ASSESSMENT OF SERUM GROWTH DIFFERENTIATION FACTOR 15 (GDF15) LEVEL AS EARLY BIOMARKER FOR DETECTION OF PREDIABETES

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ABSTRACT

Background: Diabetes Mellitus (DM) is a group of metabolic disorders in which there are high blood sugar levels over a prolonged period and it is due to either the pancreas does not produce insulin or cells of the body do not respond to insulin production. Prediabetes is an intermediate state of hyperglycemia with glycemic parameters above normal but below the diabetes threshold. It is considered to be an at risk state, with high chances of developing diabetes. Prediabetes is associated with insulin resistance and is risk factor for the development of type 2 diabetes mellitus. Those in this stratum (IGT or IFG) are at increased risk of cardiovascular disease. Of the two, impaired glucose tolerance better predicts cardiovascular disease and mortality. it is necessary to identify prediabetic patients earlier with the use of a biomarker. Several markers for predicting prediabetes have been proposed. Acute phase proteins (C-reactive protein [CRP], plasminogen activator inhibitor-1) and coagulation factors (fibrinogen, D-dimer) are considered markers that can predict prediabetes. Growth Differentiation Factor 15 (GDF15) is a divergent member of the transforming growth factor B (TGF B) superfamily. The putative role of GDF 15 is that of a stress or inflammation responsive cytokine. As one of the aspects of the inflammatory diseases of T2DM, elevated levels of GDF 15 were found to be associated with the presence of T2DM and the future development of T2DM.

Key words: Diabetes Mellitus (DM), Prediabetes, Growth Differentiation Factor 15 (GDF15).
I. DIABETES MELLITUS:

Diabetes mellitus is not one disease. It is defined as chronic hyperglycemia that may be caused by one or more of numerous underlying processes. Some of these cause diabetes directly by interfering with beta cell function or through significant defects in insulin action. In other cases diabetes is part of a more general disorder affecting many other organs or systems. Examples include some endocrinopathies, drug- or chemical-induced diabetes; diabetes related to certain infections and diabetes associated with certain genetic syndromes. (1).

Epidemiology:

As of 2016, 422 million people have diabetes worldwide from an estimated 382 million people in 2013 and from 108 million people in 1980. The prevalence of diabetes is 8.5% among adults with double rate of 4.7% in 1980. Type 2 makes up about 90% of cases. Rates are equal in both sexes. But the rate of male has been found in many populations with higher type 2 incidence due to sex-related differences in insulin sensitivity, regional body fat deposition, high blood pressure, tobacco smoking and alcohol intake (1).

The World Health Organization (WHO) estimates that diabetes mellitus resulted in 1.5 million deaths in 2012 making it the 8th leading cause of death. Another 2.2 million deaths worldwide were attributable to high blood glucose, increased risks of cardiovascular diseases and other associated complications (e.g., Kidney failure) which often led to premature death and are listed as an underlying cause of death (2).

Diabetes is frequently not diagnosed until complications appear, and approximately one third of all people with diabetes may be undiagnosed. Type 2 diabetes frequently goes undiagnosed for many years because hyperglycemia develops gradually and at earlier stages is often not severe enough for the patient to notice any of the classic symptoms of diabetes (3).

* Criteria for the diagnosis of diabetes (3)
  - HbA1C > 6.5%. The test should be performed.
  OR
  - Fasting plasma glucose (FPG) > 126 mg/dl (7mmol/l). Fasting is defined as no caloric intake for at least 8 h.
  OR
  - 2h plasma glucose > 200 mg/dl (11.1mmol/l) during an OGTT. The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.
  OR
  - In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose >200 mg/dl (11.1 mmol/l). In the absence of unequivocal hyperglycemia, criteria 1–3 should be confirmed by repeat testing.

Prediabetes

Prediabetes is an intermediate state of hyperglycemia with glycemic parameters above normal but below the diabetes threshold. It is considered to be an at-risk state, with high chances of developing diabetes (4).

