Glycemic control, dyslipidemia and endothelial dysfunction in coexisted diabetes, hypertension and nephropathy

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Abstract: Diabetes mellitus is a chronic metabolic disorder that can lead to serious cardiovascular, renal, neurologic and retinal complications. Diabetes clustered with hypertension and nephropathy has become the leading cause of end-stage renal disease globally. This study describes diabetes, hypertension and nephropathy with reference to glycemic control, dyslipidemia and endothelial dysfunction indicating the foremost basis of morbidity and mortality world wide and rapidly progressing in Pakistan. Study subjects selected and divided in four groups (60 each) followed by institutional ethical approval and informed consent. Group 1: non-diabetic, normotensive control subjects; Group 2: diabetic, normotensive patients; Group 3: diabetic, hypertensive patients and Group 4: diabetic, hypertensive patients with nephropathy. Their fasting blood samples analyzed for the estimations of blood glucose, HbA1c, serum triglyceride, cholesterol, LDL-cholesterol, HDL-cholesterol, urea, creatinine, nitric oxide and sialic acid levels. Results showed that all the groups showed significant rise in fasting blood glucose. Similarly HbA1c levels were also significantly high in all the patients as compared to controls. Group 2 showed significantly high serum cholesterol and LDL levels and low HDL levels. Group 3 and 4 showed significantly high serum triglyceride, cholesterol and LDL levels where as low HDL levels as compared to controls. Group 3 showed significantly high serum creatinine. Group 4 showed a significantly high serum urea and creatinine as compared to controls. Persistent albuminuria was characteristic in Group 4 patients. Significantly low production of serum nitric oxide with high concentration of serum sialic acid was observed in Group 3 and 4 as compared to controls. Results indicate a clear relationship of declining renal function with poor glycemic control, abnormal lipid metabolism, endothelial dysfunction and initiation of acute phase response in tissues affected from the microvascular complications of diabetes like hypertension and nephropathy. It must be taken into account while screening diabetic patients to get them rid of progressive renal impairment leading to end stage renal disease.

Keywords: Diabetes, hypertension, nephropathy, hyperglycemia, dyslipidemia, endothelial dysfunction.

INTRODUCTION

Diabetes mellitus is a metabolic syndrome characterized by collection of disorders of which hyperglycemia is the hallmark (Hasan *et al.,* 2004). According to an estimate there are 15% Pakistanis with diagnosed diabetes and million more who remain unaware that they have the disease. This number is expected to increase to 49 % by the year 2050 (Shera *et al.,* 2004).

Diabetes mellitus is a complex metabolic disease associated with a large variety of complications. Principle diabetic complications were found to be cardiovascular diseases, ophthalmic diseases, nephropathy, esthetic diseases, plant nerve diseases, cerebral blood vessel diseases, limbs blood vessel diseases and athletic nerve diseases (Shi *et al.,* 2004). This presents a serious challenge to the health care system because people with diabetes have an increased mortality and a reduced life expectancy compared with those without diabetes (Morgan *et al.,* 2000). A study conducted by World Health Organization (WHO) indicated that the estimated world wide burden of diabetes will be more than 300 millions by the years 2025 if the present trend continues. This burden is more in developing countries as 85% of diabetic patients live there and suffers from one or more complication of the disease by age 55 years (WHO, 2004).

The development and progression of chronic complications of diabetes are known to be related to certain factors such as glycemic control, increased age, longer duration of diabetes, less physical activity, history of smoking, hypertensio and obesity (Williamson *et al.,* 2000; Khuwaja *et al.,* 2004; MRFITR, 1993; Wannamettee *et al.,* 2000).

Hypertension is an extremely common comorbidity of diabetes affecting 40-60% of people with diabetes. Hypertension is also a major risk factor for cardiovascular events, such as myocardial infarction and stroke as well as for microvascular complications such as retinopathy and nephropathy (Carlos *et al.,* 2002). Hypertension frequently coexists with diabetes mellitus, occurring twice as frequently in diabetic as in non-diabetic persons (El Atat *et al.,* 2004). Hypertension is the major risk factor for the development of cardiovascular disease and for progressive renal insufficiency in diabetes mellitus (Braam *et al.,* 2004; Jeremy, 2003).

