INTERVENTION TRIAL OF MICROALBUMINURIA IN T2 DM WITH ANTIOXIDANT, ANTITHROMBOTIC AND ACE INHIBITOR AGENTS

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Abstract

The present study was undertaken in T2DM patients aged between 40 and 50 years, from outpatient department (OPD) of BIRDEM Hospital, Shahabagh, Dhaka.

A total of 1605 T2DM patients examined, 390 were found microalbominuric (ì-albumin >30mg/g) giving a prevalence rate were 22.92%. and nonmicroalbuminuric (ì-albumin d"30 mg/g). The microalbuminuric subjects were put on intensive intervention with ACE inhibitor (Captopril 25 mg, twice daily), Antioxidant (Vitamin C- 200 mg, Vitamin E- 50 mg and Vitamin A- 6 mg daily), and Antithrombotic (Aspirin- 75 mg daily). Both microalbuminuric and nonmicroalbuminuric subjects were put on strict diet control and antidiabetic therapy. The following tests FBG, HbA1c, BP, T-Cholesterol, HDLc, ì-albumin, ACR, were done in both groups at 3 months intervals up to 18th months.

The mean (years) ±SD age were 45.67 ± 3.98, and 45.45 ±3.94 and duration of diabetes 3.78 ± 3.14 and 3.72±3.07 in microalbuminuric and nonmicroalbuminuric subjects respectively. The microalbuninuric group showed satistically significant difference (p<0.005) in the following parameters FBG, HbA1c, SBP, T-choles, ACR, U.Creatinine in comparison to nonmicroalbuminuric group.

Nonmicroalbuminuric subjects showed the deterioration in TG, HDLc, and a conversion rate of 21.7% from nonmicro-albuminuric to microalbuminuric.

The present intervention with ACE inhibitor, antioxidant and antithrombotic improved glycaemic control, lipid profile and renal functions by 68% in microalbuminuric T2DM subjects.

Introduction:

Diabetes is one of the chronic diseases. Economic development has resulted in urbanization, industrialization and thereby improvement in quality of life with advancement of nutrition and sanitation. These food choice have improve in over nutrition and over eating resulting in diabetes mellitus increasing rapidly in developed and developing world.[1] In South- East Asia and the Western Pacific the incidence of DM, particularly T2DM is rapidly escalating[2]. Diabetes, hyperinsulinemia and coronary risk factors are more prevalent in Bangladeshis compared with other South-Asian migrants (India, Pakistan) settled in United Kingdom[3] and with native population[4]. Epidemiological studies in different population unequivocally showed that other than genetic predisposition, increasing age, obesity and sedentary habits are the most important risk factors for T2DM[5,6] The early detection of diabetes mellitus and monitoring of glycemic control is the key to diabetes care and always desirable for prevention of diabetic complications and thereby reducing burden of healthcare cost of citizen, especially of Bangladesh a developing country.It has been shown that maintenance of normal blood glucose unequivocally reduced mortality from acute complications.[7] In Bangladesh majority of diabetes mellitus are T2DM. The over all prevalence of T2DM was found to be 8.1%, and the sex wise prevalence for men and women were 7.7% and 8.5% respectively[8.] Most of the T2DM patients develop macro and micro vascular complications with time. The micro vascular lesion encompasses retinopathy, nephropathy and neuropathy. The macrovascular complications are related to atherosclerosis–includes mainly coronary artery disease (CAD), peripheral vascular disease (PVD) and cerebro vascular disease (CVD or stroke)[9]. Two most world- famous prospective studies– Diabetes Complications Control Trial (DCCT)[10] and United Kingdom Prospective Diabetes Study (UKPDS)[11] concluded that strict monitoring and maintenance of normal blood glucose certainly prevents micro angiopathy or macro vascular complications. Micro albuminuria is a marker for diabetic CAD[12,13.] Increased level of cholesterol, blood pressure and HbA1c were the main factors associated with the decrease in renal function given rise to microalbuminuria[14.] Mogensen[15] showed that mortality rate 14% higher in patients with raised albumin concentration than in the normal controls in their 9 years longitudinal study. Microalbumin is a term used to indicate that there is a small increases in the excretion of serum albumin in the urine (<30 mg/g)[16.] The earliest clinical evidence of nephropathy is the appearance of low but abnormal level of albumin (>30mg/g of urine) in the urine, reffered to as microalbuminuria and patients with microalbuminuria are reffered to as incipient nephropathy[17.] Values above 300 mg/g of urine are considered to represent overt nephropathy[18,19.] The pathogenesis of diabetes nephropathy is and involve direct effects of high intracellular glucose on glomerular, tubular, vascular and interstitial cell function. The Diabetic Control and Complication Trial (DCCT) established hyperglycemia as a critical determinant of progressive nephropathy[20]. Althrough there are a variety of postulated pathways by which high extracellular glucose levels mediate these effects, including activation of protein kinase C

