Type 2 Diabetes Mellitus: A Review

Duha Awad AlAwad

Abstract:
The body's inability to maintain constant normal level of blood glucose could lead to fatal consequences. Diabetes is a condition characterized by chronic hyperglycemia. The high levels of glucose could lead to complication such as nephropathy, neuropathy and retinopathy among many more. The umbrella of diabetes includes type 1 diabetes, type 2 diabetes and gestational diabetes. Although a brief preamble is presented about the three types of diabetes, this paper will emphasize on type 2 diabetes by virtue of its high prevalence. The pathogenesis of type 2 diabetes, screening methods and relevant biomarkers are also discussed in this paper.

Keywords: Type 2 diabetes, diabetes, Haptoglobin, Oxidative stress, Inflammatory markers.

1. Introduction:

According to WHO the number of people diagnosed with diabetes has increased by almost four folds in the duration between 1980 and 2014 and is expected to further increase(1). Diabetes is a prevailing non-contact disease that results in many life-threatening complications. Symptoms of diabetes include frequent urination (polyuria), thirst (polydipsia), fatigue, ketoacidosis (in type 1 diabetes) (3, 4), and eating more frequently (polyphagia)(5).. Exposure to high levels of glucose for long periods of time results in oxidative stress(6) and low grade chronic inflammation(7), both of which contribute to the complications of diabetes. The most common complications of the chronic hyperglycemia in diabetic patients include nephropathy, neuropathy, retinopathy and cardiovascular risks(8). As a result of their contribution to the development of diabetic complications, biomarkers for oxidative stress and inflammation can provide useful markers for early detection and monitoring of diabetes. Moreover, proteins like haptoglobin and enzymes like LDH and aminotransferases are also potential biomarkers for diabetes.

2. Gestational Diabetes:

During normal pregnancy women develop insulin resistance to decrease glucose uptake by the maternal cells to ensure that enough glucose is reaching the fetus. Adequate levels of glucose are important for the normal growth of the fetus, as glucose stimulates the production of fetal insulin which plays a role in the fetal growth. Insulin resistance normally occurs during the second trimester. However, if glucose levels exceeded the normal ranges for a pregnant woman, there is a likelihood that she is suffering from gestational diabetes mellitus (GD). GD is defined as the inability to tolerate carbohydrates resulting in hyperglycemia during pregnancy, but the condition ceases to exist after delivery. The risks associated with GD includes the likelihood of cesarean section, macrosomia (large baby with birthweight exceeding 4kg), and a higher risk for developing type 2 diabetes and cardiovascular diseases. GD can be screened for by oral glucose tolerance test (OGTT). The recommended treatment for GD is lifestyle changes and in some cases the use of oral hypoglycemic drugs such as metformin or insulin therapy (the administration of insulin through needle injection)(9).

3. Type 1 Diabetes

Type 1 diabetes (T1D) is an autoimmune disease where the immune system attack and destroy the cells responsible for the production of insulin, the Beta-Islet of Langerhans. This

type of diabetes constitutes less than 10% of total diabetic individuals and is prevalent among children. Both genetic factors related to HLA, and environmental factors (such as viral infections that destroy the beta islets) contribute to this type of diabetes.
In the pathogenesis T1D, the T cells of the immune system fail to recognize Beta islets cells as self-cells. The failure of the processes that mediate immune cells' natural tolerance causes these cells to produce autoantibodies which attack products of the Beta islets such as insulin, proinsulin and ZNT8 (Zinc transporter 8) (10). The autoantibodies are produced before the onset of the disease and can be used for its early detection. Moreover, the close position of the Beta islets to the endothelial cells plays a role in the exposure of the beta islets cells' autoantigen, which are then recognized and attacked by the T cells (3).
The complications of T1D are a result of the destruction of the beta islets which leads to the reduction of insulin production. Low insulin levels disable the cells' ability to uptake glucose in the blood, which leads to the starvation of the cells. The low levels of insulin could be compensated for by the administration of insulin therapy(4).

