International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 14, Issue 1, 2022

Review Article

A REVIEW ON ROLE OF MARKERS IN DIABETES MELLITUS AND ASSOCIATED MICRO AND MACROVASCULAR COMPLICATIONS

KAMALESH D. R.[1,2], GEETHA K. M.[2]

[1]Drug Testing Laboratory, Bangalore 560001, Karnataka, India, [2]College of Pharmaceutical, Sciences, Dayanand Sagar University, Shavige Malleshwara Hills, 1st Stage, Kumaraswamy Layout, Bengaluru, Karnataka 560078
[*]Email: kamaleshdr.res-shs-pharmacy@dsu.edu.in

Received: 14 Sep 2021, Revised and Accepted: 17 Nov 2021

ABSTRACT

Diabetes mellitus is one of the leading metabolic disorders in the last few decades, affecting the larger population of the world in both developed and developing countries.

In diabetes mellitus there is reduced secretion and/or action leading to disturbance in the metabolism of glucose. The prolonged hyperglycemia causes several microvascular and macrovascular complications, which are the leading cause of death. Although the prevalence of diabetes is high, the majority of the people remain undiagnosed, which leads to complications. The diagnosis of diabetes involves the measurement of blood glucose levels. Several biochemical and body components regulate the secretion and action of insulin. Therefore, they serve as biomarkers for the diagnosis of diabetes mellitus. The biomarkers like HbA1C, glycated albumin, fructosamine, ferritin, fetuin-A, ceramides, HDL cholesterol, calprotectin, Acylcarnitine and micro RNA are some of the important biomarkers for diabetes mellitus. Through this review, we have made an attempt to describe the role and significance of biomarkers for diabetes mellitus.

Keywords: Insulin, Diabetes mellitus, Biomarker, Glycated haemoglobin, Fructosamine, Ferritin

DOI: https://dx.doi.org/10.22159/ijcpr.2022v14i1.44108 Journal homepage: https://innovareacademics.in/journals/index.php/ijcpr

INTRODUCTION

Diabetes mellitus (DM) is one of the largest global health emergencies of 21st century and it is a major risk factor for cardiovascular diseases. Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, hyperlipemia, negative nitrogen balance and ketonemia resulting from irregularity in insulin secretion, insulin action or both. It is the oldest disease known to man. It is also referred as Black Death since 14th century [1].

The terms "Diabetes" and “Mellitus” are derived from Greek. “Diabetes” denotes "a passer through; a siphon" whereas "Mellitus" denotes "sweet". It is thought that the Greeks named it so due to the excessive amounts of urine produced by diabetics attracted flies and bees. The traditional way of diagnosing diabetes mellitus in ancient Chinese was by observing whether ants are attracted to a person's urine or not.

Diabetes Mellitus is a global public burden; the prevalence has been increased worldwide. The global prevalence of diabetes in 2017 was 425 million and expected to rise to 7.7% by 2030 and the number of diabetes patients is likely to be raised to another 200 million by 2040. The chronic hyperglycemia can damage various organ systems, leading to impairment of normal physiological process and life-threatening micro and macrovascular complications.

Despite the high prevalence of diagnosed diabetes mellitus, as many as half of the people diagnosed with diabetes mellitus is unaware of their disease. China, India and USA are the top three countries with largest number of people diagnosed with diabetes. The WHO has predicted that with the aged people, children and adolescents in both the developed and developing countries affected with this disease. The incidence of type 2 diabetes is higher in males compared to females, which may be due to sex-related differences in sensitivity, obesity and excess accumulation of fat and other causative factors like raised blood pressure or habits like smoking and alcohol consumption. The WHO assumes that diabetes will be 7th leading cause of fatality in 2030 [2-7].

Based on the etiology and clinical presentations, DM is classified into 3 types, namely Type I, Type II and Gestational Diabetes.

Type I diabetes (IDDM-Insulin Dependent DM, Juvenile onset DM) is most common in children, accounts 5-10% of the cases, it is an autoimmune disorder characterized by auto-destruction of beta cells of islets of pancreas, leading to less/no insulin production and secretion of insulin from pancreas. It arises due to genetic and environmental causes such as viral infection, toxins and dietary factors.

Type II Diabetes (NIDDM-Non Insulin Dependent DM, Maturity onset DM)) is the most effective type with a higher degree of predisposition than any other type of diabetes, mainly occurs in adults and constitutes about 90% of all cases of diabetes, in this type, there is there is diminished response to the insulin by the cells which is called insulin resistance. Due to insulin resistance, the insulin production in the beta cells increases initially to balance the glucose level, but over the period, the insulin production decreases leading to insulin deficiency. The over secretion of hyperglycemic hormones (Ex: glucagon), obesity and hyperlipidemia are the main contributors for insulin resistance.

Gestational diabetes occurs in pregnancy, also known as hyperglycemia in pregnancy. Usually, it affects in the second and third trimesters of pregnancy. It complicates about 7% of all pregnancies, which accounts for about 2,00,000 cases per annum and it is estimated that about 18.9% of the cases are from India. There will be an increased risk of developing type 2 diabetes in both mother and child. The fetus may have increased weight and congenital abnormalities. The complications to the child after birth include respiratory syndrome and obesity. The main contributors are placental hormones, particularly human placental lactogen, progesterone cortisol, growth hormone and prolactin. The risk factors of gestational diabetes include obesity, gestational weight gain, old age, genetic factors, history of previous children with congenital abnormalities and family history of diabetes.

Double diabetes (DD)

It is a complex phenomenon exhibiting characteristics of both type 1 and type 2 diabetes mellitus. The epidemiological studies revealed that about 25.5% of the type 1 diabetes patients additionally presented metabolic syndrome. The common symptoms of DD

includes obesity, insulin resistance, type of latent autoimmune disease in youth (LADY) and autoantibodies (GAD56, IA2 an insulin autoantibodies). DD is an event in young-onset of diabetes patients (usually 11-19 y old) due to weight gain and insulin resistance which is caused by the side effect of insulin treatment. DD potentiates the risk of micro and macrovascular complications of diabetes. As the insulin resistance and weight gain are the main clinical features of DD, the treatment regimen would include the weight reduction and insulin titration approach to use a proper dose of insulin [8-11].

Complications of diabetes mellitus

Most of the complications of Diabetes mellitus are similar regardless of the type of diabetes. These complications are responsible for the morbidity and mortality associated with both type I and type II Diabetes Mellitus. The complications of Diabetes increase with the severity of the disease. The diabetic complications are categorized broadly into microvascular and macrovascular complications.