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(PKC) isoforms, stimulation of the polyol pathway, formation of advanced glycosylation end products, the presence of an intact renin-angiotensin system (RAS) progressively augments proteinuria and accelerates decline renal function[21,22] In T2DM patients, diabetic micro angiopathy produces widespread anatomical and functional small vessels impairment which particularly affects renal glumeruli functions. Measures to prevent progression of overt nephropathy in T2DM[23] i. Achieve glycemic control ii) Maintain blood pressure iii) Reduced the level of proteinuria. The positive screening test should be confirmed by a measurement of albuminuria of the albumin-to- creatine ratio in spot morning urine samples[24.] Angiotensin converting enzyme (ACE) inhibitor is an ecto-enzyme and a Glycoprotein. The ACE inhibitor is a drugs, which block the formation or activation of angiotensin II from angiotensin I in the lung by an enzyme called converting enzyme.[25] Angiotensin II also increased lipid per oxidation and oxyradical formation and stimulates the expression of proimflamatoy genes, such as chemoattractant protein and leucocyte adhesion molecules, resulting in endothelial dysfunction[26.] ACE inhibitors protect the kidney better than other blood pressure lowering agents, due to ACE inhibitor specifically lower the intrarenal pressure[27.] Inuki[28] They showed that the ACE inhibitor possessed scavenging effect of superoxide in leukocytes in T2DM patients and that those scavenging effects were positive to suppress the progression of diabetic nephropathy in diabetic patients. The study performed by Hirsch[29] in 1998, showed that insufficient levels of vitamin C may contribute the development of heart diseases in diabetes who suffer from nephropathy. Patients with nephropathy show lower serum levels of vitamin C due to increased renal clearance of vitamin C. This reduction may allow more oxidant damage to occur to the vascular system and kidneys thus perpetrating the cycle[30.] Antioxidant such as ascorbic acid, vitamin E are all decreased in diabetes. Vitamin E, a fat – soluble vitamin that may prevent the glomerular dysfunction and helps prevents damage to lipids by oxygen free radicals. When highly reactive species attack lipids within membranes or lipoproteins, they set off the chain reaction of lipid peroxidation. Vitamin E halts this chain reaction i.e. it acts as a chain breaking inhibitors of lipid per oxidation[31.]With the existing relationship between microalbuminuria and nephropathy how angiopathy (macro-microvascular) can be reverted back is the current question. Therefore we studied to measure the effective management of nephropathy with ACE inhibitor, antithrombotic and antioxidant to established a good relief of nephropathy.

Methodology:

From outpatient department (OPD) of BIRDEM Hospital, Shahabagh, Dhaka, we consecutively selected 1605 T2DM patients between the age 40 and 50 years. The patients were selected from this age range because vast majority patients of BIRDEM hospital OPD are from these age group. This helped to maintain homogeneity among study subjects. For interventional trial we divided the total 1605 T2DM patients into two groups, namely microalbuminuric (μ-albumin >30mg/g of urine, n=390) and nonmicroalbuminuric (μ-albumin >30 mg/g of urine, n=1215). Microalbumin was tested by Nephielometry Kinetic Method. During study period 22 subjects from microalbuminuric group and 587 subjects from nonmicro-albuminuric group dropped out.The microalbuminuric subjects were put on intensive intervention with ACE inhibitor (Captopril 25 mg, twice daily), Antioxidant (Vitamin C- 200 mg, Vitamin E- 50 mg and Vitamin A- 6 mg daily), and Antithrombotic (Aspirin-75 mg daily). Both microalbuminuric and nonmicroalbumi-nuric subjects were put on diet control and antidiabetic therapy. The following tests FBG, HbA1c, BP, T-Cholesterol, HDLc, ì-albumin, ACR, were done in both groups at 3 months intervals up to follow up visits. The laboratory examinations were done by established methods. In every visit dietary and diabetes control were emphasized.

