Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 5728 - 5743
Received 25 April 2021; Accepted 08 May 2021.

Diabetic Nephropathy; Over view and use of Urinary Fetuin-A Diagnostic and Prognostic Marker

Kerollos Hakim Abdelnour Morcous[1]Hoda Saeed Abd El Rahman [2],Jehan Saeed Abdo soliman [3], Sally Mahmoud Saeed Shalaby[4]

[1] Internal Medicine resident, Kobri El-Kobba Military Hospital
[2] Professor of Internal Medicine, Zagazig University, Sharkia, Egypt.
[3] Professor of Internal Medicine, Zagazig University, Sharkia, Egypt.
[4]Professor of Medical Biochemistry, Zagazig University, Sharkia, Egypt
Corresponding author:Kerollos Hakim AbdelnourMorcous
Email:koko.vip@gmail.com

Abstract
Background: Type 2 diabetes mellitus (DM) is the most common type of diabetes, accounting for around 90% of all cases of diabetes.Diabetic nephropathy (DN) is the largest single cause of end-stage kidney disease, therefore, there is an urgent need to identify more sensitive and specific biomarkers than microalbuminuria for early detection of DN. Diabetic nephropathy is one of the most common secondary complications of diabetes mellitus and is becoming a major cause of morbidity and mortality among diabetic patients .It isa microvascular complication occurring in approximately 20-40% of patients with type 2 diabetes mellitus (T2DM), is characterized by the progressive impairment of glomerular filtration and the development of Kimmelstiel-Wilson lesions leading to end-stage renal disease (ESRD).There are many markers that may be more sensitive than urinary albumin, (the current gold Standard, in the detection of incipient nephropathy and risk Assessment of cardiovascular disease); however, the sensitivity of these markers compared with albumin requires further investigation.It is believed that elevated circulating concentrations of fetuin-A may be a risk factor for conditions such as type 2 diabetes mellitus, metabolic syndrome and non-alcoholic fatty liver disease.
Key words: Diabetes Mellitus (DM), Diabetic Nephropathy (DN), Urinary Fetuin-A.

1.Introduction:

Diabetes is a true epidemic with 415 million adults with diabetes worldwide. One third of these patients develop severe microvascular complications such as diabetic kidney disease (DKD), sight threatening diabetic retinopathy (DR), and diabetic neuropathy. Furthermore, individuals with diabetes carry an increased risk of cardiovascular disease (CVD), a risk that is particularly high in those with DKD. Consequently, those that develop end stage renal disease (ESRD) requiring dialysis or kidney transplant for survival have 18 times higher premature mortality compared with the general population **(1)**.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 5728 - 5743
Received 25 April 2021; Accepted 08 May 2021.

1.1. Macrovascular complications:

Atherosclerotic cardiovascular disease (ASCVD) defined as coronary heart disease, cerebrovascular disease, or peripheral arterial disease presumed to be of atherosclerotic origin is the leading cause of morbidity and mortality for individuals with diabetes and results in an estimated $37.3 billion cardiovascular-related spending per year associated with diabetes **(5).**

Diabetes also contributes to earlier mortality from cardiac disease. Age of disease onset is important in determining survival in type 1 diabetes: loss of life years is increased in those diagnosed under the age of 10 years compared with those diagnosed aged 26–30 years **(8).**

Common conditions coexisting with type 2 diabetes (e.g., hypertension and dyslipidaemia) are clear risk factors for ASCVD, and diabetes itself confers independent risk. Numerous studies have shown the efficacy of controlling individual cardiovascular risk factors in preventing or slowing ASCVD in people with diabetes. Furthermore, large benefits are seen when multiple cardiovascular risk factors are addressed simultaneously. Under the current paradigm of aggressive risk factor modification in patients with diabetes **(3)**.

Heart failure is another major cause of morbidity and mortality from cardiovascular disease. Recent studies have found that rates of incident heart failure hospitalization (adjusted for age and sex) were two-fold higher in patients with diabetes compared with those without **(5)**.

Diabetic ketoacidosis (DKA) is a state of metabolic decompensation in which insulin deficiency (relative or absolute) causes both hyperglycemia and excess production of ketoacids, resulting in metabolic acidosis **(6).**

In patients with diabetes hypoglycaemia is defined as all episodes of an abnormally low plasma glucose concentration that expose the patient to potential harm. No single threshold value was assigned to define hypoglycaemia since this value may differ among patients. An alert value of <70 mg/dL (<3.8 mM/L), however, was chosen to draw the attention of patients and caregivers and also for use as a cut off value in the classification of hypoglycaemia in diabetes as outlined in Table 1 **(1)**.

1.2. Microvascular complications:

Diabetic retinopathy is characterized by microaneurysms, retinal haemorrhages, cotton wool spots (from ischaemia and microinfarction), hard exudates (from protein and lipid leakage), intraretinal microvascular abnormalities and venular dilation and tortuosity **(2).**

Diabetes affects the retina, peripheral nerves and renal glomeruli leading to microvascular complicationof diabetes. The cells in these structures are unable to downregulate glucose uptake leading to an overproduction of superoxide by the mitochondrial electron transport chain and resultant oxidative stress. Microvascular complications are specific to diabetes, while macrovascular complications are not; however, people with diabetes are at a higher risk than the general population**(3).**

Diabetic neuropathies cover a range of symptoms and can be divided into diabetic peripheral neuropathies including painful neuropathy, and autonomic neuropathy **(4).**

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 5728 - 5743
Received 25 April 2021; Accepted 08 May 2021.

Diabetic neuropathy can involve the somatic and autonomic nervous systems. The most common type is diabetic sensorimotor polyneuropathy, also referred to as peripheral neuropathy. **(5).**

2.Classification of diabetic neuropathy:

Generalized symmetric polyneuropathies: acute sensory, chronic sensorimotor and autonomic.

