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Review Article

DIABETES MELLITUS AND ITS COMPLICATIONS: A REVIEW

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ABSTRACT

Insulin resistance associated with type 2 DM is the cause for the development of syndrome X, which further deteriorates the quality of life by giving rise to other disorders such as CVD, NAFLD, PCOS, many types of cancers and complications. Complications associated with DM are again subject to ethnic variation, proved based on exclusive study of XbaI(-) allele of GLUT1 gene. Study of the relative expression rates of such GLUT transporters is of prognostic importance towards certain condition associated DM as can be seen with gestational DM. Immunocytochemistry and flow cytometry detected the additional presence of GLUT4 in the lymphocyte of type 2 diabetic patient but not in type1diabetes patient, thus acting as a marker for type 2 DM. Presence of tumor in diabetics can worsen diabetic complications because of the liberation of HMGB1 protein, capable of binding with RAGE, which further activates MAPK signaling pathway. A thorough study of AGE-RAGE interaction suggests that expression of RAGE is the rate limiting step in the development of diabetic complications because of its non selectivity and ability to activate inflammatory mediators, thus holding RAGE responsible for diabetic complications; where as another study suggests that pathological or beneficial role played by RAGE depends on its isoform expressed on human tissue as esRAGE was found to exert a cytoprotective role by its ability to capture AGE outside cell. Further, a question is raised on the protective role of C peptide because of its capacity to activate MAPK pathway, which is associated with diabetic neuropathy.

Keywords: Diabetes mellitus, GLUT, C peptide, AGE, RAGE, Diabetic complications.

INTRODUCTION

Diabetes mellitus(DM) a globally prevailing syndrome characterized by hyperglycemia, glycosuria, polyuria, polyphagia, polydypsia, ketonaemia and negative nitrogen balance, is the major life threat of present times.[1,2] Irrespective of its types(type 1 and type 2) it can cause irreversible damage or complications if not checked in time; complications may arise in the form of neuropathy, retinopathy, nephropathy, vascular damage, erectile dysfunction, non-alcoholic fatty liver disease and many more.[3,4, 5,6,7,8]A study reveals that type 2 DM is the commonest form of DM constituting 90% of the diabetes population and the prevalence of complications associated with it varies from one population to the other.[9] The worldwide population of diabetic patients is increasing alarmingly with India claiming number one position in the chart with 50.8 million diabetics; a prediction made by the WHO stated that developing countries would face the impact of the epidemic in the 21st century; the population which was predicted to come under this syndrome by 2010 was 285 million people (6.4% of world's adult population) and expected to exceed to 438 million by 2030 (7.8% of the world's adult population).[10] Data from the 2011 National Diabetes Fact Sheet (released Jan. 26, 2011) reveals that 25.8 million children and adults in the United States have diabetes which is 8.3% of the population.[11] This article aims to review the role of GLUT transporters, C peptide, AGE–RAGE interaction and complications associated with this metabolic disorder.

Pathophysiology of diabetes mellitus

Type 1DM

Type1DM is a T cell mediated (CD4+ and CD8+), catabolic disorder in which circulating insulin is very low or absent, plasma glucagon is elevated, and the pancreatic beta cells fail to respond to all insulin-secretory stimuli. The pancreas shows lymphocytic infiltration and destruction of insulin-secreting cells of the islets of Langerhans. Approximately 95% of patients with type 1 DM have either human leukocyte antigen HLA-DR3 or HLA-DR4. HLA-DQs are considered specific markers of type 1 DM susceptibility, thus representing class II type of peptide display system of adaptive immunity.[12, 13,14,15]Immune dysregulation, caused by genetic susceptibility and environmental modifiers, leads to development of autoantibodies against various islet cell components, including glutamic acid decarboxylase antibodies (GAD-65 Ab), islet cell antibodies (ICA512/IA-2 Ab) and insulin antibodies (IAA) , thus the best predictor for future development of type 1DM is the expression of multiple autoantibodies. Type1 DM patients may exhibit diabetic dyslipidemia, characterized by low HDL and high TG rich particles (such as VLDL, chylomicrons); as the concentration of free fatty acid increases more and more production of acetyl –CoA will occur leading to over production of ketone bodies like acetone, acetoacetate and β-hydroxy butyrate leading to diabetic coma . Due to lack of intracellular glucose, amino acids are targeted and converted to glucose by gluconeogenesis leading to decreased muscle mass. Extracellular hyperglycaemia produces hyperosmotic plasma leading to hyperglycemic coma. The cardinal features of the syndrome are an elderly person with severe dehydration and depression of the state of consciousness, with hyperglycemia but usually without evidence of keto-acidosis. As the concentration of glucose exceeds normal level it starts appearing in the urine with subsequent manifestations.[16,1,17]