Data Collection

Selection Criteria:

By estimating microalbuminuria (>30 mg/g of urine) on morning spot urine and identified 368 T2DM patients, eligible for the microalbuminuric group. The first voided morning urine was analyzed for microalbuminuria as, there is little water intake during the night and no physical activity while asleep[29.]

Inclusion Criteria:

Known case of T2DM patients, Age between 40 and 50 years, Microalbuminuria >30 mg/g of urine and ACR changes.

Exclusion Criteria:

Age- between younger than 40 years and older than 50 years, Known diabetic kidney disease patients, Pregnancy and complicated cardiovascular disease. Informed consent was obtained from all participants. The protocol was in accordance with the Helsinki declaration and was approved by the Bangladesh Medical Research Council (BMRC) ethical committee.

Clinical Assays : The examinations were planed at baseline and after 18th months of follow up. Blood pressure were measured in the supine position after 20 min rest with a Hawksley random – zero sphygmomanometer.*Biochemical Assays:* All blood samples were taken at 0800 AM after an overnight fast. Then 5 ml of venous blood sample was collected for biochemical analysis. Fasting blood glucose were

measured by a colorimetric method. HbA1c was measured by Nyco Card HbA1c READER M by Boronate affinity assay method. Serum total cholesterol and serum HDL cholesterol concentration were measured by Enzymatic Colorimetric method. Triglycerides were measured by Colorimetry method. Urinary AER was measured by nephielometry kinetic methods.

End Points:

The primary end points – micro vascular endpoint were microalbuminuria and nephropathy (>30-300 mg/g and > 300 mg/g of urine) in morning spot urine collection.

Statistical Analysis:

A tabulation plan was prepared and necessary programs were developed using statistical software package SPSS/PC+ and EPI Info.Socio-economic, clinical and bio-clinical data were analyzed by using SPSS software package.

Result :

The patients aged between 40 and 50 (45.53±3.96) years, 62.3 percent were literate and duration of diabetes was 3.74 ± 3.09 years indicating both groups are comparable (table1). Table 2 showed that baseline Systolic BP, Diastolic BP, Urinary Creatinine, ACR, Total Cholesterol, HbA1c and Fasting blood glucose level of nonmicroalbuminuric group were not homogenous (significantly different, P<0.000) when compared with the microalbuminuric group. Average age, duration of T2DM, HDL, LDL of nonmicroalbuminuric group were homogeneous (not significantly different, P>0.05) at 5 percent level when compared with the microalbuminuric group. Actually

Table-I: *Socio-demographic characteristics of the subjects*

Average age	
Nonmicroalbuminuric group	45.45 ±3.94
Microalbuminuric group	45.67 ±3.98
Both	45.53 ± 3.96
Education	
Illiterate	37.70%
Literate	62.30%
Microalbuminuric Status	
Nonmicroalbuminuric	10582 ± 8067
Microalbuminuric	11342 ± 9559
Both	10863 ± 8652
Duration of Diabetes T_2 DM (year)	
Nonmicroalbuminuric (¼–albumin d 30) n=628	03.72±3.07
Microalbuminuric (¼ -albumin > 30) n=368	03.78±3.14
Both	03.74 ± 3.09

Table-II: *Comparison of baseline variables between nonmicroalbuminuric (µ- albumin d 30) and microalbuminuric group (µ-albumin > 30)*

