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Diabetes mellitus: A review of some of the prognostic markers of response to treatment and management

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Background: The WHO defined 'diabetes mellitus' (DM) as a metabolic disorder characterised by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from the defect in insulin secretion, or inaction, or both. When not identified early and controlled, acute and chronic life-threatening consequences may result. Identifying DM early for treatment and management, as well as clinically monitoring recovery and improvement during treatment, involves the assessments of biomarkers. The types, choice, sensitivity and descriptive information trends of these biomarkers are very important.

Aim: Some prognostic biomarkers and parameters that this review identified include glycated haemoglobin, white blood cells, mean neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, total leukocytes and neutrophils, plasma low-density lipoprotein, high-density lipoprotein and very low-density lipoprotein, platelet, fibrinogen, D-dimer and C-reactive proteins.

Results: These parameters display increases in DM, while red blood cell, haemoglobin concentration, activated partial thromboplastin time, prothrombin time and partial thromboplastin time are decreased.

Conclusion: With sound knowledge of the variations of these markers and parameters, observed reversal during treatment and management of DM and its complications can be better monitored, and guided decisions can be made.

Introduction

Diabetes mellitus (DM) can be said to be a metabolic disorder characterised by abnormalities in the metabolism of carbohydrates, protein and lipids, resulting from inadequate insulin release (type 1 diabetes) or reduced insulin sensitivity and elevated blood insulin (type 2 diabetes). The underlying culprit being severe chronic hyperglycaemia, leading to metabolic dysregulation and cardiovascular complications especially in type 2 DM (T2DM).[1] Some of the possible pathophysiological and biochemical causes of hyperglycaemia, which occur in isolation or in combination, have been proposed as mechanisms underlying T2DM[2]. These mechanisms include: (1) reduced insulin secretion from pancreatic βcells, (2) elevated glucagon secretion from pancreatic α-cells, (3) increased production of glucose in the liver, (4) neurotransmitter dysfunction and insulin resistance (IR) in the brain, (5) enhanced lipolysis, (6) increased renal glucose reabsorption, (7) reduced incretin effect in the small intestine and (8) impaired or diminished glucose uptake in peripheral tissues such as skeletal muscle, liver and adipose tissue. DM encompasses a wide range of disorders characterised by hyperglycaemia (high blood sugar) associated with multiple disorders including metabolic, cellular, organ and blood disturbances leading to vascular complications.[3]

When DM is not properly controlled, this condition can result in severely increased glucose levels (hyperglycaemia) and consequent increase in the risk of atherosclerosis and cardiovascular diseases.[4] Other complications that arise as a result of uncontrolled DM could include retinopathy, nephropathy and neuropathy,[5] as well as cellular and tissue damage through glycosylation of amino acid residues, resulting in the formation of advanced glycation end products like glycated haemoglobin (HbA1c), glycated low-density lipoprotein (LDL), glycated connective tissues and so on.

In the treatment and management of DM, a single drug therapy using an oral medication should be started along with intensive lifestyle modifications. The major classes of oral anti-diabetic drugs

could include biguanides, sulphonylureas, meglitinides, thiazolidinedione, dipeptidyl peptidase-4 inhibitors, sodium-glucose cotransporter inhibitors and α-glucosidase inhibitors. If after administration of the single drug therapy the plasma level of HbA1c still rises to 7.5%, or if the initial HbA1c is ≥9%, combination therapy with two oral agents, or with insulin, may be considered.[6,7]

Basically, DM treatment and management are usually based on either insulin (hormone) therapy and/or oral hypoglycaemic (drug therapy, especially for type 1 DM, T1DM), which has many side effects such as weight gain, hypoglycaemia, gastrointestinal disturbances, hypersensitivity reactions and so on.[8] The side effects of these drugs have generated great interest among health practitioners, DM researchers and medical biochemists alike. Of even more controversy is the monitoring of the disease during the course of drug administration, specifically to see are the most appropriate predictive markers and how sensitive are these markers in aiding and guiding medical practitioners and researchers in making appropriate decisions for and giving advice to these DM patients.

Alternative botanical compounds or extracts and diet-based therapies for the management of DM have long been suggested and more works are ongoing to support the reported claims of their efficacy.[9] It has been said that these plants contain important phytochemicals, phytonutrients and dietary fibres (the fibre hypothesis), which serve as nutraceuticals and ingredients for functional foods.[9,10] However, in diagnosing the onset and monitoring the level of improvement of the DM condition during the course of treatment and management, some important blood-based markers have been reported to be very relevant and sensitive prognostic markers.

Biomarkers discussed in this review include standard and conventionally utilised biomarkers such as fasting blood glucose, HbA1c, fasting blood insulin, homeostatic model assessment of IR (HOMA-IR) and perhaps the triglyceride-to-high-density-lipoprotein (HDL) ratio, which remain the major preclinical and clinical diagnostic interventions. Nonetheless, some other biomarkers, like white blood cells (WBCs), mean neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), total leukocytes and neutrophils, plasma LDL, HDL, very low-density lipoprotein (VLDL), plasma platelets, plasma fibrinogen, plasma D-dimer, C-reactive proteins (CRP), red blood cells (RBCs), haemoglobin concentration, prothrombin time (PT) and partial thromboplastin time (PTTK), et cetera may provide vital information, especially the markers of immunity (autoimmune antibody markers in T1DM). This review identifies some of these markers and their biochemical basis as key markers of DM and their ready availability in at least moderately equipped health facilities. Monitoring these markers and parameters can help inform treatment decisions by healthcare workers and lead to better patient health.