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The primacy of renal structural disease in the pathogenesis of hypertension in diabetes mellitus demonstrates a close relationship between microalbuminuria and blood pressure elevation (Tarn and Drury, 1994). Microalbuminuria is associated with structural lesions of kidney, including increased basement membrane thickness and arteriolar and glomerular accumulation if extracellular matrix. Blood pressure is higher in diabetic patients with albuminuria than in those with out it, and blood pressure increases as urinary albumin excretion rises. Microalbuminuria precedes a rise in blood pressure, and hypertension is no more prevalent in diabetic patients without albuminuria than in non diabetic population (Bakris *et al.,* 2000). About 30-40% of diabetic patients develop overt diabetic nephropathy which additionally impairs lipid metabolism. Lipid metabolism in diabetes may also be altered when renal replacement therapy is instituted (Thomas *et al.,* 2006). The endothelial dysfunction associated with diabetes has been attributed to a lack of bioavailable nitric oxide (NO) (James *et al.,* 2004). The serum sialic acid (N-acetyl neuraminic acid) concentration is a marker of the acute phase response, since many of the acute phase proteins (e.g. al-acid glycoprotein, fibrinogen and haptoglobin) are glycoproteins with sialic acid as the terminal sugar of the oligosaccharide chain (Crook *et al.,* 2001). Circulating serum sialic acid, an inflammatory marker has recently been shown to be a strong predictor of cardiovascular mortality (Sriharan *et al.,* 2002).

By keeping in mind the above mentioned facts, present study was designed to cluster the investigations regarding coexistence of diabetes mellitus, hypertension and nephropathy and the association of glycemic control, dyslipidemia and endothelial dysfunction in the progression of disorders.

SUBJECTS AND METHODS

Study Population

Already registered patients with type 1 and type 2 diabetes mellitus of either sex admitted in diabetic wards or visiting out patient departments of various hospitals and medical centers in Karachi were selected. The aim and procedures were explained to patients and/or attendant and informed consent was obtained. The mean age of patients was 48.48±11.52 (mean±SEM) years. Their diabetes age was more than five years. The diagnosis of diabetes was made according to the World Health Organization's (WHO) criteria. The study protocol was approved by the regulations of institutional ethical committee for the use of human subjects in research. The patients were divided into four groups as follows. Each group contained 60 subjects:

Group 1: Normal healthy individuals with no known history of hyperglycemia, hypertension or renal insufficiency as controls.
Group 2: Diabetes mellitus patients with no known history of hypertension and renal insufficiency.
Group 3: Diabetes mellitus patients with known history of hypertension or taking any antihypertensive drug without any known history of renal isufficiency.
Group 4: Diabetes mellitus patients with known history of hypertension or taking any antihypertensive drug with persistent albuminuria.

Sample Collection

The fasting blood samples of patients and control subjects were collected following aseptic techniques after the patients have been taken no drugs for the last 12 hours or more. An aliquot was taken separately in order to get serum. An informed consent was obtained for analysis of blood samples. Blood samples were processed the same day for estimations, in accordance with the ethical guidance and regulation of institution and with generally accepted guidelines governing such work.

Analytical Methods

The fasting blood glucose, serum triglyceride, cholesterol, LDL, HDL, urea, and creatinine were estimated by routine enzymatic colorimetric methods. The HbA1c levels were estimated by fast ion exchange resin separation method. Serum nitric oxide was measure by means of its metabolites nitrite and nitrate by spectrophotomerty (Smarason *et al.,* 1997) and serum sialic acid was estimated by Ehrlich's method (Crook, 1993).