Impaired fasting glucose and impaired glucose tolerance are two forms of pre diabetes that are similar in clinical definition (glucose levels too high for their context) but are physiologically distinct. Insulin resistance, the insulin resistance syndrome (metabolic syndrome or syndrome X), and prediabetes are closely related to one another and have overlapping aspects (5).

Diagnosis:

The World Health Organization (WHO) has defined prediabetes as a state of intermediate hyperglycemia using two specific parameters, impaired fasting glucose (IFG) defined as fasting plasma glucose (FPG) of 6.1-6.9 mmol/L (110 to 125 mg/dL) and impaired glucose tolerance (IGT) defined as 2 h plasma glucose of 7.8-11.0
mmol/L (140-200 mg/dL) after ingestion of 75 g of oral glucose load or a combination of the two based on a 2 h oral glucose tolerance test (OGTT) (2).

The American Diabetes Association (ADA), on the other hand has the same cut-off value for IGT (140-200 mg/dL) but has a lower cut-off value for IFG (100-125 mg/dL) and has additional hemoglobin A1c (HbA1c) based criteria of a level of 5.7% to 6.4% for the definition of prediabetes (3).

Prevalence:
There have been reports of increased mean FPG and prevalence of diabetes in developed as well as developing countries (6).

The Centers of Disease Control and Prevention National Diabetes Statistics Report suggested that 37% of United States adults older than 20 years and 51% of those older than 65 had prediabetes in 2009-2012 defined by fasting glucose or HbA1c levels. The worldwide prevalence of IGT in 2010 was estimated to be 343 million (7.8%) ranging from 5.8% in Southeast Asia to 11.4% in North American and Caribbean Countries of the nation’s population (7).

Insulin resistance and prediabetes:
In healthy individuals, the plasma glucose concentration typically ranges between 70 and 100 mg/dl after an overnight fast and usually does not exceed 120–140 mg/dl after ingestion of a meal. In the post absorptive state, glucose is released into the circulation primarily from the liver, either by the breakdown of previously stored glycogen or by the process of gluconeogenesis the synthesis of new glucose from precursors such as lactate, pyruvate, glycerol and the gluconeogenic amino acids, predominantly alanine and glutamine. Insulin is the primary hormone inhibiting the release of glucose from the liver and, after an overnight fast, circulates at concentrations ranging from about 3–10 mU/ml, depending on the age, weight and other demographic characteristics of the individual. Approximately half of all glucose produced in the fasting state is utilized by the brain, which does not require insulin for glucose uptake or metabolism (7).

After the ingestion of glucose or a mixed meal, nutrients are absorbed from the intestinal tract and stimulate the release of insulin from the pancreatic beta cells into the portal vein. Insulin initially reaches the liver where its major physiologic effects include stimulation of glycogen and triglyceride synthesis, inhibition of gluconeogenesis and suppression of lipolysis and ketogenesis (8).

Approximately 50–60% of insulin secreted by the pancreas is removed by the liver, primarily by receptor-mediated clearance, before reaching the systemic circulation. Systemic levels of insulin vary widely after nutrient ingestion and may range from 20 to over 100 mU/ml, depending on the content and size of the meal as well as the metabolic characteristics of the individual (8).

In response to the systemic hyperinsulinemia:
1) Glucose is removed from the circulation, primarily by muscle, and either stored as glycogen or oxidized to meet ongoing energy requirements
2) Lipolysis is inhibited and fatty acids are re-esterified in to triglycerides.
3) Amino acids are taken up by a wide variety of tissues for tissue growth and repair.

After about 3–5 hours, most of the nutrients have been cleared from the circulation and the basal state is restored (9).