Markers of anaemia

When reference is made to anaemia, the effect of glucose on the biochemical integrity of haemoglobin or cellular morphology of RBCs and components of haemoglobin synthesis are the points of focus. Several haematological changes affecting the RBCs, WBCs and the coagulation factors are shown to be directly associated with DM.[11,12] Haematological abnormalities reported in DM patients include RBCs, WBCs and platelet dysfunction.[13,14] Anaemia can be defined as a haemoglobin level <13.5 g/dL in men and 12.0 g/dL in women.[15]

The degree of anaemia in diabetes patients can be associated with a number of factors, including glomerular filtration rate, urinary albumin excretion rate and HbA1c levels.[16,17] Anaemia has been reported to be a result of diminished erythropoietin production by failing kidneys (in the nephrotic syndrome characterised by oedema, hypoalbuminaemia, dyslipidaemia and urine protein-to-creatinine ratio ≥3) and increased non-enzymatic glycosylation of RBC membrane proteins.[16,18] In research carried out by Erukainure et al.[16] the authors reported alterations in the RBC, haemoglobin and packed cell volume levels of the diabetic rats, suggesting the occurrence of anaemia.

Diabetes does not directly cause anaemia, but certain complications and conditions associated with diabetes can contribute to it. For example, both diabetes-related kidney disease (nephropathy; in people who have a type of neuropathy called *autonomic neuropathy*, the body may not be able to properly signal the kidneys to produce more erythropoietin in response to anaemia) and nerve damage (neuropathy) can contribute to the development of anaemia. In addition, taking certain oral diabetes drugs can raise the risk of developing anaemia. People with diabetes can also have anaemia as a result of not eating well, or of having a condition that interferes with the absorption of nutrients.

Metformin is the most widely prescribed treatment for people with T2DM. This drug has been recognised to cause malabsorption of vitamin B12 and long-term use (12–15 years) leads to vitamin B12 deficiency in 30% of people who use it. Vitamin B12 deficiency can cause anaemia and also peripheral neuropathy (nerve damage in the feet, legs, hands and arms). In addition, thiazolidinediones, which include pioglitazone (Actos) and rosiglitazone (Avandia), can also cause mild anaemia by slightly decreasing haemoglobin levels and haematocrit, a measurement of the proportion of blood that is made up of RBCs. According to Mehdi and Toto,[19] Marathias et al.[20] and Mohanram et al.,[21] angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor antagonists may cause a reversible decrease in haemoglobin concentration in patients with diabetes. The mechanisms by which ACE inhibitors and angiotensin receptor blockers lower haemoglobin include a direct blockade of the pro-erythropoietic effects of angiotensin II on red cell precursors, degradation of physiological inhibitors of

haematopoiesis and suppression of insulin-like growth factor – 1 (IGF-I). Long-term administration of losartan in 50 mg to 100 mg doses once daily in patients with diabetes and albuminuria is expected to lower haemoglobin by approximately 1 g/dL. Importantly, this effect does not diminish the renoprotective effect of losartan. It should be recognised that these classes of agents may induce or worsen symptomatic anaemia in nephropathy patients.

Mehdi and Toto[19] report that the major causes of anaemia in chronic kidney disease patients are iron and erythropoietin deficiencies and hypo-responsiveness (i.e. a requirement for high doses of erythropoietin in order to raise the blood haemoglobin level in the absence of iron deficiency, which is believed to represent impaired anti-apoptotic action of erythropoietin on pro-erythroblasts, being possibly caused by systemic inflammation and microvascular damage in the bone marrow) to the actions of erythropoietin. An observed improvement of the anaemic condition during the course of treatment and management of the DM condition through a better regulated glycaemic level is thus a good guiding prognostic information of the condition.

Immune components

Palacios et al.[22] and Egunyom et al.[23] report altered levels of WBCs, platelets and lymphocytes in diabetic rats, suggesting immune system suppression. These cells identify and eliminate pathogens, either by attacking larger pathogens through contact or by phagocytosis.[16] The reduced immunity can contribute to the various complications associated with DM.

According to Xu et al.[24] clinical researchers and medical biochemists alike have demonstrated that non-infectious systemic inflammation in T1DM patients with diabetes ketoacidosis (DKA) do have highly expressed pro-inflammatory cytokines, increased peripheral WBC count, very high total leukocytes and neutrophils but fewer eosinophils, which they claimed significantly correlated with the DKA and DK conditions of the patients. They submit that leukocyte counts of DM patients could be a powerful prognostic biomarker that could reflect the presence of hyperglycaemic crisis and acute infection.