Macrovascular complications of diabetes mellitus

The atherosclerotic changes in the larger blood vessel lead to macrovascular complications. The identified macrovascular complications associated with are coronary artery disease, cerebral and peripheral vascular disease. The mechanism of atherosclerotic changes involves chronic inflammation and injury to the arterial wall in the peripheral and coronary vascular system resulting in accumulation and rupture of oxidized LDL particles in the endothelial wall of the arteries.

Coronary Artery Disease (CAD) is a leading cause of death in individuals with type 2 diabetes. It is asymptomatic, usually leads to sudden death of the patient. The myocardial infarction is the main CAD and it accounts for about 60% of all diabetes-associated mortality. Cerebral Vascular Disease arises due to atherosclerotic changes in cerebral blood vessels. It involves the formation of embolus in vascular system, which blocks the blood flow to the cerebral region, which causes transient ischemic attack and stroke. Recovery from stroke is difficult in diabetic patients because of high blood glucose level.

Peripheral Vascular Disease is referred as Lower Extremity Arterial Disease (LEAD), occurs due to atherosclerosis in larger blood vessels of lower extremities of the body such as legs and feet. It is clinically identified by the absence of a peripheral pulse in the lower extremities. It is responsible for gangrene in diabetic patients.

Microvascular complications

These complications arise due to the thickening of the basement membrane in the capillaries and arterioles of blood vessels. The retinopathy, nephropathy and neuropathy are the main complications.

In Diabetic retinopathy, friable and poor quality blood capillaries developed in the retina as well as macular edema and grown with the progression of the disease, which leads to loss of vision or blindness. Retinopathy may start to develop as early as 7 y before the diagnosis in patients with type 2 diabetes mellitus. It is a leading cause of blindness in USA. Diabetic Nephropathy is a leading cause of renal failure in diabetes. It is the major cause for kidney failure worldwide. The structural abnormalities of nephropathy are, hypertrophy of the kidney, increased glomerular basement thickness, nodular and diffuse glomerulosclerosis, tubular atrophy and intestitial fibrosis that cause increased glomerular filtration rate with intraglomerular hypertension, proteinurea and loss of renal function.

Diabetic Neuropathy is a life-threatening complication involves both peripheral and autonomic nervous systems, affecting nearly half of diabetic patients. Chronic hyperglycemia is the primary cause for neuropathy in diabetes, as it causes the accumulation of polyols in nerves. In hyperglycemic neurons the sensory neuron mitochondria are the source of production of reactive oxygen species, which can damage their DNA and membranes; impair cell function leading to nerve degeneration. In this, the patients complain burning, irritating and stimulatory pain [12].

Biomarker

A biomarker has been defined as 'A biological molecules found in blood or other body fluids or other body fluids or tissue which represents a sign of the normal or abnormal process of a condition or disease. Therefore a biomarker may be used to see how well the body responds to a treatment for a disease or condition [13]. The biomarkers help to identify the disease at the subclinical stage and enable to apply the preventive measures at the subclinical stage and to monitor the responses of preventive measures and to decide the therapeutic strategy for the disease. The biomarkers also help to study the mechanism or pathogenesis of disease and assessment of new preventive and therapeutic measures [14].

The biomarkers may be direct or indirect markers depending on the extent of the disease, but usually, they lie outside the casual pathway. They ate used to monitor or control the burden of clinical or subclinical disease. For example, elevated thyroglobulin levels in thyroid cancer cells provide the direct measure of cancer burden [15].

In clinics, an ideal biomarker should be easily accessible by using a minimally invasive sampling procedure, making routine blood, urine, or saliva examination an excellent source of choice [16].

Some of the biological agents/biochemical or components of the cell because of their role in the maintenance of blood glucose level serve as biomarkers for diabetes mellitus and assessment of these biomarkers helps to identify, manage and to choose an appropriate therapeutic strategy for diabetes mellitus. These biomarkers also help to develop new effective and potent anti-diabetic drugs. In this paper we have made an attempt to explain some important biomarkers used in the management of diabetes and its associated complications.

Glycated haemoglobin (HbA_{1c})

It is a form of Haemoglobin that is covalently bound to glucose. When Haemoglobin is exposed to glucose in the blood, they are bound together through the glycation process. HbA_{1c} is a measure of the beta-N-1-deoxy fructosyl component of Haemoglobin [17].

It is measured primarily to determine the three-month average blood sugar level and can be used as a diagnostic test for diabetes mellitus and as an assessment test for glycemic control in people with diabetes. The test is limited to a three-month average because the average lifespan of a red blood cell is four months. Normal levels of glucose produce a normal amount of glycated haemoglobin. As the average amount of plasma glucose increases, the fraction of glycated haemoglobin increases in a predictable way. In diabetes, higher amounts of glycated haemoglobin, indicating poorer control of blood glucose levels, have been associated with cardiovascular disease, nephropathy, neuropathy, and retinopathy [18, 19].

The mechanism involves that the glycated haemoglobin causes an increase in the levels of highly reactive free radicals in the blood cells which in turn alter the blood cell membrane properties. This causes the aggregation of blood cells and increases the viscosity of blood; as a result the blood flow will be impaired. In another way the glycated haemoglobin causes inflammation in blood vessels which results in the formation of atherosclerotic plaque (atheroma). The formation of atheroma is because of free radical formation, which promotes the excitation of Fe^{2+}-Hb through Fe^{3+}-Hb into abnormal ferryl Hb (Fe^{4+}-Hb). Since, $Fe^{4+}$is unstable it tries to regain its oxidation state, in doing so, it reacts with specific amino acids present in haemoglobin. This would result in the cross-linking reaction between the haemoglobin molecules to form Hb clumps. The Hb clumps promote cell damage and the release of Fe^{4+}-Hb into the matrix of innermost layers (subendothelium) of arteries and veins. These series of reactions results in increased permeability of endothelium and production of monocyte adhesion proteins, which promote macrophage accumulation in blood vessels leading to the formation of plaques in blood vessels. The glycated Hb also inhibit the vasodilator action of Nitric oxide in the endothelium by the formation of the complex with it. Nitric oxide is a potent vasodilator which prevent the formation of plaque LDLs (i.e. "bad cholesterol") oxidized forms [20, 21].

