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Review Article

Genetics Section

Role of Mitochondrial Dynamics in Type 2 Diabetes Mellitus Dysfunction

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ABSTRACT

Type 2 Diabetes Mellitus (T2DM) is influenced by the action of both hereditary and environmental factors. These factors are comprehended in the disease interaction with β cells and insulin sensitivity to the receptors.The main cause of T2DM development is ageing, obesity, and oxidative stress. The dysfunction of the β cells of the pancreas leads to insulin insensitivity in the liver, muscle and fat metabolism. These occur due to the result of oxidative stress. Lipid peroxidation is a key initiating factor in the development of oxidative stress, which results in T2DM and its related micro-and macro-vascular problems. These are initially compensated for the generation of excess insulin synthesis, thus ensuring normal glucose tolerance. When these compensatory processes are disrupted, the majority of persons develop T2DM. Although the gene is the primary cause of T2DM, the environment also plays an important role in the disease's progression. Particularly, a sedentary lifestyle characterised by excessive food consumption and physical inactivity is a known risk factor for obesity and type 2 diabetes. This review has briefly discussed the significance of numerous causal factors, and genomic and biochemical pathways which are responsible for increasing the production of oxidative stress, a crucial component for the etiology and development of T2DM. The conclusion drawn was that the analysis of oxidative stress markers may be one of the potential methods for the diagnosis and prognosis of T2DM. Furthermore, this review will be helpful to establish the broaden conceptual framework for future studies on oxidative stress with relation to etiology of type 2 diabetes and therapeutic development.

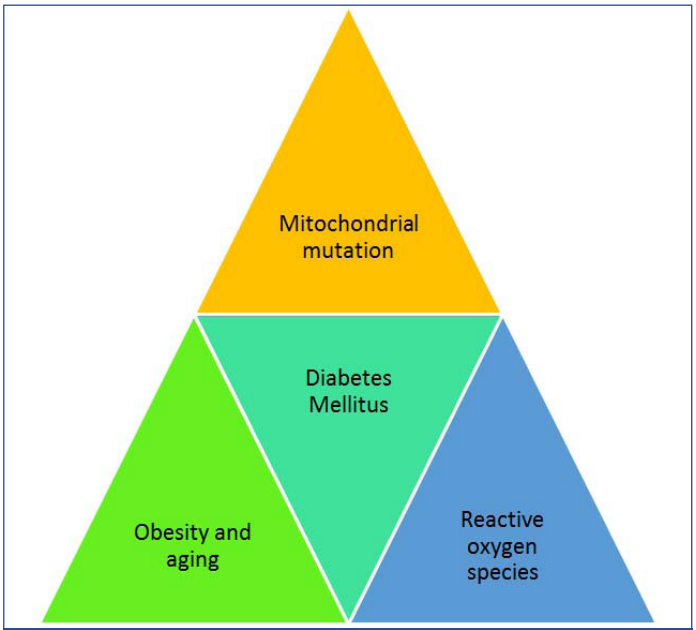
Keywords: Insulin sensitivity, Oxidative markers, Oxidative stress

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a major public health concern in the global population.It makes a significant contribution to morbidity, mortality, and healthcare costs worldwide. According to International Diabetes Federation 2021, its prevalence in low-income and middle-income countries has increased dramatically. Low-income countries are more likely to have the disease than high-income countries. It also reported that diabetes has impacted 90 million people in Southeast Asia in 2021, with 77 million of them living in India, making it the world's largest diabetes-affected country. These have increased over the last three decades; by 2030, the number of people with diabetes will have risen to 643 million, and by 2045, it will have risen to 783 million, with diabetes accounting for approximately 747,000 deaths in 2021 [1]. The statistics of the World Health Organisation (WHO) 2019 state that deaths caused by diabetes are 1.5 million and 48% of deaths occur before attaining the age of 70 years [2].

T2DM is a chronic non communicable disease caused due to insulin deficiency and a prolonged period of hyperglycaemia. It is linked to the inactivity of beta cells in the Langerhans' islets, as well as insulin synthesis and action [3]. Insulin is the primary regulator of blood sugar levels; however, the sensitivity of insulin receptors is also altered and found to be lower in those with T2DM. T2DM is caused by a malfunction of metabolic pathways in lipolysis, excessive insulin synthesis or insulin resistance, and obesity [4]. T2DM damages every organ, and the severity of the damage is determined by the organ's participation during the length of the disease. Hyperglycaemia, an elevated level of blood sugar, has an effect on the mitochondria, which leads to oxidative stress, which causes cell damage. Oxidative stress causes dysfunction in a variety of tissues, including pancreatic beta cells, liver cells, and cardiovascular cells, and that also leads to the ageing process. Reactive oxygen species (ROS) are the primary cause of insulin malfunction [5,6].