Li et al.[25] support the claims of Xu et al.[24] when they report that the mean NLR was significantly higher in DM patients with high carotid artery intima-media thickness (cIMT) than the DM patients with low cIMT, who in turn showed a significantly higher NLR compared to non-DM subjects, indicating that acute phase inflammatory response increased with the degree of the disease. The cIMT is significantly greater and more pronounced in type 2 DM patients than in non-DM subjects. This serves as a well-established biomarker of subclinical atherosclerosis, which is a risk factor for cardiovascular disease and thus can be used to predict cardiovascular events.[25] The NLR has been reported to be a potential biomarker of inflammation in tumours,[26,27] cardiovascular conditions[28] and diabetes and its complications.[29,30] The precise mechanisms that lead to this higher level of NLR in DM are not quite clear, but Li et al.[25] claim that it might be a result of inflammatory responses. According to Hillson[31], poorer glycaemic control can be linked to a greater risk of infection. Hilson[31] and Woo et al.[32] claim that DM patients may have higher WBC and neutrophils than non-DM subjects.

It is important to suggest that the array of biochemical and physiological events that are initiated during the formation of atheroma in the endothelium of blood vessels through the influence of HbA1c and other advanced glycation end products (AGEs), including glycated LDL and reactive oxidative species, which attract macrophages that eventually form foam cells with a consequent release of inflammatory pharmacological chemicals, may also trigger the increased mobilisation and recruitment of these immune components. This further precipitates the immune response in response to the level and severity of the blood glucose. Demirtas et al.[33] in their study support my suggestions when they were able to demonstrate that altered WBC count, platelet count, PLR and NLR are closely associated with HbA1c levels in individuals with and without DM. Some of these parameters are closely associated with diabetic microvascular complications.

The over-expressed levels of WBC, mean NLR, PLR, total leukocytes and neutrophils in DM thus suggest and confirm that these parameters could avail as support and complementary information in monitoring the level of compliance of the condition of these patients to medications.

Markers of dyslipidaemia

T2DM is a part of the metabolic syndrome (MS), which comprises dyslipidaemia, hypertension and impaired haematological indices.[12,34] Abnormalities of lipid metabolism, particularly hypertriglyceridemia and low levels of HDL, are almost invariably found in patients with impaired glucose homeostasis (hyperglycaemia). Hypertriglyceridemia can lead to triglyceride-rich VLDL that potentiates platelet activity, an effect that is mediated partly through apolipoprotein E and an interaction with the platelet–LDL receptor.[35,36] The administration of reconstituted HDL to DM patients has been reported to promote cholesterol efflux from platelet membranes, which suppress aggregation.[37,38] In addition, the interaction between lipids and glucose that results in the formation of glycated LDL has been said to lead to impaired nitric oxide (NO) production and increased intra-platelet calcium concentration, further contributing to platelet hyper-reactivity,[38,39] which further complicates the DM condition. Thus, laboratory assessments of plasma LDL, HDL and VLDL can be a positive prognostic marker in the monitoring of DM.

Advanced glycation end products

Hyperglycaemia results in disturbances in cellular metabolism because of increased production of reactive

oxygen species and non-enzymatic glycation of many macromolecules, which lead to changes in cellular structure and function and formation of AGEs.[38] Recurrent episodes of hyperglycaemia lead to the non-enzymatic interaction between the carbonyl group of the reducing sugar and the primary amino group of a protein, leading to a cascade of reactions, the final result of which is a heterogeneous group of compounds known as *AGEs*.[38,40] The formation of AGEs enhances metabolic disturbances and also increases reactive oxygen species production via interaction with the specific receptor for AGE (RAGE).[41,42] HbA1c is one of the AGEs that involves the complex glycosylation of haemoglobin. HbA1c increases the formation of highly reactive free radicals inside the RBCs, thus altering its cell membrane properties, leading to blood cell aggregation and increased blood viscosity, with a concomitant impaired blood flow in severe cases like DM.[43] In addition, these events cause biochemical changes in structure and biophysical properties of the basement membrane, which further causes changes in permeability and vasodilatation of blood vessels.[44]

HbA1c also triggers inflammatory processes that lead to the formation of atherosclerotic plaque (atheroma). The build-up of free radicals leads to the stripping off of electrons from Fe^{2+}-haemoglobin through Fe^{3+}-haemoglobin forming abnormal ferryl-haemoglobin (Fe^{4+}-haemoglobin). The unstable Fe^{4+} further reacts with specific amino acids in the haemoglobin chain to regain its Fe^{3+} oxidation state. The haemoglobin molecules clump together via cross-linking reactions and this complexly cross-linked haemoglobin promotes cell damage and the release of Fe^{4+}-haemoglobin into the matrix of the sub-endothelium of arteries and veins, thus causing an increased permeability of the endothelium and further production of pro-inflammatory monocyte adhesion proteins, which promote macrophage accumulation in blood vessel surfaces, ultimately leading to the formation of harmful plaques in and around these vessels.[43]

Also, HbA1c goes through vascular smooth muscle layer and inactivates acetylcholine-induced endothelium-dependent relaxation, possibly through binding to NO, preventing its normal function. NO is a potent vasodilator and also inhibits the formation of plaque promoting the oxidation of LDL.[43] This overall degradation of blood cells also releases haem molecules from these cells, and these loose haem molecules can cause oxidation of endothelial and LDL proteins, also causing the formation of plaques.[43]

Normal levels of glucose produce normal levels of HbA1c. As the average amount of plasma glucose increases, the fraction of HbA1c also increases in a dose-dependent and positively correlated fashion. This serves as an indicator that blood sugar is progressively increasing and that necessary health actions should be taken.