Statistical Analyses

Results are presented as mean±SEM. Statistical significance and difference from control and test values evaluated by Student's *t*-test. The parametric one-way analysis of variance (ANOVA) was used to compare means of a quantitative variable between two or more groups when equal variances were assumed. p-values of <0.05 were considered significant. All statistical analyses were done by using statistical package for social sciences (SPSS) version 14.0 for Windows (Chicago, IL. USA).

RESULTS

The results are compiled in tables 1-3. In group 2 (diabetic patients) fasting blood glucose, HbA1c, serum cholesterol and LDL-cholesterol were found significantly high ($p<0.01$) where as HDL-cholesterol was significantly low ($p<0.01$) as compared to group 1 (controls). No significant change was observed in case of serum triglyceride, urea, creatinine, nitric oxide and sialic acid levels (table 1).

Table 1: Comparison of glycemic status, dyslipidemia and endothelial dysfunction in groups 1 and 2

Parameters	Group 1 (Controls)	Group 2 (Diabetic patients)
Fasting Blood Glucose (mmol/L)	5.38±0.45	9.1±4.0*
HbA1c (%)	4.54±1.29	7.41±2.14*
Serum Triglyceride (mmol/L)	0.97±0.18	1.02±0.23
Serum Cholesterol (mmol/L)	4.84±0.57	6.74±0.88*
LDL-Cholesterol(mmol/L)	2.57±0.43	2.92±0.46*
HDL-Cholesterol(mmol/L)	1.35±0.19	1.06±0.12*
Serum Urea (mmol/L)	10.78±2.46	9.62±2.01
Serum Creatinine (μmol/L)	111.82±45.97	116.52±32.59
Serum NO_2/NO_3 (μmol/L)	18.13±2.65	17.5±2.9
Serum Sialic acid (mmol/L)	1.7±0.35	1.98±0.25

n=60, Values are mean±SEM, *p<0.05 as compared to controls

Group 3 (Diabetic hypertensive patients) showed significantly high (p<0.01) levels of fasting blood glucose, HbA1c, serum triglyceride, cholesterol, LDL-cholesterol, creatinine and sialic acid levels where as HDL-cholesterol and serum nitric oxide levels were significantly low (p<0.01) as compared to controls. No significant difference was found in the levels of serum urea (table 2).

Table 2: Comparison of glycemic status, dyslipidemia and endothelial dysfunction in groups 1 and 3

Parameters	(Group 1) Controls	Group 3 (Diabetic hypertensive patients)
Fasting Blood Glucose (mmol/L)	5.38±0.45	9.46±2.26*
HbA1c (%)	4.54±1.29	8.15±1.65*
Triglyceride (mmol/L)	0.97±0.18	1.38±0.24*
Serum Cholesterol (mmol/L)	4.84±0.57	6.82±0.87*
LDL-Cholesterol (mmol/L)	2.57±0.43	3.22±0.49*
HDL-Cholesterol (mmol/L)	1.35±0.19	0.99±0.13*
Serum Urea (mmol/L)	10.78±2.46	11.24±2.5
Serum Creatinine (μmol/L)	111.82±45.97	155.08±43.8*
Serum NO_2/NO_3 (μmol/L)	18.13±2.65	13.01±2.49*
Serum Sialic acid (mmol/L)	1.7±0.35	2.1±0.37*

n=60, Values are mean±SEM, *p<0.05 as compared to controls

The diabetic nephropathy patients (Group 4) showed significantly high levels (p<0.01) of fasting blood glucose, HbA1c, serum triglyceride, cholesterol, LDL-cholesterol, urea, creatinine and sialic acid where as significantly low levels (p<0.01) HDL-cholesterol as well as serum nitric oxide as compared to controls (table 3).

