DIABETIC NEPHROPATHY AND DIABETIC RETINOPATHY AS MAJOR HEALTH BURDENS IN MODERN ERA

*Tapan Behl¹, Ishneet Kaur², Dr. Heena Goel³, Dr. Rajesh K. Pandey⁴

¹Senior Research Fellow, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi
²Department of Pharmacy, Chandigarh College of Pharmacy, Mohali, Punjab
³Veterinary Officer, Department of Animal Husbandry, Junga, Shimla, Himachal Pradesh
⁴Senior Scientist, All Excel Inc. 135 Wood St., West Haven, CT-06516, USA

ABSTRACT
Diabetes mellitus is a chronic endocrine disorder characterized by abnormally high levels of glucose in the blood – a condition called hyperglycemia, which exposes the body to a wide number of other medical complications which adversely affect the body. Among these complications, the ones which are the most detrimental and unwholesome include Diabetic Nephropathy and Diabetic Retinopathy. Both of these are referred to as the microvascular impediments bestowed by the repercussions of diabetes mellitus. These two disorders have become a paramount ground of fret owing to the large population of the world suffering from diabetes today. Such complications of diabetes are even more menacing than the disease itself. Large masses being inevitably getting affected by these is a major reason of concern for the health associates worldwide. Therefore, there is an utmost need to comprehend and perceive the causes, risk factors, pathophysiology and outcomes of both these medical conditions so that everyone could be made aware of the devastating effects that such conditions exert on their lives. This would help every individual, who is at risk of developing these, realize the obligation of taking the adequate measures and preventive steps in order to eradicate and grub out these dire disorders which could prove to be extremely catastrophic.

INTRODUCTION
Diabetic Nephropathy and Diabetic Retinopathy are the two most notorious outcomes of Diabetes Mellitus. According to the statistical data given by the American diabetes
Association, 25.8 million people are affected by diabetes in the US at present, out of which 28.5% people were diagnosed with diabetic retinopathy in a span of four years and 44% of cases of diabetic nephropathy were reported in a single year. Both of these conditions are a result of various microvasculature alterations occurring as a result of disruption in the homeostasis of glucose in the body. These alterations further cause the conditions of angiopathy in the blood capillaries supplying blood to kidneys and retina, resulting in various noxious outcomes. Both of these outcomes are a serious reason to worry as they are leading causes of preventable blindness (due to the various events which occur in diabetic retinopathy such as – vitreous hemorrhage, formation of microaneurysms etc) and chronic end-stage renal disorders (due to the outcomes of diabetic nephropathy such as diminished rate of glomerular filtration, complete impairment of the normal physiology of kidneys owing to extensive damage to the nephrons etc) in the world. Hyperglycemia, being the common reason of cause, suggests that the pathophysiology of the development and progression of both these complications is somewhat similar. There are various common pathways leading to the advancement of both the disorders. Thus, understanding the pathophysiology of these complications would give a better understanding of their progression and would help in developing various methods of treating them [1-4].

Diabetic Nephropathy

Diabetic Nephropathy is one of the major complications of Diabetes Mellitus and a leading cause of chronic renal dysfunctions. It refers to a condition of angiopathy caused due to the alterations caused in the microvasculature of the blood vessels (particularly the small capillaries), which are carrying blood to the kidneys because of the hyperglycemic conditions prevalent in the body. Diabetic patients of both types of diabetes mellitus are susceptible for developing this complication [5]. An estimated statistics show that about 30% of the patients of type 1 and approximately 25-40% of the patients of type 2 diabetes mellitus are vulnerable of developing diabetic nephropathy inspite of the preventions and medications [3].