T2DM is caused by prolonged hyperglycaemia and hyperlipidemia, which are caused by obesity and ageing. High levels of free fatty acids or triglycerides in the peripheral circulation and a lack of physical activity are two factors linked to T2DM [7,8]. Ignorance of symptoms results in illness exacerbation, increased risk of stroke, coronary artery disease, chronic kidney disease, chronic liver disease, chronic obstructive pulmonary disease, and lower respiratory infection [Table/Fig-1].



[Table/Fig-1]: Factors affecting Diabetes Mellitus onset (ROS, obesity, ageing and mitochondrial mutation).

MITOCHONDRIAL DNA IN TYPE 2 DIABETES MELLITUS

Mitochondria evolved from α-proteobacteria, a free-living organism, and integrated into eukaryotes via the endosymbiont phenomenon [9-12]. There are significant similarities between mitochondrial and bacterial genomes. They share common traits such as a circular,

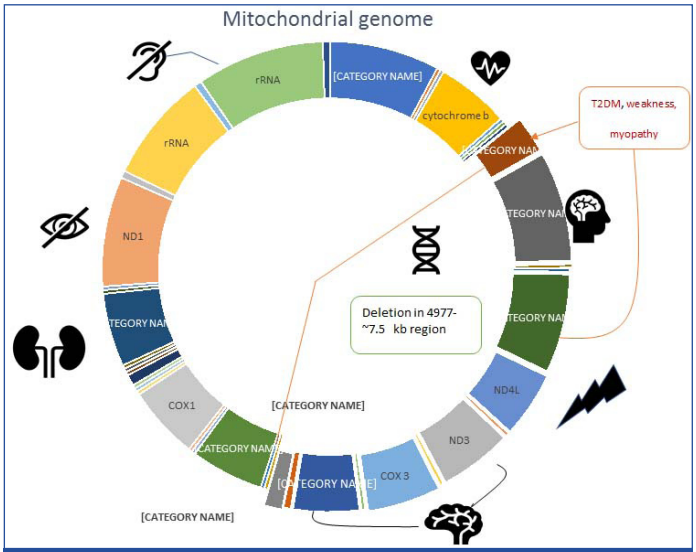
double-stranded, and supercoiled genome and are divided through binary fission. The length of Mitochondrial Deoxyribonucleic Acid (mtDNA) varies from species to species. For instance, it is roughly 75 to 80 kb in budding yeast and ~16.5 kb in humans [13]. Mitochondria play a pivotal role in the production of Adenosine Triphosphate (ATP) via oxidative phosphorylation, which serves as an energy substrate in living cells [14]. The mtDNA encodes for 37 genes, of these 13 genes are involved in the formation of multi-subunit complexes that are essential for the synthesis of energy production through Electron Transport Chain (ETC) pathways [15,16]. This complex is found on the inner mitochondrial membrane. The mtDNA is classified into two strands depending upon guanine concentration; Heavy Strand (H-strand) and Light strand (L-strand). The H-strand encodes 28 genes, while the L-strand encodes 9 genes. This has distinct replication zones with the creation of a Displacement loop. Complex I has seven subunits encoded by mtDNA; NADH Dehyrogenase (ND)1, ND2, ND3, ND4, ND4L, ND5, and ND6; complex III has one component cytochrome b; complex IV has three subunits Cyclooxygenase (COX)I, COXII, COXIII; and complex V has two subunits ATP6 and ATP8 [17]. The nuclear genome (nDNA) encodes all complex II subunits and other assembly factors which are essential for oxidative phosphorylation [18]. The mtDNA is inherited from the maternal side during conception through oocytes [19]. The morphology of the mitochondria is significantly robust and they can alter their shape depending upon the metabolic requirement of the cell [20]. Human mitochondrial replication occurs independently of the normal cell cycle, which explains why the number of mitochondrial copies varies in different cell types [21]. The presence of both wild-type and mutated mtDNA is referred to as heteroplasmy. This is controlled by the mitochondrial fission and fusion mechanism. This is revived in [4,22-24]. When the ratio of wild-type to mutant mtDNA surpasses a certain physiochemical threshold, the biochemical pathways are hindered and lead to metabolic syndromes.