Table 3: Comparison of glycemic status, dyslipidemia and endothelial dysfunction in groups 1 and 4

Parameters	Group 1 (Controls)	Group 4 (Diabetic nephropathy patients)
Fasting Blood Glucose (mmol/L)	5.38±0.45	12.15±3.54*
HbA1c (mmol/L)	4.54±1.29	9.24±1.84*
Triglyceride (mmol/L)	0.97±0.18	1.42±0.22*
Serum Cholesterol (mmol/L)	4.84±0.57	7.64±2.45*
LDL-Cholesterol (mmol/L)	2.57±0.43	3.4±0.41*
HDL-Cholesterol (mmol/L)	1.35±0.19	0.88±0.14*
Serum Urea (mmol/L)	10.78±2.46	19.36±4.58*
Serum Creatinine (μmol/L)	111.82±45.97	198.44±26.41*
Serum NO_2/NO_3 (μmol/L)	18.13±2.65	10.99±2.09*
Serum Sialic acid (mmol/L)	1.7±0.35	2.2±0.38*

n=60, Values are mean±SEM, *p<0.05 as compared to controls

The parametric one-way ANOVA revealed that except fasting blood glucose, HbA1c and serum urea levels in group 3, all means were significantly different between the groups of patients (table 4).

Table 4: Comparison of means with in groups of patients by ANOVA

Parameters	Group 2 *(p-value)*	Group 3 *(p-value)*	Group 4 *(p-value)*
Fasting Blood Glucose	0.029	0.055	0.022
HbA1c	0.022	0.052	0.007
Triglyceride	0.001	0.005	0.012
Serum Cholesterol	0.009	0.009	0.042
LDL-Cholesterol	0.001	0.018	0.027
HDL-Cholesterol	0.015	0.008	0.009
Serum Urea	0.004	0.068	0.036
Serum Creatinine	0.015	0.001	0.009
Serum NO_2/NO_3	0.042	0.008	0.001
Serum Sialic acid	0.012	0.018	0.014

DISCUSSION

From the results of present study, it is evident that the progression of hypertension and nephropathy in patients of diabetes is strongly related to the degree of hyperglycemia. This progressive change is clearly indicated in all the groups (tables 1-3). The fasting blood sugar levels for current status of glycemic control where as HbA1c was used to represent the glycemic history of previous 2-3 months (the average erythrocyte life span) and the rate of its formation is directly proportional to the ambient glucose concentration. This study showed poor glycemic control in diabetic (table 1), diabetic hypertensive (table 2) and diabetic nephropathy (table 3) patients as compared to controls. The present study found this reality as in diabetic hypertensive patients the mean HbA1c levels were observed >8% and in diabetic nephropathy patients the mean HbA1c levels were found to be >9% (tables 2-3). It is also correlated with poor blood pressure control and hyperlipidemia to accelerate diabetes complications (Bhatt *et al.,* 2002).

A variety of factors contribute to the renal damage seen in diabetes. By definition, hyperglycemia is a common etiologic factor in diabetic patients with nephropathy, but most significant is the accumulation of hypertension with hyperglycemia before and after the onset of microalbuminuria (Evans and Capell, 2000). It is well established that poor metabolic control is critical in the etiology of diabetic nephropathy. Nephropathy is uncommon in patients with HbA1c consistently <7-8% (Deferrari *et al.,* 1998). The degree of which glucose toxicity itself is directly causative in the renal lesion is still debated. At the very least, glucose is a meaningful and clinically relevant marker for the metabolic abnormality that leads to nephropathy, as shown in this study that there are fasting glucose levels are involved in the formation of AGEs leading to progression of diabetic microvascular complications. Diabetic patients with ESRD had twice as much tissue AGE as patients without diabetes, and the levels correlated directly with creatinine.

Hemoglobin is one of many proteins that undergo non enzymatic glycation, and glycosylated hemoglobin is a general term for hemoglobin non-enzymatically glycosylated with glucose. Potential glycation sites of the hemoglobin-A molecule include the N-terminal amino acid valine of the four polypeptide chains and all of the free amino groups of lysine residues. The predominant glycation site is the N-terminal valine residue of β-chain, which accounts for ~60% of bound glucose (Nuttall, 1998). HbA1c represents the most prevalent glycated species. Because erythrocytes are freely permeable to glucose, the rate of formation of HbA1c is directly proportional to the ambient glucose concentration in which the erythrocyte circulates and to the duration of exposure (Krishnamurti and Steffes, 2001). The mechanisms operating below the threshold HbA1c value of less than 10% (which corresponds to prevailing blood glucose concentration of less than 200 mg/dL or 11 mmol/L seem to be influenced by other components of the diabetes mellitus for example, abnormalities in plasma insulin concentrations.