Variables	µ– albumin (mg/g)		p-value (z-test for Equality of Means)
	d 30 mg/g (Nonmicroalbuminuric) n= 628	>30 mg/g (Microalbuminuric) n= 368	
Age (year)	45.45±3.94	45.67±3.98	0.40
Duration of T_2 DM (years)	03.72±3.07	03.78±3.14	0.74
Fasting Blood Glucose(mmol/L)	11.55±5.20	13.57±4.36	0.000
Urinary Creatinine (g/24h)	1.06±0.65	02.09±0.62	0.000
ACR (mg/g)	17.17±11.08	57.75±33.47	0.000
Systolic BP (mmHg)	125.49±13.96	147.13±15.89	0.000
Diastolic BP (mmHg)	79.56±8.25	86.33±7.77	0.000
Total Cholesterol (mg/dl)	198.89±53.82	219.93±53.89	0.000
HbA1c (%)	07.18±1.76	07.93±2.04	0.000

hyperglycemia and microalbuminuria are first two companions for incoming complications of T2DM patients. Uncontrolled hyperglycemia and hyperlipidemia enhance the situation. A study by Heeg[32] showed that 61% reducing the proteinuria in T2DM patients may occur by intervention with ACE inhibitor. It was also found that 21.7 percent of nonmicroalbuminuric group exceed their normal level of μ-albumin, whereas 68.8 percent of microalbuminuric group reverted back to normal μ- albumin level (Figure 1) FBG showed more significant fall in microalbuminuric group. Reduction rates, strength of inverse relationship between the rate of change of FBG level by follow up and improvement of normal FBG level of microalbuminuric group were higher than that of nonmicroalbuminuric group (Figure-2).

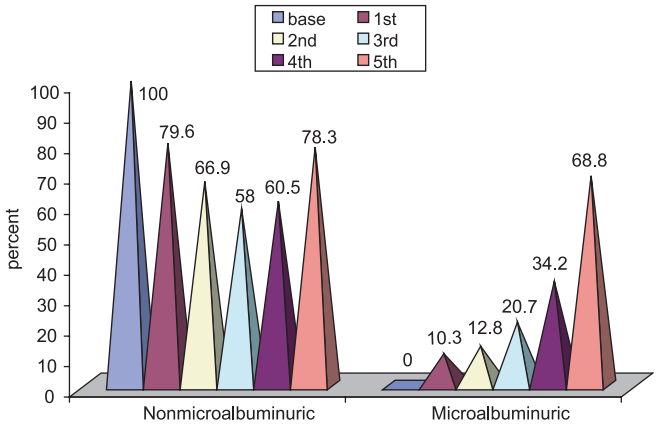

Fig.-1: *Percent of normal μ- Albumin (μ- Albumin ? 30) level*

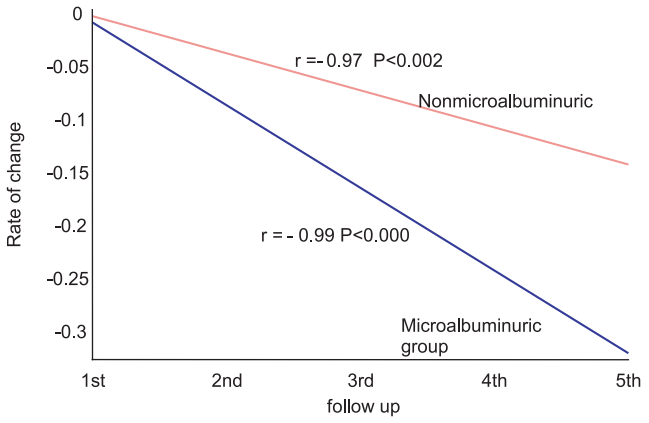

Fig.-2: *Linear trend of rate of change of fasting blood glucose level.*

The net improvement (21%) of HDLc (mg/dl) among microalbuminuric group was higher than 5.3 percent in nonmicroalbuminuric group (Figure 3).

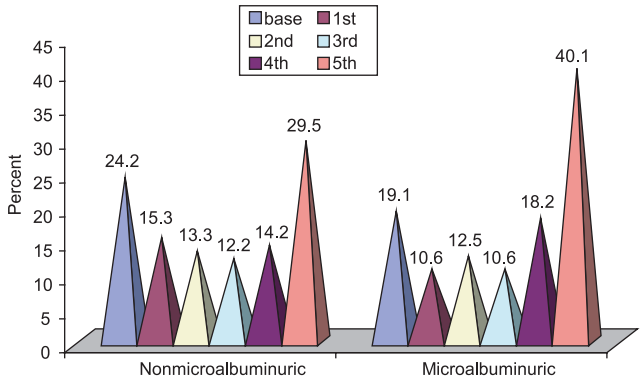

Fig.-3: *Percent distribution of normal level of HDLc after follow up.*

We observed after 5th follow up it was found that average baseline ACR (mg/g of urine) of nonmicroalbuminuric group increased to 92 percent whereas 34 percent decreased among microalbuminuric group (Table 3). During the study of U. Cretinine Showed that rate of change of U. Creatinine of microalbuminuric group gradually decreased across the follow up (Table 4).

