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Around 1900 years ago the Greek physician Aretaeus the Cappadocian describe diabetes as “a melting down of the flesh and limbs into urine.” The observation was based on increased catabolism in diabetic conditions which were reflected in urine and till date it is central to the pathology of diabetic nephropathy. Intrinsic and extrinsic factors remarkable alters glucose, lipid and protein metabolism [Moller et al 2008, Gougeon et al 2008]. This is mediated by almost all types of cells and leads to progression of diabetic nephropathy. Recently type 2 diabetes has been reported rapid progression of pre diabetic to overt diabetic conditions over an 11 year of period in addition of continuous therapeutic interventions, thus there is additional need of understanding mechanism progression of diabetic nephropathy [Fonseca 2009, Remuzzi 2006].

2.1 The Role of Intrinsic Renal Cells in the Pathogenesis of Renal Scarring

The glomerular capillary tuft is complex filtering structures which are potentially able to separate plasma components in to water, solute, cells and finally makes urine. These specialized structures have four primary constructive cell types: Parietal epithelial cells that form Bowman’s capsule, podocytes that cover the outermost layer of the glomerular filtration barrier, glycocalyx-coated fenestrated endothelial cells that are in direct contact with blood, and mesangial cells which are connects the capillary loops. The filtration process is accomplished with the equal contribution of these cells dedication for these own assignments [Vaughan et al 2008, Tryggvason et al 2005]. Therefore a strict coordination, movement and cross-talk between these cell types is essential required for the formation of a functional glomerular filtration barrier, and disruption of these processes has been suggested for the development of diabetic nephropathy [Asanuma et al 2007].

2.1.1 Glomerular Cells

2.1.1.1 The Role of the Endothelium

Endothelial cells are the essential components in the structural and functional organization of vascular beds with the integration of glomeruli [Abrahamson et al 2009].
Most probable these cells show anticoagulant, vasoactive, anti-inflammatory, and anti-proliferative properties [Rabelink et al 2010, Savage 1994, Stewart et al 1994]. Endothelial cells are the first layer which are exposed to injurious insults such as mechanical (hemodynamic/shear stress), immunological, or metabolic factors.

In diabetic patients glomerular endothelial injury is typically implicates swelling, cell-surface protrusions, and detachment from the underlying basement membrane. Recently glomerular endothelial cells have been suggested to influence podocyte function and cross-talk between the cellular compartments of the glomerular capillary walls and altered barrier of plasma protein filtering units [Ballermann et al 2007]. It has been suggested that the loss of NO-mediated anticoagulant properties would contribute to the adhesion and aggregation of platelets followed by damaged glomeruli which has been seen in experimental animals models and diabetic patients. The loss of important protective factor along with the expression of cell adhesion molecules would facilitate the infiltration of glomerular capillaries by inflammatory cells. In diabetic patients it has been seen that angiotensin II suppresses NO production through its AT1 receptor. The imbalance between angiotensin II and NO will lead to the induction of the proinflammatory transcription factor NF-κB. This is a destructive cascade which subsequently synthesizes and releases various pro-inflammatory cytokines and chemokines and the up regulation of cell adhesion molecules [Leclercq et al 2002, Wardle 2000, 2002]. In addition to this role of endothelial lipases has been observed in triglyceride metabolism and reverse cholesterol transport in to plasma in type 2 diabetic patients [Shiu et al 2010].

Recently experimental evidence imputes a role for apoptosis in the deletion of endothelial cells observed after injury [Kang et al 2002]. A study on tissue culture suggests that the sphingolipid-derived second messenger ceramide and oxidative stress are closely concerned with the induction of glomerular endothelial cells apoptosis [Huwiler et al 2001, Sanz et al 2008]. Diabetic patients continuously faces fluctuation in blood glucose and oxidative stress which slowdowns regeneration of the glomerular capillary endothelium which may rely on angiogenesis. However normal glomerular endothelium is capable of releasing angiogenic factors such as vascular endothelial growth factor (VEGF) and fibroblast growth factor-2 (FGF2). Glomerular endothelial cells also express
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VEGF receptors. VEGF is one of the factor which is concerned to mediate reactive endothelial proliferation in damaged glomeruli.

2.1.1.2 The Role of the Mesangium

Mesangial cells are other important cells in the initiation and progression of glomerulosclerosis. These specialized pericytes have contractile, phagocytic, and metabolic functions obligatory to the maintenance of glomerular integrity [Gruden et al 2005]. Several research articles describes glomerular injury, mesangial changes which are seen at the onset of diabetic nephropathy, followed by mesangiolysis, apoptosis, proliferation, expansion and sclerosis [Schrijvers et al 2004, Rennke et al 1989]. The phenotypic alteration of mesangial cells has been seen on AGEs stimulation which results in to ECM matrix expansion [Pozzi et al 2009]. Functional mesangial alterations are also observed in rats with experimental nephropathies. Which are characterized by the accumulation of macromolecules, including lipids, within the mesangium [Raij et al 1985, Elema et al 1988], moreover associated with glomerulosclerosis [Grond et al 1990]. Gathering of macromolecules in the sub-endothelial area supposed to contribute to glomerular hyalinosis [Olson et al 1985], which is follows by narrowing and ultimately the occlusion of glomerular capillaries occurs with remarkable sclerosis [Rennke 1994]. Accumulation of advanced oxidation protein products (AOPPs) has been found in diabetes and chronic kidney disease and linked to mesangial ECM deposition [Wei et al 2009]. Mesangial hypercellularity often precedes the development of mesangial sclerosis. It has been suggested that the stimulation of mesangial proliferation by growth factors such as platelet-derived and basic fibroblast growth factors. A variety of kinases has been shown to mediate mesangial proliferation in response to diverse stimuli. It has been reviled from several studies that the mitogen activated protein (MAP) kinases, p44/42 MAP kinase and Jun N-terminal kinase/stress-activated protein kinase (SAPK) all have been implicated in the beginning of mesangial proliferation [Krepinsky et al 2002]. Furthermore nuclear translocation of some of these kinases and their binding to activating protein-1 (AP-1) conducts the DNA synthesis and cellular proliferation. The peroxisome proliferator-activated receptors (PPAR) are nuclear receptors involved in the regulation of mesangial cell cycle and extracellular matrix processing [Guan et al 2001].
Cumulatively the turnover of mesangial cells in vivo appears to be regulated by a complex interplay of cyclin dependent kinases (CDK) and their inhibitors (CKI) [Shankland 1999].

The equilibrium between proliferative and anti-proliferative factors may determine the fortune of mesangioproliferative changes. The resolution of mesangial proliferation in experimental glomerulonephritis has been implicated to apoptosis [Baker et al 1994]. In addition to this increased glucose has been seen to promote mesangial cell apoptosis through oxidant-dependent mechanism [Kang et al 2003]. The equilibrium between mesangial cell proliferation and apoptosis may determine the severity of glomerulonephritis [Gruden et al 2005]. Several other factors like survival factors, and cell surface receptors integrins, α1β1 integrin restores mesangial survival if available properly. Mesangial cell α1β1 integrin expression seems to be a critical determinant of mesangial cell phenotype, growth and collagen remodeling capacity [Kagami et al 2000]. Addition to this β(1) integrin-mediated rescue mesangial cells collagen IV and laminin which protects basement membrane matrix. Contrary to this, collagen I, fibronectin, and osteonectin/SPARC, increased expression is observed in diabetic glomeruli, imposes apoptosis during glomerulosclerosis [Mooney et al 1999].

The requirement of bone marrow-derived cells at injured glomerulia has been seen [Ito et al 2001]. These cells are potential to migrate and differentiate into mesangial cells, with the virtue of these cells the recovery of mesangial injury is possible these cells patch the injured glomerulus towards normal function [Hugo et al 1997, Ito et al 2001]. In addition to this macrophages has been shown proliferative and antiapoptotic effects by stimulating protein (MSP) which might play a role in tubular regeneration after acute kidney injury [Cantaluppi et al 2008].

Several factors decide the unidirectionality of glomerulosclerosis, at one phase TGF-β1 induces mesangial cells to myofibroblast which potentially synthesizes collagen type III molecules [Schrijvers et al 2004]. On another phase mesangial cells release collagenases (MMPs) which are capable of degrading glomerular collagen type IV molecules and these MMPs does not digest collagen type III, therefore imbalance occurs in the composition of GBM membrane. This widely reported as mesangial expansion and
formation of extracellular matrix in diabetic nephropathy [Drummond et al 2002] and chronic glomerulonephritis [Schrijvers et al 2004].

2.1.1.3 The Role of the Epithelium

The glomerular visceral epithelial layer is composed of specialized cells known as podocytes. Podocytes consists of specialized structures pericyte which holds the glomerular capillary wall tightly and resists centrifugal hydrostatic force from glomerular filtration. These cells have shown less regeneration capacity. Regeneration capability of podocytes are suggested to be linked with the cell cycle regulatory proteins and its association with up regulation of cyclin kinase inhibitors (CKI) in response to the immune mediated injury [Shankland 1999]. These observations suggest lost podocytes are less likely to be recovered. However facilitated regeneration is possible but the lack of supporting factors for mitosis podocytes are seen binucleated. Podocytes has been also reported to lose their differentiated phenotype and proliferate states in collapsing glomerulopathies [Asanuma et al 2007].

The studies on animal model describes the tendencies of epithelial cells which forms cytoplasmic blebs, along with focal retraction, simplification, and flattening of foot processes. Fusion and renunciation of epithelial podocytes, also seen in glomeruli of patients with nephrotic syndrome, which is presumed to altered glomerular permeability. The understanding of mechanism could be explained by the localized denudation of the basement membrane followed by the stretching and rearrangement of foot processes, this phenomenon is accelerated with the degree of glomerular hypertension, the hydraulic flux, protein trafficking, and charge neutrality permeability of macromolecules, ultimately proteinuria [Rennke 1994].

Recently there is immersing observations which suggest podocyte depletion is central to the pathogenesis of glomerulosclerosis [Thorner et al 2008, Spurney et al 2008, Bariety et al 2006], similarly early podocytes damage foregoes as glomerulosclerosis starts even in the absence of any mesangial abnormalities [Gassler et al 2001]. A more severe condition has been seen in which podocyte depletion results in to the shedding of viable podocytes into the urine [Petermann et al 2003].
Podocyte associated irregularities has been seen within foot process effacement in hypertrophied glomeruli of type 2 diabetic patients and implicated in defects in the GBM size permselectivity and followed by subsequent development of “macromolecular shunts” in the glomerular barrier [Lemley et al 2000, Rask-Madsen 2010]. Furthermore number of podocytes in glomerular tuft at per glomerulus was associated with the progression of nephropathy and scanty podocyte predicts rapid progression. Similar observations were also seen in IgA nephropathy patients [Lemley et al 2002].