Type 2DM

The onset of type 2 DM has been most common in middle age and later life, although it is being more frequently seen in adolescents and young adults due to an increase in child obesity and inactivity; the reason being insulin resistance due to oxidative stress, down regulation of insulin receptors in the peripheral tissue or reduction in the number of insulin receptors. Due to insulin resistance an inadequate insulin response is observed, leading to a compensatory hyperinsulinaemia; reason behind syndrome X. Syndrome X leads to cardio vascular disorders (CVD) like heart attack, stroke, hypertension, and other problems like fatal non alcoholic fatty liver disease (NAFLD), polycystic ovary syndrome (PCOS), liver cancer, colorectal cancer, breast cancer, prostate cancer, impaired cognitive function etc . Other causes of type 2 DM are abnormality of glucose receptor of β cell, responding to a relatively higher concentration of glucose or relative β cell deficiency and excess hyper glycaemic hormones.[19,20,21,1]β cell dysfunction is initially characterized by an impairment in the first phase of insulin secretion during glucose stimulation and may antedate the onset of glucose intolerance in type 2DM. Coupling of glucose to the glucose sensor of β cell, following its transmembranous transport, induces an increase in glucokinase, serving as the first step in linking intermediary metabolism with the insulin secretory apparatus. As glucose transport in β -cells of type 2DM patients is greatly reduced, hence there occurs a shift in the control point for insulin secretion from glucokinase to the glucose transport system. Subsequently the second phase release of newly synthesized insulin is impaired. This secondary phenomenon, termed desensitization or β-cell glucotoxicity, is the result of inhibitory effect of glucose upon insulin

release and may be attributable to the accumulation of glycogen within the β-cell as a result of sustained hyperglycemia. Other candidates that have been proposed are sorbital accumulation in the β-cell or the nonenzymatic glycation of β -cell proteins.[22,23,24,25,26]