Principle of diagnosis: The principle is based on the fact that the glucose molecule present in the blood attaches to the haemoglobin present in the red blood cell. The higher amount of glycated haemoglobin reflects that more amount of glucose has been bound o the haemoglobin, which indicates the condition of prolonged hyperglycemia. Once, the Hb is glycated, build of glycated haemoglobin occurs in red cells. Therefore, it reflects the average level of glucose to which the cell has been exposed during the life span. Measuring glycated Haemoglobin assesses the effectiveness of therapy by monitoring long-term serum glucose regulation.

A1c is a weighted average of blood glucose levels during the life of the red blood cells (117 d for men and 106 d in women). Therefore, glucose levels on days nearer to the test contribute substantially more to the level of A1c than the levels in days further from the test.

This is also supported by data from clinical practice showing that HbA1c levels improved significantly after 20 d from the start or intensification of glucose-lowering treatment.

Measurement: several techniques have been used for the measurement of HbA1c, which includes HPLC, immunoassay, enzymatic assay, capillary electrophoresis and several affinity chromatographic techniques [22-24].

The American Diabetes Association, European Association for the Study of Diabetes, and International Diabetes Federation have agreed that, in the future, HbA_{1c} is to be reported in the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) units.

Interpretation of Results: Laboratory results may differ depending on the analytical technique, the age of the subject, and biological variation among individuals.

Higher levels of HbA_{1c} are found in people with persistently elevated blood sugar, as in diabetes mellitus. A diabetic person with good glucose control has an HbA_{1c} level that is close to or within the reference range.

Lower-than-expected levels of HbA_{1c} can be seen in people with shortened red blood cell lifespans, such as with glucose-6-phosphate dehydrogenase deficiency, sickle-cell disease, or any other condition causing premature red blood cell death.

Blood donation will result in rapid replacement of lost RBCs with newly formed red blood cells. Since these new RBCs will have only existed for a short period of time, their presence will lead HbA_{1c} to underestimate the actual average levels. There may also be distortions resulting from blood donation, which occurred as long as two months before due to an abnormal synchronization of the age of the RBCs, resulting in an older than normal average blood cell life (resulting in an overestimate of actual average blood glucose levels). Conversely, higher-than-expected levels can be seen in people with a longer red blood cell lifespan, such as with vitamin B_{12} or folate deficiency.

Results can be unreliable in many circumstances, for example, after blood loss, after surgery, blood transfusions, anemia, or high erythrocyte turnover; in the presence of chronic renal or liver disease; after administration of high-dose vitamin C; or erythropoietin treatment

Range: Normal: HbA1c below 5.7%

Prediabetes: HbA1c between 5.7% and 6.4

Diabetes: HbA1c of 6.5% or higher

For Diabetics, American Diabetes Association recommends keeping HbA1c levels below 7%

Glycated Haemoglobin test provides several advantages over glucose testing as it does not fluctuate significantly and can be performed at any time of the day. HBA1 test does not require any pretest preparation like fasting [25].

The HbA1c test is also a good indicator of lipid profile which shows a direct correlation with cholesterol, triglycerides and LDL cholesterol and an inverse correlation with HDL cholesterol. Therefore HbA1c test can identify the type 2 diabetic patients with increased risk of cardiovascular and related complications [26].

Glycated Haemoglobin measurement is not appropriate where a change in diet or treatment has been made within 6 w. Likewise, the test assumes a normal red blood cell aging process and mix of Haemoglobin subtypes (predominantly HbA in normal adults). Hence, people with recent blood loss, hemolytic anemia, or genetic differences in the Haemoglobin molecule (Haemoglobinopathy) such as sickle-cell disease and other conditions, as well as those who have donated blood recently, are not suitable for this test [27].

Fetuin A

Fetuin A is a phosphoryated glycoprotein primarily produced in the liver and secreted to plasma. It is a member of fetuin group which is comprised of three O-linked and two N-linked oligosacharides. It stimulates the production of inflammatory cytokines from adipocytes and macrophages; hence it serves as a biomarker for chronic inflammatory diseases. Fetuin A due to physiological actions, correlated with increased risk of obesity, fatty liver, type 2 DM and associated vascular complications [28-30].

Fetuin A when secreted to the plasma, it complex with the minerals like calcium and phosphorous in the circulation and prevent the sedimentation of these minerals in the serum and hence, it inhibit calcium deposition prevent the vascular calcification [31].

The genetic studies revealed that the single nucleotide polymorphism in Fetuin A gene causes type 2 DM. However, the detailed mechanism is not clear. Fetuin A inhibits the phophorylation in liver and muscle resulting in decreased insulin signaling leading to insulin resistance. Therefore, high levels of Fetuin A are associated with insulin resistance and the incidence of type 2 DM. Fetuin A also suppresses the auto phosphorylation of tyrosine kinase in muscle and liver and insulin receptor substrate proteins [32].

Fructosamine

Fructosamines are the compounds that result from a glycation reaction between a sugar (glucose or fructose) and a primary amine. Biologically fructosamines are recognized as fructosamine-3-kinase, which may trigger the degradation of advanced glycation end products. Fructosamine can also refer to specific compound 1-amino-1-deoxy-D-fructose (Isoglucoamine) which was first syntheized by Herman Emil Fischer in 1886.

Fructosamine test refers to a laboratory test for diabetes; it determines the fraction of total serum proteins that have undergone glycation (Glycated serum proteins). Usually Fructosamine level refers to albumin glycation, since albumin is the most abundant protein in the blood. The half-life of albumin is 20 d, the plasma Fructosamine concentration refers to recent changes in blood glucose i. e 1-2 w. Fructosamine test is most useful for diagnosis of diabetes in animals like cat and dog.

Fructosamine test can identify poorly controlled diabetes and helps in diabetes control. In diabetic patients with HbA1c values below the lower limit of normal, a routine Fructosamine level should be performed.

In patients with hemolytic anemia or sickle cell disease, which reduces the life span of red cells, the Hb1c test is misleadingly low because of interference caused by abnormal Haemoglobinvariants. In these cases Fructosamine test can be used as a marker of blood sugar as it measures serum albumin instead of Haemoglobin. However, Fructosamine test is clinically not used much because

a) Diabetic care is rarely changed in short intervals

b) Fructosamine has higher variability than HbA1c

c) Interpretation of Fructosamine test result is difficult

d) Fructosamine test is not well standardized and not used universally like HbA1c test

e) There is no standard reference range is available for this test [33-35].