The HbA1c test is an important blood test that gives a good indication of how well DM is being controlled. In addition to the fasting plasma glucose test, the HbA1c test is one of the main ways in which T2DM is diagnosed and monitored during treatment. HbA1c test is not the primary diagnostic test for T1DM but may sometimes be part of the laboratory regimen. Blood HbA1c concentration below 42 mmol/mol (6.0%) indicates non-DM, between 42 and 47 mmol/mol (6.0% – 6.4%) indicates impaired glucose regulation pre-DM and HbA1c of 48 mmol/mol (6.5%) or over indicates established T2DM.[43,45]

HbA1c is a chronic marker of hyperglycaemia and reflects the patient's blood glucose level over a period of 3–4 months, coinciding with the lifespan of the RBCs. However, in 2009 after its standardisation, the International Expert Committee recommended it to be used in diagnosing T2DM but not in T1DM and gestational diabetes.[6,46] The main advantage of the HbA1c test over other blood glucose tests is the convenience it offers to patients, as it does not require overnight fasting and can be done at any time of the day. However, HbA1c test is more expensive.[46] HbA1c may give a false positive result in conditions such as anaemia, haemolysis and other haemoglobinopathies like sickle cell disease and haemoglobin (Hb) variants like HbC, HbE and HbD, as well as elevated foetal haemoglobin. Thus, HbA1c assay in people of South Asian, Mediterranean or African origin merits taking these issues into account.[46,47] In physiological and pathological conditions associated with increased RBC breakdown, such as in the advanced trimesters of pregnancy, recent haemorrhage, intravascular haemolysis or transfusion or erythropoietin treatment, only blood glucose concentration estimation is used to diagnose DM. There is a paucity of data supporting the use of HbA1c in the diagnosis of T2DM in children and adolescents. Though HbA1c is not the routine marker suggested for the diagnosis of diabetes in children with cystic fibrosis or symptoms that suggest the development of acute onset of T1DM, HbA1c has been recommended for diagnosis of T2DM in children and adolescents.[46]

In order to accurately diagnose DM and in the absence of frank hyperglycaemia (plasma glucose > 200 mg/dL) or hyperglycaemic crisis, it is useful to repeat the same diagnostic test for confirmation. In situations where there are two different tests with conflicting results, the test that is positive should be repeated and a diagnosis of DM be made after a confirmatory test has been done.[6,46] For individuals whose test results returned negative for diabetes, repeat testing at 3-year intervals is suggested.[46,48]

Self-monitoring of blood glucose concentration and HbA1c level are the integral components of the standards of care in DM. They are designed to assess and monitor the effectiveness and progress level of a treatment plan, as well as to provide a guide in selecting appropriate drugs and dosages.[46,48] Optimal blood glucose control is achieved when the fasting blood glucose level is between 70 mg/dL and 130 mg/dL, 2 h postprandial level less than 180 mg/dL and bedtime glucose level between 90 mg/dL and 150 mg/dL. However, testing six to eight times daily may burden patients and may result in non-compliance. Therefore, it is recommended to ensure

that patients are properly instructed and are given regular evaluation and follow-up.[46,48] According to the current guideline, the HbA1c level should be assessed regularly in all patients with DM. The frequency of HbA1c testing is flexible and depends primarily on the response of patients to therapy and the physician's judgement. HbA1c testing is performed at least every 6 months for patients who are meeting treatment goals, but for patients who are far from achieving their glycaemic goals HbA1c testing may be performed more frequently.[46,48]

Markers of blood coagulation

Plasma platelets

The role of hyperglycaemia in platelet hyperactivity in DM is not fully clear.[49] The entire coagulation cascade is dysfunctional in DM; increased levels of fibrinogen and plasminogen activator inhibitor 1 favour both thrombosis and defective dissolution of clots once formed.[49] Platelets in T2DM individuals adhere to vascular endothelium and aggregate more readily than in physiologic conditions. Loss of sensitivity to the normal inhibitory signal exercised by prostacyclin (PGI-2) and NO generated by the vascular endothelium presents as the major defect in platelet function.[49] Insulin is the natural antagonist of platelet hyperactivity as it sensitises the platelet to PGI-2 and enhances endothelial generation of PGI-2 and NO. Thus, the reduced or lack of insulin sensitivity action in DM creates a complex array of disordered platelet activity conducive to macrovascular and microvascular events.[49]