Diabetic Nephropathy is defined and diagnosed in the terms of elevated levels of albumin being excreted out in the urine. Relentlessly abnormal amount of this protein being excreted leads to a condition known as proteinuria, which in diabetic nephropathy is divided into two stages – microalbuminuria (in which the cutoff value of albumin in urine is upto 199 microgram per minute) and macroalbuminuria (in which the level of albumin present in the urine is above 200 microgram per minute) [6].
Risk factors of Diabetic Nephropathy
Since from above it is clear that diabetic nephropathy delineated on the basis of the measurements of microalbuminuria, we need to know the risk factors which are responsible in causing this condition. Microalbuminuria is a symbol of renal endothelial damage which is caused mainly due to occurrence of following in the body

✓ Hyperglycemia is responsible for renal endothelial injury because it decrease the formation of nitric oxide (which is a very potent vasodilator), thus inhibiting one of the most effective compensation for hypertension. This leads to all the noxious effects caused by hypertension itself. Also, high levels of glucose in the blood lead to the formation of oxygen free radicals and activation of protein kinase C which further worsens the condition by causing cell death of the renal endothelial cells [7-8].

✓ Hyperinsulinemia is responsible for renal endothelial injury because it is a factor which increases oxidative stress, thus further leading to free radical induced cell death [9].

✓ Hypertension is responsible for renal endothelial injury because it increases the pressure on the arteries carrying blood to the kidneys. The walls of small arteries could withstandthis pressure only for a small period of time. After a certain time limit necrosis of the arterial cells occur resulting in renal endothelial cell injury [10].

Butgenetic factors are also preliminary responsible for predisposing a person to the risk of diabetic nephropathy which is exaggerated in the presence of conditions such as hyperglycemia, hyperlipidemia, hypertension and proteinuria. Other environmental factors such as smoking, dietary intake (factors such as excess amounts of proteins and fats) are also considered as some of the risk factors in the progression of this disease. Apart from these, any reason responsible for glomerular hyperfiltration could also be responsible for this disorder [3, 11].

Pathogenesis of Diabetic nephropathy
Hyperglycemia: - Hyperglycemia caused due to the impaired metabolism of glucose (owing to insulin deficiency or resistance) in diabetes is, undoubtedly, the most crucial factor which leads to the progression of diabetic nephropathy. It is involved in its pathogenesis in a number of ways which lead to one or more events responsible for the advancement of this disease. The various mechanisms which are activated by hyperglycemia lead to some changes
such as – mesangial cell proliferation, modulation in the glomerular filtration rate, hypertrophy of the mesangial cells, cell apoptosis, tissue damage of the renal endothelium, alterations in its membrane permeability, thickening of the basement membrane, activation of inflammatory responses, accumulation of extracellular matrix and increased cell matrix production. The various mechanisms by which hyperglycemia leads to the progression of diabetic nephropathy are given as follows

➢ Activation of Aldose Reductase pathway: - Hyperglycemia leads to the activation of Aldose Reductase pathway which is the first step of the glucose metabolism in the polyol pathway, converting glucose into sorbitol – which is responsible for cell apoptosis due to osmotic and other changes like reduced Na⁺/K⁺-ATPase activity, decreased NADPH levels and increased formation of NADH – which creates a pseudohypoxia like condition in the cells which alters the cellular metabolism in many ways and ultimately the cells could no longer withstand these conditions and get damaged. Some studies also suggest that the activation of aldose reductase pathway may also have a role in the increased production of advanced glycation products which ultimately lead to microalbuminuria [22]

➢ Activation of Protein Kinase C: - Hyperglycemia is said to be responsible for the activation of protein kinase C (probably due to the increased concentrations of diacylglycerol – DAG) which is involved in the advanced development of diabetic nephropathy. PKC occurs in the form of three isoforms – PKC-alpha, PKC-beta, PKC-gamma. The activation of one or more isoforms of PKC further leads to a variety of events such as:

✓ Protein kinase C is responsible for the activation of several prostanoids, kinases and signal pathways which lead to an increase in the membrane permeability of the renal endothelium. Thus, accounting for macular edema.

✓ It is responsible for activating transforming growth factor-beta, collagen and fibronectin that leads to the thickening of the basement membrane of renal endothelium, which is a trademark of diabetic nephropathy.

