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Review Article

PATHOPHYSIOLOGY OF SECONDARY COMPLICATIONS OF DIABETES MELLITUS

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ABSTRACT

Diabetes mellitus (DM) is the most common endocrine metabolic disorder, characterized by hyperglycemia. The cause of this hyperglycemia is either insufficient or inefficient insulin that leads to the imbalance in the metabolism of not only carbohydrates but also protein and lipids. DM is associated with various kinds of abnormalities which affects almost all the parts of the body including eye, kidney, brain, foot, etc. Hyperglycemia is not the only reason which gives DM a tag of most apocalyptic disease; it is the complications that arise from the higher concentration of glucose or metabolites come from its variant metabolic pathways. DM causes both microvascular and macrovascular complications. Microvascular complications, caused by the damage of small blood vessels, include nephropathy (kidney disease), retinopathy (eye damage) and neuropathy (nerve damage), whereas macrovascular complication, caused by the damage of large blood vessels, includes blood vessels arteries and veins. There are six metabolic pathways are there which normally leads to these complications. These pathways are sorbitol pathway, advanced glycation pathway, Hexosamine pathway, protein kinase C pathway, ketoaldehyde pathway, and oxidative stress.

Keywords: Complications, Retinopathy, Neuropathy, Nephropathy, Glycation, Sorbitol.

INTRODUCTION

The disease, diabetes mellitus (DM), was known to humankind from the very beginning. The ancient Hindu Vedas and other old Indian Sanskrit texts explained in the 6th century AD the sweetness of urine. The Hindu physician Charak (200 AD), mentioned most of the clinical features of this disease in Charak Samhita as Madhumeha. In this book, he also described the relationship between obesity and diabetes, the tendency of the disease to be transmitted from generation to generation. Sushruta (500 AD) gave the description of the disease as Madhumeya, Ikhumeha, or honey urine, as the urine of the patients tasted sweet. He also described and mentioned two varieties of Madhumeya, of which one group of patients were lean and thin and wasted to death and another group of patients were obese with complications and died a slow death [1,2].

DM is not a single disease; rather it is a group of abnormalities characterized by abnormally hyperglycemia resulting from insufficient or inefficient insulin or both [3]. An increase in blood glucose because of food intake, insulin secretion from the pancreatic beta cells has been triggered. Insulin works on hepatocytes to increase the mobilization of blood glucose and on muscle and fat cells to use blood glucose, causing a reduction in blood glucose level to the normal level. DM causes secondary complications such as blindness, kidney damage, cardiovascular disease, and lower-limb amputations and increases the mortality and morbidity. Diabetics can decrease the prevalence of these complications by managing their blood glucose, blood pressure, and blood lipid level within the normal range [4,5].

BIOCHEMISTRY OF DIABETES

DM is an endocrine multifactorial metabolic disorder which is characterized by two major defects: Decrease in insulin production by the pancreatic beta cells and resistance to the action of insulin at different target tissues (muscle, liver, and adipose), which ultimately leads to an impaired glucose uptake. The exact molecular mechanism of insulin resistance is not well-known precisely, but defects in post-insulin receptor intracellular signaling pathways are believed to play an important role here [6,7]. There are a number of factors responsible for insulin resistance, which is usually present before the onset of diabetes such as genetics, age, obesity, and hyperglycemia itself [8]. Most important contributor for the fasting hyperglycemia during diabetes is the unregulated hepatic glucose production [9].