Pathogenesis of glomerulosclerosis has been noted down on the basis of podocytes per unit area/podocyte density [Kim et al 2001]. The denuded GBM is extensively prone for the capsular adhesions which may initiate segmental glomerulosclerosis. These studies suggest that such adhesions would form bridges between the glomeruli and the surrounding periglomerular interstitium, to facilitating the extravasation of several cells like interstitial myofibroblasts into scarred glomeruli. Secondly it should not misconceptulated that ultra filtrate of glomerular filtrate into the periglomerular interstitium. During the last decade type 2 diabetic nephropathy has been focused on podocyte cells. Slit diaphragms are essential apparatus of the glomerular filtration. It consists specialized proteins which unite the structural moieties these adheres are unique membranous proteins (e.g., nephrin, podocin, and Neph1) and typical adherens junction proteins (e.g., P-cadherin, FAT, and catenins). Loss of these proteins leads to severe proteinuria [Fukasawa et al 2009] several other cytoplasmic, cell surface, slit diaphragm-associated proteins contributes to maintaining the integrity of podocyte with glomerular barrier. The nephrin which is a slit diaphragm connecting protein, and CD2-associated protein (CD2AP) an adaptor protein that binds to the cytoplasmic domain of nephrin [Shaw et al 2001], are strong pillers of kidney functioning and nephropathy. Podocin is an integral podocyte membrane protein. Alpha-actinin-4 is also localized to podocytes and might provide the crosslink between cytoplasmic actin filaments. Recent studies suggests that podocyte cells communicates through foot processes [Weide et al 2009], derangements in this signaling may lead to proteinuric renal diseases [Giardino et al 2009]. Epithelial cells assign themselves in glomerulosclerosis through the expression of class II antigens of the major histocompatibility complex, and the release of chemo tactic factors such as complement components, with increased intake of lipids. The epithelial
cells also reported to synthesize several cytokines and growth factors such as platelet-derived growth factor [Pavenstadt 2000].

2.1.2 Tubular Epithelial Cells
Proximal tubular cells express HLA class II antigens [Brady 1994] up on insult and behave like antigen-presenting cells. These cell-adhesion molecules increase interstitial inflammatory reactions. The proximal tubular cells are also releases chemotactic factors such as complement, NF-κB and IL-6 [Eddy 2001, Morcos et al 2002], nitric oxide [Cattell 2002], fatty acid-derived chemotactins as well as chemokines such as MCP-1 [Zeisberg et al 2000, Eddy 2001, Anders et al 2003].

Increased protein metabolism in proximal tubular epithelium increases ammoniagenesis, interstitial complement activation and inflammation [Nath et al 1985]. In addition to this, proteinuria is a factor which activates the proximal tubular cells and modulates physical properties of phenotype. It has been observed that the proximal tubular cells are proficient to synthesize the fibrogenic growth factors like platelet-derived growth factor and transforming growth factor-β1 [Zeisberg et al 2000, 2001]. Beside these factors, proximal tubular cells can release vasoactive and profibrotic autacoids like angiotensin II and endothelin1. Proximal tubular cells also reported to express the angiotensin II AT1 receptors. Glucose influences release of TGF-β1 from proximal tubular cells significantly when these cells were incubated with angiotensin II [Wolf 2001]. Proximal tubular cells have been recognized for the release of transforming growth factor-β1 through Angiotensin II-mediated hypertrophy [Wolf 2001]. Sensitized tubular cells faces endoplasmic reticulum stress which leads to impaired protein folding and tubular-interstitial injury [Kimura et al 2008].

Numerous macromolecules (albumin, Immunoglobulin, transferring and LDL/ox-LDL) ascertained the signaling and induction to tubuloepithelial cells on overproduction of extracellular matrix. On the otherhand angiotensin II and endothelin1 both are potent to induced increase synthesis of collagen IV by proximal tubular cells [Wolf 2001].

The one of the most impotent capability of tubular epithelial cells is to synthesize matrix metalloproteinases (gelatinases/collagenases, MMPs) along with inhibitor of tissue metalloproteinases (TIMPs). Therefore it makes a balance between epithelial cells’
synthesis and breakdown of extracellular matrix which is an important phenomenon in progression of tubulointerstitial fibrosis.

It has been shown that glomerular tubular junction scared with amorphous material separating damaged tubular cells from the basement membrane. These results suggest how glomerular injury produces scares at tubule neck region, which is central and localized and propagate in to chronic nephropathy if remains untreated [Javaid et al 2001]. Tubular basement membrane has been detected with the immune complex deposition this fact supports drug induced tubulointerstitial nephritis [Chang et al 2006].

2.1.3 Fibroblasts and Myofibroblasts

Fibroblasts and myofibroblasts contribute in the pathogenesis of renal fibrosis by any of the following four pathways 1. Stimulation by growth factors ("auto- and paracrine"), 2. Direct cell-cell contacts, 3. Extracellular matrix via integrins, and 4. Environmental conditions such as hyperglycemia or hypoxia in renal disease [Zeisberg et al 2000, Qian et al 2008]. It is suggested that the activation of renal fibroblasts can be driven by the release of cytokines and growth factors by glomerular cells, tubular cells as well as interstitial inflammatory cells [Strutz et al 2006]. Renal fibroblasts themselves secrets IL-1 and FGF2 capable of autocrine stimulation [Zeisberg et al 2000]. These cells supposed to secrete transforming growth factor-β1 and platelet derived growth factor [Yamamoto et al 1994] and produce fibrillar collagen. Fibroblasts cells are also capable of releasing matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) [Ban et al 2008, Norman et al 1996].

Over all renal fibroblasts and myofibroblasts are the cells which actively involved in metabolism of ECM and its homeostasis. Active fibroblasts and myofibroblasts provide control over progression of renal scares [Wiggins et al 1993]. Myofibroblasts have been detected in the interstitium of experimental animals and humans [Ban et al 2008, Johnson et al 1991, Alpers et al 1992, Goumenos et al 1994] with progressive glomerulonephritis. In man, their presence within the interstitium is a sensitive marker of progressive renal disease [Goumenos et al 1994]. These cells may infiltrate the glomeruli through adhesions or holes in Bowman’s capsule, and contributes to glomerulosclerosis [Lan et al 1992]. It has been reported that the glomerular and tubular epithelial cells which were
isolated from microdissection of diabetic biopsies has shown increased TRAIL-induced apoptosis [Lorz et al 2008].

Recently diabetic patients have been reported to over express Smad 7 in proximal tubular cells results in a marked inhibition of TGF-β-induced trans-differentiation into myofibroblasts. And Smad 1 which causes mesangial expansion suggested to be the putative marker of diabetic nephropathy [Kato et al 2008].

2.1.4 Vascular Sclerosis

Diabetic nephropathy and cardiopathies are correlated secondary complication because at the onset of complications both microvascular as well as macrovascular complications shares some common pathways of sclerosis [Fowler 2008]. Arteriolar hyalinosis has been seen in the development of glomerulosclerosis in diabetic patients, those patients who develops early lesions of arteriolar hyalinosis has shown severer glomerulosclerosis even in the absence of revert hypertension. Patients with systemic lupus erythematosus, vascular lesions were correlated with a significantly higher risk of end stage of nephropathy within 5 to 10 years when compared to those without vascular lesions [Kitada et al 2010].

2.1.4.1 Peritubular and Microvascular Injury and Angiogenesis

Recently Rabelink and colleagues describe the loss of peritubular capillaries in the progression of interstitial fibrosis which leads the diabetic nephropathy [Rabelink et al 2010, Bohle et al 1981, 1987]. Several experimental studies have shown that an initial phase of peritubular endothelial proliferation results in to the loss of cells and peritubular rarefaction [Kang et al 2002]. Kidney consists of large as well as small vessel web changes in these blood vessels supposed to develop renal interstitial fibrosis through ischaemia and hypoxia.

Thrombospondin-1 (TSP-1) is induces loss of the microvasculature endothelium through inhibiting endothelial cell proliferation and inducing apoptosis [Hugo et al 1997, Kang et al 2002]. TSP- 1 is released by tubular cells, macrophages and fibroblasts. Another anti-angiogenic factor SPARC (secreted protein acidic and rich in cysteine) is also upregulated in remnant kidneys [Wu et al 1997]. These results have been collected from numerous experiments on animal models of interstitial fibrosis associated with the loss of
peritubular capillaries [Kang et al 2002]. The extent of renal fibrosis is depends upon change in qualitative and quantitative ECM and angiogenesis which is solely dependent up on pathological factors hyperglycemia, dislipidemia and hypertension [Ban et al 2008].

2.1.4.2 Glomerulosclerosis vs Tubulointerstitial Fibrosis
It was observed that in diabetic nephropathy, glomerular injury is leading factor and nephropathy starts from ultra filtrate of a variety of cytokines, chemokines and growth factors [Schrijvers et al 2004, Wang et al 2000]. Afterwards these factors potentially mediate inflammatory, fibrogenic reactions and the synthesis of extracellular collagenous matrix [Wang et al 2000]. On the other hand, glomerular injury occurs it alters the attitude of the efferent arterioles. The postglomerular capillaries are suggested to participate in reduced peritubular perfusion which leads to local ischaemia and hypoxia which results in to severe complication like tubular atrophy/ interstitial fibrosis [Fine et al 2000]. Moreover proteinuria is a central connecting link between glomerular damage and tubule interstitial inflammation and fibrosis. It has been seen that glomeruli with epithelial injury and focal capsular adhesions are prone to detach from their tubules resulting in to atubular glomeruli [Javaid et al 2001].

2.2 Mechanisms of Progressive Kidney Scarring
Several hypotheses have given to explain the mechanisms of diabetic nephropathy and its progression to end stage. Some have focused on the progression of glomerulosclerosis; others have examined the mechanisms underlying the progression of tubulointerstitial scarring or vascular sclerosis [Fioretto et al 2007].