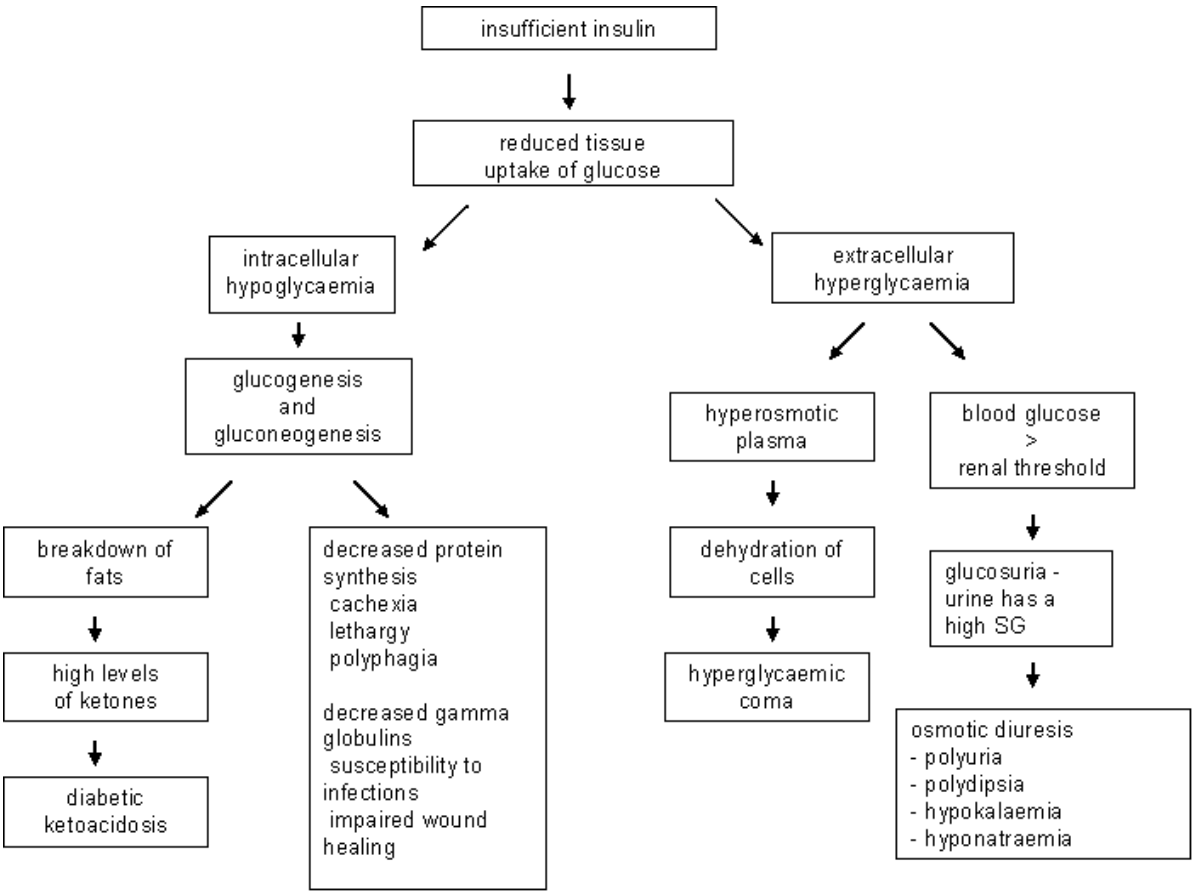

Fig 1.1: Pathophysiology of type 1 DM [18]

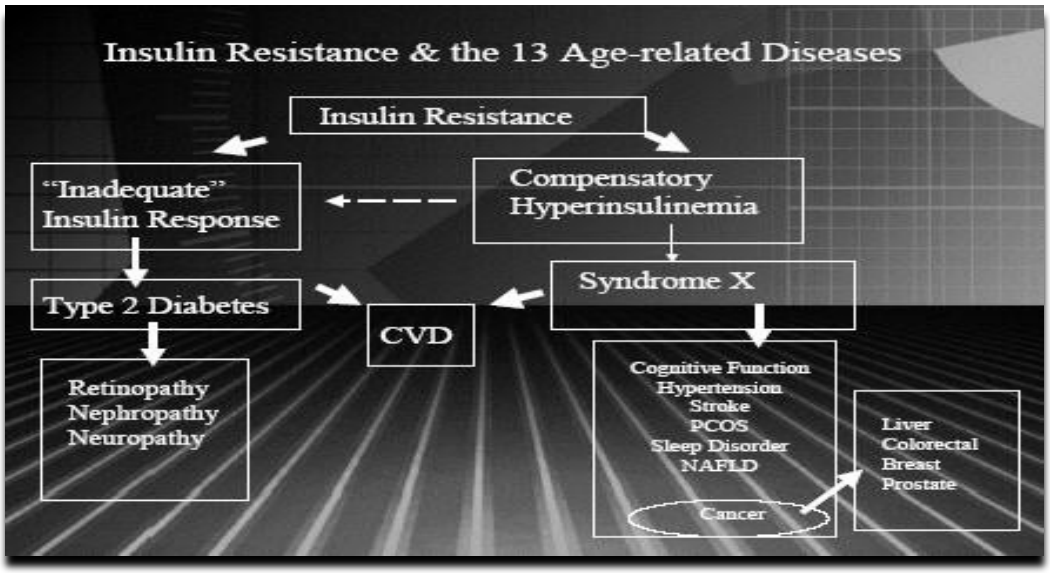

Fig 1.2: Pathophysiology of type 2 DM[27]