In patients with insulin resistance, several defects are evident. In the fasting or post absorptive state, insulin is no longer able fully to restrain hepatic glucose production, resulting in a small rise in the fasting plasma glucose concentration. This induces a compensatory increase in insulin secretion so that a new steady state is achieved with mild fasting hyperglycemia and hyperinsulinemia – the classic finding in insulin resistant states. In the post-prandial state, insulin also is not able fully to stimulate glucose uptake into muscle so there is an excessive rise in the plasma glucose concentration leading to even greater hyperinsulinemia. Similar abnormalities in lipid metabolism also occur, with elevated fasting and post-prandial levels of free fatty acids and triglycerides.

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Although the pancreas initially is able to compensate for the higher levels of glucose, the continued exposure to hyperglycemia eventually leads to beta-cell failure – a process commonly known as glucose toxicity (9).

The first phase of insulin secretion is particularly sensitive to this process and typically becomes impaired when the fasting plasma glucose concentration exceeds 100mg/dl, leading to more prolonged post-prandial hyperglycemia and even greater demand on the beta cell. Once the fasting plasma glucose concentration is consistently above 120 mg/dl, the second phase of insulin secretion also begins to fail. And the patient often progresses overt type2 diabetes shortly thereafter (10).

II. COMPLICATIONS:

1 Progression to diabetes:

The conversion rate of individuals from prediabetes to diabetes changes with population characteristics and the criteria used to define prediabetes (10). In a meta-analysis evaluating the progression of prediabetes to diabetes published in 2007, the annual incidence rate of diabetes was found to be 4%-6% for isolated IGT, for isolated IFG 6%-9% and for both IGT and IFG was 15%-19%. This meta-analysis only consisted of studies published prior to 2004. In subsequently reported major studies, the annual incidence rates of conversion from prediabetes to diabetes were similar. In the Diabetes Prevention Program (DPP) Outcomes Study, the incidence of diabetes was noted to be 11% in the control group (11).

2 Nephropathy and kidney disease:

Several studies have shown an association of increased risk of chronic kidney disease and early nephropathy with prediabetes. The causal nature of this relationship remains unclear as this association may be due increased incidence of diabetes in this group or the presence of other factors associated with hyperglycemia and nephropathy rather than the effect of prediabetes itself (12).

3 Neuropathies:

Prediabetes is found to be associated with dysfunction of cardiac autonomic activity, reflected by reduced heart rate variability, decreased parasympathetic modulation of the heart and increased prevalence of male erectile dysfunction in individuals with prediabetes. There is also increasing evidence to demonstrate a higher frequency of idiopathic polyneuropathy, painful sensory neuropathy and small fiber neuropathy among prediabetic individuals with IGT. These findings suggest an involvement of the small unmyelinated nerve fibers that carry pain, temperature, and regulate autonomic function during prediabetes, prior to development of diabetes (13).

4 Retinopathy:

Nearly 8 percent of participants with prediabetes in the DPP study were found to have evidence of diabetic retinopathy. While prediabetes has been associated with an increased risk of diabetic retinopathy in some studies, these findings vary depending on the method used for detection (14).

5 Macrovascular disease:

Prediabetes has been associated with increased risk of developing macrovascular disease but whether this elevated risk is due to prediabetes itself or due to development of diabetes remains unclear (15).

However, Cross sectional studies have shown an increased prevalence of coronary heart disease in individuals with prediabetes, but this relationship may be confounded by the common risk factors present between cardiovascular diseases and prediabetes (16).

Prevention:

Prediabetes can be prevented by maintaining a normal body weight, engaging in physical activity and consuming a healthy diet. Higher levels of physical activity (more than 90 minutes per day) reduce risk of diabetes by 28%. Dietary changes known to be effective in helping to prevent diabetes as food rich in whole grains and fibers and choosing good fats such as polyunsaturated fats found in nuts, vegetable oils and fish. Reducing sugary drink consumption and eating less red meat and other sources of saturated fat can also help to prevent diabetes. Tobacco smoking is also associated with an increased risk of diabetes and its complications so smoking cessation can be an important preventive measure as well. The World Health Organization recorded Relationship between

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type 2 diabetes and main modifiable risk factors (excess weight, unhealthy diet, physical inactivity and tobacco use) are similar in all regions of the world (2).