✓ It leads to the expression of vascular endothelial growth factor which is responsible for angiogenesis and further increase in the membrane permeability, the result of which is the accumulation of excess extracellular matrix in that area.
It also has a role in increasing the production of advanced glycation end products which bind to the proteins of the basement membrane of the endothelium, further leading to various changes that increase its permeability.

It is also responsible in increasing the expression of adhesion molecules such as ICAM-1, thus further assisting the inflammatory response [18-21].

**Formation of Advanced Glycation End Products**: The advanced glycation end products (AGEs) are the fluorescent adducts formed when glucose reacts non-enzymatically with the amino groups present in nucleic acids, lipids or proteins. The accumulation AGEs is also found in normal people but in diabetic patients, they are found to accumulate at a much faster rate because of the easy and excess availability of glucose in the body. They affect the cells because

- They associate with the modified serum proteins in the form of adduct
- Lead to the formation of endogenous adducts after the process of glucose-metabolism
- Accumulate in the form of immobilized modifications of the extracellular matrix
- Induce the production of free radicals, thus leading to oxidative stress
- Decrease the levels of nitric oxide in the body
- They produce changes in the coagulation factors such as anti-thrombin-III, tissue factor and thrombomodulin and affects the stability of fibrin leading to fostering of a procoagulant state

Various factors affect the formation of AGEs – such as availability of transitional metals, type and concentration of sugar available. Every sugar has a different rate of forming AGEs. Glycation rate of glucose is very slow whereas that of fructose is quite high [24-32].
Decreased Formation of nitric oxide:

Hyperglycemia is responsible for the decreased levels in the formation of nitric acid, which is a very potent vasodilator. Thus, this leads to the inhibition of a very powerful inhibitory mechanism of hypertension in the body.
Role of Inflammatory mediators: - Hyperglycemia leads to the activation of many inflammatory mediators, particularly cytokines (such as IL-1, IL-6, IL-18 and TNF-alpha), which have a role in the progression of diabetic nephropathy. Studies suggest that

IL-1 is responsible for the increased expression of chemotactic agents and adhesion molecules such as ICAM-1, vascular cellular adhesion molecule -1 and E-selectin. This leads to the initiation of an inflammatory response which causes damage to the renal endothelium.

IL-6 is also said to be responsible for a number of unknown factors which result in the increase in the membrane permeability of the renal endothelium and making alterations in the movement of fluids and extracellular matrix surrounding the cells of the endothelium.

IL-18 is found to be responsible for the production of IFN-gamma (interferon-gamma), which is further accountable for the expression of chemokine receptors. Also, this interleukin leads to an increased production of other cytokines such as IL-1 and TNF-alpha, thus upregulating their subsequent effects.

TNF-alpha is responsible for the activation of transcription factors, secondary messengers, growth factors, cell adhesion molecules, certain enzymes which are responsible for the synthesis of other inflammatory mediators and also leads to the production of major histocompatibility complex proteins. All these constitute various events which result in endothelial injury and changes in the permeability of the membrane of the cells constituting renal endothelium [12-17].

Diabetic Retinopathy refers to a condition in which damage occurs to the blood vessels which carry blood to the retina of the eye because of the prevailing high levels of glucose in the
body. It is one of the leading causes of blindness in the world. The prediction of the occurrence of this disorder can be made from the duration of diabetes itself. The epidemiology of retinopathy has been found to increase with increase in the duration of time for which the patient has been enduring with diabetes mellitus. The chances of developing diabetic retinopathy of the patients having diabetes from the past 5 years are around 17% and 97.5% (for having suffered with diabetes for 15 years or more) [33].

Diabetic Retinopathy is of two types

**Non-Proliferative Diabetic Retinopathy:** - It is also called background retinopathy. It is an early phase of the much larger complication of diabetic retinopathy. In its initial stage, the affected blood capillaries become vulnerable to leakage of blood and fluids from them into the retinal cells. Progression in this phase leads to profound retinal hemorrhages, formation of microaneurysms, loop formations in veins and exudate liberation (which may either be hard or soft). According to the stage of the disease, non-proliferative diabetic retinopathy is classified into – mild, moderate and severe type.