Role of GLUT in diabetes mellitus

Glucose is transferred across the plasma membrane by facilitated diffusion along a concentration gradient with the help of transport protein called GLUT. In human tissue and cell 14 isoforms of the transporter had been described: GLUT1 - GLUT 12, GLUT14 and HMIT.

Any qualitative or quantitative change in the expression of GLUT in the circulating lymphocyte may be an indication towards type 2 DM.

Immunocytochemistry and flow cytometry detected the additional presence of GLUT4 in the lymphocyte of type 2 diabetic patient but not in type1diabetes patient, thus acting as a marker for type 2 DM.[28,29] Reduced GLUT1 protein expression in the skeletal muscle is the inherent defect associated with type 2 DM and establishes the link between type 2DM and decreased basal leg glucose uptake.[30] An alteration in the activity of GLUT1 in the glomerular mesangial cells may stimulate extra cellular matrix production even under normoglycemic condition which may be a cause of damage of the nephron. A study examined six GLUT1 single nucleotide polymorphism and confirmed homozygosity of the XbaI A allele was associated with nephropathy in type1 DM.[31,32] Another study on Chinese patients shows that the frequency of XbaI (+/-) genotype and XbaI (-) allele was significantly higher in type 2 DM patient with diabetic nephropathy than those without nephropathy. Thus the presence of XbaI (-) allele of the GLUT1gene could be considered as the genetic marker for type 2 DM with nephropathy but the predictability of this study is again subject to ethnic variation as the same XbaI (-) allele reduces the chances of diabetic nephropathy in Caucasian patients.[33,34]GLUT 1 mRNA was found significantly correlated with maternal age and placental weight; placental weight is inversely correlated to GLUT1 not to GLUT3 mRNA. Among the diabetic patients GLUT1 and GLUT3 mRNA were lower in gestational DM and type 2DM than in type 1 DM. GLUT3 was significantly lower in diabetic women than non diabetic women in late gestation.[35]

Role of C peptide in diabetes mellitus

C- peptide, a cleavage product of the proinsulin molecule plays both physiological and protective roles by its ability to bind in nanomolar concentrations to a cell surface receptor which is most likely to be G-protein coupled, initiating multiple cellular effect evoking a rise in intracellular calcium, phosphatidylinositide-3-kinase activity, stimulation of Na^+ / K^+ATPase activity, increased endothelial nitric oxide synthase transcription and activation of mitogen activated protein kinases (MAPK) pathway.[36] But studies conducted on adult rats confirmed the involvement of MAPK pathway in diabetic neuropathy both via direct effect of glucose and via glucose induced oxidative stress.[37] Thus raising question on the protective role of C-peptide. A study confirmed that acidic residues on the N-terminus of proinsulin C-peptide are important for the folding of insulin precursor. In vitro refolding experiments have shown serious aggregation during refolding involving C-peptide mutant proinsulin genes with both alanine replacement mutation and deletion of three highly conserved acidic residues at the N-terminus of the C-peptide.[38]Refolding experiments confirmed the importance of normal C-peptide; it not only facilitates the folding of human proinsulin but also governs its kinetic folding pathway of human proinsulin.[39]A C-peptide test can be done when diabetes has just been found and it is not clear whether it is type 1 diabetes or type 2 diabetes. Type 1 DM has a low level of insulin and C-peptide where as type 2 DM has a normal or high level of C-peptide.[40]