Our findings have implications that the rate of decline of renal function bears a clear relationship with prolonged hyperglycemia as the progression of microalbuminuria to overt proteinuria.

In addition to hypertension, glycemic control and genetic influence, diabetic dyslipidemia seems to play an important role in the pathogenesis and progression of vascular disease in the diabetic patient and it is under discussion if it plays a role in the evolution of diabetic nephropathy. In present study the higher levels of triglyceride and triglyceride-rich lipoproteins with cholesterol in diabetic, diabetic hypertensive and diabetic nephropathy patients were observed. This atherogenic profile becomes more apparent when diabetic nephropathy is present (Zimmermann *et al.,* 1999) indicating the associated factors in the process of atherogenesis. Atherosclerosis is the main cause of mortality in diabetic patients and, therefore, a better understanding of lipid abnormalities and their pathophysiology in diabetes is a prerequisite for successful prevention of cardiovascular diseases (Colhoun *et al.,* 2001). The degree of glycemic control is an important determinant of serum lipoprotein concentrations in diabetes mellitus. Cholesterol concentrations fall by 0.1 mmol (2.2 %) and triglycerides by 0.08 mmol (8%) for each percentage-point fall of glycohaemoglobin. Intensive insulin treatment improves even the normal concentration of serum lipoproteins. Conventional insulin therapy results in peripheral hyperinsulinemia whereas insulin concentration is less than normal in portal circulation.

In the present study renal impairment was measured to estimate the renal deterioration in various stages of diabetic patients progressing towards ESRD. Serum urea levels were found to be high in diabetic nephropathy patients (table 4) during the present study. That shows that serum urea alone is not a significant marker to evaluate the renal damage in diabetic kidney patients. It is also suggested in previous study that the laboratory marker that has long served as the mainstay for detecting impaired kidney function is serum creatinine (Star *et al.,* 2002). That is indicated in present study as serum creatinine levels were found to be high in diabetic hypertensive as well as diabetic nephropathy patients (tables 2-3). Increased UAER is widely accepted as the first clinical sign of diabetic nephropathy. However it is possible that some diabetic patients could first manifest reduced GFR with hypertension (Caramori *et al.,* 2003).

The RAAS has been implicated in the pathogenesis of diabetic renal disease, based mainly upon the ability of angiotensin converting enzyme inhibitors (ACEI) and formation of angiotensin II. This is evidenced by the increase in osmolality due to the abnormal activity of aldosterone cascade (Carey and Siragy, 2003). Deletion of ACE causes a striking reduction of blood pressure, serum electrolyte abnormalities and renal pathology, indicating the many crucial roles of the RAAS. The beneficial effects of ACEI or angiotensin receptor blockade in the prevention of diabetic renal disease suggest that angiotensin II is a major mediator of progressive renal injury (Lewis, 2002). However, measurement of the activity of the circulating components of the RAAS has largely indicated suppression in diabetic complications.

