Chapter 8

In last few decades type 2 diabetes became one of the fastest growing epidemics worldwide. Diabetic nephropathy is a major secondary complication of diabetes and develops in variable time/diabetic duration. The progression rate also varies among diabetic patients. The major factors which could lead the fast progression of diabetic nephropathy; less control of metabolic factors and/or opportunistic metabolic drifts leading to sudden loss of normal homeostasis. Such drift in metabolic control are more disappointing in addition to the pitfalls in diagnostic and/or therapeutic modalities, resultants of such losses less likely to recover and cumulatively leads to progression rate faster and faster. The absolute situation has been reported in numerous recent research articles on epidemiology of diabetes and its secondary complications. In these reports diabetic nephropathy accounts major portions of mortality in over all diabetic population from all caused mortality.

The primary aim of the thesis was to study the IgG (a putative biomarker of nephropathy) in type 2 diabetic patients to obtain conclusive results for daily clinical practice. The urinary IgG was screened for its idealism in type 2 diabetic patients and compared with microalbumin. The thesis is based on the analysis of potentials of IgG, its association to hazards pool of factor affecting diabetic nephropathy, impact of co-complications, and its indexes for predictive probabilities which were compare to the microalbuminuria. The study was designed to discriminate those type 2 diabetic patients who are at higher risk of developing diabetic nephropathy among over all type 2 diabetic patients’ population. The different aspect of IgG analyzed and discussed in different section of the thesis, are summarized here.

First chapter of the thesis provides general introduction to the subject and chapter two mention review of literature and background for the thesis. Chapter four focuses on the miscellaneous factors of type 2 diabetic nephropathy. The chapter discussed the illustration why it is still unclear that some patients frequently develops diabetic nephropathy while others not, even on the appearance of microalbuminuria. Specifically some of the patients shows decline in renal function without appearance of
microalbuminuria. Recent therapeutic modalities are potentially able to increase the life expectancy in type 2 diabetic patients, therefore microvascular complications are slowly becoming important in addition to macrovascular complications and leading to the poor quality of life. Factors determining the occurrence and progression of diabetic nephropathy include hyperglycemia, hypertension, hyperlipidemia and genetic factors. The pathophysiology and risk factors of diabetic nephropathy are not completely understood. It is not clear which types of cells are involved in the initiation, propagation and progression process of renal scars. Glomerular, endothelial and mesangial cells seem to proliferate in treated circumstances, but Podocytes do not proliferate, however healthy podocytes and several other cells promote stimulation, proliferation and migration of glomerular endothelial cells recovering the capillary growth. The markers originate from renal tissue are subject of long debate and requires further confirmation. The causative mechanisms by which impaired kidney function contributes to type 2 diabetic nephropathy and other causes of mortality are not fully elucidated. Therefore it is better to access the malignancy of building blocks and there relative risk/ essential contribution in deterioration. Diabetic nephropathy has been observed to be initiated with glomerulosclerosis, plasma molecules cross the dismantled GBM subsequently appears in urine. Therefore proteinuria is the sensitive criteria to diagnose progressive renal impairment and large molecules like Immunoglobulin are potentially able to predict severity of nephropathy. Directly or indirectly hyperglycemia induces vascular lesions which leads to proteinuria, and high urinary excretion of IgG with progression of glomerular injury, but the extent of impact of the factors which leads to the glomerular lesions is unknown. In this study we consider the carbohydrates, lipids and proteins which are main constituting bricks of cellular organization. During the diabetes, uncontrolled blood sugar and oxidative stress is tremendously increased and deliver its deleterious effect on all these primary constituents. The thesis describes relative risk of all three factors for the increased glomerulosclerosis which ultimately progressed to diabetic nephropathy or proteinuria. The progression rate of nephropathy is also influenced by the fact that absolute management of energy (calories taken vs. physical exercise vs. impact of medication) are most critical and complicated. In chapter four study was designed for type 2 diabetic patients, firstly classified in to three groups
according to urinary albumin creatinine ratio (UACR, <29, 30-299, >300 mg/g creatinine) and each group was further sub classified in to low and high urinary excretion of IgG creatinine ratio (UIgGCR) on the basis of median value. At present there is no direct measurement of malignancy in building blocks or factors; however over all hyperglycemia/AGEs formation can be related to the malignancy. Therefore Advanced Glycated Endproducts (AGEs), Advanced Oxidized Protein Products (AOPP), and Advanced oxidized Lipid Endproducts (ALEs) were analyzed. In addition, anti-oxidative status of plasma (FRAP, Plasma thiol content) and antioxidant enzyme activity (Catalase, Glutathion, SOD, and Paraoxonase) were also analyzed. The relative risk was calculated for the AGE, AOPP and ALEs. The adjusted odds ratio estimates from this study might help in making decisions to the clinicians for the status of diabetic patients for progressive status of diabetic nephropathy.