Glycated albumin

In last few years Glycated albumin (GA) has been gained much significance in monitoring in Diabetes Mellitus. GA is one of the fructosamines, it is not being influenced by other serum proteins and glycation is specific to albumin. Unlike other tests fasting is not required for measurement and it reflects short-term glycemia as the half life time of the albumin is short, approximately 3 w. It is also not affected by the presence of hemolytic processes and abnormal Hb. It is a better glycemic marker than A1C, in conditions such as anemia, pregnancy, postprandial hyperglycemia and DM using insulin.

GA can be measured by ion-exchange high-performance liquid chromatography (HPLC), boronate affinity chromatography, immunoassays (radioimmunoassay and Enzyme-Linked Immuno Sorbent Assay), and colorimetric method with thiobarbituric acid and enzymatic methods using proteinase and ketamine oxidase. The reference intervals described for GA depend on the method used since GA levels may vary according to the glycation sites analyzed by the assay employed, and also if the method of analysis considers the GA molecule for measurement and not its glycated amino acids. All these methods are older and have their own limitations. An enzymatic methodology with a shorter operational time and easier to perform both manually and automatically was proposed to evaluate the GA levels in order to overcome the limitations of the previously existing techniques. Despite this difference, all methods available agree that the proportion of GA in patients with DM increases 2 to 5 fold compared to normoglycemic patients.

GA test is useful during the pregnancy since the glycation level remain the same during the pregnancy, whereas during the last months of pregnancy there is an increased demand for iron, which directly reflects on changes in the A1C throughout the pregnancy.

Compared to A1C, GA is more suitable to monitor the beginning of drug therapy in DM, and also to control the dose and change of medication since its levels diminish faster than A1C in intensive treatment.

Limitations: The GA values are influenced by the conditions which alters the albumin metabolism. Also, the increased protein metabolism indicates lower GA levels. In conditions such as hyperthyroidism, hypothyroidism, liver cirrhosis, nephrotic syndrome with massive proteinuria, or other specific disorders, the GA results may be misleading [36-39].

Ceramides

Ceramides are important bioactive lipids belonging to sphingolipid family produced from a fatty acid and sphingosine or by sphingomylein hydrolysis. Ceramides in biological membrane stabilize the cell membrane structure and modulate the distribution of receptors and signaling molecules. These regulate the activity of many enzymes like kinases or phosphatases and also alter the activity of transportation factors.

Several human studies indicated the relation between Ceramides and insulin resistance. The Ceramides antagonizes insulin signaling by inhibiting the transmission signals through phosphotidylinositol-3-kinase (PI3K) and blocking the activation of anabolic enzyme Akt/PKB. By these actions, Ceramides interfere with glucose uptake and impair the storage of nutrients such as glycogen or triglyceride, active protein phosphatase 2A(PP2A) and active proinflammatory cytokines. In pancreatic beta cells Ceramides activate multiple stress-related pathways to induce apoptosis. Plasma Ceramides are in obese children and diabetes adults; ceramides level inversely correlates with insulin sensitivity [40, 41].

HDL cholesterol

HDL is a major lipoprotein, which promote insulin secretion. The studies have been indicated that low level of HDL cholesterol is one of the risk factor for type 2 diabetes. Therefore, the increase in plasma HDL cholesterol can be a therapeutic strategy to reduce the risk of type 2 diabetes. The HDL cholesterol level significantly plays a role in the pathogenesis of diabetes mellitus by its direct effect on plasma glucose levels and also stimulating the secretion of insulin from beta cells of pancreas and modulates the glucose uptake in skeletal muscles [42, 43].

Micro RNA

Blood carries hormones, nutrients, proteins and several biomolecules secreted by cells which are known for their specific biological functions in the body. Recently the circulating nucleic acids and miRNA are found to be the biomarkers for the identification of various pathological conditions [44, 45].

Micro RNA are small endogenous RNAs found in serum, plasma, urine, saliva and breast milk, which regulate the gene expression post-transcriptionally [46]. Under some conditions, the cells release miRNAs as free or microvesicles which can be taken up by other cell types. Thus released miRNAs are mediators of cell to cell communication and coordinate several biological functions including angiogenesis, tumour cell invasion and immune response. Under the stress conditions, the miRNAs are released from islets, which have been identified by several techniques [47, 48].

There are several types of miRNA with diverse biological functions, the altered levels of miR-9, mir-29A, mir 30d, mir 124a, mir 34a, mir 146a and mir375 play a significant role in beta-cell function. These miRNAs negatively regulate insulin expression, production and secretion [49].

The circulating levels of miR-126 are decreased in type 2 diabetes, miR-126 levels are increased with diet and exercise. The miR-15a levels are also significantly reduced in type 2 diabetes as miR-15a is involved in the regulation and promotion of insulin biosynthesis by inhibiting the endogenous uncoupling protein 2 gene expressions and stimulating the insulin secretion [50-52].

Phospholipase A2

Phospholipase A2 is the group of enzymes which hydrolyzes the phospholipids at SN2 position to form fatty acid and lysophospholipid products. Phospholipase A2 is of high pharmacological significance because of its role in the release of arachidonic acid from the membranes, followed by its action in the conversion of fatty acids to leucotrienes and prostaglandins, which are the main mediators of inflammation [53].

So far, several isoenzymes of Phospholipase A2 have been identified in mammals which are classified into 12 major groups and several subgroups. The two major types are high molecular weight cytosolic Phospholipase A2 and low molecular weight secretory Phospholipase A2. The cytosolic phospholipase A2 migrates from cytosol to perinuclear membraneand other intracellular compartments of the stimulated cell. The secretory phospholipase A2 are calcium-dependent, stored in cytoplasmic granules and releases as inflammatory mediators in the extracellular compartment upon stimulation and activation of the cell [54, 55].

The studies show that cytosolic phospholipase A2 plays a role in insulin secretion and in the maintenance of dense cored secretary vesicles. However, the detailed mechanism is yet to be established to use it as a biomarker for diabetes [56].

Calprotectin

Calprotectin is a stable mammalian heterodimer belonging to S100 protein family composed of two calcium-binding cytoplasmic calgranulins, which are expressed in activated human granulocyte and macrophages in inflammatory condition [57]. Calprotectin is capable of sequestering with transition metals such as iron, manganese and zinc to form a stable complex, which is known for antimicrobial activity. Calprotectin is the only antimicrobial agent that acts through manganese sequestration [58, 59]. The calprotectin is secreted during inflammation; the mechanism of secretion is still unknown. Fecal calprotectin is an important biomarker for inflammatory bowel disease and rheumatoid arthritis [60, 61].