The last twenty (20) years have brought about a lucid realization that the vascular endothelium is not a mere barrier between intravascular and interstitial compartments. In fact, the vascular endothelium has received the status of an organ, albeit a widely spread one, which is responsible for the regulation, hemodynamic, angiogenic vascular remodeling and metabolic, synthetic, inflammatory, antithrombogenic, and prothrombogenic processes. As any other organ, the vascular endothelium is a subject for dysregulation, dysfunction, insufficiency and failure in diabetic nephropathy (Goligorsky *et al.*, 2001). Diabetes is associated with altered endothelial vascular and inflammatory, acute phase responses. This present study finds support in the observation that diabetes affects basal NO metabolism as a successive and significant decrease was observed in the level of endothelial NO at the onset of diabetic complications such as hypertension and nephropathy. The NO is a paracrine mediator acting as a potent vasodilator in various vascular beds. In the kidney, NO controls both afferent and efferent vascular tone, the ultrafiltration coefficient and medullary blood flow (Komers *et al.*, 2000). NO is synthesized as a by product of conversion of its physiological precursor L-arginine to L-citrulline. This reaction is catalyzed by a family of enzymes known as NO synthases (NOS). The decrease production of NO during diabetic complications supposed to be the consequence of reduced production of NO by NOS and inactivation of NO by reactive oxygen species produced either by glycosylated proteins or directly from vascular endothelium as higher levels of HbA1c was observed in diabetic nephropathy patients during the present study. However, this only incompletely explains reduced relaxant responses of microvessels to agonists such as bradykinin in the presence of HbA1c (Vallejo *et al.*, 2000). Several mechanisms could account for a reduced responsiveness of the diabetic renal vasculature to NO-dependent vasodilation: 1) inactivation of NO and/or 2) a reduced sensitivity of the vascular smooth muscles cells (VSMC) to NO, 3) diminished autoregulatory adjustment in renal vasculature resistance, 4) baroreflex-mediated alterations in renal sympathetic nerve activity, and 5) increased production of NO antagonists such as endothelin 1, and quenching of NO by AGEs during micro and macrovascular complications (Pflueger *et al.*, 1999). These effects were observed during hypertension in normoglycemic patients in present study due to variety of factors involved in impairment of NO metabolism (table 2).

An increasing trend of serum sialic acid in diabetic patients with the progression of complications such as nephropathy was indicated in this study. Serum sialic acid is a marker of acute phase response. Acute phase glycoproteins with sialic acid as a component of the oligosaccharide side chain being produced by liver, stimulated by proinflammatory cytokines (Shahid and Tabassum, 2006). Diabetic vascular complications tend to cause tissue injury that results in stimulation of local cytokine secretion from cells involved in the complications such as endothelium and macrophages and this induces an acute phase response. The diabetic process stimulates cytokine production from cells throughout the body, and these cytokines play a direct role in the causation of vascular complication. The latter is supported by evidence that proinflammatory cytokines cause endothelial dysfunction by increasing capillary permeability, inducing prothrombotic properties and promoting leukocyte recruitment by synthesis of adhesion molecules and chemoattractants (Mantovani and Bussolino, 1997).

CONCLUSION

The findings of this study have implicated that the rate of decline of renal function bears a clear relationship with hypertension, poor glycemic control, abnormal lipid metabolism, endothelial dysfunction and production of acute phase response in tissues affected from the microvascular complications of diabetes for example hypertension and nephropathy. Various lipid fractions are strongly associated with progression of diabetic kidney disease, but the relationship is not very much same at all stages. This finding has implications for the design of renoprotective strategies and the interpretation of clinical trials in patients with progressive diabetic micro and/or macrovascular complications.

It is also concluded that endothelial dysfunction and acute phase response are the major indicators for micro and macrovascular complications of diabetes mellitus such as hypertension and nephropathy. These should be taken into account during screening procedures regarding identifications of the diabetic patients to get them rid of progressive renal impairment to end stage renal failure. These slightly neglected abnormalities should be taken into account during screening procedures regarding indentification of diabetic patients prevent them and to

delay the progressive renal impairment leading to end stage renal disease.

REFERENCES

Bakris GL, Williams M, Dworkin L, Elliott WJ, Epstein M, Toto R, Tuttle K, Douglas J, Hsueh W and Sowers J (2000). Preserving renal function in adults with hypertension and diabetes: A consensus approach: National Kidney Foundation Hypertension and diabetes Executive Committee Working Group. *Am. J. Kidney Dis.*, **36**: 646-661.

