JPAFP Journal Academy of Family Physicians Pakistan

Editorial Article

Hypertensive Nephropathy

Naheed Nadeem
Association of Family Physician of Pakistan

ABSTRACT

Introduction: The objectives of the present research work are to study the biochemical parameters and levels of protein biomarkers affecting to hypertensive diabetic nephropathy in the Pakistani population.100 hypertensive nephropathy diabetic patients and 50 age, sex-matched normal healthy controls were recruited from Sheikh Zayed Hospital, Lahore, Pakistan.

Methodology: Individuals were equally divided into three different groups, group 1 was control, group 2 was diabetic hypertensive without nephropathy and group 3 was diabetic hypertensive with nephropathy. Blood and 24hrs urine were collected and stored for further analysis. Biochemical parameters related to the hypertensive diabetic nephropathy and specific proteins markers were analysed by 2-D liquid chromatographic system followed by mass spectrometric standard referred protocols.

Results: The proteins which showed variation between test and control samples were identified by MALDI TOF TOF analysis. The biochemical data showed significantly higher in values of fasting blood sugar, diastolic and systolic blood pressure, total serum and urinary proteins in the diabetic groups with hypertensive nephropathy as compared to group 2od without nephropathy and control in group 1.

Conclusion: The levels of proteins act as biomarker like albumin is highly up-regulated in diabetic hypertensive with nephropathy group as compared to normal and without nephropathy patients in the Pakistani population.

Key words: biomarker, hypersentivity, patient, Diabetes, biochemical's

How to cite this:

Nadeem N, HYPERTENSIVE NEPHROPATHY. J AcadFamlPhys Issue 1, volume 12(1),Pak. 2019. Page 2-13.

Corresponding author:NaheedNadeem **Email**: naheed@jpafp.org

INTRODUCTION

Diabetes mellitus is projected to become one of the world's main disablers and killers within the next twenty-five years. Pakistan will become 4th in number in increasing the diabetes worldwide. Without primary prevention, the diabetes epidemic will continue to grow. As the number of people with diabetic hypertensive nephropathy grow world wide, the disease takes an ever-increasing proportion of national health bare budgets. Glomerular and tubular damage resulting

from diabetes occur over several years, and it is possible that the excretions of glomerular and tubular proteins antedate the development of macro-albuminuria and perhaps even the development of microalbuminuria and ultimately kidney failure. The advent of novel, highly sensitive technologies such as proteomic profiling may identify urinary proteins associated with development of diabetic nephropathy well before any clinically identifiable alteration in kidney function or urine albumin excretion occur. Therefore to test this hypothesis, goals of present research work conduct a urinary proteomic analysis for determination and characterization of protein markers in the local population of Pakistan. This will certainly contribute in early detection and perhaps a possible treatment of this complication in hypertensive diabetic nephropathy and kidney failure.

EXPERIMENTAL

The hypertensive nephropathic diabetic patients and same age, sex-matched normal healthy controls were recruited from Sheikh Zayed Hospital, Lahore, Pakistan. Individuals were equally divided into three different groups, group A was control, group B was diabetic hypertensive with nephropathy and group C was diabetic hypertensive without nephropathy. Biochemical parameters related to the hypertensive diabetic nephropathy and specific proteins markers were analysed by 2-D liquid chromatographic system followed by mass spectrometric standard referred protocols. Total proteins and Albumin excretion rate was estimated by plotting the standard curve shows in Fig. 1a and b. The biochemical data showed significantly elevated levels of the fasting blood sugar, diastolic and systolic blood pressure, total serum proteins, total urinary proteins and albumin excretion rate in the diabetic group 3 with hypertensive nephropathy as shown in Fig. 2 and Table 1. The other groups control (group. 1) and group 2 showed the non significant results in the albumin excretion rate and blood pressure as shows in Fig. 2 and Table 1.

PROTEIN PROFILING

SDS-PAGE and 2D Liquid Chromatography analysis: Figure 3a. shows the Protein profiling of human urine analysis by SDS-PAGE analysis in which albumin is more present in hypertensive patients as compared to normal in the commassie staining. The albumin is major abundant protein present or observed in the patients urines samples of hypertensive nephropathy and less was observed in the normal control and without nephropathy diabetic patients. Figure 3 b. shows the 2D liquid chromatographic analysis and comparison of the control urine sample with diabetic sample and shows the elevated levels of some proteins in diabetic sample and not much expressed in normal one. Identification and purification was done by

chromatofocusing followed by reverse phase analysis and proteins are separated subjected to further analysis.