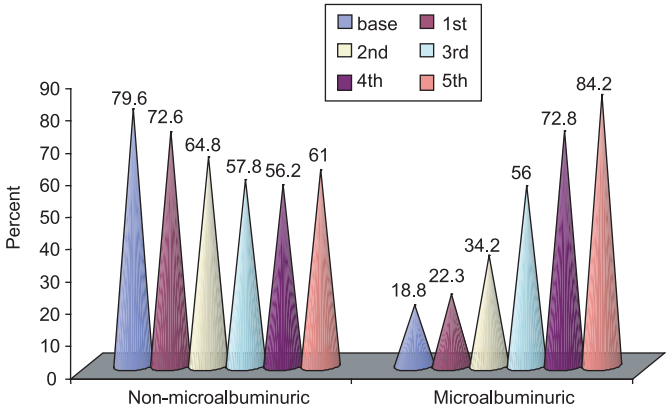

Fig.-4: *Percent of normal U. Creatinine level (1.0-1.5 g/24 h) after follow up.*

In our finding after 5th follow up we observed that average systolic blood pressure (SBP) of nonmicroalbuminuric group was higher than that of baseline whereas average SBP of micro- albuminuric group was found lower than that of baseline. Positive trend between rate of change of SBP from baseline and 5th follow up among nonmicroalbuminuric group was also found.On the other hand inverse trend among microalbuminuric group was found (Table 5). In our study we observed that the baseline total cholesterol level was statistically significant.

Table-III: *Average ACR (mg/g) and its rate of change by follow up*

Source of variation	Follow up	Mean ± SD	Rate of change of ACR (%)
	Base	17.17±11.09	From base line
Nonmicroalbuminuric group (n = 628)	1st	26.51±21.06	54(+)
(µ- Albumin £ 30 mg/g)	2nd	27.92±20.55	63(+)
	3rd	30.37±20.89	71(+)
	4th	33.14±22.99	93(+)
	5th	32.91±22.71	92(+)
	Base	57.76±33.47	From base line
Microalbuminuria group (n = 368)	1st	65.88±39.34	14(+)
(µ- Albumin > 30 mg/g)	2nd	61.58±33.10	7(+)
	3rd	56.78±31.53	2(-)
	4th	48.48±26.68	16(-)
	5th	38.08±21.64	34(-)

Table-IV: *Average U.Creatinine (g/24h) and its rate of change by follow up*

Source of variation	Follow up	Mean ± SD	Rate of change of U. Creatinine (%)
	Base	1.06±.65	From base line
Nonmicroalbuminuric group (n = 628)	1st	1.09±.71	3(+)
(µ- Albumin £ 30 mg/g)	2nd	1.27±.81	20(+)
	3rd	1.42±.79	34(+)
	4th	1.42±.71	34(+)
	5th	1.27±.78	20(+)
	Base	2.09±.63	From base line
Microalbuminuric	1st	1.97±.66	6(-)
group (n = 368)	2nd	1.77±.72	15(-)
(µ- Albumin > 30 mg/g)	3rd	1.40±.61	33(-)
	4th	1.13±.63	46(-)
	5th	0.84±.63	60(-)

Table-V: *Average systolic blood pressure (mmHg) and its rate of change by follow up*

TSource of variation	Follow up	Mean ± SD	Rate of change (per unit) of systolic blood pressure
	Base	125.49±13.96	From base line
Nonmicroalbuminuric group (n = 628)	1st	132.35±15.37	0.05(+)
(µ Albumin £ 30 mg/g)	2nd	133.63±18.67	0.06(+)
	3rd	135.03±19.86	0.08(+)
	4th	135.32±59.82	0.08(+)
	5th	129.02±17.99	0.03(+)
	Base	147.13±15.89	From base line
Microalbuminuric group (n = 368)	1st	141.14±15.82	0.04(-)
(µ Albumin > 30 mg/g)	2nd	141.94±69.84	0.04(-)
	3rd	134.18±12.52	0.09(-)
	4th	129.08±12.00	0.12(-)
	5th	123.26±11.56	0.16(-)

Discussion:

The diabetes mellitus together with its complications is gradually raising world wide. Repeated measure analyses revealed that after follow up the U.Creatinine significant decreased from baseline in microalbuminuric group (Figure 4).