Focal and multifocal neuropathies: Cranial, Truncal, Focal limb, Proximal motor (amyotrophy) and Coexisting chronic inflammatory disseminated poly neuropathy (CIDP) **(6).**

Acute sensory neuropathy, is rare, tends to follow periods of poor metabolic control (e.g. ketoacidosis) or sudden change in glycemic control (e.g., "insulin neuritis") and is characterized by the acute onset of severe sensory symptoms with marked nocturnal exacerbation but few neurologic signs on examination of the legs **(7).**

Chronic sensorimotor DPN. This is the most common presentation of neuropathy in diabetes, and up to 50% of patients may experience symptoms, most frequently burning pain, electrical or stabbing sensations, paresthesia, hyperesthesia, and deep aching pain. Neuropathic pain is typically worse at night, and the symptoms are most experienced in the feet and lower limbs **(7).**

The diagnosis of DPN can only be made after a careful clinical examination, and all patients with diabetes should be screened annually for DPN by examining pinprick, temperature, and vibration perception, pressure sensation at the distal halluces, and ankle reflexes **(7).**

Focal and multifocal neuropathies:

Mononeuropathies may have a sudden onset and can occur as a result of involvement of the median (5.8% of all diabetic neuropathies), ulnar (2.1%), radial (0.6%), and common peroneal nerves **(8).**

Cranial neuropathies are extremely rare (0.05%); involve primarily cranial nerves III, IV, VI, and VII and are thought to occur due to a microvascular "infarct," which, in the majority, resolves spontaneously over several months. Electrophysiological studies show a reduction in both nerve conduction and amplitude suggestive of underlying demyelination and axonal degeneration **(8).**

In contrast, up to one third of patients with diabetes have an entrapment, Common nerves involved are the ulnar, median, peroneal, and medial plantar nerves. Spinal stenosis is also common in people with diabetes and needs to be distinguished from the proximal neuropathies and amyotrophy **(8).**

Diabetic amyotrophy typically occurs in older patients with type2 diabetes, and in some cases, an immune-mediated epineurial micro-vasculitis has been demonstrated in nerve biopsies. Clinical features of amyotrophy include severe neuropathic pain and unilateral or bilateral muscle weakness and atrophy in the proximal thigh muscles **(9).**

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 5728 - 5743
Received 25 April 2021; Accepted 08 May 2021.

Diabetic autonomic neuropathy (DAN) results in significant morbidity and may lead to mortality in some patients with diabetes. Autonomic neuropathy may involve the cardiovascular, gastrointestinal, genitourinary systems and the sweat glands**(11).**
Patients with generalized autonomic neuropathies may report ataxia, gait instability, or near syncope/syncope. In addition, autonomic neuropathies have further symptoms that relate to the anatomic site of nerve damage, gastrointestinal, cardiovascular, bladder, or sudomotor **(10)**
Cardiovascular autonomic neuropathy may produce persistent sinus tachycardia (≥100 bpm), Orthostatic hypotension (fall in systolic blood pressure ≥ 20 mmHg upon standing), Sinus arrhythmia, Decreased heart variability in response to deep breathing and Near syncope upon changing positions from recumbent to standing **(11).**
Bladder neuropathy (which must be differentiated from prostate or spine disorders) may produce poor urinary stream, feeling of incomplete bladder emptying and straining to void **(11).**

Cognitive decline: Diabetic individuals are more likely to have brain atrophy than cerebrovascular lesions with patterns resembling those of preclinical Alzheimer disease **(12).**
Type2 diabetes was associated with hippocampal atrophy; temporal, frontal, and limbic gray-matter atrophy and to a lesser extent, frontal and temporal white-matter atrophy **(12).**
Patients with type 2 diabetes were more likely to have gray-matter atrophy in several bilateral regions of the cortices, especially in the left hemisphere, like the distribution of cortical atrophy described in early Alzheimer disease **(12)**
The foot should be inspected, pulses palpated and proprioceptive, vibratory and monofilament sensation should be assessed. Peripheral neuropathy may also be assessed using the self-administered questionnaire Michigan Neuropathy Screening Instrument **(5).**

Diabetic nephropathy is one of the most common secondary complications of diabetes mellitus and is becoming a major cause of morbidity and mortality among diabetic patients **(13)**.
Diabetic nephropathy can be defined by:

- increased urine albumin excretion (>300mg/day),
- decreased glomerular filtration rate (GFR),
- diabetic glomerular lesions
- and raised arterial blood pressure **(14)**.

Diabetic nephropathy has been described as a glomerular disorder with the following phases: glomerular hyperfiltration, incipient nephropathy, microalbuminuria, overt proteinuria and end-stage renal disorder **(15)**.
Chronic kidney disease (CKD) is a major public health concern worldwide, affecting 11 to 13% of global population, and it is associated with adverse clinical events such

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 5728 - 5743
Received 25 April 2021; Accepted 08 May 2021.

as cardiovascular disease (CVD) and progression to end-stage renal disease (ESRD) **(16).**

Diabetic nephropathy is the major cause of end stage renal disease. Renal involvement usually occurs more than 10 years after the onset of diabetes, but studies have shown that it starts even in the early stages of diabetes. Renal tubulo-interstitial damage has an important role in the pathogenesis of early diabetic nephropathy **(17)**.

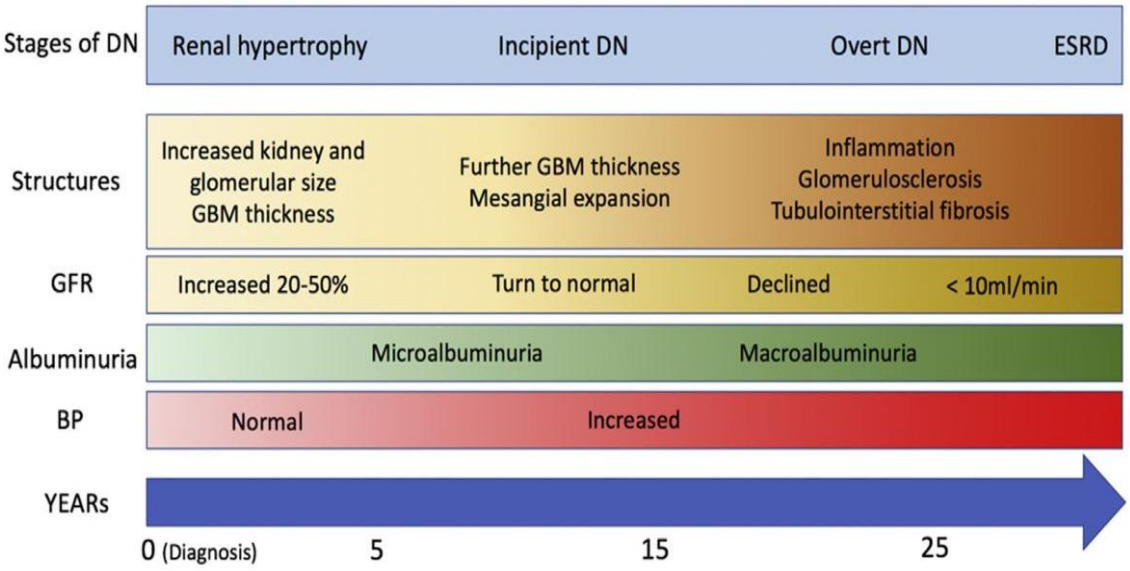
Diabetic kidney disease occurs in 20–40% of patients with diabetes **(18)**.