2.2.1 Glomerular Changes
2.2.1.1 The Changes in Glomerular Haemodynamics
Brenner and Hostetter separately proposed the hypothesis, which explains progression of glomerulosclerosis and its modifications in glomerular hemodynamics that take place after a reduction in functional renal mass [Hostetter et al 1981, Brenner and Meyer 1982, Brenner 1985]. The important factors which are potentially involved in glomerulosclerosis, glomerular hyper-perfusion, hyperfiltration and hypertension,
moderate glomerular capillary wall by stretching it which resulting in endothelial and epithelial injury. This mechanism allows crossing macromolecules into the mesangium, as much as they reached to mesangial overload and dysfunction occurs. In a recent study increased expression of VEGF has been shown in glomeruli which was further correlated to the glomerular hypertrophy and increased proteinuria [Liu et al 2007]. Morphological alteration has been seen like endothelial detachment, glomerular transudation of proteins, the formation of platelet aggregates and microthrombi within glomerular capillaries, epithelial stretching with secondary denudation of the GBM and mesangial expansion [Ichimura et al 2008, Olson et al 1985]. However these changes occur with long duration of diabetes, the progressive mesangial sclerosis, finally leads to glomerulosclerosis. In conclusion a central message from several studies is that at onset of diabetes survival of a glomeruli result into its adverse future, and with the duration of diabetes they consequentially resulted in glomerular scarring and loss of renal function.

This hypothesis also tried to explain diabetic nephropathy and other non diabetic nephropathy like abnormalities in diabetic patients. The progression of proteinuria and renal failure in individuals born with oligomeganephronia, unilateral renal agenesis, or following unilateral nephrectomy. It also covers explanations for the nephrotoxicity of a high protein intake in diet [Brenner et al 1982]. This hypothesis has been successfully applied to the therapeutic approaches and a remarkable reduction of glomerular hypertension has been observed with cessation of glomerulosclerosis. This goal has been done by applying the factor one, controlling the dietary protein restriction and second, angiotensin-converting enzyme (ACE) inhibition. ACE inhibitors had a therapeutic advantage over conventional antihypertensive agents suggested by this hypothesis. Moreover hyperfiltration was shown to be an independent predictor of diabetic nephropathy [Bloomgarden 2010] and increase in renal blood flow and glomerular filtration rate which is seen in typically at the onset of diabetes mellitus may suggested to initiate diabetic nephropathy [Lemley et al 2000].

2.2.1.2 The Changes in Glomerular Hypertrophy

Several researchers has observed adaptive glomerular hypertrophy (enlargement) at the onset of diabetes and its relation to the progression of glomerulosclerosis [Agarwal et al 2005, Fogo et al 1991, Johnson 1994, Fogo 2001(a)], but failed to observe a correlation
between the degree of glomerular hyperfunction detected by micropuncture and the subsequent sclerosis. However contradictory to this, [Fogo et al 1990] a close correlation between glomerular enlargement and early glomerulosclerosis was found in patients as well as animal model. Age-related mesangial sclerosis [Doi et al 1991].

In this section glomerular hypertrophy is suggested to be the sole determinant of the development of glomerulosclerosis in diabetic patients at the time of onset of diabetes. The further development of diabetic nephropathy is dependent on several factors, like genetic susceptibility and oligomeganephronia [Brenner et al 1994]. And type IV collagen synthesis by endothelial cells, mesangial cells, and podocyte cells [Abrahamson et al 2009].

2.2.2 Tubular Changes

In addition to the glomerular alterations it is suggested that the tubular functions also changes and ‘maladaptive’. Active tubular changes have been concerned with the pathogenesis of tubulointerstitial scarring [Tang et al 2006]. AGE dependent increased expression of adhesion molecule and chemokine has been reported which leads to transmigration of inflammatory cells into the interstitial space during diabetic tubulopathy. Intra-renal RAS activation and high glucose has been suggested to increase tubular apoptosis in diabetes, independent of systemic hypertension [Liu et al 2008].

2.2.2.1 The Changes in Tubular Growth and Hypermetabolism

One of the important factor is increased sodium reabsorption and oxygen consumption at proximal tubular cells. It has been experimentally proved in vivo a three fold increase consumption of oxygen occurs by the remnant kidney [Thomson et al 2008, Schrier et al 1994], suggested that the increase in tubular sodium reabsorption and the activation of sodium/hydrogen exchange not only led to proton extrusion and increased cellular pH, but also increases the generation and utilization of Na$^+$ – K$^+$ ATP, which can generate oxygen free radicals, causing peroxidation of lipid carbohydrates and more severe to DNA [Kamijo et al 2007].

2.2.2.2 Tubular Oxidative Stress

Recent research connects hypertrophy of the proximal tubules to the generation of reactive oxygen species (ROS) and followed by tubular injury. Renal proximal tubular
cells are actively involved in receptor mediated endocytosis of plasma proteins which escape from GBM barrier [Saito et al. 2010], this view is further supported by the fact that the glycated proteins are more likely to escape from GBM. It has been also observed that glycation of protein increases its acidic nature [Monteleone et al. 2000], at acidic pH binding of protein to FcRn receptors is higher when compared to physiological pH [Sarav et al. 2009], and metabolic acidosis act as catalyst in progression of nephropathy [Frassetto et al. 2009]. Moreover excessive internalization of proteins generates excessive H$_2$O$_2$ and ammonia [Wang et al. 2007]. Abnormal IgG-bound sugar chains have been reported in increased positive charge and active involvement in the etiology of SLE nephritis [Kinoshita et al. 2006, Davin et al. 1997]. In proximal tubular hypertrophy transforming growth factor-β1 (TGF-β1) is released at stimulation of Angiotensin II, [Wolf 2001], and ROS [Hannken et al. 2000], and nitric oxide (NO) increases. Interactions between ROS and NO lead to the formation of peroxynitrite (ONOO-); which results into the loss of NO homeostatic effects and peroxynitrite-induced cytotoxicity [Welch et al. 2000]. Reactive oxygen species is also suggested to play a key role in tubulointerstitial inflammation and fibrosis. The generation of ROS is capable of the activation of the transcription factor nuclear factor-κB (NF-κB) which induces synthesis of pro-inflammatory cell adhesion molecules, cytokines and chemokines [Wardle 2000]. On the other hand, inhibition of NF-κB protects against proteinuria-induced interstitial scarring [Rangan et al. 1999]. Along with cytotoxicity and pro-inflammatory property, ROS also has shown a direct fibrogenic effect [Houglum et al. 1991]. This pro-inflammatory phenotype may be partially modified by PPAR-γ ligation through STAT-1 inhibition independent of NF-κB transcriptional activity and MAPK signaling [Tang et al. 2006]. The lipid peroxidation product malondialdehyde accumulates progressively within the cortex of remnant kidneys [Nath et al. 1994, Haugen et al. 1999]. These results suggest ROS play a pivotal role in kidney scarring furthermore recent evidence also points out towards the upregulation of oxidative stress mediators and nitric oxide synthesizes in diabetic kidneys. Diabetic kidney has been reported for the decreased expression of mRNA and anti-oxidant enzymes including glutathione peroxidase, superoxide dismutase, catalase, and glutathione S transferase during disease progression [Maser et al.
To investigate whether reactive oxygen species stimulate renal growth and promote injury numerous studies has been carried out it has been deduced that the renal scarring, is somewhat extinct rebated with supplement of dietary protein, phosphate restriction, thyroidectomy, and chronic treatment with calcium antagonists, these potentially reduce oxygen consumption [Schrier et al 1988]. Vitamins C and E and with combination of magnesium, zinc, supplementation has been reported for improvement in glomerular function in type 2 diabetic patients [Farvid et al 2005]. The indirect investigations were also evidence from a study carried out on rats, which were fed a diet deficient in antioxidants (vitamin E and selenium). The antioxidant amino acid taurine and the drug ‘probucol’ have been shown to be protective in experimental models of proteinuria and kidney scarring [Trachtman et al 1992].

2.2.2.3 Tubular Ammoniagenesis

It has been well established that the remnant nephrons and the proximal tubules increase the secretion of acid by two manner one, by enhanced reabsorption of bicarbonate and secondly, by the generation of ammonia. A postulation was given by Nath and coworkers states that that the increased concentrations of ammonia can initiate chronic tubulointerstitial inflammation through the activation of the alternate pathway of the complement system [Nath et al 1991]. Furthermore overload of protein at proximal tubules is associated to the pathogenesis of tubulointerstitial inflammation and fibrosis. In type 2 diabetic patients lipotoxicity has been reported to the impaired Na\(^+\)/H\(^+\) exchange and NH\(_4\)\(^+\) secretion in the proximal tubule [Bobulescu et al 2008]. Moreover excessive reabsorption of proteins by the proximal tubular cells and their catabolism leads to increased ammoniagenesis. Simultaneously, angiotensin II, which stimulates ammoniagenesis, may also be involved in the pathogenesis of tubulointerstitial inflammation and fibrosis. Recently several studies on diabetic nephropathy have reported that the ACE inhibition is significantly associated with remarked reduction in proteinuria and renal ammoniagenesis. It is equally important to note that ammoniagenesis play a central key role for the explanations of nephrotoxicity by proteinuria and angiotensin II. To put forward this hypothesis of tubular dysfunctioning diabetic and non-diabetic nephropathy patients has been reported to excrete of C5b–C9 in their urine [Schulze et al 1991]. The progression of membranous nephropathy has been
correlated with the urinary excretion of this membrane-attack complex [Kon et al 1995]. A remarkable reduction has been reported in ammoniagenes and protein catabolism within proximal renal tubular cells of chronic renal failure patients. These patients were showing a good correction of metabolic acidosis with oral sodium bicarbonate and decrease level of in markers of proximal tubular injury [Rustom et al 2001].

2.3 Role of Factors in Progression of Diabetic Nephropathy

2.3.1 The Role of Systemic Hypertension

Systemic hypertension is one of the coupled factor of end-stage of diabetic nephropathy and has long been recognized to accelerate the progression of chronic nephropathies diabetic patients [Thomas et al 2009, Fox et al 2008]. Progressive glomerulosclerosis develops in experimental nephropathies associated with systemic hypertension [Bakris et al 2003]. A better explanation of mechanism by which systemic hypertension initiates glomerular scarring can be study from experimental models of hypertension in the rat [Wilson et al 1941, Hill et al 1968]. It has been suggested that the transmission of systemic hypertension to the glomerular capillary bed, normally prevented by autoregulation, determines the severity of glomerular scarring. In experimental models of hypertension where effective autoregulation and afferent vasoconstriction protect glomeruli from the transmission of systemic hypertension, glomerulosclerosis does not occur. On the other hand, when autoregulation is impaired, glomerular hypertension and glomerulosclerosis occur. The baseline systolic blood pressure is stronger predictor of developing diabetic nephropathy in type 2 patients then diastolic blood pressure [Bakrish et al 2003].