The analysis of type 2 diabetic patients in chapter four has shown that the AGEs, ALEs actively imparts in higher UIgGCR group, whereas AOPP is less significant relatively. The results have shown the highest adjusted odds ratio for the lipid peroxidation factors. These results indicated membranous deterioration is central to the pathology of diabetic nephropathy. In addition the antioxidant content/capacity was estimated with antioxidant enzyme activity. The plasma total antioxidant capacity and thiol content was found at reduced level when compared to healthy control. The antioxidant enzyme activity decreased in all diabetic patients with normo, micro and macro UACR group when compared to healthy control. There was no statistically significant difference in antioxidant enzyme activity (Catalase, Glutathion, SOD), when compared to lower vs. higher U1gGCR in normo, micro and macro UACR group respectively. Only paraoxonase enzyme activity decreased in to lower vs. higher U1gGCR in normo, micro and macro UACR group respectively. This might be due to glycation dependent threat to paraoxonase activity as reported by others. These results indicate U1gGCR as an increased relative risk, and threat for rapid progression of diabetic nephropathy.

The microvascular and macrovascular complications which are common to type 2 diabetes mellitus were considered in chapter five. This chapter is based on the background that the progressions of these complications face similar deleterious effects
of hyperglycemia and related to each other in a very complex metabolic web. After glycemic and blood pressure control inflammation and localized lesions are the main factors in development of complication. Proteinuria is putative marker of numerous pathologies from endothelial dysfunction to renal deteriorations. According to current concept, progressive nephropathy is accessed on the bases of microalbumin and when compounded with reduced eGFR may significantly increase the risk of events. But numerous studies are unable to answer that why patients with reduced eGFR and without microalbuminuria are at increased risk for all-cause mortality. Reduced eGFR is a solely marker of kidney functionality and without microalbuminuria it may decline, or microalbuminuria may appear without decline in eGFR, due to influence of co-complications associated metabolic factors. These factors are also influenced greatly with the conditions like age distribution, gender segregation, other habits e.g. smoking, are clearly the important contributors. Smoking has been shown to be related to urinary albumin excretion, and it is a risk factor for incident early development of diabetic nephropathy; therefore, the effects of cigarette smoking on urinary albumin excretion may be in the causal pathway for diabetic nephropathy progression. This factor should not ignore because male as well working female are found of smoking habit frequently in our society. The miscellaneous nature of microalbumin and its association to other co-complications reduces its faith as a predictive biomarker for diabetic nephropathy. Few researchers have suggested that the presence of both low GFR and microalbuminuria reduces the potential misclassification in identifying those individuals who truly have higher risk of diabetic nephropathy. This approach could extend a little in clinical decisions.

In chapter five only those type 2 diabetic patients were consider for study shown (1st and 2nd stages of nephropathy out of 5 stages), eGFR ≥60 ml/min/1.73m². The three major groups of current study, diabetes without complications, diabetic retinopathy complication and diabetic cardiovascular complications were sub classified in to two subgroups eGFR 60-74 ml/min/1.73m² and eGFR ≥75 ml/min/1.73m². Adjusted odds ratio was calculated for the UACR and U1gGCR. Plasma markers of anti-oxidative status (FRAP, Plasma thiol content) were analyzed along with antioxidant enzyme activity (Catalase, Glutathion, SOD and Paraoxonase).
In chapter five our finding suggests that microalbuminuria is associated with micro and macro vascular complications of diabetes. The increased urinary albumin might be due to mild microangiopathy (Class I and Class II glomerular lesions) and increased blood pressure. Whereas larger molecules like IgG are restricted from the small lesions until unless shunt like pores, nodular lesions or Kimmelstiel–Wilson lesions are not created in GBM. In this section, type 2 diabetic patients were discriminated and relative risk was calculated for the UACR and U IgGCR. The result suggests that the adjusted odds ratio for the urinary microalbumin increases with micro as well as macro vascular complications whereas odds ratio for the urinary IgG was selectively associated with decline renal function. Moreover antioxidant level of plasma was found at decreased level in diabetic patients with and without micro/macrovascular complications when compared to the healthy control, in addition decreased antioxidant level of plasma was also found in diabetic patients with micro and macro vascular complications when compared to diabetic patients without complications. These results suggest that the micro and macro vascular complications are associated with decreased antioxidant enzyme activity and antioxidant capacity in chronological manner which leads to proteinuria in a complex micro-environment. The subtle change influences UACR frequently where as U IgGCR is more stagnant and seems to be increased only in serious lesions. In this section it was found that the association of progressive diabetic nephropathy is strictly related to IgG then microalbuminuria.