There is a high-risk of DNA damage due to a lack of histone, frequent exposure to ROS and a weak repair system [25]. This causes a mutation in mtDNA. The mutation causes mtDNA alternation and leads to the accumulation of base substitution, missense, point, deletion and duplication mutations and large-scale rearrangement. It is determined by the probability of homoplasmy (the presence of all identical copies of mitochondria in a cell that may be normal or mutated) and heteroplasmy in the mitochondria. Different levels of deletion in different cells result in varying degrees of dysfunction [26]. Chomyn A et al., and Hayashi J et al., confirmed that transfer Ribonucleic Acid (t-RNA) mutation or deletion, can sustain deletion or mutation impact, 10% wild type is sufficient to regulate the normal homeostasis of the cell [27,28]. To exhibit a clinical phenotype, the heteroplasmy pathogenic threshold level must be greater than 90% [28].

A homoplasmic condition leads to a variety of point mutations, while a heteroplasmic condition leads to large-scale deletion [29]. The prevalence of large-scale deletion is minimal under normal conditions but elevated during mitochondrial disorders. The mitochondrial deletion occurs in regions ranging from 1.8 to 8 kb, resulting in the loss of protein-coding genes, tRNA, and ribosomal RNA (rRNA) genes [30]. As a result, some places are more likely to become deletion hotspots. Ding Y et al., confirmed that the t-RNAleu(URR) A3243G and ND6 T14502C point mutation are associated with Maternally Inherited Diabetes and Deafness (MIDD) which affects glucose metabolism [31]. The person suffering from MIDD has various clinical symptoms such as hearing loss, cardiomyopathy, neuropathy, and nephropathy. Prior detection of this mutation is critical to provide clinical aid to MIDD patients. These sites are also more prone to other mitochondrial disorders also such as neurodegenerative disorder (Alzheimer's and Parkinson's disease), MELAS (Mitochondrial Encephalopathy with Lactic Acidosis and Stroke-like episodes) and myopathy [32].

MITOCHONDRIAL REPAIR SYSTEM IN DIABETES MELLITUS

DNA damage caused by ROS is a key source of point mutations and large-scale deletions. This occurs due to the replication machinery slippage. This is frequently present in 4977 kb to ~7.5 kb (common deletion site) [Table/Fig-2], resulting in a variety of mitochondrial-associated diseases, cancer, and ageing. Kearns-Sayer syndrome, myopathies, diabetes, and deafness are all caused by distinct deletion mutations [33]. Deletion mutation is induced not only by the replication slippage mechanism but also by dysfunction in the break repair system. Extrinsic and intrinsic factors are used to bring deletion into the cell. Ionising radiation, ultraviolet light, smoke, heavy metal ions such as Fe, Cu, Cd, Ni and As, ozone, and air pollution are extrinsic factors and ROS, dual oxidase, lipoxygenase, cyclooxygenase are intrinsic factors [34]. Both of these factors are capable of causing a Double-stranded Break (DSB) in mtDNA. As a result, the replication and repair machinery tend to fail safeguard to the mtDNA. In eukaryotes, two types of DSB repair systems are present. These are Homology-directed Recombination (HDR) and Nonhomologous End Join (NHEJ). The HDR relies on the detection and repair of mtDNA breaks by referencing the homologous template. In contrast, NHEJ, re-ligated the DSB-induced broken end of mtDNA. Another process that resembles NHEJ is known as Micro-homology Mediated End Joining (MMEJ). It ligates the blunt end of the DSB with a short homologous base pair. It causes the flap-like structure during ligation, which is then deteriorated during end joining. This results in small-scale deletion [35]. In mtDNA large-scale deletion, DSB has typically flanked with the short repeats. These short repeats confirmed the presence of a recombination event. DSB repair with HDR, NHEJ and MMEJ could promote the error-prone intramolecular recombination and the linearisation of mtDNA event results in deletion mutation [36]. The main culprit causing deletion in the mtDNA is the repair system [37]. Long-term exposure to ROS and inefficient ROS-scavenging mechanism induces the accumulation of mutations, which leads to faulty physiological and biochemical processes.