2.3.2 The Role of Proteinuria

2.3.2.1 Glomerulosclerosis

Proteinuria is considered one of the major risk factors for the progression of end stage diabetic nephropathy and plays a significant participantion in the initiation of glomerulosclerosis [Remuzzi et al 1990]. The reduction of proteinuria by any means either dietary or pharmacologically leads to reduced incident of glomerulosclerosis. Glomerular endothelial glycocalyx constitutes a barrier to protein permeability and human heparanase has shown its ability to degrade heparan sulphate glycosaminoglycans
which leads to proteinuric states [Singh et al 2007]. The traveling of plasma proteins across the endothelial and subendothelial space can initiate glomerular hyalinosis [Olson et al 1985], which may, successively, narrow and ultimately occlude the glomerular capillaries. Increased traffic of macromolecules into the glomerular mesangium may also contribute to the pathogenesis of glomerulosclerosis [Remuzzi et al 1990]. Several plasma proteins like albumin, immunoglobulin, and there fractions has been reported for altered expression of MMP3, MMP7 and MMP9 in glomerular tissue which leads to ECM remodulation and glomerular damages [Keeling et al 2005]. Thus protein overloaded mesangial cells may lead to the release of ROS including hydrogen peroxide. H$_2$O$_2$ has potential to activate the pro-inflammatory along with profibrotic pathways. ROS has been reported to activate the transcription factor NF-κB in mesangial cells with the subsequent upregulation of mesangial transcription of a wide range of pro-inflammatory cytokines [Wardle 2000]. Moreover ROS has been an important factor for the upregulation of mesangial synthesis of ECM through TGF-β1 dependent pathways. Proteinuria and hyperglycemia induce endoplasmic reticulum stress which is an important site for protein folding. This mechanism leads to altered expression of several protective/anti oxidative enzymes [Lindenmeyer et al 2008].

In turn increased glomerular permeability for proteins results distress glomerular epithelial cells, leading to significantly structural and functional changes; this may in turn further increases the passage of macromolecules across glomerular capillaries [Rennke et al 1989, Gassler et al 2001]. Moreover hyperglycemia has been reported as major determinant of albumin permeability in diabetic microcirculation. Dietary and pharmacological aspects can cease proteinuria from progressive glomerulosclerosis. And a supplementary diet with tryptophan reduces proteinuria, but functional and histological deterioration continues to progress in subtotally nephrectomized rats [Kaysen et al 1983]. On the other hand, ramipril and placebo has shown GFR decline 0.39 and 0.89 ml/min/month respectively [Remuzzi et al 2006], where as verapamil given to rats with renal ablation prevents progressive glomerular scarring without reducing proteinuria [Harris et al 1987]. In puromycin-induced nephrotic syndrome, treatment with a hepatic hydroxymethylglutaryl coenzyme-A (HMG-CoA) reductase inhibitor (statin) corrects the
hyperlipidaemia by inhibiting albumin uptake by human proximal nephron and ceases the glomerulosclerosis without reduction in proteinuria [Harris et al 1990, Verhulst et al 2004]. Similar observations were made in experimental diabetic nephropathy in which Lovastatin, reduces glomerulosclerosis without altering the amount of proteinuria [Inman et al 1999].

2.3.2.2 Tubulointerstitial Inflammation and Scarring

Urinary proteinuria has shown a close relation between experimental proteinuria and tubulointerstitial inflammation and scarring [Eddy 2001]. Proteinuria seems to play a strong driving force for the development of tubulointerstitial scarring and fibrosis. It has been seen if enhanced uptake of proteins occurs by proximal tubular cells it leads to the activation of lysosomal enzymes which causes tubular cell injury. Experimental data has suggested that exposure of proximal tubular cells to albumin in higher amount, stimulates their proliferation, which reviles the fact that how the increased trafficking of protein in tubular region induces tubulointerstitial inflammation. There are several studies which indicates role of proteinuria in the development of tubular cell apoptosis in animal model as well as in diabetic patients with protein overload nephropathy [Kamijo et al 2007, Thomas et al 1999]. Recent data clears that proteinuria have potentials to induce tubular dysfunction, with inspiration for the synthesis and release of a several cytokines, chemokines and growth factors [Eddy 2001]. Experimentally it has proven that nephrin deficiency or overload albumin-induces activation of NF-κB [Hussain et al 2009] a compensatory reduction in proteinuria-induced renal injury has been observed with the inhibition of NF-κB [Rangan et al 1999, Wardle 2002]. It is still doubtful whether proteinuria or albumininuria is associated with proteinuric states of interstitial tubulointerstitial scarring. Several filtered macromolecules/substances such as transferrin, complement components, growth factors and lipoproteins have suggested playing prognostic importance.

2.3.3 The Role of Lipids

2.3.3.1 Glomerulosclerosis

The pathogenesis induced by protein molecules implies hypothesis of hyperfiltration and nephrotoxicity subsequent the progression of experimental and clinical renal scarring
[Brenner et al 1982], On the other hand the lipid molecules explains hypothesis covers the nephrotoxicity [Moorhead et al 1982]. Recently lipids have been correlated with the glomeruli and glomerular glycocalyx damages [Rutledge et al 2010]. In an animal model it has been seen that diet with high cholesterol-supplement rapidly progressed to nephropathy which is correlated with the age and experimental [Keane 2000]. On the contrary, hyperlipidemia is seems to be less associated dietary with diabetic nephropathy and shown a protective role in nondiabetic experimental glomerulosclerosis [Keane 2000].

The fore most roles of lipids in glomerular toxicity are the induction of glomerular capillary pressure, functional and structural alterations in endothelial and mesangial cells. Glomerular endothelial and mesangial cells have receptors for both LDL and oxidized-LDL (ox-LDL). Low-density lipoproteins deposition induces oxidative stress and inflammation, which cumulatively forwarded in to glomerulosclerosis. Moreover this mechanism gives feedback enhancement to inflammatory changes by monocyte chemoattractant protein 1, which leads to the influx of monocytes in to the glomeruli and increased glomerular injury. The infiltration of cells has been observed in several glomerulosclerosis with or without inflammation [Bloomgarden 2010].

Oxidized low-density lipoproteins exert cytotoxic effects on endothelial, mesangial, and epithelial cells and stimulate in vitro the proliferation of mesangial cells [Prato 2009, Wasserman et al 1989, Nishida et al 1999]. Oxidized and glycated lipoproteins interacts with LDL receptors on mesangial cells and activates Ras and mitogen activated protein kinase (MAP kinase) and cyclin/cyclin dependent kinases [Kamanna 2002]. Activation of kinases leads to the proliferation of mesangial cells [Kamanna 2002]. An alternate explanation to this observation could be that, the proliferative responses at the onset of diabetic nephropathy flourishes in to high expression of TGFβ1 in mesangial cells, which leads to over production of extracellular matrix at the later stage of diabetes mellitus [Okada et al 2002]. In other words activation of pathways in mesangial cells from proliferation to extracellular matrix synthesis potentially participates in the development of glomerulosclerosis in diabetic nephropathy. In conclusion qualitative changes in low-density lipoproteins significantly contribute nephrotoxicity. It has been seen that diabetic patients with familial hyperlipidaemia more prone for cardiovascular complications
rather than nephropathy. Lipoprotein thrombi formation is scanty incidence been described within the glomerular capillaries of dyslipidemic patients [Saland and Ginsberg 2007] and associated with deformity of apoprotein E [Saito et al 1989, 1999]. It has been observed that apolipoproteins A and B100 tends to accumulate in glomerular region of diabetic as well as non diabetic patients at chronic glomerulonephritis [Saland and Ginsberg 2007]. It is therefore reassuring that the apoE act as inhibitory factor of mesangial proliferation and apoptosis of mesangial cells which is potentially induced by LDL [Chen et al 2001]. It is feasible to say that mesangial expansion is more appropriately caused by ApoE deficiency rather than hyperlipidemia.

2.3.3.2 Tubulointerstitial Scarring
Recent research suggests that lipids actively initiates and participates in the progression of tubulointerstitial scarring. Diabetic patients has reported oxidized LDL dependent damage of proximal tubular cells, these cells actively internalized oxi- LDL in vitro; these abnormal non enzymatically glycated lipoproteins are capable to induce proliferation of proximal tubular cells in culture as well as the synthesis of extracellular matrix components such as fibronectin. Moreover, LDL causes phenotypic alterations in proximal tubular cells in culture. On the other hand accumulation of particles of native low-density lipoprotein within tubular cells is nontoxic. The balance between oxygen free radicals and antioxidants supposed to be sole determinant of low-density lipoproteins uptake by proximal tubular cells and its resulting nephrotoxicity [Ong et al 1994]. Therefore the studies concludes that concept of production of oxygen free radicals, ischaemia and hypoxia [Fine et al 2000], haematuria [Hill et al 1989], and proteinuria/transferrinuria with tubular iron overload [Alfrey 1994] in tubular cells are integrated with its hypermetabolism [Schrier et al 1994]. The reabsorption of lipid by proximal tubular cells explains the nephrotoxicity of lipids related to fatty acids uptake associated with albumin. It has been suggested that free fatty acids potentially induces lipid chemotactic factor which attracts monocytes and initiates tubulointerstitial inflammation [Kamijo et al 2007]. This concept is further supported by several sutdies in which protein (BSA)-loaded rats shows monocytic infiltrate surrounding to the proximal tubular cells at instant onset of the nephropathy [Thomas et al 1999, Bobulescu et al 2008]. Further, in contrast to these results administration of fatty
acid-depleted albumin failed to induce the interstitial inflammation [Thomas et al 1999]. Summation to this, apoptosis was seen in overloading rats with fatty acid-replete albumin [Thomas et al 1999]. In primary cultures of human proximal tubule cells fatty acids interact with peroxisome proliferator-activated receptors (PPAR) which results in enhanced apoptosis [Liu et al 2008]. The role of lipids in activation and progression of human diabetic nephropathy are based on the observation assumption that very few patients with primary hyperlipidaemia develop diabetic nephropathy and secondly prolonged hyperlipidaemia in some nephrotic patients is not always associated with progression. And forward to this few frequencies of incidences can be explain by the presence of qualitative nature of circulating lipids and circulating oxidized immune complexes [Wong et al 2010]. Furthermore we cannot ignore the genetic predisposition, often environmental factors, are concerned to the initiation of lipid-associated nephropathies.