Platelet activation plays a key role in athero-thrombosis in T2DM and increased *in vivo* platelet activation with enhanced thromboxane biosynthesis has been reported in patients with impairment of glucose metabolism even in the earlier stages of disease and in the preclinical phases.[50]

In healthy subjects, without DM, the induction of acute hyperglycaemia can lead to increased platelet reactivity and platelet activation as evidenced by increased markers such as soluble P-selectin and CD40-ligand.[35,38] Exposure of platelets to hyperosmolar solutions may also lead to increased reactivity, suggesting that hyperglycaemia may have a direct osmotic effect.[35,38] Both chronic and acute hyperglycaemia have been reported to cause *in vivo* activation of protein kinase C (PKC), a transduction pathway mediator for many pro-aggregatory platelet agonists.[35,38] Platelets from patients with DM, unlike those from healthy individuals, also manifest a short-term activation of the calcium-sensitive PKCβ isoenzyme by acute hyperglycaemia even *in vitro*, in the absence of additional stimuli, indicating that DM has an inherent diabetes-related dysregulation of this pathway.[35,38] A study of patients with T2DM undergoing percutaneous coronary intervention (PCI) found that improvements in glycaemic control were associated with reduced platelet reactivity.[35,51] The observed clinical correlation of these changes is that even mild elevations in pre-procedural fasting glucose are associated with increased risk of mortality following PCI and, conversely, optimal pre-procedural glycaemic control (HbA1c < 7%) in T2DM patients is associated with improved clinical outcome.[35]

Some of the AGEs formed in DM cause externalisation of platelet membrane phosphatidylserine that leads to surface clotting factor activation and so directly enhance the thrombogenic state.[35,52] Similarly, the platelets of patients with diabetes have increased glycation levels of surface membrane proteins, which cause decreased membrane fluidity and increased platelet sensitivity to agonists.[35,53,54,55] Several studies have reported that increased platelet reactivation in patients with diabetes may confer less cardiovascular protection with antiplatelet therapy, particularly aspirin.[56,57] It has already been demonstrated that IR and hyperinsulinaemia are associated with the stimulation of erythroid progenitors and increased levels of inflammatory markers.[58, 59]

Low-dose aspirin still remains the major antiplatelet therapy by reducing the risk of heart attack, stroke or cardiovascular death in intermediate to high risk patients with established vascular disease by up to about 20%.[35] Even with this intervention, some patients' platelets still remain activated when assessed, despite aspirin therapy. Multiple studies have found these patients to be at higher risk of atherothrombotic events.[35] This problem is particularly prominent and severe in DM patients, 10% – 40% of whom display high residual platelet reactivity on biochemical testing despite aspirin therapy.[35]

Though aspirin has been reported to effectively block the eicosanoid thromboxane A2 positive feedback loop, platelets of affected patients continue to manifest high on-treatment platelet reactivity, which places them at increased risk of subsequent thrombotic events.[35] The inability of aspirin monotherapy to sufficiently prevent platelet reactivity thus supports the claim for dual antiplatelet therapy in certain patients at high risk of thrombotic events, such as patients with acute coronary syndrome or undergoing PCI,[35,60] and drives the search for more potent and specific antiplatelet agents with a more consistent platelet-suppressing effect. This thus suggests that an anti-DM therapy that is able to manage the hallmark of the disease condition, that is, severe hyperglycaemia, and reduce platelet level in the blood will be most favourable, supporting the assertion that the blood platelet level could demonstrate a significantly positive correlation with plasma glucose and also be a beneficial prognostic marker to monitor the extent of improvement of the disease condition.

D-dimer

D-dimer is a fibrin degradation product, a small protein fragment present in the blood after a blood clot is degraded by fibrinolysis. It is so named because it contains two D fragments of the fibrin protein joined by a cross-link.[61] D-dimer concentration may be determined by a blood test to help diagnose thrombosis. Since its introduction in the 1990s,

it has become an important test performed in patients with suspected thrombotic disorders. While a negative result practically rules out thrombosis, a positive result can indicate thrombosis but does not rule out other possible or potential causes, for example pulmonary embolism, deep vein thrombosis, malignancy, pregnancy and so on.[62] Its main use, therefore, is to exclude thromboembolic disease where the probability is low. In addition, it is used in the diagnosis of the blood disorder disseminated intravascular coagulation.[61] According to Kanani et al.[63] plasma D-dimer can serve as a predictive marker for the risk of coronary artery diseases and DM nephropathy patients, which they claimed may suggest an increased thrombogenic state that is related to the increased susceptibility to vascular disease in these patients.

Nwose et al.[64] reported changes in D-dimer levels in DM progression to macrovascular complications, as was earlier stated. Coban et al.[65] reported significantly higher plasma D-dimer levels in patients with impaired fasting glucose.

Alteration of blood coagulation and fibrinolysis along with poor glycaemic control in DM has also been implicated in the development of diabetic vascular complications. Plasma level of D-dimer has been reported to reflect the amount of lysed cross-linked fibrin, thus making it an accepted marker of hypercoagulability in disease conditions.[63]

The observation of decreased plasma D-dimer in patients under anti-diabetic drugs may thus indicate better control of plasma glucose in DM patients, improvement of the underlying DM conditions and declined development of vascular complications, following decreased amount of lysed cross-linked fibrin and platelet coagulation. It would not be out of place to predict a significant positive correlation between plasma D-dimer and glucose level; El Asrar et al.[66] report that D-dimer level significantly correlate with the disease duration.