[Table/Fig-2]: Schematic representation of mitochondrial genome with deletion at the 4977 bp, region shows the dysfunction in various organs. Region encodes ND6 and ND4 subunit dysfunction leads to diabetes, weakness and myopathy. ATPase 6 and ND3 cause neuropathy, cytochrome b and ND3 dysfunction related to cardiomyopathy. rRNA region dysfunction leads to deafness.

In mitochondria, Base Excision Repair (BER) processes operate with remarkable fidelity. The mitochondria and the nucleus both contain sub-compartmentalised forms of the majority of BER enzymes. These are fulfilling the needs of replication and repair activities and are cross-communicating with one another via mitochondrial and nuclear localisation signalling. The mitochondria include a number of proteins that are linked with BER [38]. For instance, the Uracil-DNA Glycosylase (UNG)1 isoform, which is unique to the mitochondria, and

the protein variations 8-Oxoguanine–DNA Glycosylase (OGG)-1b, OGG1-1c, and OGG1-2a, AP Endonuclease1, Flap Endonuclease (FEN)1 are other BER proteins with mitochondrial localisation signalling encoded in their isoforms [39]. The ExoG nuclease and the enzyme responsible for deleting flaps produced by long-patch BER are the primary examples of proteins with unique mitochondrial localisation. In cells lacking BER proteins, there are higher concentrations of mtDNA with deletion mutations. For instance, mice lacking the glycosylase Nei like DNA Glycosylase 1 demonstrated metabolic syndrome, and their liver cells exhibited higher mtDNA damage and indications of mtDNA deletions and/or rearrangements [40]. There is compelling evidence that the mitochondrial BER operates properly and mitigates the harm caused by large-scale deletion. However, a decreased level of mitochondrial BER causes mtDNA deletions. The maintenance of the genetic information encoding the proteins of the electron transport chain is ensured by the repair of abasic sites and oxidised bases by BER, which may lower the probability of developing mtDNA mutations [36].

ROLE OF REACTIVE OXYGEN SPECIES (ROS) IN THE DEVELOPMENT OF T2DM

During inefficacious oxidative phosphorylation, ROS are produced, which are metabolic hazardous compounds capable of causing cell stress and mutation, resulting in disorder. When it is produced in a controlled manner it acts as a signalling molecule [41]. In biological systems, ROS are a category of highly reactive chemicals. It is made up of many reactive species, some of which are produced from oxygen and others from nitrogen [42].The mitochondria are the primary sites of oxidation, where oxidation enzymes convert molecular oxygen to radical oxygen; NADPH oxidase (NOX), and as well as ROS produced in other non-mitochondrial loci i.e., peroxisomes by cytochrome P450 [43]. Superoxide (O2.-), hydroxyl radical (.OH), peroxyl (.RO2), and hydroxyl peroxyl (.HRO2-) are examples of oxygen-derived free radicals, while H_2O_2 and hypochlorous acid (HOCl) are examples of oxygen non-radicals. RON, like ROS, is made up of free radicals such as nitric oxide (.NO) and nitrogen dioxide (.NO2-), as well as non-radical species like alkyl peroxy-nitrite (RONOO-), nitrous oxide (HNO2), and peroxy-nitrite (ONOO-). The key contributions of products in metabolic pathways are superoxide, nitric oxide, and peroxy-nitrite. The thiyl radical (RS.) and tyrosyl radical (TR) are two more reactive species formed in the metabolic pathways of ROS and RON [44].

$H+ + O2 + e- \rightarrow HO2\bullet$ (hydroperoxyl radical)

$HO2\bullet \rightarrow H+ + O2\bullet-$ (superoxide radical)

$O2\bullet- + 2H+ + e- \rightarrow H_2O_2$ (Hydrogen Peroxide)

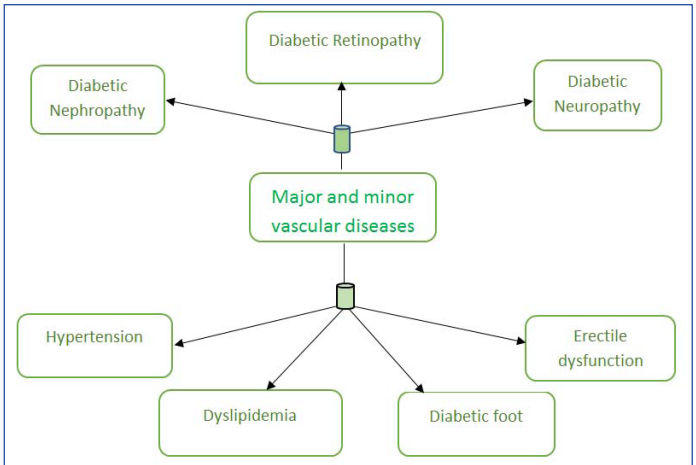
$H_2O_2 + e- \rightarrow OH- + OH\bullet$ (hydroxyl radical)