4. **Type 2 diabetes**

Type two diabetes (T2D) is the most prevalent. It constitutes 90% to 95% of the total cases of diabetes. In T2D diabetes insulin is normally produced by the beta islets of Langerhans, but the cells' response to insulin is impaired (this is termed as insulin resistance)(4). A feedback loop between beta islets and insulin sensitive tissues (liver, adipose tissue and muscles(11)) control glucose levels in the blood. Insulin is released in response to elevated blood glucose to stimulate tissue uptake of glucose. Sometimes, as a result of normal physiological changes (pregnancy and ageing), tissues begin to develop insulin resistance. Insulin resistance reduces the effect of insulin on tissues hence decreasing their ability to uptake glucose. When such a phenomenon occurs, beta islets increase the production of insulin to compensate for the deficiency in glucose uptake(12). T2D develops when the need of insulin exceeds the capacity of insulin production by beta islets, and the function of beta islets decline(11).
It is worth mentioning that a fourth classification of diabetes exists and is rarely mentioned or discussed. This classification is termed 'Specific types of diabetes due to other causes' and it includes drug/chemical induced diabetes, neonatal diabetes and MODY (maturity onset diabetes of the young) among many more(13). This paper will not further discuss this class of diabetes as its main concern is T2D.

4.1 Pathophysiology of Type 2 diabetes

The development of T2D diabetes requires the presence of two conditions, insulin resistance (IR) and beta islets abnormality. Obese individuals are very likely to suffer from insulin resistance and therefore develop T2D because of the accumulation of adipose tissue in their body, since adipocytes play a role in the development of insulin resistance. Insulin resistance is caused mainly by the release of non-esterified fatty acids (NEFAs)(11) and proinflammatory cytokines (interlukein-6 and tumor necrosis factor)(14) from the adipocytes. An increase in NEFA leads to an increase in intracellular metabolites of fatty acids. These metabolites result in the phosphorylation of insulin receptor substrate-1 (IR1) and insulin receptor substrate-2 (IR2) by serine/threonine kinase. This process results in the declination of of the insulin-receptor signaling mechanism(11). Proinflammatory cytokines also cause the phosphorylation of IR substrates, thereby inducing IR(14).
Healthy beta islets are able to recompense for insulin resistance by excreting more insulin. Dysfunctional beta islets on the other hand cannot. One mechanism through which hyperglycemia can cause the dysfunction of beta islets is through oxidative stress. Excess glucose leads to excess free reactive oxygen species (ROS) such as superoxide anion (O_2^-) which cause the auto destruction (apoptosis) of beta islets(14). This leads to impaired glucose

tolerance and impaired fasting glucose (prediabetes) and finally to the development of T2D. Beta islets become unresponsive and their function decrease by 75% in diabetic patients. Thus, measurements of beta islet function are of significant in determining individuals at risk of developing diabetes, especially if they exhibit risk factors such as obesity (adipose tissue produces proinflammatory cytokines and NEFAs which contribute to insulin resistance)(11). New studies suggest that alpha islets of Langerhans (glucagon producing cells) play a role in the pathogenesis of type 2 diabetes(15). The pathophysiology of diabetes is summarized in figure 1.1.