In our study, the prevalence of microalbuminuria was 22.99. The incidence of complications of T2DM with microalbuminuric patients were statistically significant difference between the groups. The comparison of the clinical, biochemical and haemodynamic variables significantly associated with hyperglycemia and hypertension. Both, microalbuminuric and nonmicroalbuminuric groups, showed no between microalbuminuric and nonmicro- albuminuric groups showed not significant difference between the groups by age, duration of diabetes mellitus, TG, HDLc and LDLc (Table 2). FBG level was statistically significant in microalbuminuric group ($p<0.000$) after 5th follow up. The study found that (Figure 2) the rate of change of FBG level and follow up of the microalbuminuric group significantly negative correlation ($r= -0.99$, $p< 0.000$) was observed, whereas the rate of change of FBG level nonmicroalbuminuric group were significantly correlated ($r= -0.97$, $p< 0.002$). The rate of change of ACR of microalbuminuric group gradually decreased across the follow up and reached normal level by 20 percent whereas, rate of change of ACR tends to increase as number of follow up increased in nonmicro-albuminuric group (Table 3).

Positive trend between rate of change of SBP from baseline and 5^{th} follow up among nonmicroalbuminuric group was also found. On the other hand inverse trend among microalbuminuric group was found (Table 5). Mean difference between the two groups were statistically significant ($p < 0.000$).

Conclusion:

By controlling microalbuminuria in patients with T2DM, prevention of the development of macro and micro angiopathy, in the age between 40 and 50 years is Possible. The overall improvement of nephropathy was due to the combined effects of the three interventional agents about 68.8 percent from baseline.

References:

1. King H, Rewers M. Global estimates for prevalence of diabetes mellitus and impaired glucose tolerance in adults. WHO Adhoc Diabetes reporting group. *Diabetes care* 1993; 16:157-177
2. Weir MR. Albuminuria predicting outcome in diabetes: Incidence of microalbuminuria in Asia-Pacific Rim. *Kidney International* 2004; 66: S38-S39.
3. Mckeigue PM, Marmot MG, Syndercombe court YD, Cottier DE, Rahman S, Riemersma RA. Diabetes hyperinsulinemia and coronary risk factors in Bangladeshi in East London. *Br. Heart J* 1988; 60: 390-396
4. McKeigue PM, Shah B, Marmot MG. Relation of central obesity and insulin resistance with high diabetes prevalence and cardiovascular risk in South Asians. *Lancet* 1991; 337: 382-386.
5. World health Report 1998, WHO Genava; Life in the 21st Century- A vision for all.
6. Vaaler S. Optimal glycemic control in type 2 diabetic Patients. *Diabetic Care* 2000; 23: B30-B34.
7. Rahim MA, Vaaler S, Ali SMK, Azad Khan AK, Hussain A, Nahar Q. Prevalence of Type 2 Diabetes in Urban slums of Dhaka, Bangladesh. *Bangladesh Med Res Counc Bull*. 2004; 30: 60-70
8. Khanam PA. Senior research officer. Deptt. of Epidemiology and Biostatistics. *Research Division*, BIRDEM hospital, Shahabag, Dhaka.
9. The Diabetes Control and Complications Trial (DCCT) Research group. The effect of intensive treatment therapy of diabetes on the development and progression of long- term complications in insulin-dependent diabetes mellitus. *N. Eng J Med* 1993; 329: 977-986
10. UK Prospective Diabetes Study (UKPDS-33) Group. Intensive blood glucose control with sulphonylurias or insulin compared with conventional treatment and risk of complications in patients with Type 2 diabetes. *Lancet* 1998; 352: 837-853.
11. Gall M-A, Haugaard P, Borch-Johnsen K, Parving H-H. Risk factors for development of incipient and overt diabetic nephropathy in patients with non-insulin dependent diabetes mellitus: Prospective, Observational study. *BMJ* 1997; 314: 783
12. Mogensen CE, Keane WF, Bennett PH, Jerums G, Parving H-H, Passa P, et al. Prevention of diabetic renal disease with special reference to microalbuminuria. *Lancet* 1995; 346: 1080-1084
13. Mogensen CE. Long-term antihypertensive treatment inhibiting progression of diabetic nephropathy. *BMJ* 1982; 285: 685-688.
14. Jervell J. An update on Diabetes. *Axis-shield PoC As*, Oslo, Norway. *1st edition* 2000; 30-35
15. Mogensen CE. Microalbuminuria Predicts Clinical Proteinuria and early mortality in maturity onset diabetes. *N Eng J Med* 1984: 310: 356-360