Bhatt DL, Marso SP, Hirsch AT, Ringleb PA, Hacke W and Topol EH (2002). Amplified benefit of clopidogrel versus aspirin in patients with diabetes mellitus. *Am. J. Cardiol.*, **90**: 625-628.

Braam B, de Koning EJ and Mees EJ (2004). Diabetic nephropathy: the role of blood pressure and extracellular volume in its pathogenesis and treatment. *Ned. Tijdschr. Geneeskd.*, **148**(5): 212-217.

Caramori ML, Fioretto P and Mauer M (2003). Low glomerular filtration rate in normoalbuminuric type 1 diabetic patients. *Diabetes*, **52**: 1036-1040.

Carey RM and Siragy HM (2003). The intrarenal renin angiotensin system and diabetic nephropathy. *Trends in Endcrionol. Metab.*, **14**: 274-280.

Carlos AP, Parrot MA and Raskin P (2002). The treatment of hypertension in adult patients with diabetes. *Diabetes Care*, **25**: 134-147.

Colhoun HM, Lee ET, Bennet PH, Lu M, Keen H, Wang SL, Stevens LK and Fuller JH (2001). Risk factors for renal failure: the WHO multinational study of vascular disease in diabetes. *Diabetologia.*, **44**: S46-S53.

Crook M (1993). The determination of plasma Sialic acid. *Clin. Biochem.*, **26**: 31-37.

Crook MA, Pickup JC, Lumb PJ, Georgino F, Webb DJ and Fuller JH (2001). Relationship between Plasma sialic acid concentration and microvascular and macrovascular complications in type 1 diabetes. *Diabetes Care*, **24**: 316-322.

Deferrari G, Repetto M, Calvi C, Ciabattoni M, Rossi C and Robaudo C (1998). Diabetic nephropathy: from micro- to macroalbuminuria. *Nephrol. Dial. Transplant.*, **13**(Suppl 8): 11-15.

El Atat F, McFarlane SI and Sower JR (2004). Diabetes, hypertension and cardiovascular derangements: pathophysiology and management. *Curr. Hypertens. Rep.*, **6**(3): 215-223.

Evans TC and Capell P (2000). Diabetic nephropathy. *Clin. Diab.*, **18**: 1-15.

Goligorsky MS, Chen J and Brodsky S (2001). Endothelial cell dysfunction leading to diabetic nephropathy: Focus on nitric oxide. *Hypertension*, **37**: 744-748.

Hasan ZU, Zia S and Maracy M (2004). Baseline disease knowledge assessment in Patients with type 2 diabetes in a rural area of Northwestern of Pakistan. *J. Pak. Med. Assoc.*, **54**(2): 67-73.

James PE, Lang D, Tufnell-Barret T, Milsom AB and Frenneaux MP (2004). Vasorelaxation by red blood cells and impairment in diabetes. *Circ. Res.*, **94**: 976.

Jeremy WT (2003). Treating hypertension in diabetic nephropathy. *Diabetes Care*, **26**(6): 1802-1805.

Khuwaja AK, Rafique G, White F and Azam SI (2004). Macrovascular complications and their associated factors among persons with type 2 diabetes in Karachi, Pakistan – A multi center Study. *J. Pak. Med. Assoc.*, **54**: 60-66.

Komers R, Lindsley JN, Oyama TT, Allison KM and Anderson S (2000). Role of neuronal nitric oxide synthase (NOS1) in the pathogenesis of renal hemodynamic changes in diabetes. *Am. J. Physiol. Renal Physiol.*, **279**: F573-F583.

Krishnamurti U and Steffes MW (2001). Glycohemoglobin: A primary predictor of the development or reversal of complications of diabetes mellitus. *Clin. Chem.*, **47**: 1157-1165.

Lewis EJ (2002). The role of angiotensin II receptor blockers in preventing the progression of renal disease in patients with type 2 diabetes. *Am. J. Hypertens.*, **15**: 123S-128S.

Mantovani A and Bussolino F (1997). Cytokine regulation of endothelial cell function: from molecular level to the bedside. *Immunol. Today*, **18**: 231-240.