AGE-RAGE interaction and role of RAGE in DM

AGE (advanced glycation end product) formation was marked with the identification of generation of brown colored substances by a nonenzymatic reaction between reducing sugars and amino acids, where linkage took place between the carbonyl group and the amino group to form Schiff bases and then Amadori compounds, finally yielding irreversibly cross-linked products.[41] AGE has the ability to bind with multiple number of receptors such as lactoferrin, scavenger receptors types I and II, oligosaccharyl transferase-48 (OST-48), 80K-H phosphoprotein, galectin-3, and CD36.[42]Elevated blood glucose levels contribute to the glycation of proteins and lipid resulting in the formation of AGE. Binding of AGE to its receptor(RAGE) leads to the generation of reactive oxygen species, which in turn activates nuclear factor κB (NF-κB) resulting in the activation of many inflammatory proteins and mediators like TNF-α, TNF-β, IL-1, IL-6, IL-8, IL-18, nitric oxide(NO) and interferon-γ. Combination of NO with super oxide leads to the formation of toxic peroxynitrite with subsequent micro and macro vascular complications.[19]Presence of tumor in diabetic patient can initiate an inflammatory condition and neuropathy because of the ability of the tumor cell to produce high mobility group protein(HMG), specifically HMGB1,capable of binding with RAGE, which activates MAPK signaling pathway and NF-κB.[43]RAGE has been identified as a receptor for many other ligands like amyloid-beta peptide (Aβ) & β-sheet fibrils, S100/calgranulins and Mac-1.[42]These evidences suggest that expression of RAGE is the rate limiting step for developing diabetic complications. A study confirmed that AGE-RAGE interaction on endothelial cells plays an important role in the development of complication like atherosclerosis, accounting for 80% of all deaths among diabetic patients, mediated by NF- κB and vascular cell adhesion molecule 1 (VCAM 1).[44] AGE along with some other senescent macro protein derivatives are involved in the development and progression of many types of cancers.[45] AGE-RAGE interaction is considered to play an important role in disease like multiple sclerosis, as it was confirmed by a pilot study using immunohistochemical technique, where an abundance in advanced glycation end product N-epsilon-carboxy methyl lysine (CML) and its receptor (RAGE) was detected using monoclonal antibodies for CML and human RAGE.[46]Animal study involving RAGE transgenic mice exhibited the exacerbation of the indices of nephropathy and retinopathy ; also showed the presence of extracellular signals and nuclear factors that induce the transcription of human RAGE gene and are the risk factors of diabetic complications. Three major splice variants of RAGE mRNA were identified after carefully analyzing poly(A)$^+$RNA isolated from polysomes of human endothelial cell and pericytes; described as full-length membrane-bound form, a novel N-terminally truncated membrane-bound form, and a novel C-terminally truncated soluble form. The C-truncated form was named as endogenous secretary RAGE (esRAGE) and was described as naturally occurring soluble RAGE in human and would be cytoprotective, because it is able to capture AGE outside cells. Thus we can predict that RAGE has both physiologic and pathologic roles to play based upon its isoform expressed on human tissue.[41,47,48]

Diabetic complications

Diabetic complications are the challenges associated with diabetes in the form of micro and macro vascular complications; microvascular compications include retinopathy, nephropathy and neuropathy whereas macrovascular compications include coronary artery disease (CAD), peripheral vascular disease (PVD) and cerebrovascular events (CVA).[9]