The first step in screening for DN is to measure albumin in an isolated urine sample. The results of albuminuria in an isolated sample can be expressed as albumin concentration (mg/l) or as albumin/creatinine ratio (mg/gm). Although albumin concentration may be influenced by urine dilution/concentration, this measure appears to be the best choice, considering its cost and accuracy. Every abnormal albuminuria test should be confirmed in two of three samples collected at a three to six-months interval, due to the daily variability of UAE **(19).**

Although the Kidney Disease Outcomes Quality Initiative (KDOQI) and Kidney Disease: Improving Global Outcomes guidelines recommend assessing micro albuminuria for DKD screening, they also underscore the insufficient sensitivity of this test. The intra individual variability in urinary albumin excretion depends on numerous factors, including coexisting diseases, exertion, body mass, and temperature, making this type of screening potentially unreliable. Although diabetic retinopathy (DR) in the presence of micro albuminuria according to the KDOQI guidelines on DKD is considered an equivalent marker of DN (overt albuminuria) **(20).**

Intensive diabetes management and early institution of anti-proteinuric therapy have been shown to slow the progression of nephropathy and improve micro and macro-vascular complications of diabetes leading to decreased morbidity and mortality **(21)**.

3.Pathophysiology of diabetic nephropathy:



Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 5728 - 5743
Received 25 April 2021; Accepted 08 May 2021.

Figure 1 | Renal changes in type 1 diabetes mellitus. Type 2 diabetes mellitus may not follow this time course. DN, diabetic nephropathy; ESRD, end-stage renal disease; GBM, glomerular basement membrane; GFR, glomerular filtration rate; BP, blood pressure.

4.Pathophysiology: The transformation in nature of the glomerular basement membrane (GBM) from its normal collagen chains, α 1(IV) and α 2(IV) to more restricted collagen chains, α 3(IV) and α 4(IV) results in the accumulation of type IV collagen in the lamina rara interna of the GBM **(22)**.

Research suggests that increased vascular resistance in renal interlobar arteries can damage glomerular and non-glomerular nephron structures and contribute to the onset and progression of non-proteinuric DN **(23).**

As the disease progresses, more layers of the GBM are filled with these extracellular matrix components, which further expand the GBM by approximately twice the normal size. This affects the compositional quality and function of the GBM leading to proteinuria or macromolecular leakiness **(24)**.

Histopathological changes: Three major histological changes occur in the glomeruli of persons with diabetic nephropathy. First, mesangial expansion is directly induced by hyperglycaemia, perhaps via increased matrix production or glycosylation of matrix proteins. Second, thickening of GBM occurs. Third, glomerular sclerosis is caused by intraglomerular hypertension (induced by renal vasodilatation or from ischemic injury induced by hyaline narrowing of the vessels supplying the glomeruli). These different histologic patterns appear to have similar prognostic significance **(25).**

Diabetic nephropathy begins with glomerular hyperperfusion and renal hyperfiltration and then progresses to microalbuminuria and a lowered glomerular filtration rate (GFR). guidelines define DN using main criteria: a decline in renal function, proteinuria, and a reduction in GFR **(26).**

Chronic kidney disease (CKD) is diagnosed by the persistent presence of elevated urinary albumin excretion (albuminuria), low estimated glomerular filtration rate(eGFR), or other manifestations of kidney damage **(27)**.

Microalbuminuria is the earliest non-invasive marker of diabetic nephropathy (DN) that occurs because of diabetes-induced glomerular damage. Role of tubulo-interstitium has also been increasingly appreciated in progression of DN **(28)**.

Persistent albuminuria in the range of 30–299 mg/24 h has been shown to be an early stage of DN in type 1 diabetes and a marker for development of DN in type 2 diabetes **(28)**.

Microalbuminuria is being used as the gold standard for the detection of early diabetic nephropathy. Albuminuria is a marker of glomerular damage. However, a Japanese study has shown that typical histopathologic changes of diabetic nephropathy may occur in patients without albuminuria. Hence, urinary albumin may not be enough to identify patients with early diabetic nephropathy. **(28)**.

With advanced cases of DN, the kidney biopsy shows mesangial hypercellularity and expansion, thickening of the basement membranes, arteriolar hyalinosis, and interstitial fibrosis. In some cases, Kimmelstiel-Wilson lesion seen in DN kidney

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 5728 - 5743
Received 25 April 2021; Accepted 08 May 2021.

biopsies correlate with an increased risk of worsening renal function and retinopathy **(28).**

Diabetic nephropathy is viewed as a spectrum of presentations with many authorities arguing for expanding the current pathological classification of DN to improve treatment strategies and outcomes (**29).**

Compared with patients with type II diabetes and DN, patients with type 1 diabetes and DN with normo-albuminuria had more of glomerular lesions, such as increased glomerular basement membrane thickness and more Kimmelstiel-Wilson nodules, and more frequent progression of DN **(21).**

DN can progress to low GFR without evidence of microalbuminuria **(21)**.

Ultrasound technology is one alternative which has provided opportunities for diagnosing and monitoring the progression of DN. Unlike renal biopsies, ultrasound represents an inexpensive and non-invasive method for examining and grading the progression of DN and other related renal pathologies, such as renal cysts or stones **(30).**

Specifically, an increase in the Renal Resistive Index (RRI), which measures renal vascular resistance, has been shown to reliably detect and monitor the progression of DN and NP-DN **(31).**

Ultrasound sonography provides an effective method to screen, identify, and monitor hemodynamic and morphologic changes in DN patients **(32).**

Biomarkers for early detection of DN:

For type 2 diabetic nephropathy, it is reported that urinary cystatin C and NAP (non-albumin protein) could reflect the progression of type 2 diabetic nephropathy **(33)**.