2.3.4 Association between Microvascular and Macrovascular Diseases

Diabetic secondary complications are related to each other because at the onset of diabetes disturbed metabolic profiles affect the later stage development of secondary complications.

Macrovascular as well as microvascular complications follows some common pathways of destruction, like similar cytokine for inflammation, extravasation, receptors for signaling pathways and membranous lesions and mechanisms of scaring [Fowler 2008]. The pathogenic role of lipids in glomerulosclerosis has provoked to find similarities between atherosclerosis and glomerulosclerosis. The endothelial injury, smooth-muscle cell/mesangial proliferation, extracellular matrix deposition within the vascular wall, and ultimately sclerosis are the common processes in both the cases. An initial hemodynamic or mechanical insult is frequent to glomerulosclerosis and atherosclerosis.

The both are accelerated by systemic hypertension. This hypothesis also incorporates a role for lipids as they are implicated in the pathogenesis of atherosclerosis. Furthermore this hypothesis explains the common input of cytokines, chemokines and growth factors to glomerulosclerosis and atherosclerosis. Likewise, the oxygen free radicals [Haugen et al 1999] and nitric oxide [Cattell 2002] are common player in both the atherosclerosis and glomerulosclerosis.
2.4 Mediators of Renal Scarring

2.4.1 Extracellular Matrix Turnover

2.4.1.1 Glomerulosclerosis

The glomerular ECM homeostasis is maintained by degradation as well as synthesis of its components by all three lines of glomerular cells. Several MMPs and their TIMPS have been suggested to play important roles in type 2 diabetic nephropathy. The increased synthesis of extracellular matrix components such as collagens, fibronectin, and proteoglycans by glomerular cells is thought to contribute to glomerulosclerosis. Moreover, mesangial cells release numerous metalloproteinases which digest extracellular matrix components. The regulation of MMP activity is done by releasing tissue inhibitors of MMPs (TIMP-1, 2 and 3). Diabetic glomerulosclerosis is significantly correlated to the imbalance between mesangial MMPs and TIMPs. It has been observed that incubation of mesangial cells with high concentrations of glucose and TGFβ1 reproduces diabetic nephropathy [Singh et al 2001], moreover, endothelin1 down regulates the release of MMP2 and upregulate TIMP2. In patients with type 2 diabetic nephropathy, urinary MMP9 and MMP2 releases were shown to be significantly increased [Zaoui et al 2000]. Recent evidence suggests that modulation of ECM is done selectively and there is an observation in which several ECM components were not digested with MMPs. Therefore it is suggested that ECM protects itself from proteolytic action of MMPs within scarred glomeruli [Johnson et al 1997, Skill et al 2001]. Recently, podocyte cells have been reported to express FcRn molecules for removing IgG from the GBM, impairment of the clearance machinery leads to glomerular diseases and proteinuria [Akilesh et al 2008].

2.4.1.2 Tubulointerstitial Fibrosis

Similar to the glomerulosclerosis, tubulointerstitial fibrosis is also resultant of disturbed synthesis and breakdown of extracellular matrix. The evidence of tubulointerstitial fibrosis in diabetic patients is less likely to be occur however it has been seen tubular cells, infiltrating macrophages and interstitial fibroblasts all cells are potential of synthesizing extracellular matrix. Numerous studies have shown increased deposition of...

Plasminogen-plasmin system is another mechanism of matrix demodulation. It is inhibited by plasminogen activator inhibitor-1 (PAI-1) which has shown in numerous studies on interstitial fibrosis. PAI-1 synthesing levels have been seen increased by fibrotic mediators such as angiotensin II and TGF-β1 [Fogo 2001(b)]. Therefore tubulointerstitial fibrosis is suggested from increased synthesis and decreased breakdown of extracellular matrix.

2.4.1.3 Key Mechanisms of Kidney Scarring
The diabetic nephropathy has two main scleroses 1. glomerulosclerosis and 2. tubulointerstitial fibrosis both involves similar mechanisms. At first step, an injury takes place and induces fibrogenesis by the activation of glomerular/tubular cells and afterward activated cells releases chemokines and cytokines attracting inflammatory cells. The final fibrotic phase is likely to result from an imbalance between the synthesis and degradation of ECM it causes irreversible fibrosis.

2.5 Modulating Factors of Progressive Kidney Disease
The progression of diabetic nephropathy is influenced by a numerous factors.
1. Susceptibility factors determining tendency towards specific nephropathy progression.
2. Progression factors these factors affect the rate of decline of renal function after the onset of diabetic nephropathy. Progression factors are divided into the modifiable and non-modifiable. Non-modifiable factors include the patient’s genetic and age and gender. Modifiable factors include systemic hypertension, proteinuria, dyslipidemia and lifestyle factors such as smoking, alcohol, caffeine and the use of prescribed drugs.

2.5.1 Non-Modifiable Risk Factors
2.5.1.1 Genetic /Familial History of Diabetes
Genetic factors are known to affect the susceptibility of diabetic patients to various types of diabetic nephropathies but they influence the natural history and progression of diabetic nephropathy too. It has been observed from several epidemiological studies; individuals likely to have increased chance of developing diabetes and its complications, who has reported first order, close relatives, and positive familial history of diabetes. In
addition ethnic variations also play important role like south Asians have higher HbA1c levels than whites Europeans [Likhari et al 2009].

### 2.5.1.2 Gender-related Factors

It has been seen that end-stage renal failure are more common in males [US Renal Data System 2001, National Kidney Foundation K/DOQI 2002]. Male gender is more prone for end stage of renal functioning seen in numerous major studies; these observations suggested a faster rate of decline in GFR in males [National Kidney Foundation K/DOQI 2002]. Many of the studies use univariate analysis and gender as risk factors. In multivariate analyses gender-related risk factor have not always confirmed independence. One study from Japan reported that the females had a faster rate of progression [National Kidney Foundation K/DOQI 2002]. Simultaneously a very high incidence of diabetic renal failure has been described in postmenopausal African-American women. A study from France has shown that diabetic women’s have higher odds ratio for the mortality ratio then diabetic man patients and diabetic patients over all have higher mortality rate then non diabetic nephropathy patients [Villar et al 2007].

### 2.5.1.3 Age-related Factors

It has been observed in numerous studies that the incidence of end stage renal failure increase with age [US Renal Data System 2001]. The etiology of renal failure is different in the elderly with a higher incidence of hypertension (including renovascular hypertension), type 2 diabetes and obstructive uropathy accounting for 40 to 60% of patients with diabetic nephropathy. Recently south Asian population has been reported to develop type 2 diabetes at earlier age [Sharp et al 2008]. One notable exception is type 1 diabetic nephropathy where young age at diagnosis is associated with a faster rate of GFR decline. The faster rate of progression of diabetic nephropathy in the elderly may be influenced by higher levels of blood pressure, underlying atherosclerotic renal ischaemia as well as the associated cardiopathy.
2.5.2 Modifiable Risk Factors

However nothing can be improve to influence the non-modifiable risk factors, therefore
growing focus is towards the modifiable factors affecting the rate of progression of
diabetic nephropathy. These include systemic hypertension, proteinuria, and metabolic
factors such as hyperglycemia, dyslipidemia, and possibly hyperuricaemia. Along with
these clinical factors, recent interest has focused on the contribution of cigarette smoking,
alcohol and drugs to the risk of development of end stage of diabetic nephropathy.

2.5.2.1 Hypertension

From numerous studies systemic hypertension was identified as potential mediator for
initiating and progressing for diabetic and other nephropathies. Recent studies provide
faster progression of diabetic nephropathy in the presence of systemic hypertension.
[Bloomgarden 2010, Pohl et al 2005]. A study on Australian population has shown that
the diabetic patients with poor glycemic control (HbA1C ≥ 8) faces systolic hypertention
(SBP≥140) [Thomas et al 2009]. Some studies have suggested the significant
contribution of nocturnal hypertension (loss of night-time fall/dip) may be an
independent risk factor for the progression of renal failure. Others suggest that the
elevation of pulse pressure may be an additional factor in the initiation and progression of
diabetic nephropathy in diabetic and non-diabetic nephropathies [Fox et al 2008].

2.5.2.2 Proteinuria

In diabetic nephropathy it has been shown that an association between heavy proteinuria/
microalbuminuria and a fast rate of GFR decline by regression analysis. Heavy
proteinuria or highmolecular weight proteinuria has been associated with a faster rate of
progression of diabetic nephropathy [National Kidney Foundation K/DOQI 2002,
Bakoush et al 2002]. The Modification of Diet in Renal Disease (MDRD) and other
studies showed that baseline proteinuria was a strong predictor of subsequent decline in
renal function and other associated macrovascular complications [Klahr et al 1994].
Numerous studies have reveled positive correlation between excretion of large molecular
weight proteins in urine and fast progression of diabetic nephropathy [Narita et al 2006].
On the other way low dietary protein intake shown reduced impact on nephropathy [El
Nahas et al 1984] and ACE inhibition predicts a better outcome in diabetic nephropathy.
and nondiabetic nephropathies. In type 2 diabetic patients proteinuria has been reported for dyslipidemia and continue declining renal function in macroalbuminuric patients [Yang et al 2008]. It is further clarified that dietary [El Nahas et al 1984] and pharmacological strategies were able to improvement renal function in proportional to the extent of the reduction in proteinuria. Proteinurias (small as well as larger molecules) have been shown a good predictive potential for the future cardiovascular incidences in diabetic and non diabetic nephropathies.

2.5.3 Metabolic factors

2.5.3.1 Glycaemia

Poor glycemic control directly influences initiation, progression and severity of diabetic nephropathy. Several pathways and mechanisms have been suggested to explain role of glycemia in the progression of diabetic nephropathy [Kitada et al 2010]. Uncontrolled glycemia has shown faster rate of decline in GFR in diabetic patients On the other hand intervention studies shown that tight glycemia was associated with improved prognosis however short term glycemic control does not improve microcirculation in type 2 diabetic patients [Meyer et al 2009]. The United Kingdom Prospective Diabetes Study (UKPDS) found supportive results that progression of diabetic nephropathy in type 2 diabetic patients was significantly improved on good glycemic control for longer period.