Microvascular compications

Retinopathy

Diabetic retinopathy (DR) is a well recognized complication occurring both in type 1 and type 2 diabetes mellitus and has been shown that nearly all type 1 and 75% of type 2 diabetes will develop DR after 15 years duration of diabetes.[49]DR is classified into non proliferative diabetic retinopathy (NPDR) or background retinopathy and proliferative diabetic retinopathy (PDR). NPDR is characterized by vascular closure with the earliest visible signs as microaneurysms and retinal hemorrhages. Progressive capillary nonperfusion is accompanied by development of cotton-wool spots, venous beading and intraretinal microvascular abnormalities. PDR which occurs with further retinal ischemia is characterized by the growth of new blood vessels on the retina and posterior surface of the vitreous.[9, 50] Patients with mild NPDR show a 3% incidence of diabetic macular edema (DME) but those with moderate to severe NPDR have a 40% incidence of DME. In the presence of proliferative diabetic retinopathy (PDR) there is a 71% incidence of DME.[51]Visual impairment in diabetic retinopathy is the consequence of diabetic macular edema and PDR.[49,52] Risk for developing retinopathy is greater in diabetics with poor control of blood sugar level , high blood pressure, high cholesterol, pregnancy, smoke etc.[53]

Nephropathy

Nephropathy is the leading cause of chronic renal failure, the initial marker being microalbuminurea, which can be screened by measurement of albumin to creatinine ratio in a random spot collection.[9]Initial microalbuminurea associated with diabetic nephropathy was observed in the range of 30-299 mg/24 hours in a 24 hours urinary collection, 20-199 µg/min in a timed urine collection or 30-299 µg/mg creatinine in a spot urine collection on at

least two occasions within a three-to-six month period. A greater proportion of patient with type 2 DM compared with type 1 DM develop microalbuminuria.[54]The abnormal value of microalbuminuria based on 24 hr urine collection method is 150-300 mg/day whereas for macroalbuminuria it is more than 300mg/day.[55] Even though diabetic nephropathy can be categorized into stages: micro and macro albuminuria based on the values of urinary albumin excretion, yet it has been seen that the risk for developing diabetic nephropathy and cardiovascular disease starts even when urinary albumin excretion values are within the normoalbuminuric range.[56] It has been postulated that fluctuations (increase in HbA1C i.e glycosylated haemoglobin of more than 2% between two consecutive measurement within 3 months interval ± 2 weeks) in HbA1C is the predictor of nephropathy in type1DM.[57]It has been seen that hypertension is an adverse factor in all progressive renal diseases and seems especially so in diabetic nephropathy, which shows histological change as diabetic glomerulosclerosis, characterized by glomerular basement membrane thickening and mesangial expansion with increased extracellular matrix deposition. Imaging study confirmed that in the early stage of nephropathy the size of kidney increases due to hyperfiltration but as the time proceeds kidney diminishes in size from glomerulosclerosis.[55,58]

Neuropathy

Diabetes mellitus is the most common cause of neuropathy worldwide. Neuropathies are classified into symmetrical or asymmetrical (focal or multifocal) forms, the symmetrical form is primarily sensory and autonomic where as the asymmetric form can be sensory, motor or both as well as affecting the individual cranial or peripheral nerves. Diabetic peripheral neuropathy is defined as stocking- glove neuropathy or somatic and/or autonomic neuropathy which affects the longest nerve first before progressing proximally.[59,60,61,62]Distal symmetrical form of diabetic peripheral neuropathy otherwise known as diabetic sensorimotor peripheral neuropathy is the primary risk factor for the development of diabetic foot ulcer, responsible for 85% of lower extremity amputation in diabetes patient.[63]Thus it is necessary to monitor neuropathy if at all present and to find its significance level to take the proper treatment strategy. A standard neuropathy disability score (NDS) of over 6 indicates the presence of significant neuropathy.[9]

Macrovascular compications

Coronary artery disease (CAD)