In chapter two it is mentioned that the type 2 diabetic nephropathy is multicentre disease therefore its treatment strategies could not be as simple as these are assuming at presently. And this could be why we observing increased number of patients ever seen. Current therapeutic interventions potentially sustain the life of diabetic patients but unable to decrease the progressing of diabetic nephropathy. For an effective diagnostic modality all factors must be considered which could have association to the putative biomarker including their extent of influence. Chapter six provides the results of odds and matrix analysis. Numerous other researchers have reported that the other type of nephropathies could not stop by simple treating hypertension and proteinuria. The localize inflammations and active matrix metallo-proteases (MMP) are important factor
which leads to the progression of nephropathy even though these patients were taking therapies.

Chapter six reported odds ratio for factors which potentially leads loss of macromolecules in urine. The key check post biomarkers were selected vise; physiochemical properties of filtrating mechanism, normal glomerular basement membrane which has primary protective layer of glycosaminoglycans that makes a barrier to prevent adherence of plasma proteins to the GBM. Secondly proteins themselves do not adhere on the epithelial wall of macro and micro circulations due to the negative charge on surface proteins of cells. The loss of this sialic acid content is suggested to increased adherence of proteins to the GBM. Sialic acid is frequently passed out from glomerular and tubular passage of nephron, unlike proteins/peptides which are up taken through receptor mediated mechanism or other sugars moieties which are reabsorbed through epithelial cell. Loss of sialic acid in urine reflects vulnerability of GBM. The peeled GBM with increased blood pressure has been suggested for forced permeation of these proteins in to the urine. The hydroxy proline content of the urinary proteins is putative indicator of increased renal remodulation and collagen turnover. The protein/peptide bound hydroxy proline content of urinary proteins is also an indicator of enhanced catabolism of collagen proteins. Keeping all these facts in account, the odds ratio were analyzed for the selected biomarkers; urinary sialic acid to creatinine ratio (USACR), urinary glycosaminoglycans to creatinine ratio (UGAGCR), urinary protein/peptide bound hydroxy proline to creatinine ratio (UHPCR), glycated hemoglobin and blood pressure for the all four quartiles of urinary IgG excretion (UIgGCR). The highest odds ratio was observed for the UGAGCR, followed by UHPCR, a markers of collagen catabolism, USACR, in addition odds for glycated hemoglobin and systolic blood pressure were significantly higher from second to fourth quartile. These results indicate that urinary excretion of IgG is significantly influenced by corrosion of glomerular basement membrane then the loss of charge selectivity of proteins on GBM. The result also supports the stagnant nature of urinary IgG. The main finding suggests that the increased blood pressure might force small molecules to pass out in the urine upon subtle charge loss, whereas larger molecules could not escape until unless shunt like pores are not created in GBM. The markers of glomerular membrane integrity have significant impact on GBM porosity
increment, increment in these factors leads to risks for scarcity of GBM, loss of macromolecules in urine. Therefore a level of urinary IgG represents the extent of serious lesions in kidney. Secondly an ideal biomarker must be non invasive in nature and must be free from influence of other metabolic drifts. The matrix relation to the IgG was also reported to its idealism for the diabetic nephropathy.