Urinary biomarkers like neutrophil gelatinase associated lipocalin (NGAL) and cystatin-C can help in the detection of renal tubular damage that has been found to occur in the early stages of diabetic nephropathy **(34)**.

Cystatin C, a cysteine protease inhibitor, is a low-molecular-weight protein produced by all nucleated human cells is regarded as a marker of glomerular as well as tubular damage. It is freely filtered by the glomerulus and reabsorbed primarily by proximal tubular cells. Its levels are not influenced by renal factors, inflammation, infections, liver diseases, dietary factors or constitutional factors that could influence the production rate **(35)**.

Studies have found that urine Cystatin C and NGAL levels increase with progression of DN and are positively correlated to UACR and negatively correlated to GFR **(33)**.

NGAL is a ubiquitous glycoprotein (25kd) produced by neutrophils and epithelial cells including kidney tubular cells. It is considered as a marker of renal tubular injury. Synthesis and urinary excretion of NGAL increase in many pathological conditions, such as ischemia, infections, inflammation, intoxication, cancer, cardiac surgery and renal injury **(34)**.

Urinary transferrin and retinol-binding protein (RBP) were discovered as potential biomarkers with excellent diagnostic accuracy. Transferrin is an iron-binding glycoprotein that regulates iron levels and is responsible for iron transport in serum.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 5728 - 5743
Received 25 April 2021; Accepted 08 May 2021.

Urinary transferrin increases gradually as patients progress through the stages of DN **(35**).

5.Fetuin-A

Several studies showed that hepatokines, liver-derived hormones, can regulate systemic energy metabolism and insulin sensitivity through integrated organ crosstalk **(36).**

5.1. Structure:

Fetuin-A is a glycoprotein structured molecule which weighs 60kDa and has a serum concentration ranging between 0.5 and 1.0g/L **(37)**.

Fetuin-A includes a N-terminal heavy chain of 321 amino acid residues, which is bound by disulphide bonds to the C-terminal light chain of 127 amino acids. About 20% of the circulating HFA is phosphorylated at serine-120 and serine-130 **(38)**.

Synthesis:

Among hepatokines, fetuin-A is a circulating plasma glycoprotein, which secretes mainly from liver and to lesser extent from other organs such as tongue, placenta, and adipose tissue **(39).**

Fetuin-A, also known as a 2 -Heremans–Schmid glycoprotein (AHSG), is a systemically acting inhibitor of ectopic calcification **(40)**.

It is believed that elevated circulating concentrations of fetuin-A may be a risk factor for conditions such as type 2 diabetes mellitus, metabolic syndrome and non-alcoholic fatty liver disease **(41).**

In human studies, fetuin-A is associated with various metabolic dysregulations. Serum fetuin-A concentrations correlate positively with liver fat in nondiabetic individuals, and high fetuin-A concentrations are associated with subjects with metabolic syndrome, insulin resistance, impaired glucose tolerance, and diabetes **(42).**

The dual physiological functions of fetuin-A in inhibiting vascular calcification and insulin signalling may be critically important to cardiovascular outcomes. In the general population, fetuin-A is strongly associated with increased risk of myocardial infarction and ischemic stroke independent of standard risk factors **(43)**

In addition to its effect on insulin sensitivity, fetuin-A is also an important inhibitor of ectopic calcification acting on the systemic concentration. It increases the blood solubility of calcium and phosphorus and prevents spontaneous mineral precipitation in the vasculature **(44).**

Low fetuin-A concentrations predict greater risk for CVD mortality in non-diabetic subjects, but reduced risk of CVD death in those with diabetes. Furthermore, fetuin-A concentrations are also associated with various markers of subclinical CVD. Previous studies have indicated that fetuin-A concentrations are positively associated with carotid intima-media thickness (IMT), but negatively related to coronary arterial calcification severity **(45).**

Human Fetuin-A plays an anti-inflammatory role by counteracting the production of proinflammatory cytokines. Apart from inhibition of calcification, fetuin-A can also attenuate inflammatory response and inhibit neutrophil stimulation by hydroxyapatite crystal **(46)**.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 5728 - 5743
Received 25 April 2021; Accepted 08 May 2021.

Fetuin-A and Insulin resistance:

Fetuin-A inhibits insulin receptor tyrosine kinase in the muscles and liver resulting in decreased insulin signalling and insulin resistance **(47)**.

Posted records suggest that most novel biomarkers do not enhance hazard prediction while brought to fashions based totally on traditional hazard scores. Yet, associations of novel biomarkers such as fetuin-A with metabolic markers or complications do assist to understand their position within the pathophysiology of the vascular disease **(48).**

Fetuin-A may decrease insulin sensitivity by suppressing adiponectin production in adipose tissue **(49)**.

In mediating insulin resistance, Fetuin-A inhibits the transmembrane tyrosine kinase activity of the insulin receptor. The interaction of HFA with the insulin receptor's ectodomain 95-kDa b-subunit reversibly inhibits the insulin-stimulated insulin receptor's auto phosphorylation, which plays a role in insulin resistance **(50).**

Fetuin-A is known to bind to the tandem fibronectin type 3 domains present in the extracellular portion of the transmembrane β -subunit of the insulin receptor, further away from the high-affinity pocket between the two α -subunits which comprise the binding site of insulin **(51)**

Higher fetuin-A and lower adiponectin levels may contribute the development of insulin resistance, diabetes and subsequent obesity-related CKD and diabetic nephropathy **(44)**.

Fetuin-A can be synthesized in the kidneys, choroid plexus and all major organs during fetal development as well as liver tissue **(45)**.