Uncontrolled blood sugar leads to severe toxicity through glycation of proteins, and AGEs formation which have potential for triggering several pathways leading to diabetic nephropathy. Low level of antioxidative enzymes, increased level of glucotoxins, altered tissue and plasma haemostasis has been observed with hyperglycemia. Glycation of plasma proteins and GBM glycocalix leads to GBM deposition and glomerulosclerosis and anionic proteinuria. Human proximal tubular epithelial cells are free to uptake glucose from primary urine in patients with type 2 diabetes [Rahmoune et al 2005]. High sugar level has been reported for activation of apoptosis, [Susztak et al 2006] altered mitochondrial morphology and density volume and increases intracellular nitrotyrosine content [Prato 2009], glucose fluctuations, which might occur in pre-diabetic conditions, may accelerate loss of β-cell function and mass. Type 2 diabetic patients have shown increased endoplasmic stress in β-cells when exposed to higher glucose levels [Poitout et al 2008]. Glucotoxicity induces inflammatory and oxidative stress responses [Uribarri et
al 2007], which leads to insulin resistance and reduce pancreatic β-cell function by several different mechanisms. It important to note that elevated plasma glucose may occur at relatively later stages of diabetes, however strict control of insulin action and insulin secretion occurs under homeostasis. Glucotoxicity causes initial lipotoxicity and reversible lesion of diabetic nephropathy. However podocytes are insulin sensitive and increased insulin resistance causes podocyte depletion at onset stages.

2.5.3.2 Lipids
Lipids molecules have been suggested to the initiate and progress the diabetic nephropathy with similar mechanism as in atherosclerosis, Hyperlipidaemia (triglyceridaemia) has shown wide asymmetrical distribution and positive relation with increased risk of end stage renal failure [Muntner et al 2000]. In type 2 diabetes, macroalbuminurina predicts high triglyceride-cholesterol and high LDL-cholesterol, HDL with GFR from normal to lower range [Yang et al 2008, Foggensteiner et al 2001].

Relation between dietary lipid and improved renal function has been observed in type 2 diabetic nephropathy. In a meta-analysis of fifteen major studies concluded that dyslipidemia is a risk factor for a faster rate of progression, confirmed by multivariate logistic regression analysis [National Kidney Foundation K/DOQI 2002]. Hypercholesterolaemia has been associated with a faster rate of decline in GFR in diabetic nephropathies [Krowleski et al 1993, Bonnet et al 2000, Yang et al 2008]. Similarly observation were made, in non diabetic nephropathies, and elevated plasma cholesterol, triglycerides with oxidized forms of lipids seems to be associated with a faster rate of progression when compared with controlled dislipidaemic patients. Oxidized lipoproteins and lipid hydroperoxides has been reported for apoptosis signaling [Mshelia 2004]. Moreover the significant association of lipid hydroperoxides with type 2 diabetic nephropathy patients has been shown [Mehrotra et al 2001]. It has been observed that lipid saturated monocyte derived macrophages cells exhibits increased lipid peroxidation and hydroperoxide formation due to high lipid content in the cell [Firth et al 2007].

A study on type 2 diabetic patients has reported improvement in plasma lipid profile was related to the beneficial impact on diabetic nephropathy [Teixeira et al 2004]. Several
studies have reported increased free fatty acids and its deleterious effect on insulin secretion and action. Pancreatic β-cell lipotoxicity may play an important role in the progression from normal glucose tolerance to overt hyperglycemia. Hyperglycemia induces several violent pathways will maintenance as well as worsens the diabetes. It has been also reported that chronic exposure of pancreatic β cells to free fatty acids induces toxicity through multiple pathways. Free fatty acids leads to accumulation of malonyl-CoA and long-chain fatty-acyl-CoA, increased fatty acid oxidation and etherification, accelerated ceramide synthesis, fatty acid-induced apoptosis and activation of endoplasmic reticulum stress. Moreover human pancreatic islets cells when incubated with high glucose, elicited triglyceride levels which leads to reduction of insulin content and glucose-stimulated insulin release. These alterations were causes reduced utilization and oxidation of glucose. Free fatty acids have been shown to directly inhibit glucose transport because of accumulation of diacylglycerol and fatty-acyl CoAs that ultimately reduce insulin signaling. Elevated free fatty acids have been seen in peripheral tissues insulin resistance. Insulin resistance is suggested a major risk factor for nephropathy in type 2 diabetic patients [Prato 2009].

2.5.3.3 Miscellaneous-Smoking

Many possible mechanism and pathways have been suggested, the effect of smoking on the progression of diabetic and non-diabetic nephropathies [Orth 2002]. Smoking (cigarette/bidi) causes an increase in systemic blood pressure and affects renal hemodynamic. Smoking increases progression rate up to six fold depending up on numbers of cigarette smoken per day. Smokers with glomerulonephritis appear to have more severe vascular scares when compared to non-smokers [Lhotta et al 2002]. Smoking has been reported to be associated with dyslipidemia and declining GFR in the diabetic nephropathy [Sahid and Mahboob 2007]. Smoking significantly increases the risk of end stage diabetic nephropathy with odds 2.24 for diabetic patients with hypertension [Yacoub et al 2010].

2.6 Management of Diabetic Nephropathy

Several strategies have been suggested to improve understanding and pathophysiology of diabetic nephropathy. The pathways and mechanisms which are central to the progression
are suggests new therapeutic approaches. However there are no simple solutions to prevent type 2 diabetes and diabetic nephropathy [Cefalu 2009], therefore management of diabetes focuses on the mechanisms of action of dietary and pharmacological interventions [Fraser et al 2007].

2.6.1 Therapeutic Interventions in Diabetic Nephropathy

Till date treatment of patients is given on the basis of his/her clinical characteristic, and concept of personalized medicine is lacking in present treatment strategies. Therefore human data on diabetic nephropathy is missing, however animal model interpreted the pathology and pharmacological interventions but at the reality ground these data deviates in many aspects in population. Another approach “meta analysis” is seems to be a ray of hope in clinical studies to conclude something concrete clinical decisions. It has been frequently observed the uncontrolled situations over the treatment. This factor is due to (1), Genetic variation in population and there response towards treatment. (2), Lack of understanding of factors that are centre to the pathogenesis and its relative risk. Presently following aspects/factors are treated which are suggested as central to the pathology of diabetic nephropathy.

2.6.2 Blood Pressure Control

Systemic hypertension has been well characterized in diabetic patients and its role in progression of diabetic nephropathy. Type 2 diabetic patients taking anti hypertensive medicine were reported less likely to develop diabetic nephropathy and reduce renal events [Fisman et al 2002]. Several outcome has been seen which cure or slowdowns progression of diabetic nephropathy, protenuria and rate of declining GFR [Adamczak et al 2002]. Furthermore well control on systemic hypertension in later stages of diabetic nephropathy is potentially able to rescue worsening the conditions.

2.6.3 Clinical Controversies

Some researchers express controversies on systemic hypertension control and decline GFR in diabetic as well as non diabetic patients [Marcantoni et al 2000, Adamczak et al 2002]. Another controversy in blood pressure control is treatment strategies of drug or the type of antihypertensive agents used. Few studies addressed blood pressure control as one
of the important goals in relation to progressive diabetic nephropathy. A meta-analysis of antihypertensive intervention studies from selected major studies in diabetic as well as non-diabetic nephropathies suggest a slowing of the rate of decline in GFR proportional to the reduction in mitogen activated kinase (MAP). It has been observed from a study on normotensive type 2 diabetic patients; blood pressure control (~128/75 mmHg) was protective factor against the progression of incipient and overt diabetic nephropathy [Schrier et al 2002]. In the normal population, having normal kidney function, without proteinuria blood pressure was aimed to achieve 140/90 mmHg. Patients at higher risk of faster progression or near to the end stages of nephropathy, hypertension control was strictly recommended the goals in guidelines. Patient’s without or with mild proteinuria is known to have a better prognosis.

2.6.4 Metabolic Control
Hyperlipidaemia (high plasma cholesterol and triglycerides level) may contribute to the progression of diabetic nephropathy. Numerous lipid lowering agents like cholestyramine, halofenate, clofibric acid, and the 3-hydroxy-3-methylglutaryl coenzyme-A (HMG CoA) reductase inhibitor lovastatin reduces the severity of age-related and ablation-induced glomerular sclerosis in animals with diabetic nephropathy [Keane 2000]. Statins have several virtual properties like immuno-modulatory, anti-proliferative, anti-inflammatory as well as anti-fibrotic effect [Martin-Ventura et al 2003]. There is no well known observation with reduction in hyperlipidaemia and restored GFR in diabetic nephropathy. It has been suggested from numerous studies simvastatin, an HMG CoA reductase inhibitor, restores amount of proteinuria but unable to slow the rate of progression of nephropathy over a two year time [Thomas et al 1993]. In a meta-analysis 12 lipid lowering agents were analyzed and it was concluded that overall impact on restoration of progressive nephropathy was very small [Fried et al 2001].

2.6.5 Advanced Stage Treatments
The numbers of studies documented that type 2 diabetes with increased platelet abnormalities. Intraglomerular platelet aggregation and microthrombosis are implicated in the pathogenesis of progressive glomerulosclerosis. Therefore antiplatelet and
Anticoagulants are suggested to protect progression of renal scarring. Heparinoids which are known for their anticoagulant properties plays protective role in glomerular scarring. Heparin which is polyanion, supposed to alter negative electrical potential of the GBM and suppresses the proliferation of cultured mesangial cells. Heparin also shows the activation of serum lipases and lipid-lowering effects. Heparin-like glycosaminoglycans (GAG) has been reported to inhibit glucose-induced mesangial upregulation of TGF-β1 expression [Ceol et al 2000]. These findings indicate a potential for GAG and heparinoids in the prevention of diabetic glomerulosclerosis [Ceol et al 2000]. A great proportion of diabetic nephropathy patients die with cardiovascular complications, platelets aggregation have been significantly correlated with coronary artery disease. Numerous studies were designed to examine the potential impact of antiplatelet therapy and aspirin has shown therapeutic antiplatele benefits in type 2 diabetic patients [Ferroni et al 2004]. Other antiplatelet drugs such as indobufen, dipyridamole and picotamide have been investigated in clinical practice for several years. Aspirin and dipyridamole for overt type diabetic nephropathy demonstrated that a combination of the two drugs significantly reduces proteinuria [Khajehdehi et al 2002].