ROS damages a nucleic acid structure, proteins, and lipids due to its strong reactivity, and volatility. It is a major component in the development of diabetes, Alzheimer's disease, ageing, cardiovascular disease, cancer, and sarcopenia [45]. ROS damages the Kreb's cycle and the ETC, both of which are essential for complete glucose oxidation. The release of hydrogen ions or protons during ETC is primarily responsible for the synthesis of ATP by inner mitochondrial complex V, as well as the formation of oxygen and nitrogen reactive metabolites (ROS and RON). Short-lived free radicles like superoxide anion (O-) are produced when an electron is lost during ETC through NADPH oxidase (NOX). Immune cells also use the same mechanism to counter the foreign particles by the production of ROS via the NOX 2 system [46]. It is also important in cell signalling; cytokine and growth factor stimulation initiate the downregulation cascade and ROS acts as a secondary messenger, regulating gene expression. An increase in ROS levels not only impairs cell integrity, but also has an impact on cell proliferation, growth, and dysfunction of antioxidants leading to apoptosis [47]. To reduce the effect of ROS and RON in cells, there is a compensating mechanism in place. There are two types of antioxidants enzymatic and non-enzymatic. The enzymatic antioxidants have the capacity to counter the oxidative stress directly acting upon the reactive species. This enzymatic antioxidant contains Superoxide Dismutase (SOD), bilirubin, Catalase (CAT), and Glutathione Peroxidase (GPX). Other important antioxidant enzymes like Glutathione Reductase (GR), Peroxiredoxins (Prx), Thioredoxin (Trx), Thioredoxin Reductase (TrxR), and Glutaredoxins (Grx) also aim to safeguard cells by reducing oxidised critical thiols in important enzymes and proteins and by preserving the proper intracellular redox state [45-48]. Endogenous substances including reduced Glutathione (GSH), uric acid and bilirubin, are included in the nonenzymatic system, along with vitamins (vitamins A, C, and E), beta-carotene, flavonoids, polyphenols, zinc, and selenium [49]. Superoxide Dismutase (SOD) converts reactive superoxide into H_2O_2, which is less reactive, more stable, and permeable to the plasma membrane [50,51]. This H_2O_2 is diffused to the cytosol, where the cytosolic antioxidant such as catalase, glutathione peroxidase, and thioredoxin peroxidase catalysed the neutralization of H_2O_2 into H_2O (water) and O_2 (oxygen). Cu-Zn-SOD and Mn-SOD are antioxidant enzymes of the SOD family. The quantity of H_2O_2 is increased by the Fenton reaction and the Haber-Weiss reaction, and it is eliminated from the cell by cytosolic and peroxisome antioxidants [52,53]. Down-regulation of these enzymes results in irregulated neutralising reactive species pathways (ROS and RON).

Catalase is a peroxisome-present enzyme that catalyses the reduction of H_2O_2. The catalase gene (CAT gene) is found on the 11th chromosome in humans [54]. Variation in the CAT gene causes various diseases. The single nucleotide polymorphism (SNPs) in the transcription site causes low expression in the CAT gene which gives rise to diabetes, hypertension, asthma, carcinoma, osteoporosis and insulin resistance [55]. These diseases are caused by various SNPs sites on promoter regions such as -262C/T, -844G/A or -844C/T and 1167T/C. In the CAT gene at loci of -262C/T polymorphisms caused by oxidative stress which manifested the clinical syndromes vary along the specific ethnic group. This polymorphism results in decreased activity and the induction of various disorders such as gestational diabetes, diabetic retinopathy, nephrotic syndrome, coronary heart disease, and cardiovascular disease, among many others [56]. Diabetes alters the morphology of the organs which drastically lowers the rate of catalase expression and also increases oxidative stress protein. In a 2004 experiment on model animals, it was confirmed that the catalase enzyme reduces the effect of ROS in diabetic myocytes. Catalase overexpression in a model organism provides a compensatory mechanism for the effect of ROS. It has also been reported that the recovery of myofibrils and preserves the composition of myocytes and the contractility of the heart [57].