ProteoVue and Delta Vue analysis: The ProteoVue software imports the data from the 2D liquid chromatography and give the pI map of the all proteins separated into various fractions in the Fig 4a. DeltaVue software compares the both ProteoVue maps of control as in red colour and diabetic as in green colour shows in the Fig 4 b. The prominent peak shows the presence of human albumin in the pI range of 6.38-6.68.

MASS SPECTROMETRIC ANALYSIS

Mass Spectrometric (MALDI TOF TOF) analysis: The fraction selected from the 2 D analysis was purified and subjected to further mass spectrometric analysis as shown in Fig. 5a. The intact mass if the human albumin which was identified by MALDI TOF TOF is shown in Fig 5 b. While the tryptic digest of the human albumin protein was confirmed by the MASCOT analysis as shown in the Fig. 5c.

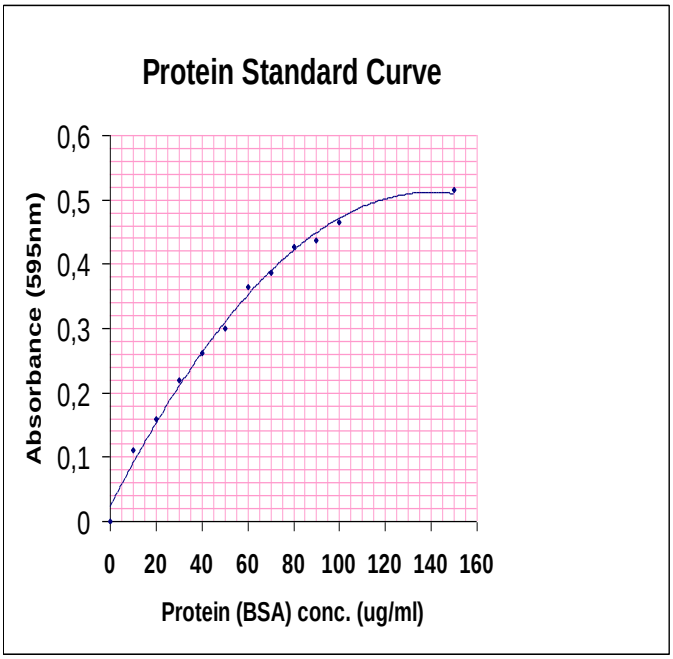
CONCLUSION

It may be concluded by the above research works as the prevalence of diabetic hypertensive nephropathy is much more common as compared to without nephropathy in diabetic local population of Pakistan. Albumin act as protein biomarker for this disease and the albumin excretion arte and level of albumin is significantly higher in these patients as compared to normal and without nephropathy diabetic individuals. So there is need to do more research work in this field and to control the hypertensive nephropathy in the diabetes and prevent the kidney failure.

Acknowledgement: This research work is funded by the University of the Punjab, Lahore and Higher education commission (HJEC), Islamabad.

Table 3.1Comparison of physical and biochemical parameters of control with that of diabetic hypertensive nephropathy patients.

Categories	FBS (mg/dl)	Uric Acid	BUN	Serum Creat	Urine Creat	Alb Exc Rate (mg/24hrs urine)	GFR	Total urine volume (liter)	Total proteins mg/ml	Blood pressure	
										Diastolic mm/Hg	Systolic mm/Hg
Group no. 1	93.7±2.74	14.58± 1.2	10.2± 0.65	0.79±0.05	49.635± 1.96	9.40635± 4.96	118.505 ± 16.96	2.711± 0.96	53.6± 1.96	110± 41.96	75± 1.96
Group no. 2	179.7692** ± 18.53	4.938* ± 1.9	13± 0.67	0.8846 15± 0.06	42.5923 ± 1.92	9.40635± 4.96	105.908 ± 15.96	1.157.5* ± 0.96	93.4**± 1.95	125± 42.96	79± 1.96
Group no. 3	193.9**± 22.51	4.0*± 1.95	13± 0.61	0.875± 0.065	42.175± 1.92	55.8985* **± 21.96	95.2515 *± 12.96	1.11*± 0.96	148.45* **± 1.98	*147.7± 41.96	*96.5± 1.96



Data are means ± SD. *=p>0.05 (statistically non significant),**b=p<0.01 (statistically significant), ***=p<0.001
(statistically highly significant) comparison of control with diabetic baseline. Group 1 is control, group 2, is without nephropathy diabetic hypertensive and group 3 is with hypertensive nephropathy diabetic individuals.