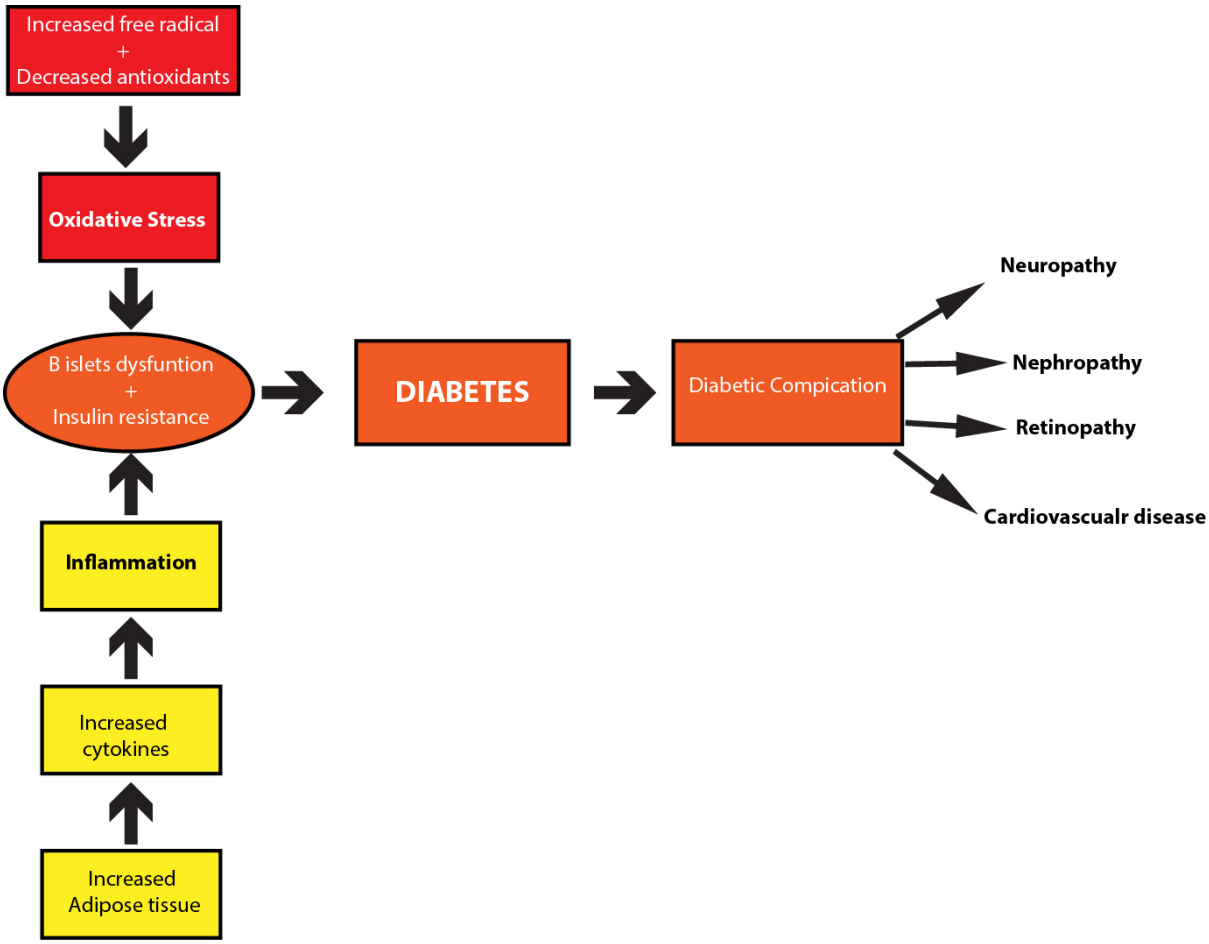

Figure 1.1: This diagram exhibits the relation between oxidative stress, inflammation and diabetes. Oxidative stress damages cellular components and can directly result in the destruction of B islet cells. Inflammation contributes to insulin resistance and endothelial dysfunction in diabetes(2). Further discussion of these processes and the ability to use their indicators as biomarkers for diabetes is found in later sections.

4.2 Screening and diagnosis of type 2 diabetes

According to the American Diabetes Association (ADA), screening(15) and diagnosis of diabetes (as well as prediabetes) can be performed in two ways, either by measuring the plasma glucose or HbA1C(13). The criteria for the diagnosis of diabetes for the based on plasma glucose and HbA1c (proposed by ADA) is summarized in table 1.1.

Table 1.1 – Diagnostic criteria for diabetes

FPG	Greater than or equivalent to 126mg/dL
2hPG	Greater than or equivalent to 200mg/dL
HbA1c	Greater than or equivalent to 6.5%
Presence of symptoms (polyuria, polydipsia, etc.)	Greater than or equivalent to 200mg/dL

Table 1.1: A summary for the criteria of diagnosis of diabetes proposed by the ADA(13). If a patient's results meet any criteria the patient is diagnosed as diabetic. If the results are at the equivalent to the range provided (equivocal), the test should be repeated and confirmed by performing a second test.

4.2.1 Plasma Glucose

The plasma glucose assessment includes measuring fasting plasma glucose (FPG) or 2-h plasma glucose (2-h PG) after the ingestion of 75g of oral glucose during an OGTT(13). OGTT stands for glucose tolerance test, which is the method used to diagnose diabetes based on changes in plasma glucose levels following the ingestion of a glucose load. To perform the test, blood is drawn from a fasting patient to determine FPG. The patient is then given 75g of oral glucose, and their blood glucose is measure after two hours (2hPG)(16).