Nwose et al.[64] investigate the possible discriminatory potential of D-dimer in identifying the variations in the fibrinolytic and/or coagulation component of diabetes-associated with disease progression to macrovascular complications. They report that the changes in D-dimer levels may indicate diabetes disease progression to macrovascular complications and that using D-dimer in conjunction with other biomarkers may identify stages of disease progression. They identify a steady significant increase in D-dimer levels that became increasingly higher than the normal levels as the disease progressed from prediabetes to cardiovascular complications. Nwose et al.[64] report changes in D-dimer levels in DM progression to macrovascular complications as reported earlier. Using D-dimer in conjunction with other biomarkers to identify stages of disease progression, commencing from prediabetes and continuing to development of asymptomatic and clinical cardiovascular disease in DM, could be a beneficial approach in monitoring the extent of compliance of the disease condition to anti-DM drugs.

Plasma fibrinogen

Studies have shown that the formation of an occlusive thrombus, on a damaged atherosclerotic lesion, is the most common precipitating factor of acute myocardial infarction. Evidence also suggests that fibrinogen has a role, both in the early stages of plaque formation and late complications of cardiovascular disease.[67] Fibrinogen itself is determined by several modifiable and non-modifiable determinants like age, sex, smoking, body mass index (BMI), hypertension, alcoholism, glycaemic control, lipid profile and urine albumin excretion rate.[68,69] Several studies have shown that a haemostatic factor especially hyperfibrinogenaemia is implicated as a source of atherosclerosis and its complications. Studies have also reported that fibrinogen levels were higher in DM patients than in controls.[70] According to Bembde,[70] patients with T2DM had a high prevalence of hyperfibrinogenaemia. Plasma fibrinogen levels were independently correlated with HgA1c values, in which a positive observation suggested that fibrinogen may be involved in the increased cardiovascular risk of patients with T2DM. In a study carried out by Coban et al.,[65] the levels of plasma fibrinogen in patients with T2DM, impaired fasting glucose, and normal subjects were 449 (306–605) mg/dL, 348 (264–468) mg/dL and 216 (179–260) mg/dL, respectively, suggesting a significant correlation between fibrinogen and glycaemia. Sapkota et al.[71] report that approximately 71% of DM patients had plasma fibrinogen < 351 mg/dL, whereas all non-DM persons had fibrinogen in the range of 151 mg/dL–350 mg/dL; they also report mean fibrinogen values of DM patients and non-DM persons to be 389 mg/dL and 321 mg/dL, respectively.

Thus, it would not be out of place to claim that plasma fibrinogen could be a very prognostic marker in monitoring the response of DM patients to medical interventions and drugs.

Activated partial thromboplastin time

Patients with DM have a high risk of atherothrombotic events. Venous thrombosis has also been found to occur more frequently in DM patients.[72] Nearly 80% of patients with DM die from thrombosis, and 75% of these deaths have been attributed to cardiovascular complications.[72] Plasma fibrinogen levels influence thrombogenesis, blood rheology, blood viscosity and platelet aggregation. Epidemiological studies have found a significant association between fibrinogen levels and insulin levels. While the clinical relationship between shortened activated partial thromboplastin time (APTT), increased fibrinogen levels and the risk of venous thrombosis is supported by the literature, the exact biological mechanisms of thrombosis in DM are likely to be multifactorial and are not clear yet. Endothelial abnormalities play a critical role in the enhanced activation of platelets and clotting factors that occur in DM patients. In such patients, coagulation activation markers are elevated and coagulation abnormalities seem to be directly linked to hyperglycaemia, involving all stages of coagulation.[72]

In an experiment to support the above claims, Sapkota et al.[71] report that approximately 74% of DM patients and all non-DM persons had APTT in the range of 26–40 s; mean APTT values of the DM patients and non-diabetic persons were approximately 30 s and 32 s, respectively. The observation of Sapkota et al.[71] regarding shortened APTT strongly agree with the findings of Zhao et al.[72] and Sauls et al.[73] but were prolonged for DM patients under treatment. It would thus be safe to predict that APTT and plasma fibrinogen exhibit a negative correlation and that further prolonging the APTT of DM patients in response to treatment and management of the disease could be important in monitoring the patients.

Prothrombin time and partial thromboplastin time

PTTK and APTT are parameters that are used to detect coagulation factor deficiencies and to monitor replacement therapy in patients who are at risk of bleeding (haemorrhagic complications). A correlation has been reported between short APTT values and the risk of thrombosis.[73] A significantly prolonged PT and PTTK of DM patients compared with non-DM subjects has been reported. Sauls et al.[73] report shorter clotting times in T2DM patients (11.3 s) compared to the non-DM subjects (11.9 s). Abdulrahman and Dallatu[74] report an improvement of these clotting parameters in treated DM subjects. These findings suggest that despite the popular notion of a pro-thrombotic tendency in diabetes, diabetics may also be prone to developing haemorrhagic complications. It is helpful to bear this in mind and to incorporate PT and PTTK assays as routine investigations for better management of these patients.[75] The control of the underlying glycaemia in DM through anti-diabetic drugs would thus restore the PT and PTTK level to normal, suggesting improved haemorrhagic conditions in times of injuries to blood vessels or tissues.