**Growth Differentiation Factor 15**

There has been a striking increase in the prevalence of type 2 diabetes mellitus (T2DM) as well as that of prediabetes. In the prediabetic stage, lifestyle modifications and the use of some drugs such as metformin and α-glucosidase inhibitor can modify insulin resistance (IR) and hence delay or reduce the progression to T2DM. For achievement of this outcome, it is necessary to identify prediabetic patients earlier with the use of a biomarker. Several markers for predicting prediabetes have been proposed. Acute phase proteins (C-reactive protein [CRP], plasminogen activator inhibitor-1) and coagulation factors (fibrinogen, D-dimer) are considered markers that can predict prediabetes (16).

However, these parameters are more correlated with cardiovascular risk than IR. Therefore, a novel marker that is based on the pathogenesis of prediabetes and T2DM is necessary. IR is already present in the prediabetic stage. T2DM develops when the IR becomes more severe, and the pancreatic β-cells fail to compensate for IR (9).

The sequential progression from normal glucose tolerance (NGT) to T2DM through prediabetes is associated with multifactorial components. Chronic inflammation may be a contributing factor for the development of T2DM in a nondiabetic population. In a large cohort study, patients with prediabetes who had high levels of acute phase proteins (e.g., CRP, plasminogen activator inhibitor 1) converted to T2DM more frequently than those who had lower levels of acute phase proteins (17).

Because T2DM is an inflammatory disease, treatment with salicylates and interleukin-1 (IL-1) antagonists lowered blood glucose levels and attenuated the T2DM-associated complications. Inflammation was associated with increased IR rather than decreased insulin secretion. Inflammation in the prediabetic stage accentuated the cardiovascular risk by more than 1.56 times that in the NGT group (17).

Growth Differentiation Factor 15 (GDF15) is a divergent member of the transforming growth factor B (TGF B) superfamily. The role and the downstream mechanism of GDF 15 have not been yet clearly elucidated. According to many studies, GDF 15 is associated with cancers, cardiovascular diseases and inflammatory diseases. The putative role of GDF 15 is that of a stress or inflammation responsive cytokine. As one of the aspects of the inflammatory diseases of T2DM, elevated levels of GDF 15 were found to be associated with the presence of T2DM and the future development of T2DM. However, the relationship between GDF 15 and prediabetes has not yet been investigated (18).

**Nature and Functional mechanism of GDF 15:**

Growth/differentiation factor 15 (GDF15) was first identified as Macrophage inhibitory cytokine-1 or MIC-1. Under healthy conditions, GDF15 is expressed at low levels in all organs, whereas it is expressed in high concentrations in the liver, kidney, heart and lungs in response to stress signals throughout adult life (19).

Macrophages play a key role in both normal and pathological processes involving immune and inflammatory responses, to a large extent through their capacity to secrete a wide range of biologically active molecules. To identify some of these as yet not characterized molecules, a subtraction cloning approach designed to identify genes expressed in association with macrophage activation had been used.

One of these genes, designated macrophage inhibitory cytokine 1 (MIC-1), encodes a protein that bears the structural characteristics of a transforming growth factor β (TGF-β) superfamily cytokine. Although it belongs to this superfamily, it has no strong homology to existing families, indicating that it is a divergent member that may represent the first of a new family within this grouping. Expression of MIC-1 mRNA in monocytoid cells is up-regulated by a variety of stimuli associated with activation, including interleukin 1β, tumor necrosis factor α (TNF-α), interleukin 2, and macrophage colony-stimulating factor but not interferon γ, or lipopolysaccharide (LPS). Its expression is also increased by TGF-β. Expression of MIC-1 in CHO cells results in the proteolytic cleavage of the propeptide and secretion of a cysteine-rich dimeric protein of Mr 25 kDa. Purified recombinant MIC-1 is able to inhibit lipopolysaccharide -induced macrophage TNF-α production, suggesting that MIC-1 acts in macrophages as an autocrine regulatory molecule. Its production in response to secreted proinflammatory cytokines and TGF-β may serve to limit the later phases of macrophage activation (19).
Macrophages are key cells in immune and inflammatory responses, participating in many normal biological processes including wound healing and resistance to tumors and infections. These cells are also important mediators of the pathology of a range of chronic inflammatory and fibrotic disorders such as rheumatoid arthritis, atherosclerosis and pulmonary fibrosis. The macrophage’s role in these processes is accomplished in large part through its capacity to secrete bioactive molecules including enzymes, lipids, and a wide range of cytokines. To a large extent, it is the secretion of a complex mixture of cytokines into the surrounding milieu that mediates the effects of the macrophage on surrounding cells such as lymphocytes, fibroblasts, and endothelial cells. Production of these cytokines is usually under stringent control with a requirement for cell activation prior to their local production. (19).