DEFECTIVE METABOLIC PATHWAYS IN T2DM

Dysfunction in mitochondrial not only involves declines in oxidative phosphorylation, but also in various processes such as regulation of redox reaction, Ca++ homeostasis, innate immunity, phagocytosis of bacteria, and apoptosis signalling. Defective mitochondrial metabolic pathways affect both oxidative phosphorylation and insulin production or sensitivity [58]. It causes the mitochondrial macromolecule to malfunction, resulting in decreased fatty acid oxidation and lipid build-up in the viscera which also leads to major and minor vascular disease [Table/Fig-3]. Pathways that accumulate Diacylglycerol (DAG) and Ceramide (CER), decrease insulin synthesis and signalling. DAG inhibits insulin via the Protein Kinase C (PKC) pathway, while CER inhibits insulin via the Akt route. Both DAG and CER activation leads to insulin resistance and malfunction. Insulin resistance is the marker for the high deposition of fatty acids and triglycerides which are inert metabolic and interfere with insulin actions [59].



[Table/Fig-3]: Major and minor vascular diseases include diabetic retinopathy, diabetic neuropathy, diabetic nephropathy, hypertension, dyslipidemia, diabetic foot, and erectile dysfunction. This disorder develops as a result of hyperglycaemia-induced damage to the vascular system, primarily the blood vessels, which results in constriction and occlusion.

PKC is activated by phosphatidyl serine and calcium and is mainly activated by DAG. It is associated with vasoconstriction, proliferation, extracellular protein production, and smooth muscle growth, all of which are dysregulated in T2DM. Furthermore, the Advanced Glycosylation End product (AGE) is a precursor for the formation of high ROS by NOX, which also activates PKC,endothelin-1 and Nuclear Factor κB (NFκB), leading to dysregulated Mitogen Activating Protein Kinase (MAPK) signalling [60]. Additionally, polyol pathways play an important role in the formation of ROS.Through these pathways, elevated ROS triggers a number of pro-inflammatory pathways in response to ischemia, impairs angiogenesis, and results in chronic genetic variations that continue to influence the expression of genes even after glycemia returns to normal [61]. This mechanism is also involved in cells, where insulin is not necessary for engaging entry, including the retina, kidneys, and peripheral nerves, which are directly involved in late T2DM complications such as retinopathy, nephropathy, and dementia [62,63]. Adipose tissue is a metabolically dynamic tissue that plays a role in a variety of biological functions, including angiogenesis, immunity, glucose metabolism, and lipid metabolism [7,64]. There are two forms of adipose tissue present in humans. These are White Adipose Tissue (WAT) and Brown Adipose Tissue (BAT). WAT is a high-energy storage molecule, whereas BAT is a heat-generating molecule. During fasting, WAT provides energy by lipolysis, whereas BAT involves in the homeostasis of maintaining body temperature and weight through uncoupling proteins. Uncoupling proteins bypass the oxidation pathway by acting as proton carriers and creating a shunt between the ETC complex and ATP synthase [65]. WAT contain a fewer number of mitochondria while BAT contains numerous mitochondrial units.Thus, the outcome of the redox process is minimum in WAT than that of BAT due to imbalance in the number of mitochondria [66]. In normal conditions, insulin stimulates the cells to increase glucose absorption and glycogenosis. When the level of free fatty acids in adipocytes increases, it interferes with insulin signalling by impeding glucose absorption and glycogen synthesis. As a result, adipocyte dysfunction disrupts insulin sensitivity and energy generation throughout the body. Increased ROS activity in adipocytes causes mitochondrial malfunction, which impairs thermogenesis and adipogenesis [67]. Excessive dietary intake and prolonged periods of inactivity result in fat deposition in the viscera, which alters metabolic pathways via genetic and epigenetic changes. This is a major cause of faulty lipid pathways; as time passes, these effects worsen, resulting in fat deposition in the liver, which inhibits the liver's ability to operate as the primary site of metabolism [4,67]. The liver, pancreatic beta cells, and skeletal muscles are the most impacted organs in T2DM [7,45,67]. The therapeutic approach to counter DAG is reviewed in [68].