Tabur S and coworkers investigated the role of calprotectin as a biomarker of neuroinflammation in diabetic peripheral neuropathy. In the study, the serum calprotectin level was estimated in 29 patients with diabetic neuropathy and 30 diabetic patients without neuropathy and 40 healthy controls. The result indicates that the serum calprotectin levels were significantly higher in patients with

and without neuropathy than healthy controls. In fact, the higher serum calprotectin level was observed in neuropathy patients compared to diabetic patients without neuropathy. The calprotectin as reported to be an inflammatory marker, plays a role in the pathogenesis of neuroinflammation and thereby causing the destruction of nerves in diabetic patients. The authors also found that there was a positive correlation between Calprotectin and HBA1c. The serum calprotectin levels affect the glucose or glycation products and the increased serum calprotectin level is observed in diabetic neuropathy [62]. In another study, the results exhibited that the high circulating and urinary calprotectin are linked to low grade chronic inflammation and insulin resistance [63].

Acyl carnitine

Acyl carnitine is an acetylated form of L-Carnitine, which is naturally produced in the body and can also be taken through diet as dietary supplement. In the process of carnitine shuttle, L-carnitine undergoes acetylation to form acylcarnitine which is an intermediate in the fatty acid oxidation, for the transfer of long chain fatty acids from cytosol to mitochondria [64]. The several studies on the effect of lipid accumulation on insulin resistance found that through multiple mechanisms, the accumulation of intracellular lipids and their metabolites in ectopic tissues (lipotoxicity), the oversupply of dietary fats leads to insulin resistance, which is the main cause of diabetes [65]. Particularly accumulation of fatty acyl co A or its metabolites in muscle, inhibits both insulin signaling and glucose oxidation [66]. As the exact mechanism of acylcarnitine causing insulin resistance is unknown, several theories have been postulated to explain the same. One of the most popular theory states that lipid oversupply leads to the accumulation of lipid metabolites such as diacylglycerol and fatty acyl coA, which activates a serine/threonine kinase cascade leading to defects in insulin signaling through serine/threonine phosphorylation of insulin receptor substrate [67]. From the several studies reported, it can be concluded that the plasma acylcarnitine level is a potential biomarker of insulin resistance; alteration of serum acylcarnitine level is associated with type 2 diabetes and prediabetes [68-70].

Adiponectin

Adiponectin (also referred as GBP-28, apM1, AdipoQ and Acrp30) is a protein hormone and adipokine produced primarily in adipose tissue and secrete to the bloodstream, it is also found in muscle and brain. It is involved in the regulation of glucose level and fatty acid oxidation [71-73].

Adiponectin accounts for around 0.01% of all plasma proteins at around 5-10µg/ml. The plasma conc, is higher in males compare to females. Adiponectin is an adipocyte-specific protein, plays a role in the development of insulin resistance. Decreased levels of adiponectin levels play a significant role in the development of type 2 diabetes, obesity and cardiovascular diseases [74, 75].

Ferritin

Ferritin is an intracellular protein which stores the iron and release the iron in a controlled manner. It is found in most tissues as cytosolic protein, but some amount is also secreted to serum, thereby acting as an iron carrier. It acts as a buffer for iron deficiency and iron overload. The plasma ferritin is an indirect marker of iron storage in the body; therefore, estimation of serum ferritin is a diagnostic test for iron deficiency anemia [76].

Elevated serum ferritin and transferring levels is associated with an increased risk of diabetes. The iron of ferritin produces the highly reactive radical formation, which damages the DNA and cell membrane integrity of beta cells of the pancreas and high oxidative stress; as a result the insulin secretion capacity of beta cell reduces. Also, it causes the insulin resistance. The reactive oxidative species may lead to beta-cell apoptosis which suppresses insulin secretion [77-79].

CONCLUSION

Diabetes mellitus is one of the most prevalent causes for the development of several micro and macrovascular complication in larger group of population in the world. There are several studies reported to indicate the role of diabetes mellitus in chronic disorders. Though the drug treatment and modification in lifestyle is recommended for the treatment of diabetes mellitus, the diagnosis of diabetes is a challenging task, as the research indicates that the most of the people are not diagnosed with the diabetes mellitus. The reason being the lack of knowledge about the bio chemicals and genetic factors involved in the regulation of blood glucose levela. However, through the studies several biomarkers have been identified which plays a significant role in regulation of blood glucose level. The glycated Haemoglobin, glycated albumin, fetuin, ferritn, ceramides, HDL cholesterol, adiponectin, calprotectin, acylcarnitine, phospholipase A2 and microRNA are some of the important biomarkers identified. Each one has its own applications and limitations as described above. Further studies on the biomarkers to understand the mechanism of action is recommended which would help the health care professionals for accurate and precise estimation of blood glucose level and thereby helping the physician to determine the nature of treatment for diabetes mellitus.

ACKNOWLEDGMENT

The authors are thankful for the Principal and HOD, College of Pharmaceutical, Sciences, Dayanand Sagar University, Bengaluru, Karnataka for the consistent support and providing the facility.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors hereby declare that there is no any conflict of interest involved in this paper.

REFERENCES

1. Deepthi B, Sowjanya K, Lidiya B, Bhargavi RS, Babu PS. A modern review of diabetes mellitus: an uninhilatory metabolic disorder. J In Silico *In Vitro* Pharmacol. 2017;3(1):1-5.
2. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLOS Med. 2006;3(11):442. doi: 10.1371/journal.pmed.0030442.
3. Tiwari N. Therapeutic targets for diabetes mellitus: an update. Clinic Pharmacol Biopharm 2014;3(1). doi: 10.4172/2167-065X.1000117.
4. Johnson L, Strich H, Taylor A, Timmermann B, Malone D, Teufel Shone N, Drummond R, Woosley R, Pereira E, Martinez A. Use of herbal remedies by diabetic Hispanic women in the southwestern United States. Phytother Res. 2006;20(4):250-5. doi: 10.1002/ptr.1820. PMID 16557605.
5. Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. J Clin Invest. 2005;115(5):1111-9. doi: 10.1172/JCI25102.
6. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nat Rev Endocrinol. 2018;14(2):88-98. doi: 10.1038/nrendo.2017.151, PMID 29219149.
7. Lacro CRM, Barengo NC, Flores LA, Rauner M. Update on the impact of type 2 diabetes mellitus on bone metabolism and material properties. Endocr Connect. 2019;8(3):R55-70.
8. Khawandanah J. Double or hybrid diabetes: A systematic review on disease prevalence, characteristics and risk factors. Nutr Diabetes. 2019;9(1):33. doi: 10.1038/s41387-019-0101-1, PMID 31685799.
9. Mishra BK, Shukla P, Aslam M, Siddiqui AA, Madhu SV. Prevalence of double diabetes in youth onset diabetes patients from east Delhi and neighboring NCR region. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2018;12(6):839-42. doi: 10.1016/j.dsx.2017.08.016.
10. Merger SR, Kerner W, Stadler M, Zeyfang A, Jehle P, Muller Korbsch M, Holl RW. Prevalence and comorbidities of double diabetes. Diabetes Res Clin Pract. 2016;119:48-56. doi: 10.1016/j.diabres.2016.06.003.