Role of GDF 15 in human body:

Growth differentiation factor-15 (GDF-15) is a stress responsive cytokine. It is highly expressed in adipocytes cardiomyocytes, macrophages, endothelial cells, and vascular smooth muscle cells in normal and pathological condition. GDF-15 increases during tissue injury and inflammatory states and is associated with cardiometabolic risk. Increased GDF-15 levels are associated with cardiovascular diseases such as hypertrophy, heart failure, atherosclerosis, endothelial dysfunction, obesity, insulin resistance, diabetes, and chronic kidney diseases in diabetes. Increased GDF-15 level is linked with the progression and prognosis of the disease condition. Age, smoking, and environmental factors are other risk factors that may increase GDF-15 level. Most of the scientific studies reported that GDF-15 plays a protective role in different tissues. However, few reports show that the deficiency of GDF-15 is beneficial against vascular injury and inflammation. GDF-15 protects heart, adipose tissue, and endothelial cells by inhibiting JNK (c-Jun N-terminal kinase), Bad (Bcl-2-associated death promoter), and EGFR (epidermal growth factor receptor) and activating Smad, eNOS, PI3K, and AKT signaling pathways. It is a challenge for the scientific community to use GDF-15 information for patient monitoring, clinical decision-making, and replacement of current treatment strategies for diabetic and cardiovascular diseases (20).

Growth differentiation factor-15 (GDF-15) is a stress-responsive cytokine. It plays an important role in the regulation of the inflammatory response, growth and cell differentiation.

Elevated GDF-15 was found in patients with established CV diseases including hypertension, stable coronary artery disease, acute coronary syndrome, myocardial infarction, ischemic and non-ischemic-induced cardiomyopathies, heart failure, atrial fibrillation, as well as stroke, type two diabetes mellitus (T2DM), chronic kidney disease, infection, liver cirrhosis, malignancy. Therefore, aging, smoking, and various environmental factors, i.e. chemical pollutants are other risk factors that might increase serum GDF-15 level. Although GDF-15 has been reported to be involved in energy homoeostasis and weight loss, to have anti-inflammatory properties, and to predict CV diseases and CV events in general or established CV disease population, there is no large of body of evidence regarding predictive role of elevated GDF-15 in T2DM subjects (21).

GDF 15 and Prediabetes:

The putative role of GDF 15 is that of a stress or inflammation responsive cytokine. As one of the aspects of the inflammatory diseases of T2DM, elevated levels of GDF 15 were found to be associated with the presence of T2DM and the future development of T2DM. However, the relationship between GDF 15 and prediabetes has not yet been investigated (22).

However, Rajput et al (22) reported that, it is convenient to measure GDF 15 level with one fasting sample, and consistent protein stability data were observed. The GDF 15 level is based on the response to chronic inflammation and its compensatory secretion in IFG and T2DM.GDF 15 is highly associated with IR, and the levels were significantly different between the NGT and the IFG groups. Hence, GDF 15 might be a novel biomarker for detecting IFG.

Conflict of Interest: No conflict of interest.

REFERENCES