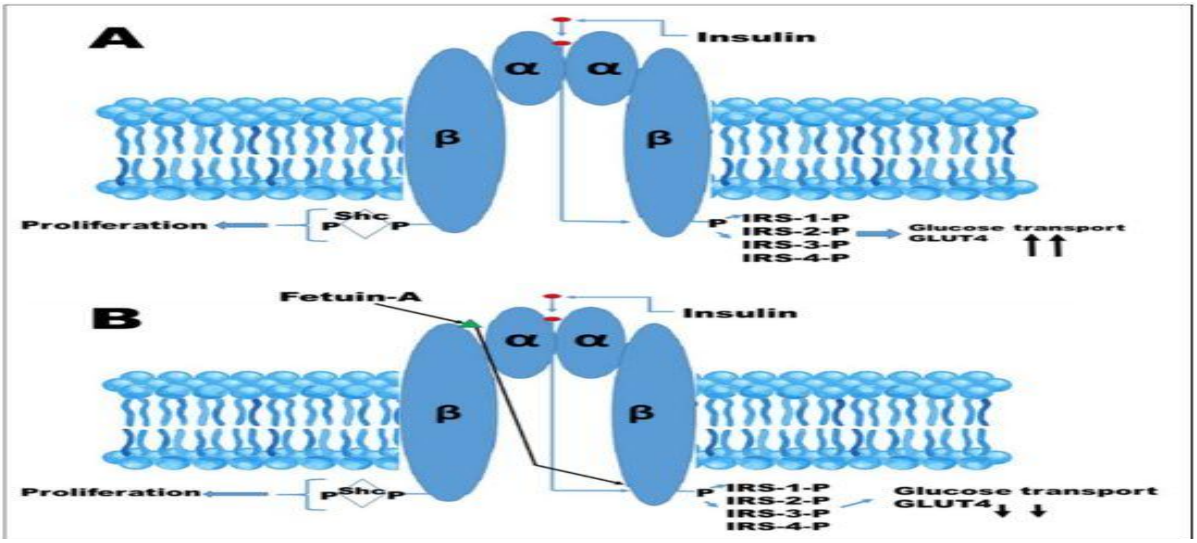


Figure 2 | Insulin signalling. (A), activation of the insulin receptor by binding of insulin to the α-subunits of its receptor, initiates autophosphorylation of the intracellular portion of the β-subunits, resulting in the recruitment and phosphorylation or receptor substrates such as IRS-1, IRS2, IRS3, and IRS4 Shc proteins. Shc activates Ras-MAP pathway, culminating in proliferation. IRS proteins activate the PI3-AKT pathway, the signal of which is transmitted to promote

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 5728 - 5743
Received 25 April 2021; Accepted 08 May 2021.

translocation of GLUT4 and glucose transport into the cell. (B), the binding of fetuin-A to the extracellular portion of beta subunit attenuates tyrosine kinase signalling, resulting in reduced glucose transport and hence a possible source for insulin resistance.

Increased secretion of fetuin-A in adipose tissue engages toll-like receptors (TLRs), contributing to pro-inflammatory state leading to insulin resistance and metabolic syndrome **(46)**.

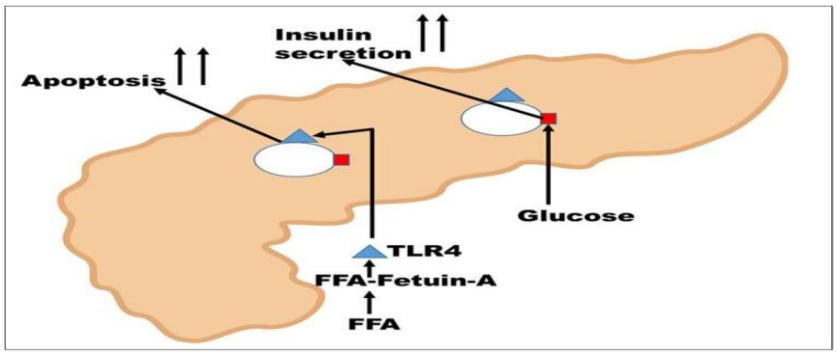


Figure 3 | Fetuin-A-mediated insulin resistance in the pancreas. High glucose initiates insulin secretion by the islet cells. Free fatty acids such as palmitate initiate their signals in the infiltrating adipocytes via TLR4, resulting in upregulation of cytokine synthesis, culminating in the apoptosis of islets cells and reduced insulin secretion.

Fetuin-A has been principally studied as an inhibitor for ectopic calcium deposition in the renal field and it is also an important promoter for insulin resistance. Fetuin-A has been shown that it acts as carrier of free fatty acids (FFAs) and they are the intrinsic ligands for Toll-like receptor 4 (TLR4), which induces adipose tissue inflammation and insulin resistance **(47)**.

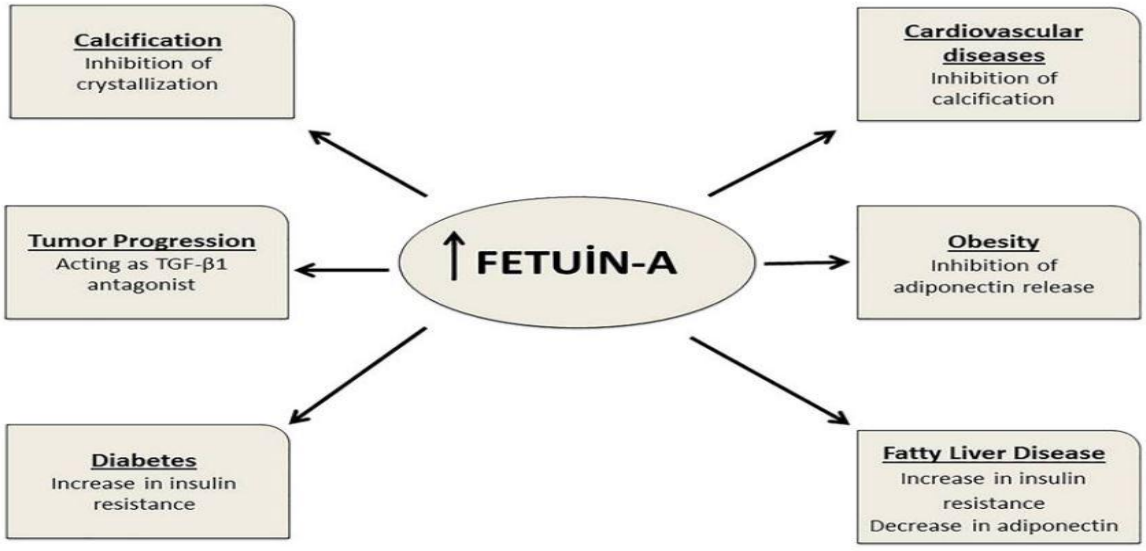


Figure 4| Biological features of Fetuin-A, Fetuin-A undertakes important biological functions in diseases such as calcification, cardiovascular diseases, tumour progression, diabetes, obesity, and fatty liver disease

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 5728 - 5743
Received 25 April 2021; Accepted 08 May 2021.