4.2.2 HbA1c

The HbA1C measures the amount of glycated hemoglobin (an irreversible attachment between glucose and the N-terminal of hemoglobin) in the blood, giving an indication about the glucose concentration in the blood over an extended period of time(17). Although HbA1c is frequently used to diagnose diabetes, an exact threshold to differentiate between diabetic and healthy individuals does not exist(15). Moreover, the sensitivity of HbA1c at the designated cutoff value (6.5%) is poor(13). However, HbA1c remains the preferred method to detect hyperglycemia as it is less affected by biological changes, resembles blood glucose levels over several months (2-3) and is more acceptable by patients than the ordinary plasma glucose OGTT(18).

5. **Biomarkers of diabetes and its complications**

The main tool used to diagnose diabetes(13) and monitor blood glucose over the course of a long period of time (about two to three months) is HbA1C. Biomarkers are biological molecules that can be used to detect diseases and monitor treatments(18). Biomarkers are either direct products of the diseased tissue or are indirect products of other tissues stimulated by the diseased tissue(19). Biomarkers are important in detecting diabetes and its associated complications. They allow early identification before the manifestation of the disease complications. Thereby giving individuals the chance to apply protective measurements to prevent the consequences of the disease(20).

5.1 Inflammatory markers

Low grade inflammation has been observed in diabetic patients(21). During inflammation, chemicals are released to regulate inflammatory responses. Since chemicals of inflammation are released during the progression of diabetes mellitus, identification and measurement of these chemicals can allow the early detection of diabetes and its complications(22). Cytokines are small polypeptides important in regulating immune responses(23). Three important cytokines released during inflammation are interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-a)(24) and high sensitivity C-reactive protein (Hs-CRP)(25). Many immune cells, such as macrophages, neutrophils, and adipocytes, produce TNF-a. TNF-a has

been linked to diabetes as a result of its contribution to insulin resistance seen in obese patients(24). IL-6, an immune protein consisting of 184 amino acids, is produced by T-cells, macrophages, and adipocytes(26). Hs-CRP, an inflammatory protein produced mainly by the liver(27), was proven to be a sensitive marker for low grade inflammation(28). Moreover, cytokines can be used for detecting individuals at of risk of developing diabetes. Studies on Turkish females(29) and Italian Caucasians(30) suggest that IL-6 and TNF-a levels increase with impaired glucose tolerance. However, a study on Korean patients opposes these results(31). Further studies are required to establish a cohort link between TNF-a, IL-6 and IGT. However, TNF-a and IL-6 are candidates, which can be utilized as biomarkers for the early detection of IGT, which if not managed appropriately could progress to diabetes(32).

5.2 Oxidative damage markers

Free radicals, the major participants in oxidative stress, are a considered the main cause of the gradual manifestation of diabetic complication due to their ability to damage proteins, DNA and lipids(33). Therefore, markers that can detect oxidative stress might be helpful in predicting the level of damage caused by diabetes. The biomarkers for oxidative stress could be divided into six main groups; proteins, lipids, vitamins, glutathione, catalase, and superoxide dismutase(34).

5.2.1 Proteins

Laboratory experiments have shown that proteins can become modified or dysfunctional after exposure to free radicles such as ROS(35). Chronic hyperglycemia in diabetic patients induces the formation of advanced glycated end products (AGEs). AGEs are formed when lipids, amino acids or nucleic acids react with reducing sugars in the absence of enzymatic catalysis(36). Elevation of AGEs have been reported in diabetic patients(37). An example of AGEs is HbA1c which is the gold standard to diagnose diabetes today(13, 34). AGEs can cause subsequent modification in protein involved in gene expression(38). Some circulating proteins have AGE receptors. AGEs bind to these proteins and modify them, in the production of growth factors and cytokines from endothelial cells(39). In addition, oxidation of proteins results in the formation of carbonyls. High levels of carbonyls were observed in diabetic patients(40). Therefore, carbonyls ca act as a useful biomarker for oxidative stress(41). High levels of carbonyls contribute the the pathogenesis of diabetic nephropathy(42).