16. Gillum RF. The association of body fat distribution with hypertension, hypertensive heart disease, coronary heart disease, diabetes and cardiovascular risk factors in men and women aged 18-79 years. *J chronic disease* 1987; 40: 421-428.

17. Stamler J, Vaccaro O, Neaton JD, Wentworth D. Diabetes, Other Risk Factors, and 12– years Cardiovascular Mortality for Men. Screened in the Multiple Risk Factor Intervention Trial. *Diabetes care* 1993; 16: 434 -449

18. DeFronzo RA, Ferrannini E. Insulin Resistance. A Multifaceted Syndrom Responsible for NIDDM, Obesity, Hypertension, Dyslipidemia, and Atherosclerotic Cardiovascular Disease. *Diabetes Care* 1991; 14: 173-194.

19. Part I: Diagnosis & Classification of Diabetes Mellitus. Report of WHO consultation. Definition, Diagnosis and Classification of Diabetes mellitus and its complications. WHO, Department of Non Communicable Diseases *Survillance*, Geneva. 1999.

20. Larkins R G, Dunlop M E. The link between hyperglycemia and diabetic nephropathy. *Diabetologia* 1992; 35: 499-504

21. Jerums G, Cooper M, Gilbert R, O' Brien R, Taft J. Microalbuminuria in diabetes. *Med J Aust* 1994; 161: 265-268.

22. Marre M, Chatelliar G, Leblanc H , Guyene TT, Menard J, Passa P. Prevention of diabetic nephropathy with enlapril in normotensive diabetics with microalbuminuria. *BMJ* 1998; 297: 1092-1095

23. Fader A. Diabetic Macrovascular Complication: Common Underlying Pathophysiology and an emerging treatment terget. Carrying for diabetes.*com;* Part of the carring for Diabetes Educational Forum 21, 2005 (Internet)

24. Wild and Associates " Global Prevalence of Diabetes". *Diabetes care* 2004; 27: 1047 -1053.

25. Willams A, Hennekens C. The role of aspirin in cardiovascular disease-forgotten benefits ? *Expert opinion Pharmacother* 2003; 4: 1-7.

26. Mogensen CE. Long-term antihypertensive treatment inhibiting progression of diabetic nephropathy. *BMJ* 1982; 285: 685-688.

27. Yudkin JS. Forrest RD, Jackson CA. Microalbuminuria as predictor of vascular disease in non-diabetic subject: Islington Diabetes Survey. *Lancet* 1988; 3: 530-533.

28. Schmitz A, Hansen HH, Christensen T. Kidney function in newly diagnosed type 2 (non- insulin dependent) diabetic Patients before and during treatment. *Diabetologia* 1989; 32: 434-439.

29. American Diabatic Association: Clinical Practice recommendation 1997: diabetic nephropathy. *Diabetes Care* 1997; 20: S24 - S30.

30. Anderson S, Rennke HG, Garcia DL, Brenner BM. Short and long term effects of antihypertensive therapy in the diabetic rat. *Kidney International* 1989; 36: 526-536.

31. Dinneen SF, Gerstein HC. The association of microalbuminuria and mortality in non-insulin dependent diabetes mellitus. A systemic overview of literature. *Arch Inter Med.* 1997; 157: 1413-1418.(Review).

32. Moris JN. Recent history of coronary disease. *Lancet* 1951; 1:1-7.