11. Braham R, Alzaid A, Robert AA, Mujammami M, Ahmad RA, Zitouni M, Sobki SH, Al Dawish MA. Double diabetes in Saudi Arabia: A new entity or an underestimated condition. World J Diabetes. 2016;7(20):621-6. doi: 10.4239/wjd.v7.i20.621, PMID 28031780.
12. Kamalesh DR, Geetha KM, Harish M. Target treatment in diabetic and microvascular complications: an updated review. Int J Pharm Sci Res. 2020;11(8):1000-8.
13. Lyons TJ, Basu A. Biomarkers in diabetes: hemoglobin A1c, vascular and tissue markers. Transl Res. 2012;159(4):303-12. doi: 10.1016/j.trsl.2012.01.009, PMID 22424433.
14. Herder C, Karakas M, Koenig W. Biomarkers for the prediction of type 2diabetes and cardiovascular disease. Clin Pharmacol Ther. 2011;90(1):52-66. doi: 10.1038/clpt.2011.93, PMID 21654741.
15. Lima N, Cavaliere H, Tomimori E, Knobel M, Medeiros-Neto G. Prognostic value of serial serum thyroglobulin determinations after total thyroidectomy for differentiated thyroid cancer. J Endocrinol Invest. 2002;25(2):110-5. doi: 10.1007/ BF03343973, PMID 11929080.
16. Chien HY, Lee T, Chen C, Chiu Y, Lin Y, Lee L, Li W. Circulating microRNA as a diagnostic marker in populations with type 2 diabetes mellitus and diabetic complications. Journal of the Chinese Medical Association. 2015;78(4):204-11. doi: 10.1016/j.jcma.2014.11.002.
17. Schnedl WJ, Liebminger A, Roller RE, Lipp RW, Krejs GJ. Hemoglobin variants and determination of glycated hemoglobin (HbA1c). Diabetes Metab Res Rev. 2001;17(2):94-8. doi: 10.1002/dmrr.186. PMID 11307174.
18. Sherwani SI, Khan HA, Ekhzaimy A, Masood A, Sakharkar MK. Significance of HbA1c test in diagnosis and prognosis of diabetic patients. Biomark Insights. 2016;11:95-104. doi: 10.4137/BMI.S38440, PMID 27398023.
19. English E, Milosevich E, John WG. *In vitro* determination of haemoglobin A1c for diabetes diagnosis and management: technology update. Pathol Lab Med Int. 2014;6:21-31.
20. Radchenko OM, Korolyuk OY. Glycated hemoglobin: mechanisms of formation and clinical significance (literature review and own researches). IEJ 2020;16(1):69-75. doi: 10.22141/2224-0721.16.1.2020.199131.
21. Guo W, Zhou Q, Jia Y, Xu J. Increased levels of glycated hemoglobin A1c and Iron deficiency Anemia: a review. Med Sci Monit. 2019;25:8371-8. doi: 10.12659/MSM.916719, PMID 31696865.
22. Weykamp C, John WG, Mosca A. A review of the challenge in measuring haemoglobin. J Diabet Sci Technol. 2009;3(A1c):439-45.
23. Ozçelik F. Comparison of three methods for measurement of HbA1c. Turk J Biochem. 2010;35:344-9.
24. Lin CN, Emery TJ, Little RR, Hanson SE, Rohlfing CL, Jaisson S, Gillery P, Roberts WL. Effects of hemoglobin C, D, E, and S traits on measurements of HbA1c by six methods. Clin Chim Acta. 2012;413(7-8):819-21. doi: 10.1016/j.cca.2011.12.019.
25. Rathod SD. Glycated haemoglobinA1c (HbA1c) for detection of diabetes mellitus and impaired fasting glucose in Malawi: a diagnostic accuracy study. BMJ. 2018;8:e020972.
26. Khan HA, Sobki SH, Khan SA. Association between glycaemic control and serum lipids profile in type 2 diabetic patients: HbA1c predicts dyslipidaemia. Clin Exp Med. 2007;7(1):24-9. doi: 10.1007/s10238-007-0121-3, PMID 17380302.
27. Radin MS. Pitfalls in hemoglobin A1c measurement: when results may be misleading. J Gen Intern Med. 2014;29(2):388-94. doi: 10.1007/s11606-013-2595-x, PMID 24002631.
28. Qatanani M, Lazar MA. Mechanisms of obesity-associated insulin resistance: many choices on the menu. Genes Dev. 2007;21(12):1443-55. doi: 10.1101/gad.1550907, PMID 17575046.
29. Mori K, Emoto M, Yokoyama H, Araki T, Teramura M, Koyama H, Shoji T, Inaba M, Nishizawa Y. Association of serum fetuin-a with insulin resistance in type 2 diabetic and nondiabetic subjects. Diabet Care. 2006;29(2):468. doi: 10.2337/diacare.29.02.06.dc05-1484.
30. Mori K, Emoto M, Inaba M. Fetuin-A: A multifunctional protein. Recent Pat Endocr Metab Immune Drug Discov. 2011;5(2):124-46. doi: 10.2174/187221411799015372, PMID 22074587.