Fetuin-A and Vascular Calcification:

fetuin-A accounts for up to one-half of the in vitro capacity of the serum to prevent the precipitation of calcium and phosphorus. It is now recognized that fetuin-A can actively regulate the cell-mediated process of osteogenesis in the vessel wall. In the presence of calcium, fetuin-A binds to cell surface proteins, annexins II and VI **(48)**.

Intracellular fetuin-A inhibits apoptosis of vascular smooth muscle cells. It is also incorporated into the secreted matrix vesicles and apoptotic bodies and therein inhibits mineralization in a concentration-dependent manner. Furthermore, it enhances the phagocytosis of apoptotic bodies by viable vascular smooth muscle cells, limiting their ability to nucleate calcium phosphate **(49)**

Fetuin-A antagonizes the action of bone morphogenetic protein-2, an important osteogenic protein that stimulates the first step in VC the trans differentiation of calcifying vascular cells **(50)**.

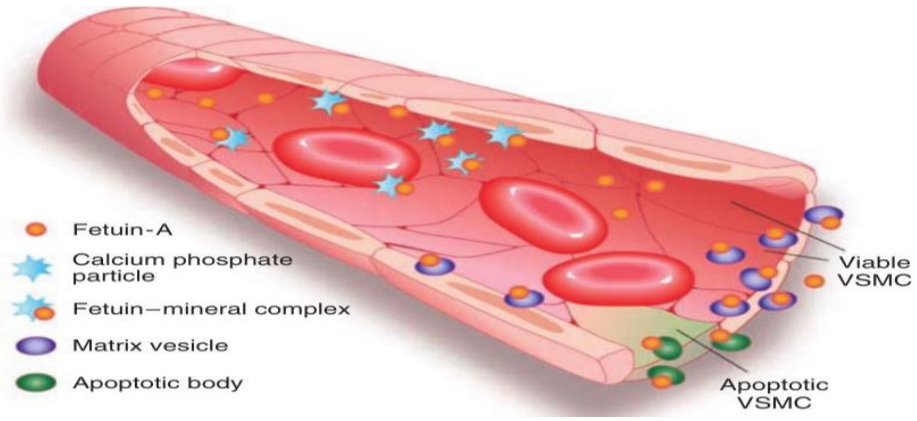


Figure 5 l Possible mechanisms for inhibition of vascular calcification by fetuin-A.
Fetuin-A can locally inhibit calcifications in the walls of the blood vessels. it is internalized by vascular smooth muscle cells (VSMCs**)** and incorporated into the matrix vesicles from apoptotic and viable VSMCs, which results in inhibition of mineral nucleation **(51).**

Fetuin-A and Diabetic nephropathy:

The association between lower fetuin-A levels and poor prognosis in CKD patients could be explained by several possible mechanisms. First, Fetuin-A can inhibit the deposition of calcium phosphate by forming soluble mineral complexes, which can be called as fetuin-mineral complex **(49).**

Low fetuin-A levels may weaken the phagocytosis of apoptotic cells and macropinocytosis by human macrophages, which led to infection or inflammation. Persistent inflammation not only itself acts as a risk factor, but also it is seen as a promoter in development of CKD **(53).**

Fetuin-A can be considered as a biomarker of nutritional status, and malnutrition is a common complication and associated with increased mortality in CKD patients **(54).**

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 5728 - 5743
Received 25 April 2021; Accepted 08 May 2021.

Fetuin-A deficiency has been directly associated with uremic vascular calcification.

Fetuin-A is a marker of the inflammatory nutritional state and acts as a protective agent because it solubilizes the calcium phosphate salt **(55)**.

In CKD patients, serum level of fetuin-A is significantly lower than in healthy control subjects **(56)**.

In a patient with chronic kidney disease (CKD) the level of fetuin-A is low, and it is associated with arterial calcification. Fetuin-A forms 'calciparticles' by binding with the calcium and phosphate **(57)**.

In the setting of chronic kidney disease (CKD), Vascular Calcification (VC) is more severe and prevalent in both the intima and the media of the blood vessels **(58)**.

Higher excretion of fetuin-A into urine has been reported to reflect the insulin resistance and inflammatory responses in obesity and type 2 diabetes and it may reflect the increase in the serum levels of fetuin-A and alterations in the changes in the permeability of glomerular capillaries **(59).**

Fetuin-A may enter proximal tubule cells by reabsorption from the tubule lumen after passing from plasma through the glomerular slit diaphragm **(59)**.

Higher urinary fetuin-A excretion demonstrated a higher risk for the development of microalbuminuria and reduction of renal function **(59).**

Urinary concentration of fetuin-A may be depending on the hepatic production, permeability variations through glomerular basement membrane by capillary damages, and changes in tubular re-absorption. Fetuin-A is reported to pass through the slit diaphragm and re-introduced to proximal tubular cells by megalin-mediated endocytosis **(59).**

Conflict of Interest: No conflict of interest.

.References

1. **Groop PH, Thomas MC, Moran JL, Wade`n J, Thorn LM, Ma¨kinen VP, Rosenga˚rd-Ba¨rlund M, Saraheimo M, Hietala K, Heikkila¨ O, Forsblom C, FinnDiane (2009).** Study Group: The presence and severity of chronic kidney disease predicts all-cause mortality in type 1 diabetes. Diabetes, 58:1651-1658.
2. **Donaghue KC, Marcovecchio ML, Wadwa RP, et al (2018).** ISPAD clinical practice consensus guidelines 2018: microvascular and macrovascular complications in children and adolescents. PediatrDiabetes; 19(Suppl. 27): 262–274.
3. **Katsarou A, Gudbjornsdottir S, Rawshani A, et al(2017).** Type 1 diabetes mellitus. Nat Rev Dis Primers; 3: 17016.
4. **Tesfaye S, Boulton AJ, Dyck PJ, Freeman R, Horowitz M, Kempler P, Lauria G, Malik RA, Spallone V, Vinik A, Bernardi L, Valensi P (2010).** Toronto Diabetic Neuropathy Expert Group: Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. Diabetes Care, 33:2285-2293.
5. **American Diabetes Association (2019).** Children and adolescents: standards of medical care in diabetes-2019. Diabetes Care; 42: S148–S64.