A study conducted on diabetic population revealed that more than 3 out of 4 diabetic patient die of causes related to atherosclerosis and in most cases (75%) because of CAD. Type 2 DM increases the risk of CAD by 2-4 times in the overall population.[64]CAD is caused by atherosclerosis characterized by the formation of plaques. With increase in size of the plaques there occurs angina; sudden rupture of the plaque leads to the acute coronary syndrome (ACS), which is a medical immergency.[65]ACS may occasionally occur in the absence of electrocardiographic changes or elevations in biochemical markers, still the main diagnostic tool of ACS, unstable angina and acute myocardial infarction is the measurement of cardiac enzymes and markers. Measurement of cardiac markers like troponin T and troponin I gives an idea regarding ACS and myocardial infarction. As per the British Cardiac Society (BCS) working group a 12 hr serum troponin T concentration of less than 0.01µg/l indicates ACS with unstable angina, serum troponin T concentration≥0.01 µg/l and <1.0 µg/l indicates ACS with myocyte necrosis and troponin T concentration ≥1.0 µg/l indicates ACS with clinical myocardial infarction.[66] Thus these values play a crucial role to the identification of the very specific problems associated with ACS.

Peripheral vascular disease (PVD)

PVD also known as peripheral arterial disease (PAD) is an occlusive disease of the large peripheral arteries (especially of the legs) excluding the coronary and intracranial vessels, primarily caused by atherosclerosis. Measurement of ankle-brachial pressure index (ABPI) has emerged as the relatively simple, non-invasive and inexpensive diagnostic tool of choice. An ABPI ≤ 0.90 is not only diagnostic of PAD even in the asymptomatic patient but also an independent marker of increased morbidity and mortality from cardiovascular diseases. It is also indicative of a haemodynamically significant arterial stenosis. Traditional risk factors of PAD include age, diabetes, smoking, obesity and hypertension, whereas non-traditional risk factors include race, chronic kidney disease and hypercoagulable states. PVD can be categorised using the Fontaine classification systems as shown in table 1.

Table 1: Staging of PVD

Stage	History
I	Asymptomatic
IIa	Mild claudication
IIb	Moderate-severe claudication
III	Ischaemic rest pain
IV	Tissue loss or ulceration

The above mentioned stages can be confirmed by measuring the ABPI, as ABPI >0.9 indicates normal but ABPI between 0.5-0.9 indicates moderate claudication where as ABPI <0.4 indicates critical limb ischaemia (rest pain/ulceration).[67,68] Thus ABPI provides clue regarding a particular type of PVD.

Cerebrovascular events (CVA)

Cerebrovascular diseases such as stroke, transient ischaemic attack(TIA) are more common in people with diabetes, it is three times more common in those with diabetes. In addition, there is increased mortality after a stroke and increased levels of disability in people with diabetes compared to those without.[69] Two principal causes of CVA in diabetic patients are small artery disease and atherosclerosis of cervical and intracranial arteries.[70] Cerebral vascular disease affects blood flow to the brain, leading to TIA and stroke. It is caused by narrowing, blocking or hardening of the blood vessels that go to the brain or by high blood pressure; such blockage changes brain function and may lead to loss of balance, confusion, blindness in one or both eyes, double vision, difficulty speaking or a severe headache.[71]A study shows that diabetes also governs the type of stroke as Ischaemic stroke is more prevalent than hemorrhagic stroke in diabetics and sub cortical infarcts are more common in diabetics than non diabetics.[72]

CONCLUSION

DM a globally prevalent syndrome if not checked leads to life threatening complications, which are again subject to ethnic variation. Lower expression of certain varieties of glucose transporter is again the predictor of certain condition associated DM, thus can be of prognostic importance. Insulin resistance associated with DM may give rise to another syndrome, which in turn is the root cause of many other crippling disorders. Cleavage product of proinsulin molecule plays an important role in the folding of proinsulin and its mutation alters the kinetic folding pathway of human proinsulin; thus any mutation may interfere with proper insulin action. Such a cleavage product also gives an useful hint for ascertaining the type of DM. Based on AGE-RAGE interaction we can predict that RAGE is the rate limiting step in the determination of diabetic complications. All these findings guide our path to the exact diagnosis and finding of the molecular cause related with the type of DM and to adapt proper treatment strategies.

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