31. Pal D, Dasgupta S, Kundu R, Maitra S, Das G, Mukhopadhyay S, Ray S, Majumdar SS, Bhattacharya S. Fetuin-a acts as an endogenous ligand of TLR4 to promote lipid-induced insulin resistance. Nat Med. 2012;18(8):1279-85. doi: 10.1038/nm.2851, PMID 22842477.
32. Aroner SA, St-Jules DE, Mukamal KJ, Katz R, Shlipak MG, Criqui MH, Kestenbaum B, Siscovick DS, de Boer IH, Jenny NS, Budoff MJ, Ix JH, Jensen MK. Fetuin-A, glycemic status, and risk of cardiovascular disease: the multi-ethnic study of atherosclerosis. Atherosclerosis. 2016;248:224-9. doi: 10.1016/j.atherosclerosis.2016.03.029, PMID 27038419.
33. Youssef D, El Abbassi A, Jordan RM, Peiris AN. Fructosamine-an underutilized tool in diabetes management: case report and literature review. Tenn Med. 2008;101(11):31-3. PMID 19024248.
34. Lee JE. Alternative biomarkers for assessing glycemic control in diabetes: fructosamine, glycated albumin, and 1,5-anhydroglucitol. Ann Pediatr Endocrinol Metab. 2015;20(2):74-8. doi: 10.6065/apem.2015.20.2.74, PMID 26191510.
35. Ribeiro RT, Macedo MP, Raposo JF. HbA1c, fructosamine, and glycated albumin in the detection of dysglycaemic conditions. Curr Diabetes Rev. 2016;12(1):14-9. doi: 10.2174/1573399811666150701143112, PMID 26126638.
36. Shimizu L, Kohzuma T, Koga M. A proposed glycemic control marker for future. J Lab Prec Med. 2019;4:23.
37. Koga M, Hashimoto K, Murai J, Saito H, Mukai M, Ikegame K, Ogawa H, Kasayama S. Usefulness of glycated albumin as an indicator of glycemic control status in patients with hemolytic anemia. Clin Chim Acta. 2011;412(3-4):253-7. doi: 10.1016/j.cca.2010.10.014.
38. Huang Y, Hu Y, Ma YU, Ye G. Glycated albumin is an optimal biomarker for gestational diabetes mellitus. Exp Ther Med. 2015;10(6):2145-9. doi: 10.3892/etm.2015.2808, PMID 26668607.
39. Dozio E, Di Gaetano N, Findeisen P, Corsi Romanelli MM. Glycated albumin: from biochemistry and laboratory medicine to clinical practice. Endocrine. 2017;55(3):682-90. doi: 10.1007/s12020-016-1091-6. PMID 27623968.
40. Haus JM. Plasma ceramides are elevated in obese subjects with type2 diabetes and correlates with the severity of insulin resistance. Diabetes. 2009;58(1):337-43.
41. Summers SA. Ceramides in insulin resistance and lipotoxicity. Prog Lipid Res. 2006;45(1):42-72. doi: 10.1016/j.plipres.2005.11.002, PMID 16445986.
42. Haase CL, Tybjærg Hansen A, Nordestgaard BG, Frikke Schmidt R. HDL cholesterol and risk of type 2 diabetes: a mendelian randomization study. Diabetes. 2015;64(9):3328-33. doi: 10.2337/db14-1603, PMID 25972569.
43. Femlak M, Gluba Brzozka A, Ciałkowska Rysz A, Rysz J. The role and function of HDL in patients with diabetes mellitus and the related cardiovascular risk. Lipids Health Dis. 2017;16(1):207. doi: 10.1186/s12944-017-0594-3. PMID 29084567.
44. Gilad S, Meiri E, Yogev Y, Benjamin S, Lebanony D, Yerushalmi N, Benjamin H, Kushnir M, Cholakh H, Melamed N, Bentwich Z, Hod M, Goren Y, Chajut A. Serum microRNAs are promising novel biomarkers. PLOS ONE. 2008;3(9):e3148. doi: 10.1371/journal.pone.0003148, PMID 18773077.
45. Etheridge A, Gomes CP, Pereira RW, Galas D, Wang K. The complexity, function and applications of RNA in circulation. Front Genet. 2013;4:115. doi: 10.3389/fgene.2013.00115, PMID 23785385.
46. Lu TX, Rothenberg ME. MicroRNA. J Allergy Clin Immunol. 2018;141(4):1202-07. doi: 10.1016/j.jaci.2017.08.034.
47. Vasu S, Kumano K, Darden CM, Rahman I, Lawrence MC, Naziruddin B. MicroRNA signatures as future biomarkers for diagnosis of diabetes states. Cells. 2019;8(12):1533. doi: 10.3390/cells8121533, PMID 31795194.
48. Beltrami C, Angelini TG, Emanueli C. Noncoding RNAs in diabetes vascular complications. J Mol Cell Cardiol. 2015;89(A):42-50. doi: 10.1016/j.yjmcc.2014.12.014, PMID 25536178.
49. Kong L, Zhu J, Han W, Jiang X, Xu M, Zhao Y, Dong Q, Pang Z, Guan Q, Gao L, Zhao J, Zhao L. Significance of serum microRNAs