As discussed before the prognostic information obtained from microalbuminuria clinically apprehensive. The progression of diabetic nephropathy is complicated by the fact that the progression parameters are time-dependent and varies over the duration of diabetes. The chapter seven was aimed to analyze the IgG as predictive biomarker for the diabetic nephropathy. A cohort was selected from random type 2 diabetic population however for homogeneity of the model patients with other co-complications were excluded with few other criterions. The patients were selected for the study presented their first eGFR > 60 ml/min/1.73m². All the patients were classified in to four quartiles <25%, 25-50%, 50-75%, and >75% urinary IgG excretion (UIGGCR). The survival probabilities for diabetic nephropathy and their duration of diabetes were analyzed by Kaplan-Meier survival analysis. After characterization of this cohort the different statistical approaches were applied and compare predictive capabilities of IgG and microalbumin. The results clearly indicate that the renal deterioration is closely associated with decline in renal function. The patients with high degree of renal function decline fall with higher excretion of UIGGCR, however these two factor lacking a linear correlation. Further analysis of data was made through ROC curve analysis, which revealed the levels of significance of different indexes of IgG and microalbumin in addition to Yoden index associated criterion. The predictive probabilities were further checked in ROC curve through sensitivity and specificity. It is important to note that the trial made previously by other researcher were unable to report the predictive capabilities of urinary IgG excretion when compare with microalbumin and these researcher only reported the superiority of IgG over the microalbumin. The main finding of the thesis states that the UIGGCR and FEIgG are better indexes then UACR and FEAlbumin. This study first time reported the potential of IgG excreted in urine with a greater sensitivity and specificity to discriminate those type 2 diabetic patients who are on the higher risk of diabetic nephropathy.
8.1 Conclusion

In conclusion, type 2 diabetic nephropathy is step wise process which proceeds rapidly when metabolic drifts associate with key factors affecting the progression rate. Results obtained in this study suggest that the uncontrolled hyperglycemia directly or indirectly leads to proteinuria, microalbumin like small molecules influenced frequently on metabolic disturbances, moreover on their flutters control. In addition microalbuminuria could revert but infect diabetic lesions remains as such. The results have suggested that IgG serves as a better marker to identify progressive diabetic nephropathy at incipient stages. IgG is stable then microalbumin. The association between urinary IgG and co-variants suggested that urinary excretion was strictly associated with renal lesions then charge selectivity loss. It was also concluded that the type 2 diabetic patient with higher urinary IgG takes lesser time to develop diabetic nephropathy. The predictive capabilities of IgG are of higher sensitivity vs. specificity in comparison of urinary albumin. Conclusively this study suggests FEIgG as one of the best predictor of renal deterioration which is potentially able to discriminate the patient at high prevalence of diabetic nephropathy. Urinary excretion of IgG is superior index then microalbuminuria.

We recommend this thesis be taken as a basis for discussion among clinicians, clinical chemists, urologist and diagnostic industries regarding the current diagnostic and /or therapeutic pitfalls, and we encourage its use it concept to facilitating daily clinical practice. This thesis might be instructive to minimize mistakes when a patient is susceptible for the risk of diabetic nephropathy.

Finally, the prevention or slowing the progression of diabetic nephropathy will significantly improve both the patient’s quality of life and reduce the public health expenditure.
8.2 Future Perspectives

Many questions regarding the urinary excretion of IgG and diagnosis of diabetic nephropathy remain unanswered after these investigations, and this thesis could be extended in numerous promising research topics. The study must be repeated with a larger sample population to confirm the present observation in type 2 diabetic patients. Further studies would be benefited if patients will be select from a wide variety of ethnic backgrounds covering all stages of nephropathy with histological characterization. The inclusion of type 1 diabetic patients would be a valuable addition to any future study. The urinary excretion of IgG at advanced stages of nephropathy increases in exponential manner and these changes happen over the time this could provide a good background for interesting research topics. Moreover moving beyond population studies, studies in animal models of diabetes would be in-valuable in understanding the mechanisms. Advanced imaging and structural information may revile many untouched aspects of diabetic nephropathy. In addition, advanced simulation studies based on geometrical computational models could be performed to analyze glomerular remodulation. For such type of studies data of odds ratio and matrix correlation from biochemical status to stages of nephropathy could be important. Hopefully such studies will potentially be able to diagnose diabetic nephropathy with higher accuracies and significantly postponing the progression of diabetic nephropathy.