Morgan CL, Currie CJ and Peters JR (2000). Relationship between diabetes and mortality: A population study using record linkage. *Diabetes Care*, **23**: 1103-1107.

Multiple Risk Factor Intervention Trial Research Group (MRFITR) (1993). Diabetes, other risk factors and 12 year cardiovascular mortality for men screened in the multiple risk factor intervention trial. *Diabetes Care*, **16**: 434-444.

Nuttall FQ (1998). Comparison of percent total GHb with percent HbA1c in people with and without known diabetes. *Diabetes Care*, **21**: 1475-1480.

Pflueger AC, Larson TS, Hagl S and Knox FG (1999). Role of nitric oxide in intrarenal hemodynamics in experimental diabetes mellitus in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **277**: R725-R733.

Shahid SM and Tabassum M (2006). Correlation between frequent risk factors or diabetic nephropathy and serum sialic acid. *Asian J. Biochem.*, **1**(3): 244-250.

Shera AS, Jawad F, Maqsood SJ, Azhar M and Ahmed U (2004). Prevalence of chronic complications and associated factors in type 2 diabetes. *J. Pak. Med. Assoc.*, **54**: 54-59.

Shi WX, Li XY and Li J (2004). The morbidity of chronic diabetic complication with logistic regression analysis of related potential risk factors. *Zhonghua Liu Xing Bing Xue Za Zhi*, **25**(1): 60-64.

Smarason AK, Allman KG, Young D and Redman CW (1997). Elevated levels of serum nitrate, a stable end

product of nitric oxide in women with preeclampsia. *Br. J. Obstet. Gynaecol.*, **104**: 538-543.

Sriharan M, Reichelt AJ, Opperman MR, Duncan BB, Mengue SS, Crook MA and Schmidt MI (2002). Total sialic acid and associated elements of the metabolic syndrome in women with and without previous gestational diabetes. *Diabetes Care*, **25**: 1331-1335.

Star R, Hostetter T and Hortin GL (2002). New markers for kidney disease. *Clin. Chem.*, **48**: 1375-1376.

Tarn AC and Drury PL (1994). Blood pressure in children, adolescents and young adults with type 1 diabetes. *Diabetologia*, **29**: 275-281.

Thomas MC, Rosengard-Barlund M, Mills V, Bonnback M, Thomas S, Forsblom C, Cooper ME, Takinen MR, Viberti G and Groop PH (2006). Serum lipids and the progression of nephropathy in type 1 diabetes. *Diabetes Care*, **29**(2): 317-322.

Vallejo S, Angulo J, Peiro C, Nevado J, Sanchez-Ferrer A, Petidier R, Sanchez-Ferrer CF and Rodriguez-Manas L (2000). Highly glycated oxyhemoglobin impairs nitric oxide relaxations in human mesenteric microvessels. *Diabetologia*, **43**: 83-90.

Wannamettee SG, Shaper AG and Alberti KG (2000). Physical activity, metabolic factors and the incidence of coronary heart diseases and type 2 diabetes. *Arch. Intern. Med.*, **160**: 2108-2116.

Williamson DE, Thompson TJ, Thun M (2000). Intentional weight loss and mortality among overweight individuals with diabetes. *Diabetes Care*, **23**: 1499-1504.

World Health Organization (2004). The Diabetes Program, available at http://www.who.int/diabetes/en/. September 21, 2004.

Yokoyama H, Jensen JS, Myrup B, Mathiesen E R, Ronn B and Decken T (1996). Raised serum sialic acid concentration precedes onset of microalbuminuria in IDDM. *Diabetes Care*, **19**: 435-440.

Zimmermann J, Wanner C and Quaschning T (1999). Lipid management in type 2 diabetes with nephropathy. In TRitz E and Rychlík I: Nephropoathy in type 2 Diabetes. Oxford Clinical Nephrology Series. Oxford University Press, Oxford, pp.137-157.