in pre-diabetes and newly diagnosed type 2 diabetes: a clinical study. Acta Diabetol. 2011;48(1):61-9. doi: 10.1007/s00592-010-0226-0, PMID 20857148.

50. Liu Y, Gao G, Yang C, Zhou K, Shen B, Liang H, Jiang X. The role of circulating micro RNA-126 (miR-126): a novel biomarker for screening prediabetes and newly diagnosed type 2 diabetes mellitus. Int J Mol Sci. 2014;15(6):10567-77. doi: 10.3390/ijms150610567, PMID 24927146.
51. Sun LL, Jiang BG, Li WT, Zou JJ, Shi YQ, Liu ZM. Micro RNA-15a positively regulates insulin synthesis by inhibiting uncoupling protein-2 expression. Diabetes Res Clin Pract. 2011;91(1):94-100. doi: 10.1016/j.diabres.2010.11.006, PMID 21146880.
52. Al-Kafaji G, Al-Mahroos G, Alsayed NA, Hasan ZA, Nawaz S, Bakhiet M. Peripheral blood microRNA-15a is a potential biomarker for type 2 diabetes mellitus and pre-diabetes. Mol Med Rep. 2015;12(5):7485-90. doi: 10.3892/mmr.2015.4416, PMID 26460159.
53. Burke JE, Dennis EA. Phospholipase A2 biochemistry. Cardiovascular Drugs Ther. 2009;23(1):49-59. doi: 10.1007/s10557-008-6132-9, PMID 18931897.
54. Triggiani M, Granata F, Giannattasio G, Marone G. Secretory phospholipases A2 in inflammatory and allergic diseases: not just enzymes. J Allergy Clin Immunol. 2005;116(5):1000-6. doi: 10.1016/j.jaci.2005.08.011, PMID 16275367.
55. Murakami M, Sato H, Miki Y, Yamamoto K, Taketomi Y. A new era of secreted phospholipase A_2. J Lipid Res. 2015;56(7):1248-61. doi: 10.1194/jlr.R058123, PMID 25805806.
56. Jones PM, Burns CJ, Belin VD, Roderigo Milne HM, Persaud SJ. The role of cytosolic phospholipase A(2) in insulin secretion. Diabetes. 2004;53Suppl 1:S172-8. doi: 10.2337/diabetes.53.2007.s172. PMID 14749284.
57. Striz I, Trebichavsky I. Calprotectin- a pleiotropic molecule in acute and chronic inflammation. Physiol Res. 2004; 53(3):245-53. PMID 15209531.
58. Brophy MB, Nolan EM. Manganese and microbial pathogenesis: sequestration by the mammalian immune system and utilization by microorganisms. ACS Chem Biol. 2015;10(3):641-51. doi: 10.1021/cb500792b, PMID 25594606.
59. Nakkashigeet AL, Human Nakashige TG, Zhang B, Krebs C, Nolan EM. Calprotectin is an iron sequestering host defense protein. Human calprotectin is an iron-sequestering host-defense protein. Nat Chem Biol. 2015;11(10):765-71. doi: 10.1038/nchembio.1891, PMID 26302479.
60. Lehmann FS, Burri E, Beglinger C. The role and utility of faecal markers in inflammatory bowel disease. Ther Adv Gastroenterol. 2015;8(1):23-36. doi: 10.1177/1756283X14553384.
61. Rheenen PF, Vijver EV, Fidler V. Calprotectin for screening of patients with suspected inflammatory bowel disease: a diagnostic metaanalysis. Br J 2010;341:c3369.
62. Tabur S, Kokmaz H, Ozkaya M, Aksoy NS, Akarsu E. Is calprotectin a novel biomarker of neuroinflammation in diabetic peripheral neuropathy? Diabetol MET Synd. 2015;7:30-6.
63. Ortega FJ, Sabater M, Moreno Navarrete JM, Pueyo N, Botas P, Delgado E, Ricart W, Fruhbeck G, Fernandez Real JM. Serum and urinary concentrations of calprotectin as markers of insulin resistance and type 2 diabetes. European J Endocrinology. 2012; 167(4):569-78. doi: 10.1530/EJE-12-0374.
64. Ramsay RR, Gandour RD, van der Leij FR. Molecular enzymology of carnitine transfer and transport. Biochim Biophys Acta. 2001;1546(1):21-43. doi: 10.1016/s0167-4838(01)00147-9. PMID 11257506.
65. Boreen J, Taskinen MR, Olofsson SO, Levin M. Ectopic lipid storage and insulin resistance: a harmful relationship. J Intern Med. 2013;274(1):25-40. doi: 10.1111/joim.12071, PMID 23551521.
66. Mynatt R. L-carnitine and type 2diabetes. Diabet Met. 2009;25:S45-S49.
67. Morino K, Petersen KIF and, Shulman GI. Molecular mechanisms of insulin resistance in humans and their potential links with mitochondrial dysfunction. Diabetes. 2006; 55Suppl 2:S9-S15. doi: 10.2337/db06-S002, PMID 17130651.
68. Mihalik SJ, Goodpaster BH, Kelley DE, Chace DH, Vockley J, Toledo FGS, DeLany JP. Increased levels of plasma acylcarnitines in obesity and type 2 diabetes and identification of a marker of glucolipotoxicity. Obesity. 2010;18(9):1695-700. doi: 10.1038/oby.2009.510.
69. Bene J, Hadzsiev K, Melegh B. Role of carnitine and its derivatives in the development and management of type 2 diabetes. Nutr and Diabetes. 2018;8(1):8-128. doi: 10.1038/s41387-018-0017-1, PMID 29549241.
70. Mai M, Tonjes A, Kovacs P, Stumvol M, Fiedler GM, Leichtle. Serum levels of acyl carnitines are altered in pre diabetic conditions. Plus One. 2013;8(12):e82459.
71. Achari AE, Jain SK. Adiponectin, a therapeutic target for obesity, diabetes, and endothelial dysfunction. Int J Mol Sci. 2017;18(6):1321. doi: 10.3390/ijms18061321, PMID 28635626.
72. Ghoshal K, Ghoshal K, Bhattacharya M. Adiponectin: probe of the molecular paradigm associating diabetes and obesity. World J Diabet. 2015;6(1):151-66. doi: 10.4239/wjd.v6.i1.151.
73. Nedvidkova, Smitka K, Kopsky V, Hainer V. Adiponectin. Adiponectin, an adipocyte-derived protein. Physiol Res. 2005; 54(2):133-40. PMID 15544426.
74. Coppola A. Effect of weight loss on coronary circulation and adiponectin levels in obese women. Int J Cardiol. 2009;134(3):414-6. doi: 10.1016/j.ijcard.2007.12.087, PMID 18378021.
75. Hara K, Yamauchi T, kadowaki T. Adiponectin: an adipokine linking adipocytes and type 2 diabetes in humans. Curr Diab Rep. 2005;5(2):136-40. doi: 10.1007/s11892-005-0041-0, PMID 15794918.
76. Wang W, Knovich MA, Coffman LG, Torti FM, Torti SV. Serum ferritin: past, present and future. Biocheim Biophys Acta. 2010;1800(8):760-9. doi: 10.1016/j.bbagen.2010.03.011, PMID 20304033.
77. Aregbesola A, Virtanen JK, Voutilainen S, Mursu J, Lagundoye A, Kauhanen J, Tuomainen T. Serum ferritin and glucose homeostasis: change in the association by glycaemic state. Diabet Met Res Rev Diabetes Metab Res Rev. 2015;31(5):507-14. doi: 10.1002/dmrr.2628.
78. Sharifi SF, Nasab NM, Zadeh HJ. Elevated serum ferritin concentrations in pre-diabetic subjects. Diabetic Vasc Dis Res Diab Vasc Dis Res. 2008;5(1):1-18. doi: 10.3132/dvdr.2008.003, PMID 18398807.
79. Akter S, Nanri A, Kuwahara K, Matsushita Y, Nakagawa T, Konishi M, Honda T, Yamamoto S, Hayashi T, Noda M, Mizoue T. Circulating ferritin concentrations and risk of type 2 diabetes in Japanese individuals. J Diabetes Investig. 2017;8(4):462-70. doi: 10.1111/jdi.12617, PMID 28060459.