6. **Abbott CA, Carrington AL, Ashe H, et al (2002).** The North-West Diabetes Foot Care Study: incidence of, and risk factors for, new diabetic foot ulceration in a community- based patient cohort. Diabet Med 19:377– 384.
7. **Miguel GA, Fernández EG, Rodríguez JCR, Pablos DL, Díaz-Guerra GM, Hawkins FG(2015).** Association Between Peripheral Neuropathy and Macrovascular Disease in Diabetic Patients. Presented at Endocrine Society's 97th Annual Meeting and Expo, March 5-8; San Diego.
8. **Bansal D, Gudala K, Muthyala H, Esam HP, Nayakallu R, Bhansali A (2014).** Prevalence and risk factors of development of peripheral diabetic neuropathy in type 2 diabetes mellitus in a tertiary care setting. J Diabetes Investig.; 5:714–21.
9. **Spallone V., D. Ziegler, R. Freeman, et al(2011).** Cardiovascular autonomic neuropathy in diabetes: clinical impact, assessment, diagnosis, and management.
10. **Moran C., Thanh G. Phan, Jian Chen, Leigh Blizzard, Richard Beare, Alison Venn, Gerald Münch,Amanda G. Wood, Josephine Forbes, Timothy M. Greenaway, Susan Pearson and Velandai Srikanth (2013).** Diabetes Care Aug; DC_130143.
11. **Gheith O, Othman N, Nampoory N, Halimb M, Al-Otaibi T (2016).** Diabetic kidney disease: difference in the prevalence and risk factors worldwide. J Egypt Soc Nephrol Transplant; 16:65e72.
12. **Motawi, T. K., Shehata, N. I., ElNokeety, M. M., & El-Emady, Y. F(2018).** Potential serum biomarkers for early detection of diabetic nephropathy. Diabetes Research and Clinical Practice, 136, 150–158.
13. **Hill, N. R., Fatoba, S. T., Oke, J. L., Hirst, J. A., O'Callaghan, C. A., Lasserson, D. S., et al(2016).** Global prevalence of chronic kidney disease - a systematic review and meta-analysis. PLoS ONE 11: e0158765.
14. **Garg V, Kumar M, Mahapatra HS, Chitkara A, Gadpayle AK, Sekhar V (2015).** Novel urinary biomarkers in pre-diabetic nephropathy. Clin Exp Nephrol;19:895–900.
15. **Afkarian M, Zelnick LR, Hall YN, et al (2016).** Clinical manifestations of kidney disease among US adults with diabetes, 1988-2014. JAMA; 316:602–610.
16. **National Kidney Foundation KDOQI (2012).** clinical practice guideline for diabetes and CKD: 2012 Update Am J Kidney Dis, 60, pp. 850-886.
17. **Malinowska A, Kistowski M, Bakun M, et al(2012).** Diffprot – software for non-parametric statistical analysis of differential proteomics data. J Proteomics; 75: 4062-4073.
18. **Holman RR, Paul SK, Bethel MA, Matthews DR, et al (2008).** 10-year follow-up of intensive glucose control in type 2 diabetes. N Engl J Med.; 359:1577–89.
19. **Chen YM, Miner JH (2012).** Glomerular basement membrane and related glomerulardisease. Transl Res; 160:291e7.
20. **Chawla V, Roshan B (2014).** Non-proteinuric diabetic nephropathy. Curr Diab Rep; 14: 529.
21. **Caramori ML, Kim Y, Huang C, Fish AJ, Rich SS, Miller ME, et al (2002).** Cellular basis of diabetic nephropathy: 1. study design and renal structural-functional relationships in patients with long-standing type 1 diabetes. Diabetes; 51:506e13.

22. **Hall JE, Henegar JR, Dwyer TM, Liu J, Da Silva AA, KuoJJ(2004).** Is obesity a major cause of chronic kidney disease?. Adv Ren Replace Ther. 2004 Jan. 11(1):41-54.
23. **Bermejo S, Pascual J, Soler MJ (2017).** The large spectrum of renal disease in diabetic patients. Clin Kidney J; 10: 255-256.
24. **Tuttle KR, Bakris GL, Bilous RW, et al (2014).** Diabetic kidney disease: a report from an ADA Consensus Conference. Diabetes Care ;37: 2864–2883.
25. **Comper WD, Hilliard LM, Nikolic-Paterson DJ, Russo LM (2008).** Disease dependent mechanism of albuminuria. Am J Physiol Renal Physiol. ;295: F1589–600.
26. **Krolewski AS, Niewczas MA, Skupien J, et al(2014).** Early progressive renal decline precedes the onset of microalbuminuria and its progression to macroalbuminuria. Diabetes Care. ; 37:226–34.
27. **Kamijo-lkemori A, Sugaya T, Kimura K(2014).** Novel urinary biomarkers in early diabetic kidney disease. Curr Diab Rep; 14:513.
28. **Schwartz MM, Lewis EJ, Leonard-Martin T, Lewis JB, Batlle D(1998).** Renal pathology patterns in type II diabetes mellitus: relationship with retinopathy. The Collaborative Study Group. Nephrol Dial Transplant; 13: 2547-2552.
29. **Piccoli GB, Grassi G, Cabiddu G, Nazha M, Roggero S, Capizzi I, De Pascale A, Priola AM, Di Vico C, Maxia S, Loi V, Asunis AM, Pani A, Veltri A(2015).** Diabetic Kidney Disease: A Syndrome Rather Than a Single Disease. Rev Diabet Stud; 12: 87-109.
30. **Jastaniah SM, Alsayed NA, Awad IR, Fida HH, Elniel H(2013).** Evaluation of renal disorders in type 2 diabetic patients using ultrsonography. Open J of Med Imaging; 03: 165-170.
31. **Spatola L, Andrulli S(2016).** Doppler ultrasound in kidney diseases: a key parameter in clinical long-term follow-up. J Ultrasound; 19: 243-250.
32. **Mancini M, Masulli M, Liuzzi R, Mainenti PP, Ragucci M, Maurea S, Riccardi G, Vaccaro O(2013).** Renal duplex sonographic evaluation of type 2 diabetic patients. J Ultrasound Med; 32: 1033-1040.
33. **Kim SS, Song SH, Kim IJ, Jeon YK, et al(2013).** Urinary Cystatin C and Tubular proteinuria predict progression of diabetic nephropathy. Diabetes Care. 2013; 36:656–61.
34. **Assal HS, Tawfeek S, Rasheed EA, El-Lebedy D, Thabet EH (2013).** Serum cystatin C and tubular urinary enzymes as biomarkers of renal dysfunction in type 2 diabetes mellitus. Clin Med Insights Endocrinol Diabetes; 6:7-13.
35. **Filler G, Bokenkamp A, Hofmann W, Le Bricon T, et al (2005).** Cystatin C as a marker of GFR -history, indications, and future research. Clin Biochem.; 38:1–8.
36. **Mishra J, Dent C, Tarabishi R, Mitsnefes MM, et al(2005).** Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. Lancet. ;365:1231–8.
37. **Al-Rubeaan K, Siddiqui K, Al-Ghonaim MA, Youssef AM, Al-Sharqawi AH, Alnaqeb D (2017).** Assessment of the diagnostic value of different biomarkers in relation to various stages of diabetic nephropathy in type 2 diabetic patients. Sci Rep; 7:1e9.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 5728 - 5743
Received 25 April 2021; Accepted 08 May 2021.