2.6.6 Miscellaneous Interventions

In diabetic and non diabetic experimental studies several miscellaneous interventions has been tried and success was achieved moderately. Aminoguanidine/advanced glycation end-products (AGE) inhibitors, heparinoids/glycosaminoglycans and miscellaneous anti-fibrotic agents such as perfenidone have also been tested in experimental animals with diabetic and nondiabetic nephropathies [Fukagawa et al 1999]. Administration of a dopamine analogue (ibopamine), Chinese herbal extracts, oral sorbents (AST-120), and the infusion of prostaglandin E1 also has been tried. Improved renal functional at the end stages of diabetic nephropathy has been observed when injected with insulin-like growth factor 1 (IGF-1), [Miller et al 1997, Vijayan et al 1999]. The use of antioxidant therapy in nephropathy shows potential in prevention of oxidative stress [Dwivedi et al 2009].

2.7 Prediction of Diabetic Nephropathy

Type 2 diabetic nephropathy is one of the mislanioust complication and its rate of progression is tightly related to the the drifts in metabolic control. It does not progresss
fast as compared to other types of nephropathies as shown in Figure 2.1 [Remuzzi et al 2006]. Due to longer latency period at present it is impossible reliably to predict who of the young type 2 diabetic patients will progress to diabetic nephropathy during their lifespan. Numerous approaches have been puted forward in addition to intensive treatment and good metabolic control reduces the risk for diabetic nephropathy significantly. But idealism is not possible practically. The prognosis would be effective if ensured risk for diabetic nephropathy could be predicted at an early stage. The special effort could be focused on those patients who are at the risk considering biomarkers mentioned in the subsequent sections.

![Figure 2.1](image)

**Figure 2.1** Progression characteristics of different type of nephropathies with the time. The figure shows high variability in GFR decline, a fast decline is observed in all nephropathies except type 2 diabetic nephropathy, which progress with variable duration of diabetes.

### 2.8 Markers Associated with Diabetic Nephropathy

Numerous hypotheses has been proposed for the propagation of diabetic nephropathy but none of them is independent and explain progression of diabetic nephropathy confidently, secondly the extent of relations is not clear on the other hand some of the hypothesis
overlaps. The following biomarker describes the factors involved in the progression of diabetic nephropathy.

2.8.1 Markers of Glycation and Glucotoxicity (Glycated Hb and AGE)

Poor glycemic control is considered as sole determinant factor for all the diabetic complications, the extent of nonenzymatic glycation can be assessed by marker of glycation, Glycated Hemoglobin and AGEs. Most adoptive mechanisms for the pathogenesis of diabetic complications include formation of advanced glycosylation end products (AGEs). Recently AGE has been correlated with decline renal function [Nagai et al 2010]. Incipient nephropathy has shown association with AGE deposition in kidney tissue, the deposition is further found to be parallel associated with increase AGE concentration in plasma, and co-localization in skin [Wautier et al 2001]. Glucose nonenzymatically reacts with proteins to form the reversible Schiff base adduct, which subsequently can rearrange to form the stable Amadori product and AGE products. These protein-bound forms of glucose and their oxidized, cleaved, and dehydrated derivatives can produce reactive intermediates. In vitro, free metal ions catalyze steps in a nonenzymatic glycoxidation pathway that generates AGE products. AGEs can damage tissues through a number of mechanisms, including generation of oxidizing intermediates, formation of immune complexes, interaction with a cellular receptor called RAGE (receptor for AGE), and promotion of cytokine release. The glycoxidation hypothesis overlaps the proposed link between oxidative stress and the production of AGE products. Poor glycemic control appears to be an independent risk factor for triggering numerous pathways directly or indirectly [Gatti et al 2009].

2.8.2 Markers of Lipid and Lipotoxicity

(PON, Lipid hydroperoxides and Lipid peroxidation products)

Almost all type of lipid could be oxidized by free radicals, the membrane of cells and cell organelles consists large amount of PUFAs, lecithin, cholesterol, and phospholipids. PUFAs are more prone for oxidation and leads to the formation of numerous types of peroxide products. Impaired lipid status is widely reported during development of secondary complication of type 2 diabetes mellitus however several mechanisms were proposed for the impaired level of lipid in diabetic patients. At early stages of diabetes
uncontrolled glucose level contributes huge free radicals generation. In addition to this glycation of proteins and enzymes, deleteriously alters the activity of molecules which maintains homeostasis. Serum paroxonase is a glycoprotein of mol weight 45-kD. It is glycation prone which is associated with HDL, PON 1 is involve in the prevention of LDL oxidation and its activity does not affects plasma cholesterol level however human PON 1 is an anti oxidative enzyme which shows numerous anti oxidative activities, it has been reported for activity of phospholipase A2 for hydrolysis of oxidized phospholipids, moreover this also exhibits activity which scavenges hydrogen peroxides lipid hydroperoxides which is similar to peroxidase activities. It is suggested that diminished activity of PON 1 play an important role in the development of diabetic nephropathy through loss of anti-oxidative properties secondly PON1 also protect HDL hence cholesterol transportation so, it maintains homeostasis of angiotensin in renal diseases Serum paraoxonase is one of the antioxidant enzyme which is prone for the glycation. Glycation of paraoxonase-1 inhibits its activity low paraoxonase activity results in to increased membrane lipid hydroperoxides. This mechanism is suggested to cause membranous-nephropathy/glomerulosclerosis. Lipid hydroperoxides formed during the glucotoxicity and high oxidant in plasma as reported previously. The basement membrane change its property with the development of interstitial fibrosis, fewer mesothelial cells, vascular wall thickening, vasodilatation and increased angiogenesis and cumulatively these are characteristic of incipient diabetic nephropathy. Advanced lipid peroxidation end product has been observed as an inflammation inducer and enhances excretion of chemo toxins by monocyte activation.

Increased cholesterol in diabetic patients is observed due to enhanced biosynthesis or diminished clearance from the blood during the normal homeostasis circulating LDL is metabolized in liver through LDL receptor mediated uptake this increased plasma-LDL may be due to oxi-LDL glycosylation of LDL or defect/loss of function in LDL receptor either through excessive glycosylation, Oxidative stress higher concentration of triglyceride in diabetic patients may be due to increased secretion of VLDL and impaired catabolism loss of lipoprotein, loss of lipoprotein lipase activity, unable to scavenge triglyceride therefore its plasma level increases. Increased lipid peroxidation in diabetic patients is a resultant of increased level of plasma phospholipids leakage and
membrane damages, the phospholipids in membrane are one of the main constituents that maintain membrane fluidity, integrity and micro-viscosity. Ultimately increased lipid peroxidation in plasma indicates loss of these functions and progression towards diabetic nephropathy and other complications. Increased lipid peroxidation is also resultant factor of unbalanced antioxidant defense mechanism may be due to diminished activity of antioxidant enzyme or enhanced free radicals generation. Excessive glycation may arrest protein folding at early stages which leads to conformational changes; altered LDL may result in differential oxidation by reactive oxygen species and increased enhanced lipid peroxidation rate and reaction.

2.8.3 Markers of Total Anti-oxidant Capacity

(FRAP and Total plasma thiol content)

Oxidative stress has been postulated as one major contributor to long-term diabetic complications. The nature, magnitude, and localization of oxidative stress in diabetes have been studied extensively. There is overlapping bridge relationship between hyperglycemia and oxidation which is unclear, however the link between these two accelerates diabetic complications. Moreover, it is not known whether oxidative stress is a primary event that occurs early in the course of the disease or whether it is a secondary phenomenon that merely reflects end-stage tissue damage. Although normal metabolism produces oxidative free radical but these are absorbed instantly by high antioxidant capacity and many anti oxidant enzyme participates in scavenging process. Alters normal homeostasis of redox balance, imposed by glucose incites various metabolic pathways with enhanced generation of advanced oxidized protein product (AOPP), advanced lipid peroxidation product (ALEs), and generation of reactive oxygen species (ROS) with a positive feedback process. The ROS seem to be most destructive factor which triggers various pathways and central to the pathogenesis of hyperglycemic injury. In the consequence of progressive diabetic nephropathy total plasma thiol and total antioxidant capacity plays an important role for providing buffering capacity of disturbed redox balance. Several research supports depletion of anti oxidant capacity and its relation to the nephropathy [Farvid et al 2005], moreover reactive oxygen species 1of antioxidant capacity are known to cause apoptosis and podocyte depletion at the onset of diabetic
nephropathy [Susztak 2006]. Recently reduction in oxidative stress has been suggested for the prevention of secondary complications of diabetes [Araki et al 2010].

### 2.8.4 Markers of Renal Function (eGFR$_{MDRD}$ and eGFR$_{GC}$)

Estimated Glomerular filtration rate has been considered independent predictor of renal function decline, several studies has shown eGFR was better marker then albuminuria. The glomerular filtration rate (GFR) was estimated using Cockcroft-Gault (CG) this equation was corrected for sex and body surface area (BSA) for Indian type 2 diabetic population. Use of body surface area corrected CG equation enabled direct comparisons with GFR estimates of Modification of Diet in Renal Disease (MDRD) equation as CG-GFR equation is better suited to estimate subnormal GFR in Indian population. eGFR was expressed in unit = ml/min/1.73 m$^2$ [Kramer et al 2003, Singh et al 2009].

### 2.8.5 Markers of Renal Remodulation

(Uranian hydroxi-proline and Urinary glycosamine glycans)

Glomerular basement membrane bears several proteoglycans and glycoproteins called the glycocalyx (10-60 nm thick), the glycocalyx is up to 90 nm in height and provides negative charge to the GBM. During uncontrolled blood sugar, anionic charge loss occurs which leads to charge depleted patches on GBM. Thus peeled-off GBM have increased vulnerability and exposed cell surface receptors for the extravasation or infiltration of cells. These cells alter remodulation and ECM accumulation, as collagen is most abundant structural component. Its metabolic/degraded end products are reflected in urinary (peptides and relatively increased Hydroxi-proline). In different studies urinary glycosaaminoglycans (GAGs)/creatinine ratio and Urinary hydroxi-proline/creatinine ratio were evaluated in diabetic and non diabetic nephropathy to investigate pathophysiology of kidney matrix.

### 2.8.6 Markers of Protein Energy Wasting

(Plasma AOPP, Urinary IgG and Urinary Albumin)

Recently attention has been given on protein energy wasting, which may occur in the form of loss of protein functionality through its oxidation or loss of these proteins in urinary excretion. Accumulation of plasma advanced oxidation protein products (AOPP)
has been found in nephropathy patients, plasma AOPP is heterogeneous in nature and continuously increases in plasma till the end stages of diabetic and nondiabetic nephropathies. Type 2 diabetic nephropathy exhibits faster progression and consequently chronic, when low as well as higher molecular weight protein loss occurs Immunoglobulins (IgG, IgA, IgM), transferrin, and ceruloplasmin.