**Proliferative Diabetic Retinopathy:** - It is an advance stage of the non-proliferative diabetic retinopathy. As its name suggests, it is associated with proliferation of new blood vessels. This occurs due to the involvement of various growth factors (such as vascular endothelial growth factor – VEGF) which lead to the process of angiogenesis. This process is also known as retinal neovascularization and involves the formation of new blood vessels in retina and into the vitreous fluid. Since the newly formed vessels are not much matured and strong, thus are fragile enough to leak out their contents very easily into the retinal cells and vitreous fluid – accounting for the cloudy vision. Further adverse condition arises when the vitreous chamber begins to constrict (due to the extremely high pressure conditions prevalent in the eye due to osmotic imbalance) resulting in retinal detachment. This accounts for blindness or loss of vision associated with this complication [34-37].

**Risk Factors of Diabetic Retinopathy**

✓ The duration for which the person has been suffering from diabetes is the most critical risk factor for the development of diabetic retinopathy. It has been concluded by many researchers that the chances of diabetic retinopathy increases with increase in the duration of prevalence of diabetes in any patient

✓ High levels of glucose prevalent in the body are responsible for the injury (caused due to oxidative stress and various other factors discussed later) to the blood capillaries of retina
Hypertension is also one of the biggest risk factors of diabetic retinopathy because it is responsible for inducing stress on the capillaries due to which hemorrhages occur.

Studies suggest that tobacco consumption might also be one of the risk factor for the development and progression of diabetic retinopathy but its reasons for involvement in it are not so well defined and are currently an area of active research [38-39].

**Pathogenesis of Diabetic Retinopathy:**

The formation of microaneurysms is the first step in the pathophysiology of diabetic retinopathy, whose formation is accounted to be a result of the release of various angiogenic factors, thickening of the basement membrane and excessive pressure on the capillary walls due to hypertension prevalent in the body. Since the root cause of all the above alterations is hyperglycemia, it is regarded as the base for all the succeeding events which finally end up causing diabetic retinopathy. So, various studies had been conducted to find out the mechanisms by which hyperglycemia leads to the various alterations in the microvasculature blood supply of the retina. The various mechanisms proposed in relation to this include

- **Increased Flux of the Polyol Pathway:** It is a biochemical pathway which leads to the metabolism of the excess glucose present in the body via aldol reductase pathway. But in this process of glucose metabolism, sorbitol is formed as an intermediate product. The cellular membranes are highly impermeable to sorbitol and thus this intracellular pathway leads to the accumulation of sorbitol inside the cells. This sorbitol is responsible for causing cellular injury due to various factors such as osmotic changes. Although sorbitol is slowly metabolized into fructose, this gives no relief to the cell because fructose itself forms advanced glycation end-products (AGEs) after undergoing non-enzymatic protein glycation. These AGEs formed are even more noxious for the cells, thus resulting in the cellular damage [40-42].

- **Non-Enzymatic protein glycation:** The formation and accumulation of AGEs also forms a part in the pathogenesis of diabetic retinopathy. Their involvement in the progress of this disorder is similar to that described above in the case of diabetic nephropathy. In fact, AGEs are a part of the pathophysiology of all the medical complications associated with diabetes mellitus. The advanced glycated end-products particularly involved in diabetic retinopathy are – CML and pentosidine, which are formed as a result of the rearrangement of the intermediate glycation product through the oxidative pathway. Chemically, CML is N-(carboxymethyl)lysine. CML is specifically formed by the oxidative cleavage of the Amadori
products whereas pentosidine is a fluorescent adduct formed a cross-linkage between the lysine and arginine residues in the protein. This accumulation of these end-products is supported by the evidence provided by a study that CML was found in an increased amount in the retinal cells and its associated vasculature [43-46].