As priorly stated, ceramide is a causative component in the dysfunction of the Akt pathway. This has been done by activating Protein Phosphatase 2A (PP2A) which dephosphorylated Akt. The Akt signalling system controls lipid and protein metabolism. It functions as a downstream effector in lipid, polypeptide, cell cycle regulator, transcription factor, vesicle trafficking, and metabolic enzymes. It is activated by phosphorylation of phosphatidylinositol 3,4,5-triphosphate (PIP3) [69]. Akt regulated downstream functions such as protein and lipid kinase by serine-threonine receptor phosphorylation and is also involved in apoptosis and cell cycle maintenance. Insulin-targeted organs or tissues, such as skeletal muscles, liver, and adipose tissue, are involved in Akt. Akt is made up of three subunits: Akt1, Akt2, and Akt3. Akt1 is found in every cell of the body, Akt2 is found in insulin-binding organs such as adipose tissue, muscles, and liver, and Akt3 is found in the testis and brain [70]. In response to insulin, the Akt pathway stimulates glycogen production. Akt2 is involved in the insulin-stimulated translocation of GLUT4 transport across the plasma membrane of skeletal muscles, which stimulates glycogen synthesis by activating glycogen synthase and inactivating GSK3 (glycogen synthase kinase 3), which converts glucose-6-phosphate to glycogen. Insulin encourages the production of proteins and the breakdown of fats. Obesity causes insulin resistance by modifying proteins. It depends on the degree of saturation of lipids in the skeletal muscles and liver [71].

EFFECTS OF OXIDATIVE MARKER IN T2DM

Oxidative stress is generated from an imbalance between ROS and the antioxidant mechanism. ROS causes oxidative stress, which activates a variety of biological processes such as apoptosis, necrosis, and autophagy. Oxidative stress and lipid peroxidation stimulate autophagy. ROS triggers autophagy by several mechanisms involving changes in the phosphorylation of Ulk1/ATG13, Beclin-1, P62/SQSTM1, AMPK and Foxo3A, leading to damage to the organ structure, especially β cell of the pancreas. The macromolecules of the cell, such as carbohydrate, lipid, protein, and nucleic acid, all undergo molecular changes during oxidative stress. These alterations result in changed macromolecule by-products that serve as oxidative stress markers. This modification is occurred due to insulin insensitivity in adipocytes dysregulation and insulin dysfunction. Oxidative biomarkers are listed in [Table/Fig-4].

Lipid peroxidation	Malondialdehyde (MDA)
	8-iso-prostaglandin F2α (8-iso-PGF2α)
	Thiobarbituric acid reactive substances (TBARS)
	Hydroxynonenal (HNE)
DNA damage oxidative markers	8-hydroxy-2′-deoxyguanosine (8-OHdG)
	8-hydroxyguanosine (8-HdG)
	8-hydroxyguanine (8-HG)
Nitrative stress biomarkers	4-hydroxy-2-nonenal glutathione (HNE-GSH)
	4-hydroxy-2-nonenal mercapturic acid (HNE-MA)
	8-nitroguanosine (8-NdG)
	8-nitroguanine (8-NG)
	3-nitrotyrosine
Inflammatory biomarkers	Chlorotyrosine
	Bromotyrosine
Metabolic disorder biomarkers	Nε-carboxymethyllysine
	Nε-carboxyethyllysine
Advanced Glycation End-Products (AGEs)	3-deoxyglucosone
	Glyoxal
	Methylglyoxal

[Table/Fig-4]: Type 2 diabetes mellitus and oxidative stress markers.

Lipid Peroxidation

Linolenic and arachidonic acids are Polyunsaturated Fatty Acids (PUFA) that are the principal targets of free radical lipid peroxidation. The lipid alteration leads to instability in fluidity, and integrity of the plasma membrane. MDA, 8-iso-PGF2, TBARS, and HNE are just a few examples of oxidative indicators that can modify lipids and generate aldehyde [5]. MDA is generated in lipid peroxidation and involved in the formation of foam cells that leads to atherosclerosis and other cardiovascular diseases [72]. MDA and TBARS together form a stable complex substance, MDA-TBRAS adduct. These are capable to react with phospholipid, protein, amino acid, nucleic acid and aldehyde which leads to loss of cell membrane integrity. Isoprostane (8-iso-PGF2) is the prostaglandin-like substance, which is formed by lipid peroxidation of arachidonic acid and present in an esterified form on phospholipid and released as free form by the action of phospholipase, which is detected in a variety of tissues and organs, as well as in body fluid [73,74]. Urine is extensively used for testing oxidative stress markers [75,76]. T2DM markers include elevated levels of MDA and isoprostane which are associated with cardiovascular complications, kidney dysfunction, and altered antioxidant activity.