5.2.2 Lipids

Another target for ROS are lipids. Major changes are observed in lipid structure and function of diabetic patients, especially those suffering from vascular complications(43). Malondialdehydes (MDA) are compounds formed from the reactions between transitions metals (such as copper and iron) and hydroperoxide. It can react with and damage the cell membrane(44). MAD is an important biomarker to detect lipid peroxidation due to oxidative stress(45, 46). An increase in MDA in diabetic patients(47) suggests its contribution to the complications of diabetes. High levels of MDA also indicate defective antioxidant activity in the body(48). Studies have linked lipid peroxidation to the development of atherosclerosis and neural complications(49, 50).

5.2.3 Vitamins

Vitamins like vitamin A, C, and E are antioxidant and can neutralize free radicles(51), hence protecting the body from oxidative stress. When the balance between free radicles and antioxidants is decreased, compilations of diabetes arise(52). In addition to its antioxidant activity, vitamin A is assumed to play a role in lipid metabolism in the liver and B islets function(53). Another vitamin that is proposed to be associated with T2D is vitamin D. B islets have vitamin D receptors (VDR). Vitamin D was shown to promote the secretion of

inulin from B islets obtained from animal tissue after it binds to VDRs(54). In addition, vitamin D deficiency was associated with inflammation, hence linking it to diabetes(55). However, a recent study (in 2015) by Liefaard et al. found no evidence for a relation between levels of CRP and vitamin D(56). Despite the discrepancies, vitamin D, is being heavily studied to determine whether it can be used to decrease hyperglycemia in diabetics(57). Measuring changes in the levels of vitamins is important, as they can be indicative of underlying oxidative damage(34).

5.2.4 Glutathione

Glutathione (GSH) is a tripeptide (58) that have many functions in the body, one of which is the protection against tissue damage(59). GSH, a competent antioxidant that is present in most of cells, can be used as a biomarker for the detection of imbalance between free radicles and antioxidants(60). A study by Calabrese et al. have reported a decrease in GSH levels in diabetic patients(61). Another study by Dinçer et al. has nominated low levels of GHS as a participant in the oxidative damage of DNA in diabetic patients(62). Glutathione disulfide (GSSG) is reduced by glutathione reductase (GSR) to GSH through a redox reaction involving the oxidation of NADPH to NADP+. GSH is regenerated by GSR, however, excess ROS can result in the disturbance of GSR function(63). Moreover, low levels of GSH were associated with an increased likelihood of infections in diabetics, and a potential use of GSH as a therapeutic agent to decrease susceptibility of diabetics to infections(64).

5.2.5 Catalase

Catalase (CAT) is an enzymatic antioxidant that catalyzes the metabolism of hydrogen peroxide. Hydrogen peroxide is a toxic molecule that can damage DNA, RNA, proteins, and lipids if present in high concertation(65). CAT neutralizes hydrogen peroxide into oxygen and water. B islets are rich in mitochondria, which can act as a source of large amount of ROS. A study has suggested that CAT deficiency leads to oxidative stress on B islets, which results in their subsequent dysfunction, contributing to the development of diabetes(66). Another study reported that hyperglycemia lead to increased hydrogen peroxide levels and reduced CAT levels by altering gene expression in addition to other effects(67).

5.2.6 Superoxide dismutase

Superoxide dismutase (SOD) is another enzymatic antioxidant that catalyzes the oxidation and reduction of superoxide anions into hydrogen peroxide and oxygen(68). SOD neutralizes the main free radical produced during oxygen metabolism, that is superoxide anion(34). There are three main forms of SOD, which are products of three different genes(69). These are; SOD1 (or Cu-Zn-SOD), SOD2 (or Mn-SOD), and SOD3 (or EC-SOD), each of these forms is found in the cytosol, mitochondria, and extracellular space respectively(70). Moreover, superoxide radicals react with nitric oxide (NO) to produce peroxynitrite radicals(71). Wang et al. suggested through a study that antioxidant supplements containing SOD or overexpression of SOD could protect against diabetes(72). Moreover, Kim has observed that patients suffering from diabetic skin have high levels of ROS and suggested that EC-SOD can be the underlying cause for the alterations observed in diabetic skin(73).