The increased lipolysis by insulin resistant adipose cells and the subsequent increased circulating free fatty acids contribute to the secondary complications of DM by impairing the b-cells function, glucose uptake in skeletal muscles, and increasing gluconeogenesis in the liver. Adipose tissues have been emerged as an important endocrine gland which produces a number of hormones collectively known as adipocytokines or "adipokines." Hormones produced by adipocytes regulate insulin sensitivity such as resistin and adiponectin, food uptake like leptin, inflammation such as tumor necrosis factor-α (TNF-α), interleukin-6 ,and factors affecting blood coagulation such as plasminogen activator inhibitor-1 (PAI-1) [10]. As diabetes progresses, insulin production and secretion slow down which results in progressive hyperglycemia. Hyperglycemia itself aggravates insulin resistance and decreases insulin secretion, which is known as called glucotoxicity. The main reason and the mechanism of progressive failure of pancreatic β-cell is not completely known, but a number of factors involved in this pathophysiology of diabetes, which include genetic determinants, glucotoxicity chronic inflammation, and the harmful effect of elevated levels of free fatty acids on the functioning of β-cell, called as lipotoxicity [10,11]. Multiple organs are affected in this case namely muscle, liver, adipose tissues, and pancreas, generate the pathogenic condition that causes diabetes.

Diabetes is undoubtedly one of the fastest growing non-communicable health problems in the current century and has emerged as a major healthcare problem in India. According to Diabetes Atlas published by the International Diabetes Federation (IDF), there are an estimated 382 million persons with diabetes in the world in 2013, and this number is predicted to rise to almost 592 million people by 2035 with 55% increase in the cases [12]. The major part of diabetic people is between the age group of 40 and 59, and some 80% of this population live in low-income and middle-income countries (IDF Atlas 2014). All the types of diabetes are on the increase; especially type 2 which might increase by 55% by 2035. Globally, the number of diabetics increase continuously due to increment in aging population, flawless urbanization, less physical inactivity, the high prevalence of obesity, and sedentary lifestyle [13,14]. In the case of developing countries also, urbanization was treated as a measure for the increased risk of DM, which is also associated with some or more other reasons such as altered diet, obesity, decreased physical activity, and increased stress [12]. The global figure on the prevalence of diabetes in the 20-79 age groups is listed in Table 1.

The transition from a traditional to modern lifestyle, consumption of fat-rich diet and high calories food and an increased level of mental stress has compounded the problem up to the next level. In addition to genetic factors, obesity due to improved economic status is a major factor in this epidemic. In many parts of the developing world, low birth weight and maternal malnutrition during pregnancy may also play a leading role in insulin resistance [12].

SECONDARY COMPLICATION CAUSED IN DIABETES

All different types of diabetes are characterized by fasting and post-prandial hyperglycemia and relatively insulin insufficiency. Diabetic peoples are at high risk for developing various kinds of disabilities and life-threatening complications. In the case of unmanaged DM, the hyperglycemia causes cause chronic microvascular and macrovascular complications such as weight gain, neuropathy, nephropathy, retinopathy, and arteriosclerosis [15,16]. It is also associated with the accelerated such atherosclerotic macrovascular disease affecting arteries which carries blood to the heart, brain, and lower extremities. In the early stages, intracellular hyperglycemia causes abnormalities in blood flow and increased vascular permeability. In the case of all high-income countries, diabetes is a leading cause of blindness, renal failure, atherosclerotic diseases, and amputations, especially in lower-limbs. Among all these complications atherosclerosis is the most important cause of mortality and morbidity in the peoples with diabetes.

There are a number of biochemical metabolic pathways and various mechanisms of action for glucose toxicity have been reported by a different group of scientists. These pathways include polyol pathway, hexosamine pathway, methylglyoxal pathway, glucose autoxidation, protein kinase C (PKC) activation, methylglyoxal formation and glycation, and oxidative phosphorylation (OXPHOS) [17] (Fig. 1).

There are many potential mechanisms whereby excess glucose metabolites traveling along these pathways might cause damage to b-cell. All such pathways have in common feature of the formation of reactive oxygen species (ROS) higher rate with growing time span [18] cause chronic oxidative stress, which promotes the development of microvascular and cardiovascular diseases [15,19,20]. This in turn results in expression of defective insulin gene and decreased insulin secretion as well as increased pancreatic beta cell apoptosis [21-25].

Houstis *et al.* have shown that the treatment of 3T3-L1 adipocytes with TNF-α or dexamethasone increases the ROS levels and results in decreased insulin secretion. Antioxidant molecules or transgene encoding ROS scavenging enzymes ameliorate the insulin resistance caused by TNF-α or dexamethasone-treated 3T3-L1 adipocytes [26].

Table 1: Estimated number of people with diabetes (age group of 20-79 years) in 2014 and 2035

Ranking	2014		2035	
	Country	People with diabetes (millions)	Country	People with diabetes (millions)
1	China	103.2	China	109.7
2	India	77.4	India	104.2
3	USA	22.6	USA	25.7
4	Indonesia	15.6	Indonesia	21.2
5	Brazil	13.3	Brazil	16.4
6	Russian federation	10.8	Pakistan	15.8
7	Pakistan	10.2	Nigeria	14.9
8	Japan	9.4	Bangladesh	13.3
9	Bangladesh	9.4	Mexico	10.3
10	Nigeria	8.0	Russian Federation	9.6

In physiologic physiological concentrations, endogenous ROS help to maintain homeostasis. However, when these free radicals accumulate in higher concentration and for longer periods of time, they cause chronic oxidative damage and other adverse effects on cells as well as biomolecules. This is particularly important and problematic for the pancreatic islet cells, which are among those tissues which contains the lowest levels of intrinsic antioxidant defenses. As minimum as six different glucose metabolic pathways are emphasized in the reports as chief contributors of ROS leading to the various effects and they are listed below (Fig. 1).

Polyol pathway

It is also known as sorbitol pathway since the intermediate of this pathway is sorbitol. It is implicated in diabetic complications, especially in the microvascular damage to the retina, kidney, and nerves [27]. Cells utilize glucose for energy production, but the unused glucose enters the polyol pathway and gets converted to the sorbitol due to the action of the enzyme, aldose reductase, and the cofactor nicotinamide adenine dinucleotide phosphate (NADPH) (Fig. 2). Sorbitol cannot cross cell membranes, and when it accumulates, it produces osmotic stress on cells by drawing water into the insulin-independent tissues [28].

This step consumes NADPH, which is required for the regeneration of reduced glutathione (GSH). This could induce intracellular oxidative stress [17]. In the next step of the reaction, sorbitol dehydrogenase oxidizes sorbitol to fructose, which also produces NADH from NAD^+. Hexokinase can return the molecule to the glycolysis pathway by phosphorylating fructose to form fructose-6-phosphate. However, if the blood glucose is more than that can be handled in the glycolysis pathway, the mass balance ultimately favors the production of sorbitol [29]. This reaction increases cytosolic NADH: NAD^+ ratio, inhibiting the

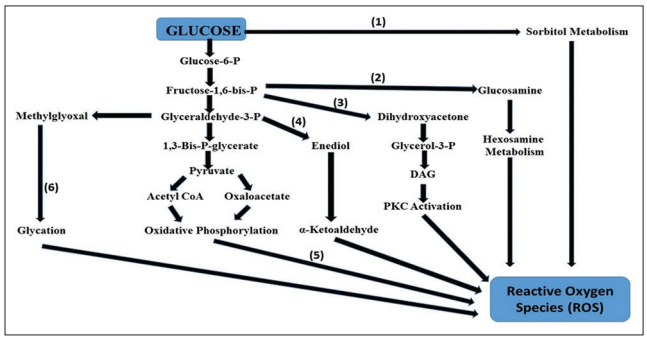

Fig. 1: Potential mechanisms by which hyperglycemia and its immediate biochemical sequelae lead to the formation of reactive oxygen species. Under pathologic conditions of hyperglycemia, excessive glucose levels can swamp the glycolytic process and inhibit glyceraldehyde catabolism, which cause glucose, fructose-1,6-bisphosphate, and glyceraldehyde-3-P to be shunted to other pathways: (1) Sorbitol metabolism; (2) hexosamine metabolism; (3) PKC activation; (4) enolization and α-ketoaldehyde formation; (5) oxidative phosphorylation; and (6) dicarbonyl formation and glycation (Robertson, 2004)

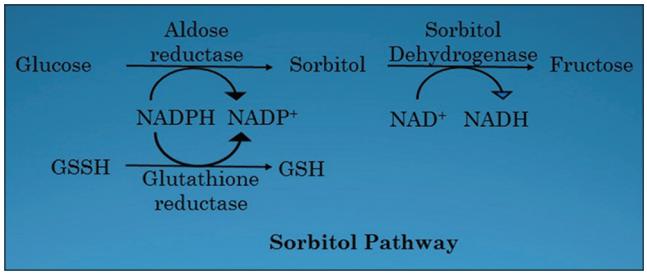

Fig. 2: Polyol pathway-induced oxidative stress

enzyme glyceraldehyde-3-phosphate dehydrogenase, and increases the concentration of triose phosphate. This increases the formation of methylglyoxal, a precursor of advanced glycation end products (AGEs) and diacylglycerol (DAG), thus activating PKC [17].

Hexosamine pathway

The excess intracellular glucose is shunted to the hexosamine pathway in many tissues during diabetes. This could contribute to oxidative stress because of the inhibition of the pentose shunt pathway, thereby diminishing the production of the cellular antioxidant leading to the reduction in reduced GSH [30]. In the hexosamine pathway, fructose-6-phosphate is converted to *N*-acetylglucosamine-6-phosphate (GlcNAc) by the rate limiting enzyme glutamine: Fructose-6-phosphate amidotransferase (GFAT). It is then converted to uridine diphosphate N-acetylglucosamine (GlcNAc). This is a substrate for *O*-linked glycosylation of the proteins, which is catalyzed by *O*-GlcNAc transferase (Fig. 3) [17]. There are reports that the increased activity of the hexosamine pathway may contribute to glucose-induced insulin resistance [31].

PKC pathway

PKC is a family of enzymes that are involved in controlling the functions of other proteins through the phosphorylation of hydroxyl groups in serine and threonine amino acid residues in the proteins. PKC enzymes in turn, are activated due to increasing the concentration of diacylglycerol or Ca^{2+} [32]. Hence, these enzymes play important roles in several signal transduction cascades [33]. There are about eleven isoforms of PKC, nine of which are activated by the DAG. Intracellular hyperglycemia increases the amount of DAG in diabetic animals [33]. Activated PKC has a number of pathogenic consequences including affecting the expression of endothelial nitric oxide synthase [34], endothelin-1 [35], and vascular endothelial growth factor [36]. It also transforms the growth factor-b and PAI-1 [37], by activating nuclear factor kappa B (NF-kB) and NAD (P) H oxidases [38]. The abnormalities caused by these actions are summarized in Fig. 4.

Autoxidation of glyceraldehyde

In addition to the classical pathway of glucose metabolism, there is a less familiar alternative pathway for the autoxidation of glyceraldehyde

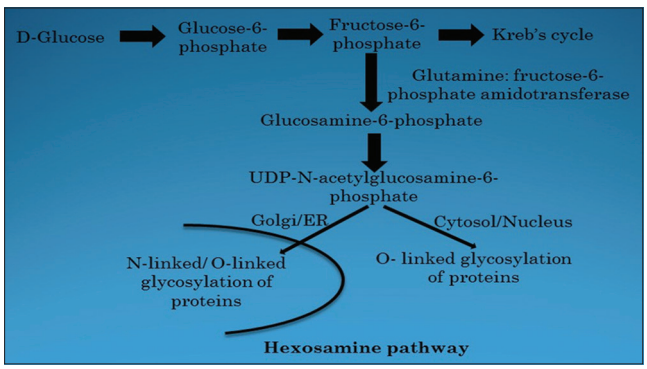

Fig. 3: Hexosamine pathway for biosynthesis of uridine diphosphate N-acetylglucosamine

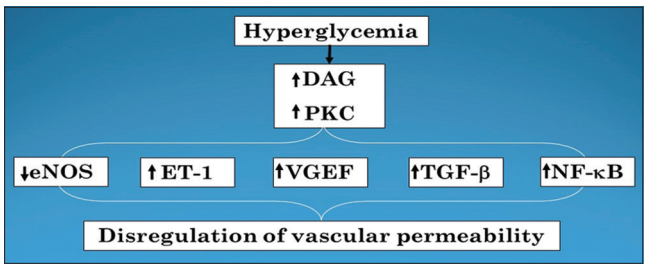

Fig. 4: Abnormalities arising out of the activation of protein kinase C caused due to hyperglycemia`

(Fig. 1, pathway 4). The autoxidation of α-hydroxyaldehydes generates hydrogen peroxide (H_2O_2) and α-ketoaldehydes [39]. In the presence of redox active metals, H_2O_2 can form the highly toxic hydroxyl radicals. This pathway forms two potentially toxic substances, α-ketoaldehydes, which contribute to glycosylation-related protein chromophore development, and the hydroxyl radical, an ROS that can cause mutagenic alterations in the DNA. Although glyceraldehyde is thought of as an insulin secretagogue, when present in excess, it may also inhibit insulin secretion [40].

Long-term exposure to high glucose concentrations decreases glyceraldehyde-phosphate dehydrogenase (GAPDH) activity in islets [41], which favors the accumulation of an excess of glyceraldehyde. Exposure of endothelial cells to high glucose concentration causes inhibition of GAPDH [42], through the mechanism of ROS-activated poly(ADP-ribosyl)ation of GAPDH by the poly(ADP-ribose) polymerase. This in turn is associated with intracellular AGE formation and activation of PKC, the hexosamine pathway, and NF-κB, a protein complex that controls transcription [18].

OXPHOS

Mitochondria are the major source of energy generation in the cells. 38% fewer mitochondria have been reported in the muscles of insulin resistant individuals when compared to control [43]. High glucose concentration increases the overproduction of electron donors by the tricarboxylic acid cycle, which in turn increases the production of mitochondrial superoxide [44] (Fig. 1, pathway 5). Lipotoxicity and glucotoxicity in obese and T2DM subjects induce the overexpression of β-cell uncoupling protein 2, which increases the proton leakage across the mitochondrial inner membrane and decreases ATP synthesis leading to insufficient secretion of insulin. Insulin resistance in the target tissues has been related to decreasing in mitochondrial content, reduced fatty acid oxidation, defective OXPHOS, and poor ATP production [45]. The decreased fatty acid oxidation, caused either by the reduced number of mitochondria or mitochondrial dysfunction, increases the levels of fatty acyl-CoA and DAG. These molecules activate PKC and finally inhibit the recruitment of GLUT4 to the membrane and insulin-mediated glucose uptake [46].

Formation of AGE

AGEs are a heterogeneous group of molecules formed from the non-enzymatic reaction of reducing sugars with free amino groups in proteins, lipids, and nucleic acids (Fig. 1, pathway 6). They are formed at a constant and slow rate in the normal body, starting in early embryonic development, and accumulating with time. However, their accumulation

Table 2: AGE-mediated effects in various macro and microvascular beds

Alter cell viability	↑↓ Vascular smooth muscle growth
	↑↓ Vascular endothelial cell viability
Thrombosis	↑ Adhesion molecule expression
	↑ Activity of transcription factor NF-κB
	↑ Tissue factor (Procoagulants)
	↓ Thrombomodulin (Anticoagulants)
	↑ Atherogenesis
Blood rheology	↑ Pro-inflammatory cytokine release
Vasopermeability	↑ Endothelial cell permeability
	↓ Basement membrane charge selectivity
Vasoregulation	↑ Quenching of NO
	↓ eNOS activity
	↓ Endothelium-dependent vasodilation
	↓ Vessel contractibility/elasticity
ECM dysregulation	↑ Mesangial cell matrix production
	↓ Skin elasticity
	↑ Thickness, cross-linking and resistance to degradation
	↑ Vascular wall stiffness

ECM: Extracellular matrix, eNOS: Endothelial NO synthase, NO: Nitric oxide, NF-κB: Nuclear factor kappa B, AGE: Advanced glycation end products

Table 3: Long-term complications of diabetes caused because of hyperglycemia (reference)

Long-term complications of diabetes		
Tissue or organ affected	**What happens**	**Complications**
Blood vessels	Fatty material builds up and blocks large or medium-sized arteries in the heart, brain, legs, and penis. The walls of small blood vessels are damaged, and they do not transfer oxygen to tissues	Poor circulation causes wounds to heal poorly and can lead to heart disorders, strokes, gangrene of the feet and hands, erectile dysfunction, and infections
Eyes	The small blood vessels of the retina are damaged	Decreased vision and ultimately, blindness occur
Kidneys	Blood vessels in the kidney thicken. Protein leaks into urine	Kidneys malfunction and ultimately kidney failure occur
Nerves	Nerves are damaged because glucose is not metabolized normally and the blood supply is inadequate	Legs gradually weaken. People have reduced sensation, tingling, and pain in their hands and feet
Autonomic nervous system	The nerves that control blood pressure and digestive processes are damaged	Swings in blood pressure occur. Digestive function is altered; Erectile dysfunction develops. Swallowing becomes difficult
Skin	Blood flow to the skin is reduced, and sensation is decreased resulting in repeated injury	Sores and deep infections (diabetic ulcers) develop. Healing is poor
Blood	White blood cell function is impaired	People become more susceptible to infections (urinary tract and skin)
Connective tissue	Glucose is not metabolized causing tissues to thicken or contract	Carpal tunnel syndrome and Dupuytren's contracture develop

is markedly accelerated in diabetes because of the increased availability of glucose [47]. The production of AGEs can cause damage to target tissues by three general mechanisms namely [48,49].

- Modification of intracellular protein by AGEs
- Modification of extracellular matrix protein and components
- Modification of plasma protein by AGEs.

Diabetes-mediated of AGEs formation and accumulation during the DM have been widely implicated in all macrovascular and microvascular complications [50] (Table 2).

The reactivity of AGEs may be less or more than the initial sugars from which they are formed. They are absorbed by the body during digestion with about 30% efficiency. Many cells in the body (such as endothelial cells, smooth muscle or cells of the immune system) from tissues such as lung, liver, kidney, or peripheral blood bear the receptor for AGEs. These bind to the AGEs and contribute to age and diabetes-related chronic inflammatory diseases including atherosclerosis, asthma, arthritis, myocardial infarction, nephropathy, retinopathy, or neuropathy [47].

Almost every organ in the human body is affected after prolonged hyperglycemia as listed in Table 3.

CONCLUSIONS

Diabetes is a chronic disorder causing due to the abnormality in the insulin synthesis or its action or both together. The most serious complication caused in the case of DM is their side effect and secondary complications which arise due to the assemblage of glucose. This accumulated glucose is harbored into different metabolic pathways which lead to the production of various compounds and metabolites and also generated free radicals, which damage the systemic organs and organelles. Medicines to control these secondary complications are unavailable in today's market. DM cannot be cured only it will be managed through proper management of food habit, physical exercises, and drugs. Long-term glucose homeostasis is required to reduce diabetes-related secondary complications. Glycemic control has a positive effect on both macrovascular and microvascular complications of DM for management and preventing the new onset of the complication and decelerating the progression these complications.

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