Protein oxidation

Protein is a major component of the cell, and it plays a major role in a variety of physio-chemical processes such as transporting organic or inorganic solutes across membranes, cell signalling, cytoskeleton, cell cycle, and catalysis, among many others. Proteins are also targeted by ROS. Protein oxidation leads to cross-linkage, fragmentation, denaturation and proteolysis [77]. Protein modification causes a variety of cellular dysfunctions since it is involved in several vital cellular functions. The various amino acids such as serine, cysteine, threonine, glutamine, and tyrosine are found in the side chains of proteins in which tyrosine is primarily affected by ROS and produces a variety of oxidative metabolites, including 3-nitrotyrosine (NY), a class of nitrative stress biomarker, and chlorotyrosine (CY) and bromotyrosine (BY), a class of inflammatory biomarkers [78]. The precursor for NY is nitric oxide, which is synthesised by nitric oxide synthase, and CY and BY are synthesised by eosinophile peroxidase and neutrophile myeloperoxidase, respectively [79]. Furthermore, advanced oxidative protein products (AOPP) are also a marker for oxidative stress. AOPP is produced by chloramine and hypochlorous acid (HOCL), also known as chloraminated oxidants. It is derived from the plasma protein albumin [80]. Plasma protein carbonylation and di-tyrosine promoted cross-linking of protein products. These are associated with ischemic heart disease, endothelial dysfunction, diabetic retinopathy, and other T2DM-related disorders [81].

DNA and RNA Oxidation

ROS causes a range of oxidative markers in DNA and RNA, the chief inducers being 8-oxo-7,8-dihydroguanine (8-oxoG), and 8-hydroxydeoxyguanosine (8-OHdG), 8-hydroxyguanosine (8-HdG), and 8-hydroxyguanine (8-HG). Transversion mutation occurs due to these oxidative metabolites resulting in an incorrect linkage between base pairs [82]. This phenomenon is highly mutagenic, resulting in diseases such as T2DM, cancer, and CVD, among others. 8-OHdG is eliminated unchanged in the urine and is also found in other body fluids [77]. The oxidation of DNA by 8-oxoG activates the base excision repair system, which salvages the DNA. The (apurinic or apyrimidinic) AP site is formed as a result of the DNA damage repair system, which acts as a replication and transcription stopping group [83].The GC content of DNA, which is primarily found in the promoter region, is required for transcriptional factor binding. When 8-oxoG is formed, it is integrated into the genome and prevents the transcriptional machinery from working, as a result, the cell cycle stalled at the point of mutation [84]. ROS also damages microsatellites, and telomerase and also affects epigenetic processes by manipulating protein binding sites. Other than 8-oxoG, there is also the presence of an unusual oxidant such as 5-hydroxymethyl uracil (5-OHMeUra) or 8,5′-cyclo-2′-deoxyadenosine (cyclo-dA). 5-OHMeUra is involved in methylation pattern abruption, while cyclo-dA causes protein interaction to be hindered, resulting in transcription failure [77].

CONCLUSION(S)

Diabetes is a metabolic condition that lasts for a long time. T2DM is not caused by a single component; additional factors like obesity, genetic factors (both inheritance and epigenetically), improper nutrition, sleep patterns, and a stagnant lifestyle all play a role. T2DM is directly influenced by mitochondrial function and alterations. Because mitochondria are an oxidation site, various diseases and treatments target them. During the oxidation process, ROS is produced. The disease is caused by an imbalance in the generation of ROS and their elimination by antioxidants. According to recent research, a high amount of fat in the body causes insulin resistance and blood vessel obstruction, which is classified as a cardiovascular disorder and is a common result of T2DM caused by ROS. Renal failure, amputation of the lower extremities, liver dysfunction, and other complications are also possible. Endogenous antioxidant identification and mechanistic information provide us with the knowledge that forms the foundation of a therapeutic approach for preventing and delaying complications. Furthermore, the detection of biomarkers aids in the research and development of target drug design.

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