38. **Iroz A, Couty J-P, Postic C (2015).**Hepatokines: unlocking the multi-organ network in metabolic diseases. Diabetologia 58(8):1699–1703.
39. **Chatterjee P, Seal S, Mukherjee S, Kundu R, Mukherjee S, Ray S, Mukhopadhyay S, Majumdar SS, Bhattacharya S (2013).** Adipocyte fetuin-A contributes to macrophage migration into adipose tissue and polarization of macrophages. J Biol Chem 288(39):28324–28330
40. **Schafer C, Heiss A, Schwarz A, Westenfeld R, Ketteler M, Floege J, Muller-Esterl W, Schinke T, Jahnen-Dechent W(2003).** The serum protein alpha 2-Heremans–Schmid glycoprotein/fetuin-a is a systemically acting inhibitor of ectopic calcification. J Clin Invest 112:357–366
41. **Haukeland JW, Dahl TB, Yndestad A, et al (2012).** Fetuin A in nonalcoholic fatty liver disease: in vivo and in vitro studies. Eur J Endocrinol; 166: 503–510
42. **Weikert C, Stefan N, Schulze MB, Pischon T, Berger K, Joost HG, et al(2008).** Plasma fetuin-a levels and the risk of myocardial infarction and ischemic stroke. Circulation; 118:2555–62.
43. **Jahnen-Dechent W, Heiss A, Schäfer C, Ketteler M** (**2011).** Fetuin-A regulation of calcified matrix metabolism. Circ Res; 108:1494–509.
44. **Ix J, Barrett-Connor E, Wassel C, Cummins K, Bergstrom J, Daniels L, et al(2011).** The associations of fetuin-A with subclinical cardiovascular disease in community-dwelling persons: the Rancho Bernardo Study. J Am Coll Cardiol ;58:2372–9.
45. **Maruo S, Mori K, Motoyama K, Nakamura M, Kawarabayashi R, Kakutani Y, et al(2018).** Correlation analysis of monocyte subsets and insulin resistance considering fetuin-A involvement in patients with type 2 diabetes. ClinTranslMed; 7:9.
46. **Hennige, A. M., H. Staiger, C. Wicke, F. Machicao, A. Fritsche, H.-U. H€ aring, and N. Stefan (2008).** Fetuin-A induces cytokine expression and suppresses adiponectin production. PLoS One 3 (3):765.
47. **Mathews, S.T. et al(2000).** a 2-HSG, a specific inhibitor of insulin receptor autophosphorylation, interacts with the insulin receptor. Mol. Cell. Endocrinol. 164, 87–98.
48. **Westenfeld, R., W. Jahnen-Dechent, and M. Ketteler (2007).** Vascular calcification and fetuin-A deficiency in chronic kidney disease. Trends in Cardiovascular Medicine 17 (4):124–128.
49. **Pal D, Dasgupta S, Kundu R, Maitra S, Das G, et al(2012).** Fetuin-A acts as an endogenous ligand of TLR4 to promote lipid-induced insulin resistance. Nat Med.
50. **Kundranda MN, Ray S, Saria M et al(2004).** Annexins expressed on the cell surface serve as receptors for adhesion to immobilized fetuin-A. BiochimBiophys Acta; 1693: 111–123.
51. **Reynolds JL ,Skepper JN , McNair R et al(2005).** Multifunctional roles for serum protein fetuin-A in inhibition of human vascular smooth muscle cell calcification. J Am Soc Nephrol; 16 :2920 – 2930.
52. **Herrmann, M., Kinkeldey, A., and Jahnen-Dechent, W(2012).** Fetuin-A function in systemic mineral metabolism. Trends Cardiovasc. Med. 22, 197–201.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 5728 - 5743
Received 25 April 2021; Accepted 08 May 2021.

53. **Miyamoto, T., Carrero, J. J., and Stenvinkel, P(2011).** Inflammation as a risk factor and target for therapy in chronic kidney disease. Curr. Opin. Nephrol. Hypertens. 20, 662–668.
54. **Perez-Sotelo, D., Roca-Rivada, A., Larrosa-Garcia, M., Castelao, C., Baamonde, I., Baltar, J., et al(2017).** Visceral and subcutaneous adipose tissue express and secrete functional alpha2hsglycoprotein (fetuin a) especially in obesity. Endocrine 55, 435–446.
55. **Coen G et al (2006).** Cardiac calcifications: fetuin-A and other risk factors in hemodialysis patients. ASAIO J; 52:150–156.
56. **Sharon MM(2009).** Vascular calcification: the three-hit model. J am Soc Nephrol ; 20:1162–1164.
57. **Kalpakian MA, Mehrotra R(2007).** Vascular calcification and disordered mineral metabolism in dialysis patients. Semin Dial; 20:139–143.
58. **Jung CH, Kim BY, Kim CH, Kang SK, Jung SH, et al (2013).** Associations of serum fetuin-A levels with insulin resistance and vascular complications in patients with type 2 diabetes. Diab Vasc Dis Res.
59. **Matsui I, Hamano T, Mikami S, Inoue K, Shimomura A, Nagasawa Y, et al (2013).** Retention of fetuin-A in renal tubular lumen protects the kidney from nephrocalcinosis in rats. Am J Physiol Renal Physiol. ;304: F751–60.