References:

1. Report of the Expert Committee on the Diagnosis and classification of Diabetes Mellitus.
2. Defronzo, R.A. The triumvirate : beta cell, muscle, liver: G collusion responsible for NIDDM. Diabetes 1992; 37:667-87
3. Jawaid SA. Proceedings of an advance cpurse in management of diabetes. P J M Sci 2002:1855-60
4. Launch of "Diabetes action Now" 5May 2004Igeneva.
5. King H, Rewers M: Global estimates for prevalence of diabetes mellitus and impair glucose tolerance in adults: WHO Ad Hoc Diabetes Report Group. Diabetes Care 16:157-177, 1993
6. World Health Organization: Diabetes Mellitus: Report of a WHO Study Group. Geneva, World Health Org., 1985 (Tech Rep Ser, no. 727)
7. Atkinson MA, Maclaren NK: The pathogenesis of insulin dependent diabetes. N Engl J Med 331:1428-1436,1994
8. Baekkeskov S, Neilsen JH, Marner B, Bilde T, Ludvigsson J, Lernmark A: Autoantibodies in newly diagnosed diabetic children with immunoprecipitate human pancreatic islet cell proteins. Nature 298: 167-169, 1982
9. Atkinson MA, Maclaren NK, Riley WJ, Winter WE, Fisk DD, Spillar RP: Are insulin autoantibodies markers for insulin-dependent mellitus? Diabetes 35: 894-898, 1986
10. Kaufman D, Erlander M, Clare-Salzler M, Atkinson M, Maclaren N, Tobin A: Autoimmunity to two forms of glutamate decarboxylase in insulin-dependent mellitus. J Clin Invest 89:283-292,1992
11. Christie MR, Tun RY, LoSSS, Cassidy D, Brown TJ, Hollands J, Shattock M, Bottazzo GF, Leslie RDG: Antibodies to GAD and tryptic fragments of islet 64K antigen as distinct markers for development of IDDM: studies with identical twins. Diabetes 41:782-787, 1992
12. Schott M, Schatz D, Atkinson M, Krischer J, Mehta H, Vold B, Maclaren N: GAD65 autoantibodies increase the predictability but not the sensitivity of islet cell and insulin autoantibodies for developing insulin dependent diabetes mellitus. J Autoimmunity 7:865-872, 1994

13. Schmidli RS, Colman PG, Harrison LC: Do glutmic acid decarboxylase antibodies improve the prediction of IDDM in first-degree relatives t risk for IDDM? J Autoimmunity 7:873-879, 1994

14. Myers MA, Rabin DU, Rowley MJ: Pancreatic islet cell cytoplasmic antibody in diabetes is represented by antibodies to islet cell antigen 512 and glutamic acid decarboxylase. Diabetes 44:1290-1295, 1995

15. Lan MS, Wasserfall C, Maclaren NK, Notkins AL: 1A-2, a transmembrane protein of the protein tyrosine phosphatase family, is a major autoantigen in insulindependent diabetes mellitus. ProcNatlAcadSci USA 93:6367-6370,1996

16. Lu J, Li Q, Xie H, Chen Z, Borovitskaya AE, Maclaren NK, Notkins AL, Lan MS: identification of a second transmembrane protein tyrosine phosphatase, 1A-2β, as an autoantigen in insulin-dependent diabetes mellitus: precursor of the 37-KDa tryptic fragment. ProcNatlAcadSci USA 93: 2307-2311, 1996

17. CantorAB, Krischer JP, Cuthbertson DD, Schatz DA, Riley WJ, Malone J, Schwartz S, Quattrin T, Maclaren NK: Age and family relationship accentuate the risk of IDDM in relatives of patients with insulin dependent diabetes. J ClinEndocrinolMetab 80:3739-3743, 1995

18. Huang W, Connor E, DelaRosa T, Muir A, Schatz D, