Causes and Complications of Diabetes - In A Biochemical View Point

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Abstract: Diabetes Mellitus is a metabolic disorder in which glucose is underutilized, producing hyperglycemia. Diabetes is now described as one of the main threats to human health in 21st century because of the role of the lifestyle factors such as diet and obesity along with genetic factors. Due to genetic susceptibility at early age, type 2 diabetes mellitus is considered as an emerging and significant problem in children and adolescents. Once this disease is established, it leads to complications related to oxidative stress which deals with imbalance between the generation of reactive oxygen species and antioxidant defense capacity of the body. This review aims to summarize the mechanisms of increased oxidative stress, obesity and gene regulation in the causes and complications of diabetes.

Keywords: Diabetes, oxidative stress, reactive oxygen species, obesity.

INTRODUCTION

Diabetes mellitus is a heterogeneous group of metabolic disorders that is characterized by absolute or relative deficiencies of insulin, derangements in carbohydrate, lipid and protein metabolism, changes in endothelial function and microvascular structure and is diagnosed by the presence of hyperglycemia(1). Despite the fact that diabetes mellitus is a controllable disorder, it is a major health threat worldwide predisposing to markedly increased morbidity and mortality arising due to chronic debilitating complications associated with it (2).Statistical analysis depicted that in diabetic patients, there is increased death rate due to poor glycemic control(3) and these patients are significantly prone to cardiovascular disease, diabetic neuropathy, nephropathy, retinopathy, non traumatic amputation and stroke in adults(4). The prevalence of diabetes is increasing worldwide. About 150 million people have type2 diabetes with the figures expected to double by 2025(5). Diabetes is a multifactorial disorder induced by genetic susceptibility in HLA genotypes and lifestyle changes such as increasing obesity, sedentariness and dietary habits. These factors have a remarkable effect on the development of the disease in both Western and Developing countries (6).This article deals with the role of oxidative stress, lipid peroxidation and protein oxidation in diabetes mellitus. Also, emphasizing the regulation of PPAR gene expression by diet and BMI and type2 diabetes in children in parallel to rising obesity rates.

DIABETES AND OXIDATIVE STRESS

Oxidative stress is an imbalance between the generation of reactive oxygen species (ROS) and antioxidant defense capacity of the body. It is seen that ROS is generated by mitochondrial sources i.e. by oxidative phosphorylation and by uncoupling of the respiratory chain and by cytosolic souces i.e. glycolysis, flux through the sorbitol pathway, advanced glycation mechanisms, uncoupling of nitric oxide synthase and xanthine oxidase (7). It is closely associated with a number of diseases including diabetes and its complications, cancer and cardiovascular diseases (8). The increased presence of ROS is also implicated in the pathogenesis of type 1diabetes (9).It is observed that increased intracellular ROS cause defective angiogenesis in response to ischemia. Atherosclerosis and cardiomyopathy in type2 diabetes mellitus are caused by selective insulin resistance, which increases mitochondrial ROS production from free fatty acids and by inactivation of two critical atherosclerotic enzymes namely nitric oxide synthase and prostacyclin synthase (10).The ROS generated damages vascular endothelial cells and stimulate the proinflammatory pathway involving transcription factor NFκB. NFκB controls the expression of inflammatory cytokines such as TNF-α, IL-1B and IL-6 which results in a chronic low grade inflammation. (11).

It is shown that in diabetes; chronic hyperglycemia sustain the oxidative stress by excessive generation of ROS in Glomerular and tubular cells, via over expression of oxidase and contributes to renal tissue injury. The activation of glomerular sterol regulating element binding protein- 1c (SREBC-1c) plays an important role in the progression of diabetic nephropathy by inducing NADPH oxidase mediated oxidative stress. Protein kinase C (PKC) is involved in the hyperglycemia induced overexpression of vascular endothelial growth factor (VEGF) in podocytes, thus, excaberating the diabetic nephropathy (12).

Oxidation results in generation of superoxides, hydrogen peroxide and hydroxyl radicals (13).

which may cause oxidative insult in diabetes. Accumulating evidence points to a number of interrelated mechanisms14 which includes increasing production of free radicals such as superoxides(15)or decreasing

antioxidant status(16), glycoxidation(17) and formation of advanced glycation end products(18), activation of the polyol pathway(19) and altered glutathione redox status(20), ascorbate metabolism changes(21)andantioxidant enzyme inactivation (22) ***(Figure I).***

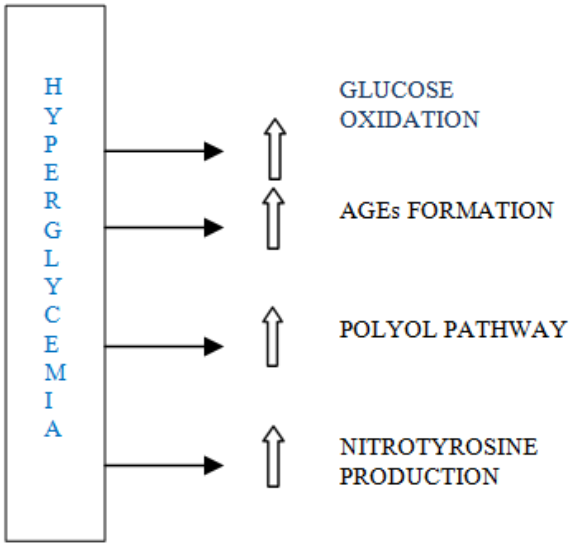

Figure I : Effect Of Hyperglycemia.

Some mechanisms are discussed as follows-

a) Glycation of Proteins-

Along with hemoglobin many proteins are glycated due to prolonged hyperglycemia in diabetic patients. As already proven glycated hemoglobin is used as a measure of the risk for the development of complications of diabetes [23]. Advanced glycation endproducts (AGEs) formed are complex products of glycation and oxidation (glycoxidation), which are postulated to be increased with age and at an accelerated rate in diabetes- mellitus [24].

b) Glutathione In Oxidative Stress-

Tissue glutathione plays a vital role in antioxidant defense [25]. Reduced glutathione (GSH) detoxifies reactive oxygen species such as hydrogen peroxide and lipid peroxides directly or in a glutathione peroxidase (GPX) catalyzed mechanism. Intracellular glutathione stores and a favorable redox status are maintained by NADPH dependent reduction of oxidized glutathione (GSSG) which is catalyzed by glutathione reductase (GRD)[26].

However, type2 diabetes patients show decreased erythrocyte GSH and increased GSSG levels [27]. There is an inverse relationship between erythrocyte GSH levels and presence of complications in type 1 and 2 diabetes patients(28).

c) Decreased Activity Of Superoxide Dismutase And Catalase –

Superoxide dismutase (SOD) and catalase are another group of major antioxidant enzymes (Figure II).

SOD exists in three different isoforms-

1) Copper and zinc-SOD is present and widely distributed throughout the cell. The gene is located on chromosome 21q 22.1. It is a homodimer in which ligands of copper and zinc are histidine side chains [29].

2) Extracellular-SOD is found in plasma and extracellular space. It is the secreted glycoprotein isoform of copper zinc – SOD which binds to proteoglycans in the vascular wall and protects against free radical injury. The gene is located on chromosome 4p15.3-p15.1.

3) Mitochondrial-SOD is located in mitochondria. The chromosome on which the gene is located is 6q25.3. The ligands of the manganese ions are 3 histidine side chains, an aspartate side chain and a water molecule or hydroxyl ligand depending on the manganese oxidation state [30].

Catalase is a hydrogen peroxide decomposing enzyme localized to peroxisomes mainly. Catalase is known to decrease cross linking and AGEs formation[31].

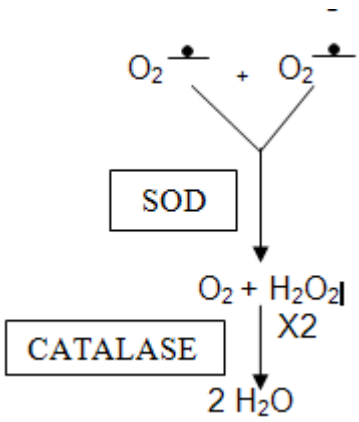

Figure II : SOD and catalase enzymes catalyzing oxidation and reduction of separate substrate molecules; both of which are highly specific for their substrates, O2 and H2O2 respectively [32].

Superoxides react with other reactive species such as nitric oxide (NO) to form peroxynitrites[33], which react with the tyrosine residues of proteins leading to nitrotyrosine production. It is an evidence of increased oxidative stress. Nitrotyrosine was found in the plasma of all type2 diabetic patients examined(34).

There is decreased activity of cytoplasmic copper and zinc-SOD and mitochondrial –SOD in neutrophils of the diabetic patients as estimated indirectly by cytochrome c reduction(35).

In a study conducted, it was shown that high levels of free

radicals together with low antioxidant activity capacity were detectedin obese diabetic adults, hence, in elevated oxidative stress. It further potentiated the risk of atherosclerotic and diabetic complications in these subjects (36).

It is implicated that there was reduction of oxidative stress, improvement in antioxidant enzymes and endothelial dysfunction in subjects undergoing treatment with both hypoglycemic drugs and vitamin E supplementation (37)

d) The Polyol Pathway-

This pathway is induced by hyperglycemia, resulting in activation of aldol reductase and production of sorbitol (38). This leads to depletion of erythrocyte NADPH and GSH (Figure III).

LIPID PEROXIDATON IN DIABETES MELLITUS-

a) ***Peroxidation of Lipids-***

Increased oxidative stress as measured by indices of lipid peroxidation is shown to be increased in both type 1 and 2 diabetes mellitus(40).

Measurement of thiobarbituric acid reactive substances (TBARS) is commonly employed to detect lipid peroxidation end products (41). Its use was pioneered in 1976(42).

TBARS level in plasma of diabetic patients is increased with the duration of disease and development of complications (43). Erythrocytes of diabetic patients are shown to be more susceptible to lipid peroxidation as measured by TBARS (44).

Serum levels of conjugated diene isomer of linoleic acid were found to be higher in diabetic patients with microalbuminuria than controls subjects (45). In a study, conducted in type2 diabetic patients as compared to age matched control subjects, there was an increase in lipid peroxide level and decrease in ascorbate level (46). Also, liposomes of erythrocyte membranes of diabetic patients were highly sensitive to superoxide induced lipid peroxidation (47).

Serum Malondialdehyde (MDA) levels were also implicated in pathophysoiology of oxidative stress in diabetes mellitus. These levels were higher in patients with newly diagnosed type2 diabetes than in matched controls (48).

The studies also showed that lipid peroxidative damage of the brain is increased in diabetes. The increased lipid peroxidation during diabetes is responsible for the formation of lipid hydroperoxides in membranes and results in damage of membrane structure (49).

Recent evidence for systemic oxidative stress includes the detection of increased circulating and urinary levels of the lipid peroxidation product F-2 isoprostane (8- epi – prostaglandin F 2α) type 2 diabetic patients and in obesity (50). In patients of type 2 diabetes mellitus, lipid peroxidation in lymphocytes was found to be higher than the control group, suggestive that hyperglycemia induces the oxidative stress condition inside the lymphocytes, which further stimulates cell death (51).Also, it was found that there was increased Interleukin-6 (IL-6) and MDA levels and decreased total antioxidant capacity, revealing the presence of inflammation with increased oxidative stress (52).

a) ***Susceptibility of LDL-Cholesterol to Oxidation-***

An increased amount of oxidized LDL-C is a common feature in diabetes. (53) Incubation of LDL-C with glucose at concentrations seen in the diabetic state increases susceptibility of LDL-C to oxidation which was measured by TBARS and conjugated diene formation (54).

b) ***Autoantibodies to Oxidised Cholesterol***- Type 1 and 2 diabetic patients have significantly higher antibody ratio than control subjects for copper oxidized LDL-C and MDA modified LDL(55). Increased ratios of oxidized LDL-C antibodies were also detected in type2 diabetics with macrovascular disease (56).

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PROTEIN OXIDATION IN DIABETES MELLITUS-

Proteins are an easy target for oxidative damage. Reactive oxygen species (ROS) modify amino acid side chains of proteins such as arginine, lysine, proline and threonine residues to form protein carbonyls. Protein carbonyl content is the most widely used marker of oxidative stress (57) and its elevated levels have been detected in both type 1 and 2 diabetes mellitus (58).

Non enzymatic condensation of free carbonyl groups of reducing sugars and their phosphate derivatives with free amino groups of proteins, lipids and DNA generates glycated species. These advanced glycation end products (AGEs) accumulate in plasma and tissues in diabetes. Autoantibodies against serum AGEs form AGE- immune complex which have also been detected in diabetic

patients. (59)

The study also shows the positive correlation of microalbuminuria levels with those of MDA, protein carbonyl content, low density lipoprotein (LDL) and triglyceride levels, suggesting the microvascular lesions in diabetes mellitus as a direct consequence of uncontrolled hyperglycemia and an indirect one from the oxidative stress (60).

DNA glycation marker, N2- carboxyethyl-2-deoxyguanosine, has been reported in kidneys and aortas of diabetic patients. Also, level of DNA- AGEs might serve as additional bio marker to evaluate initiation and progression of secondary complications of diabetes (61).

DIABETES AND OBESITY- "DIABESITY"

Diabetes and obesity are twin interrelated epidemics which threatens to engulf the world (62). Obesity is the driving force behind the diabetes epidemic (63). It is a "silent killer", a risk factor for potentially fatal conditions including heart disease, diabetes, high blood pressure, high blood cholesterol and stroke. Abdominal obesity is known to predispose individuals to insulin resistance. Abdominal fat secretes a group of hormones called adipokines which possibly impair glucose tolerance (64). Obesity is found in approximately 55% of type2 diabetic patients (65).

REGULATION OF GENE EXPRESSION BY DIET AND BODY MASS INDEX-

The peroxisomes proliferated activated receptors (PPAR's) are proteins which belong to the family of ligand activated transcription factors and they respond to the changes in dietary lipids by altering the gene expression in fat and carbohydrate metabolism (66

There are three members of this nuclear receptor superfamily-

1) PPARα for fatty acid oxidation and starvation response.

- It is expressed in liver, kidney, heart, skeletal muscle and brown adipose tissue.

2) PPARδ for fatty acid oxidation and thermogenesis.

- It acts on liver and muscle.

3) PPARγ for fat synthesis and storage and adipokine production.

- It is expressed in liver and adipose tissue. The "lipid burden hypothesis" is employed to explain the onset of type2 diabetes mellitus. Normally, the action of PPARγ on adipocytes keeps the cell ready to synthesize triacylglycerol (TAG). These adipocytes are insulin sensitive and produce leptin which leads to continuous intracellular deposition of TAG (67). But in obese individuals, adipocytes become filled with TAG and adipose tissue cannot meet demand for tissue storage. Also, adipocytes and their precursors, pre-adipocytes become less sensitive to insulin.

The gene expression associated with the development of new adipocytes (genes for the transcription factors SREBP1 and PPARγ) is downregulated but it is upregulated in skeletal muscle and liver which now store TAG. TAG is now stored "ectopically" at abnormal locations (68). Excess stored fatty acids and TAG is toxic to liver and muscle (69).

Some individuals are genetically less tolerant to handle this burden of ectopic lipids and are more susceptible to the cellular damage that leads to the development of type2 diabetes mellitus (70).

Also, TAG contained in chylomicron and VLDL-cholesterol are hydrolyzed by lipoprotein lipase (LPL) on the capillary endothelium and fatty acids hence produced are transported to adipocytes(71). There is abnormal regulation of adipose tissue LPL with a resultant increase in its activity in obese diabetic individuals (72). Recent research had revealed that adipose tissue is an important endocrine organ whichproduces peptide hormones known as **adipokines**. One of the important adipokine is leptin, which in brain acts on the receptors in the hypothalamus to curb appetite. Leptin regulates the feeding behavior and sends the message that fat reserves are sufficient. Hence, it promotes a reduction in fuel intake and increased expenditure of the energy (73).

A new study has shown that leptin plays a major role in islet cell growth and insulin secretion. In the presence of obesity, insulin resistance in the beta cell and the lack of leptin signalling leads to poor beta cell growth and function which leads to glucose intolerance (74).

In a study conducted in Japanese Americans it is found that elevated leptin is a risk factor for development of type2 diabetes in men (75).

Another peptide hormone known as adiponectin also affects blood glucose regulation. It is produced mainly in adipose tissue and sensitizes other organs to the effects of insulin, protects against atherosclerosis and inhibits inflammatory responses (76).

In patients of diabetes mellitus type 2, who are insulin

insensitive due to defective adiponectin genes clears glucose from the blood slowly as compared to non diabetic controls [77].

The drugs used in the treatment of type 2 diabetes mellitus i.e. thiazolidinediones increase the expression of adiponectin mRNA in adipose tissue and increased blood adiponectin levels in experimental animals [78].More so, the mainstays of therapy for type 2 diabetes involve drugs that are insulin centric i.e. they are designed.

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to increase insulin secretion and decrease insulin resistance. But with time evolution, the drugs for type 2 diabetes should also include glucose centric therapy such as antioxidant protection of the β- cell. This will facilitate repair of β- cell undergoing damage by oxidative stress secondary to the chronic hyperglycemia. Potent antioxidants such as N- acetylcysteine and aminoguanine have been demonstrated in vitro to provide protection of β-cells against oxidants like ribose and prolonged

exposure to the supraphysiologic concentrations of glucose and increase the expression of insulin(79).

GENETICS ASSOCIATED TO TYPE 2 DIABETES MELLITUS IN CHILDREN AND ADOLESCENTS: THE NEW SCENE-

Until a decade ago, type2 diabetes was regarded as a disease of the middle aged and elderly people, but there are alarming number of evidences of appearance of type2 diabetes in children and adolescents [80].The incidence of onset of the disease have been reported in the 20-30 years age group [81].

A sedentary lifestyle and caloric abundance have created new physiological conditions that are capable of changing expression of a number of genes involved in body metabolism and weight regulation [82].

Genes associated are gene HNF 1B [83] and Beta 3-Adrenergic Receptor Gene which determines the amount of fuel burned when body is in resting state. A specific mutation in this gene called TRP64ARG is 4 times more common in Pima Indians as compared to Europeans and hence, they show high rate of type 2 diabetes mellitus [84]. Gene HHEX-IDE plays an early role in influencing insulin resistance through its impact on body size during childhood as it contributes to childhood obesity(85).Genetic association analysis using thousands of Single Nucleotide Polymorphism (SNP) markers and wide genome screening is being applied for the search of genes predisposing to type 2 diabetes mellitus in young population [86].The Pro12Ala polymorphism in PPAR 2 genes has been associated with a reduced risk of type 2 diabetes mellitus and insulin resistance [87]. In a study, for an autosomal gene scan for genes

contributing to the development of type 2 diabetes and body mass index (BMI) in 164 families and 256 affected sibling pairs it was observed that 12 regions showed significant multipoint evidence of linkage with type 2 diabetes mellitus[88].

Also, the genes ABCC8 and KCNJ11, which encode the subunits sulfonylurea receptor1 (SUR1) and inwardly rectifying potassium channel (Kir6.2) of the β-cell ATP sensitive potassium channel (KATP), controls the insulin secretion. Common polymorphisms in these genes (ABCC8 exon 16-3t/c exon 18t/c, KCNJ11 E23K) have been invariably associated type 2 diabetes mellitus [89].

In another study conducted, meta-analysis of case control data showed that the E23K allele was associated with type 2 diabetes. These results confirm that large scale association studies are important for the identification of the disease susceptibility alleles [90].

CONCLUSION

Diabetes mellitus is associated with an increased mortality from various complications especially, coronary heart disease and oxidative stress has also been implicated in pathogenesis of micro- and macrovascular complications of diabetes. With the invent of technology, surplus food and physical inactivity, obesity has emerged as a major threat to human existence. Type 2 diabetes mellitus and obesity can be subjected to a genetic dissection of complexity based on pathogenic mechanisms, time course of the traits and the individual's age within the prediseased period. There are an increasing number of patients with type 2 diabetes mellitus all over the world with involvement of even children and adolescents age groups in it. Genetic screening for type 2 diabetes mellitus aimed at identifying subjects at disease risk for prevention studies is still limited to research settings only; hence, there is an urgent need to develop more radical preventive measures by better diet and exercise programs and global awareness at war footing level.

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