C-reactive proteins

Recent studies have reported that poor glycaemic control is significantly associated with the development of macrovascular complications of DM, and they have identified that CRP is an important risk factor for cardiovascular disease as one of the acute phase markers of inflammatory response, for example atherosclerosis. However, it was concluded that CRP is directly related to HbA1c and glycaemia in DM patients.[76] CRP is a marker for inflammation that is involved in many chronic diseases, including heart disease, stroke and DM. Scientific studies have shown not only that a high level of CRP is a risk factor for heart disease and stroke, but also that lowering CRP levels can substantially lower a person's risk of heart disease.

Mugabo et al.[77] in their review identify that apart from the role of CRP in predicting cardiovascular risk, as a marker of early events, it may represent an active participant in atherogenesis (DM vasculopathy). CRP is highly expressed in endothelial atherosclerotic plaques and both vascular cells and monocytes and/or macrophages represent significant sources of CRP during inflammatory events in blood vessel walls. The released CRP further activates the main cell types present in the atherosclerotic lesions, precipitating the development and progression of atherosclerosis. However, inflammatory and metabolic factors associated with DM, for example severe hyperglycaemia, adipokines, AGEs (HbA1c, glycated lipoproteins) and free fatty acids, were reported to trigger CRP production by endothelial cells, smooth muscle cells and monocytes and macrophages.[77] By implication, during treatment and management of DM, if the hyperglycaemic state is regulated by the normoglycaemia and the inflammatory events are reduced, HbA1c (and consequently CRP) will be reduced. This thus suggests that CRP could be a very beneficial prognostic marker (especially in complicated DM with vasculopathy and atherosclerosis) in the assessment of the level of response of DM patients to drugs and other medical interventions.

Fasting blood insulin, homeostasis model assessment of insulin resistance and C-peptide

Fasting blood insulin and HOMA-IR have been credited to be significantly powerful tools in the assessment of metabolic dysfunctions that are based on poor blood glucose control and IR associated with metabolic and hemodynamic alterations, as well as higher cardio-metabolic risks, characteristic of MS and blood glucose management.[78] MS has been widely used to describe a collection of clinical signs, including central obesity, hypertension, dyslipidaemia, impaired glucose metabolism, arthritis and elevated blood pressure, which, regardless of cause, identifies individuals at risk of atherosclerotic cardiovascular disease and T2DM.[78,79]

HOMA-IR is a tool for the assessment of IR in T2DM. IR increases atherogenesis and atherosclerotic plaque instability by inducing pro-inflammatory activities on vascular and immune cells.[78] At the other end of the divide, Simental-Mendía et al.[80] and Mohammadabadi[81] argue that using HOMA-IR could convey inaccurate results in T2DM and hyperglycaemic patients.

IR is an early and important factor in the development of T2DM and may be present for years before the emergence of any changes in glycaemic control. A practical measure of IR would be valuable for early identification of individuals at risk for T2DM and cardiovascular disease. Obesity is closely linked to IR, with BMI and waist circumference being good predictors of IR.[82]

However, a number of models, including HOMA-IR, quantitative insulin sensitivity check index and fasting IR index, were proposed as more simplified measures of insulin sensitivity.[82] Other related models use fasting insulin plus various lipids measures, such as free fatty acids (elevated), triglycerides (elevated) or high-density lipoprotein and/or total cholesterol (elevated). These models have been said to be quite simple and require only a single blood sample, but their advantages over fasting

insulin are not clear. The other models that are based on the oral glucose tolerance test (OGTT), using various combinations of glucose and insulin values from the fasting state and during the OGTT include the Matsuda index, the Stumvoll index and oral glucose insulin sensitivity, as was identified by Cobb et al.[82] More complex methods, including insulin tolerance test and the frequently sampled intravenous glucose tolerance test, require multiple blood samples and thus are complicated and not practical for routine screening purposes. Therefore, there remains a need for a simple IR test for routine screening, prospective studies, risk assessment and therapeutic monitoring.

Johnson et al.[83] identify the use of fasting insulin levels as the most robust model for assessing pre-DM, as they claim that this model may provide the most dependable utility as a clinical tool because of the highest quartiles observed in their work, which suggests a significantly greater likelihood of identifying pre-DM. Burke et al.[84] collaborate this claim in their work when they report that fasting insulin levels are positively related to measures of obesity, systolic and diastolic blood pressure, triglyceride, β-lipoprotein cholesterol and pre-β-lipoprotein cholesterol levels. According to Mack et al.[85] and Burke et al.,[84] IR is a common finding in DM and may serve as a measure of efficacy of therapies (exercise, exogenous insulin, sulfonylureas and PPAR gamma agonists) for DM, as a marker for risk of developing T2DM and as a confirmation for the presence of T1DM.