- **Activation of Protein Kinase C**: Hyperglycemia is responsible for the activation of protein kinase C due to the increased de novo synthesis of diacylglycerol. Protein Kinase C belongs to family of 10 enzymes which are involved in the regulation of the physiology of other proteins by responding to certain hormones, stimuli through neurons or growth factors in a process which involves the phosphorylation of the hydroxyl groups of serine and threonine amino acids present in these proteins. This results in certain signal transduction events. Out of all the isoforms of protein kinase C, the isoform beta1/2 is speculated to be associated in the pathophysiology of diabetic retinopathy. The activation of this enzyme leads to a series of events which result in certain alterations such as –

- Increase in the permeability of the endothelium of retinal blood capillaries
- Changes in the hemodynamics of the retinal cells and the surrounding capillaries
- Increased expression of vascular endothelial growth factor (VEGF)
- Increased activation and adhesion of leukocytes
- Synthesis of extracellular matrix proteins
- Remodeling of extracellular matrix
- Increased liberation of angiogenic factors
- Endothelial dysfunction leading to capillary occlusion [47-50]

After all these events, Protein Kinase C is responsible for the activation of various mechanisms which include:

- Inflammation
- Neovascularization
- Irregularity in hemodynamics
- Progression of Diabetic Retinopathy

Due to all these events
Hemodynamic Changes and involvement of Renin-Angiotensin-Aldosterone System:

According to The Wisconsin Epidemiological Study of Diabetic Retinopathy (WESDR) and The United Kingdom Prospective Diabetes Study (UKPDS), hypertension is greatly involved in the pathophysiology of diabetic retinopathy.

Oxidative Stress:

Involvement of ROS (reactive oxygen species) has been actively found during studies in the progression of diabetic retinopathy. Increased production of reactive species or inhibition of their scavenging mechanisms (such as detoxification with various reducing and sequestering agents e.g., thioredoxin, glutathione (GSH), and tocopherol (vitamin E) or by enzymes such as superoxide dismutases (SODs), catalase, glutathione peroxidase, and thioredoxin reductase) leads to an imbalance in the homeostasis of ROS, thus resulting in oxidative stress. The contribution of reactive oxygen species in this disease is the breaks that they cause in the DNA strands which leads to the activation of poly-(ADP-ribose)-polymerase (PARP). The activation of PARP inhibits the activity of glyceraldehyde phosphate dehydrogenase (GAPDH), thus leading to the accumulation of certain metabolites which are responsible for the activation of AGEs, whose role in the progression of this disorder have been already discussed in this context above [58-61].
Involvement of growth factors: - Various growth factors such as basic fibroblast growth factor (bFGF), insulin-like growth factor-1 (IGF-1), angiopoietin-1 and -2, stromal-derived factor-1, epidermal growth factor (EGF), transforming growth factor-beta 2 (TGF-β2), platelet-derived growth factors (PDGFs), vascular endothelial growth factor (VEGF) and erythropoietin have been studied for their involvement in the pathophysiology of diabetic retinopathy. Out of these, the most studied growth factors are – IGF-1 and VEGF. Although elevated levels of IGF-1 have been found in the vitreous fluid during various studies of diabetic retinopathy, its exact mechanism by which it is involved in its progression is still unknown. The mechanism whose role is most understood in the progression of this complication is VEGF. It is involved in the process of neovascularization. It enhances angiogenesis, leads to the breakdown of the blood-retinal barrier, stimulates the growth of endothelial cells and increases the permeability of the endothelial walls of the capillaries supplying blood to the retina [62-70].

CONCLUSION
Since diabetic nephropathy and diabetic retinopathy are the two most dreadful and griming complications of diabetes mellitus, there is an utmost need to understand the basic pathophysiology which lead to the progression of these disorders so that they can be well-studied and methods to prevent the events that occur in it could be formulated. The present need of the hour is to take every possible step that could lead to the cure and prevention of occurrence of such complications in the patients suffering from diabetes. Further studies are required to validate the exact mechanisms involved in them and additional researches are necessary to discover the perfect remedies for them in order to eradicate such complications completely.

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