5.3 Haptoglobin

Iron, although very important for the normal cell function, can be toxic to cells. It is easily oxidized and reduced. Therefore, iron can frequently contribute to the generation of free radicles, which cause cellular damage(74). Haptoglobin is a glycoprotein that plays an important role in recycling iron and preventing its toxicity (75, 76). Haptoglobin bind to free hemoglobin in the blood to prevent iron loss. Moreover, when haptoglobin binds to hemoglobin, it prevents iron in hemoglobin form enhancing the formation of free radicles such as hydroxyl radical(77), which can damage tissues and cells(78). The ability of haptoglobin to reduce the toxic effects of free radicals depends on the genotype of

haptoglobin(79). Three alleles code for three proteins, which are Hp1F, Hp1S, and Hp2(80). Two of these proteins are linked together through disulfide bonds to give a single polypeptide(81). As a result, three different genotypes of haptoglobin exists. These are: Hp1-1 dimer, Hp1-2 heterodimer, and Hp2-2 dimer, with Hp2-1 occurring the most(82). A study by Melamed-Frank et al. suggests that Hp1-1 provides the greatest antioxidant activity among the three phenotypes(79). Therefore, if diabetic patients possess the Hp2-1 phenotype, they are more likely to suffer the consequences of oxidative damage(83).
Moreover, diabetic patients with Hp2-2 genotype are more likely to suffer from cardiovascular complications(84), retinopathies(85), infections(86, 87), and neuropathies(88). Studies have shown that Hp2-2 is correlated with increased severity of myocardial infarctions and higher risks of developing cardiovascular complications (84, 89). Another study shows that individuals with Hp2-2 genotype are at a higher risk for developing sever retinopathy(85). Also, haptoglobin has been proven to prevent infectious disease by removing iron from circulations, which is a necessary nutritional requirement for some infectious pathogens(90). This benefit is applicable to Hp1-1 genotype as it has an improved function in clearing hemoglobin from circulation(79). On the other hand, Hp2-2 allows hemoglobin to remain in circulation for extended periods of time(79) thereby increasing chances of infections. Finally, although the exact mechanism of neurodegeneration that occur in diabetic patients is not fully understood, oxidative stress provides a possible explanation(91). Since Hp genotypes are related to the occurrence of oxidative stress(79), there's a likelihood that they are also associated with neurodegeneration seen diabetic patients. From here, it is clear that haptoglobin genotyping can be a valuable marker to detect risk factors associated complication of diabetes. Determining genotypes of haptoglobin is done traditionally be done through electrophoresis. However, scientists have developed another method that utilizes ELIZA to determine haptoglobin genotypes(92).

5.4 LDH and aminotransferases

Diabetes has pathophysiological effects on the salivary glands function. Measurements of products produced by these glands can be of value in clinical practice. LDH and aminotransferases increase in T1D and T2D due to the damage caused to salivary cells, releasing these enzymes. Cinquini et al(93) and Malicka et al(94) both suggested that the rise in LDH and aminotransferases is more closely linked to T1D, as they have suggested that the autoimmune disease in T1D results in the destruction of salivary cells(94). The use of LDH and aminotransferases as biomarkers of T2D diabetes remains an understudied topic and requires further improvements before it can be introduced into the clinical practice.

6. **Conclusion**

T2D is a result of insulin resistance accompanied by beta islet dysfunction. The development of T2D has both genetic and environmental factors underlying it. Early detection of T2D is very important in the prevention of its associated complication. Since inflammation and oxidative stress are observed in diabetes, biomarkers for these two conditions could be used for the early detection of the disease, delaying or preventing its complications, and for monitoring therapeutic drugs. Other Biomarkers such as haptoglobin, LDH and aminotransferases are possible candidates to be used as protein and enzymatic biomarkers for diabetes, however, more studies are required on both them.

Abbreviations:

Hp → Haptoglobin
IGT → Impaired glucose tolerance
IL-6 → Interleukin-6
IR → Insulin resistance
LDH → Lactate dehydrogenase
T1D → Type 1 diabetes
T2D → Type 2 diabetes
TNF-a → Tumor necrosis factor-alpha
ROS → Reactive oxygen species
AGE → Advanced glycation end products
VDR → Vitamin D receptor
GSSG → Glutathione disulfide
GSH → Glutathione
GSR → Glutathione reductase
CAT → Catalase
SOD → superoxide dismutase

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