yang M., Dong J., Liu H., et al., 2011: Effects of short-term continuous subcutaneous insulin infusion on fasting plasma fibroblast growth factor-21 levels in patients with newly diagnosed type 2 diabetes mellitus. **PloS One; 6:e26359**.

Yu H., Xia F., Lam S., et al., 2011: Circadian rhythm of circulating fibroblast growth factor 21 is related to diurnal changes in fatty acids in humans. **Clinical Chemistry, 57, 691–700**.

Zechel S., Werner, S., Unsicker K., et al., 2010: Expression and Functions of Fibroblast Growth Factor 2 (FGF-2) in Hippocampal Formation. **The Neuroscientist 16 (4): 357–373**.

Zhang X., Yeung C., Karpisek M., et al., 2008: Serum FGF21 levels are increased in obesity and are independently associated with the metabolic syndrome in humans. **Diabetes, 57, 1246– 1253**.

Zipes D., Camm A. and Borggrefe M., 2006: Guidelines for Management of Patients With Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death. **Circulation; 114: e385–e484**.

Zoccali C., 2002: Cardiorenal risk as a new frontier of nephrology: research needs and areas for intervention. **Nephrol Dial Transplant, 17 Suppl 11: p. 50-4**.

Zoccali C., Mallamaci F., Parlongo S., et al., 2002: "Plasma norepinephrine predicts survival and incident cardiovascular events in patients with end-stage renal disease," **Circulation, vol. 105, no. 11, pp. 1354–1359**.

Zoccali C., 2006: Traditional and emerging cardiovascular and renal risk factors: an epidemiologic perspective. **Kidney Int, 70(1): p. 26-33**.