2.8.6.1 Low Molecular Weight Proteinuria
Urinary albumin is widely accepted biomarker of proteinuria in clinical practice till now. After onset of microalbuminuria, the risk of progressing to diabetic nephropathy is about 50% [Krolewski et al 1994]. This risk may, however, vary greatly between different age groups, or at different levels of glycemic control, or after differing durations of diabetes. To identify those who will develop microalbuminuria is even more difficult. In addition microalbuminuria is also associated with endothelial dysfunction and could revert upon therapeutic interventions. These factors reduce the trust full predictive capabilities for progressive diabetic nephropathy.

2.8.6.2 High Molecular Weight Proteinuria
High molecular weight protein loss in urine is the best predictor of fast progressive nephropathy. Numerous high molecular weight proteins i.e. IgM, ceruloplasmin, β2-macroglobulin ect. have been suggested for diagnostic and prognostic purpose. Immunoglobulin G (IgG), a putative biomarker for nephritic symptoms seems to be high capabilities of predictive potentials for diabetic nephropathies. However it has been ignored from last decade research. IgG is actively involved in pathogenesis of diabetic nephropathy which is still to be revealed. The following section described the up-to-date literature on IgG and diabetic nephropathy.

2.9 Immunoglobulin G
IgG is one of the class of immunoglobulin molecule, normally present in highest concentration in both intra and extravascular spaces, with a mean normal adult serum concentration of 1200 mg/dl. This class is specifically known for its secondary antibody responses (immunologic memory) and involved in defense against infections. Other IgG isotype molecules are involved in complement fixation, opsonization and fixation with
macrophages. The fragments of IgG light chain and heavy chain (κ and λ chain previously known as Bence Jones proteins monomers of 22 kD and dimmers of 44 kD).

2.9.1 IgG in Type 2 Diabetic Patients

The glycation of IgG seems interesting for several reasons. The binding of glucose that deplete the net charge of IgG molecule, leads to a lower mobility therefore glycated IgG had increased tendency of adherence than non-glycated IgG [Bruneval et al 1985]. It has been suggested that glycation induced a conformational change in IgG with less positive charge this view is further supported by diminished reactivity upon glycation of IgG. Changes in the biological activity of the glycated IgG molecule is due to the alteration of some of its free amino groups by the non-enzymatic glycosylation.

2.9.2 Urinary Excretion of IgG

Negative charges of the endothelial surface have been proposed to partially prevent the traversal of plasma macromolecules [Singh et al 2007, Tryggvason et al 2005]. The glomerular filtration barrier has long been considered a passive size and charge-selective sieve, capable of restricting IgG and albumin on the basis of size and charge, respectively [Weinbaum et al 2007, Sarav et al 2009]. In diabetic patients high blood glucose depletes charge from several blood /plasma proteins [Budak et al 2004], this impaired charge selectivity leads to enhanced glomerular passage and urinary excretion of negatively charged molecules like albumin and IgG₄. In more advanced stages of diabetic nephropathy a concomitant defect in glomerular size selectivity is reported in a grossly increased urinary excretion of glycated IgG [Mistry and Kalia 2008]. In addition neutral-charged IgG, negatively charged IgG₄, and ceruloplasmin was selectively increased in impaired glucose tolerance patients. A study on healthy subjects has shown that increased flux of acute protein loading induces a selective increase in clearance of IgG, IgG₄ which is ultimate resultant of increased intraglomerular hydraulic pressure [Hoogenberg et al 1996]. These observations were further supported by reduced fractional clearance of the proteins upon angiotensin II blockade treatment of diabetic patients [Andersen et al 2000]. Therefore it is clarified that IgG in urine increases with hemodynamic change in kidney. Apart from glomerular pathology IgG has been reported in other malfunction. IgG in systemic lupus erythematosus patients with proteinuria had a higher positive
electric charge than that in systemic lupus erythematosus patients without proteinuria, healthy individuals, and patients with other collagen diseases [Kinoshita et al 2006]. It has been also observed that glycation of immunoglobulin protein increases its acidic nature [Monteleone et al 2000]. At acidic pH binding of protein to FcRn receptors is higher when compared to physiological pH [Sarav et al 2009] and metabolic acidosis act as catalyst in progression of nephropathy [Frassetto et al 2009]. Moreover excessive internalization of proteins generates excessive H₂O₂ and ammonia [Wang et al 2007]. Abnormalities like IgG-bound sugar chains have been reported for the increased positive charge and active involvement in the etiology of SLE nephritis [Kinoshita et al 2006]. Further more FcRn binding has been reported different for albumin and IgG, it has been shown that albumin FcRn binding decreased up to 200-fold from acidic to neutral pH, which indicated that as pH reverts from acidic to normal, albumin is saved from lysosomal degradation and urinary excretion but not IgG.

### 2.9.3 IgG Deposition in Diabetes Nephropathy

Directly or indirectly immunoglobulin has strong association with diabetic nephropathy, direct involvements appears in following manner one, that result from deposition into the kidney of intact immunoglobulin molecules and second caused by components of immunoglobulin molecules usually light chains or light chain fragments, and less frequently, heavy chains or heavy chain fragments [Bruneval 1985]. Immunoglobulin’s molecules are typically polyclonal and deposit either as pre-formed immune complexes or interact directly with kidney antigens, deposition of IgG has been observed in type 2 diabetic patient’s kidney with a good relation to nephropathy. In glomerulonephritis predominant immunoglobulin class is IgG (IgG 94%, IgA 60%, IgM 29%), and in 80% of cases involvement of both κ and λ light chains are seen [Korbet et al 2006, Komatsuda et al 2008].

### 2.9.4 IgG and its Clearance by FcRn Molecules

The plasma concentration and turnover of IgG is maintained through its Fc receptor (FcRn) mediated uptake. FcRn is expressed in adults, including in vascular endothelium, liver, spleen, lung, and kidney; hence, its gene designation as IgG Fc receptor, alpha chain transporter. FcRn binds to albumin and IgG with high affinity at acidic pH (≤6.5)
but not at physiologic pH (7.4), [Rodewald et al 1976]. Notably, the interactions of FcRn with albumin are distinct from those with IgG. A study carried out on cultured endothelial cells have shown that FcRn acts on pinocytosed IgG, saving it from lysosomal degradation and maintains homeostasis of IgG in plasma, IgG which do not bind to FcRn accumulate in the lysosomal degradation pathway [Ward et al 2003]. In the kidney, FcRn is expressed on the podocytes and the brush border of the proximal tubular epithelium [Sarav et al 2009]. FcRn is a key protein involved in systemic albumin and IgG metabolism and facilitates the loss of IgG from plasma protein pools.

2.9.5 IgG Induced Matrix Metalloproteases
The implication of IgG in the progression of several acute and chronic clinical disorders led to different mechanism. Matrix metalloprotease 3 (stromelysin-1) and matrix metalloprotease 7 (matrilysin-1) can cleave all immunoglobulin G proteins. These metalloproteases are essential part of compliment fixation, secondly these matrix metalloproteases releases immunoglobulins from the site of inflammation and scared tissue by unspecific cleavage of proteinacious moeties in structural organization [Gearing et al 2002]. It has been also reported the MMP 7 levels inhibited by AGEs which clearly indicates the imbalance of MMP levels and conditions which either supports IgG, and its immune complex deposition on GBM or degradation of extracellular matrix [McLennan et al 2007].

2.9.6 IgG as a Putative Biomarker for Kidney
IgG haemostatic is quite stable and restorable. The dramatic hike in plasma and urine appears only in metabolic disorders and diseases and it has been reported as biomarker for diabetic nephropathy. It has been previously shown that high molecular weight proteinuria (>100 kD) and IgG is associated with decline in renal function in patients with incipient nephropathy [Varghese et al 2007, Mistry and Kalia 2008]. The GBM was previously considered to represent the size and charge selective macromolecular barrier, but recent studies have emphasized the podocyte slit diaphragm as being the main size-selective filter [Fukasawa et al 2009]. Glomerular filtration barrier is composed of non-restrictive pores (non charged pores), through which α2- macroglobulin does not filter, but plasma proteins with molecular radii of, 45–55 Å selectively filter when the
intraglomerular hydraulic pressure is elevated [Weinbaum et al 2007]. Urinary IgG are useful predictors of renal insufficiency in patients with diabetic as well as non diabetic nephropathy [Branten et al 2005]. It has been reported that during the sepsis conditions urinary excretion of IgG can increased up to 26 folds. Urinary excretion of IgG is highly correlated with urinary excretion of small proteins like 1-microglobulin (27 kD), Cytatin C (13 kD) then albumin (69 kD) [Bakoush et al 2001]. Moreover, excretion of IgG and 1-microglobulin has been investigated as a potential predictor for both decrease and progression of renal function. The couple of these molecules are useful in the identification of those patients who are at the risk for progressive nephropathy and for those who are treated with immunosuppressive therapy soon after diagnosis [Bazzi et al 2001]. These studies suggest that IgG is putative marker of glomerular as well as tubular dysfunction and renal function recovery when coupled with other biomarker.

2.9.7 IgG in Diagnosis of Type 2 Diabetic Nephropathy
The increased excretion rate of IgG has been reported which is less related to albumin excretion rate in type 2 diabetic patients [Narita et al 2004]. Several studies have reported high fractional clearance of IgG in urine of type 2 diabetic patients in comparison of type 1 diabetic patients. Jain and Rajput selected type 2 diabetic patients and identify fractions of IgG proteomics approaches of 2-dimentional gel electrophoresis and mass spectrometry more over they suggested the fractions of IgG in urine may be used as early possible marker of diabetic nephropathy [Jain et al 2005], in an another proteomic study of type 2 diabetic patients, urine samples were analyzed on 2-dimentional gel electrophoresis followed by LCMS. They identify 62 unique proteins out of which these were of immunoglobulin fragments, Ig γ-1 chain C region, Ig α-1 chain C region, Ig κ-chain C region, Ig γ-4 chain C region, Ig γ-2 chain C region [Rao et al 2007]. These fragments were suggested as putative biomarkers of diabetic nephropathy.

In adult patients with type 2 diabetes, podocytes were reported in the urine of 53% and 80% of microalbuminuric and macroalbuminuric subjects respectively, but in none of the normoalbuminuric subjects. Therefore it is logical to examine large molecules (Immunoglobulins). In urine to estimate threat of near future glomerular crescents and podocyturia in normoalbuminuric diabetic patients. Previously as well as recently IgG has been suggested a genuine biomarker for glomerular and tubular dysfunction.