As discuss by Becker et al.,[86] the synthesis of insulin in the pancreatic beta cells begins with the synthesis of the insulin precursors, preproinsulin and proinsulin. After synthesis, proinsulin is cleaved with the aid of proteolytic enzymes into three protein chains, A, B and C. The C-peptide is then removed and the A chain is bound to the B chain through disulphide linkages to form active insulin protein, giving rise to equal amounts of active insulin and C-peptide, so that the measurement of C-peptide can be used to assess the patient's endogenous insulin secretion after the initiation of treatment with exogenous insulin administered intravenously. After post-translational modifications, insulin is packed into insulin secretory granules located near the plasma membrane of the beta cell and is then ready to be secreted. These biochemical events make the C-peptide one of the key markers for the assessment of onset T1DM, through the identification of a decrease in the blood level of the peptide.

T1DM is a chronic autoimmune disease in which the insulin-producing beta cells of pancreatic islets are gradually destroyed. The clinical presentation of T1DM is preceded by a prodromal phase characterised by the appearance of diabetes-associated auto-antibodies in the circulation. Both the timing of the appearance of auto-antibodies and their quality have been used in the prediction of T1DM among first-degree relatives of diabetic patients.[87]

T1DM, predisposed by the human leukocyte antigen (HLA) class II locus located at the short arm of chromosome 6, is a disease with a subclinical prodromal period characterised by selective destruction of the insulin-producing β cells in the pancreatic islets. The destruction process is manifested by infiltration of the islets by mononuclear cells and may proceed over a period of many years. This pre-DM period offers an opportunity to identify those individuals who are likely to develop T1DM later and to start intervention aimed at delaying or preventing the manifestation of the clinical disease. The presence of these circulating auto-antibodies to various islet cell proteins is one of the most thoroughly characterised immune phenomena associated with T1DM, as they are the first detectable markers of an ongoing destructive process in the islets and thus provide a potential tool for identifying individuals at risk for developing the disease in the future.[88]

Thus, the other markers discussed in this review may not offer much assistance and information in the identification of onset T1DM during this prodromal period but may only guide the medical personnel during the process of management and monitoring of treatment of the patient with the established condition.

Conclusion

It is important to note that events leading to DM may be associated with obesity, dyslipidaemia, hypertension and cardiovascular diseases; therefore, lifestyle changes such as a healthy diet, physical activity, moderate alcohol consumption and cessation of smoking, in addition to the introduction of pharmacological agents in the form of drugs, are deemed important to stop or delay the timeline of development of DM (Chaudhury et al., 2017). However, in monitoring these events, and in a situation where the DM progresses into the much more advanced stage and into stages presenting with associated complications, the markers and parameters discussed in the review could offer a great deal of guiding information, especially during treatment and management of the condition. The information obtained could guide the medical advisers of these patients on the appropriate drugs, doses and period of administration that best suit their conditions.

Standard and conventional biomarkers for the assessment of extent of improvement or progression of DM, such as fasting blood glucose, HbA1c, fasting insulin, HOMA-IR and perhaps the triglyceride-to-HDL ratio, remain the major preclinical and clinical diagnostic interventions, especially at early stages and progression when the metabolic dysfunctions of DM is still emerging. However, at more established stages of DM, the other biomarkers (NLR, APTT, PT, PTTK, markers of anaemia and so on) mentioned in this review could afford beneficial added information and indicators of the metabolic disease. For T1DM individuals in a prodromal phase, where fasting glucose, HbA1c, fasting insulin and HOMA-IR would appear to seemingly be at normal levels, these other markers, however, may provide vital information, especially the markers of immunity, but the autoimmune antibody markers are the key informative index.

Generally in DM, depending on the stage of the condition and associated complications, there is an observed significant increase in the plasma levels of HbA1c, WBC, mean NLR, PLR, total leukocytes and neutrophils, plasma LDL, HDL and VLDL, platelet, fibrinogen, D-dimer and CRP, while RBC, haemoglobin concentration, APTT, PT and PTTK are decreased. With this knowledge about the variations of these parameters, a reversal during treatment and management of DM and its complications can be better monitored, and guided decisions can be made. Thus this supports my suggestion that these parameters are very sensitive markers that can provide very important prognostic information about compliance to treatment. However, fasting glucose, HbA1c, fasting insulin or HOMA-IR, autoimmune antibodies and C-peptide (in the case of T2DM) and triglyceride-to-HDL ratio are the major markers of DM, especially at the onset of the metabolic conditions, but the other markers identified in this review corroborate those aforementioned at the established stages and serve as prognostic markers during treatment and management.

As has been stated in this review, in monitoring the biochemical, physiological and pathological events of onset DM and DM progression, these markers and parameters could offer superintendent information, especially during treatment and management of the condition. The obtained information could be a beneficial guide to the medical advisers of these patients on appropriate drugs, doses and period of administration that best suit their conditions.

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Competing interests

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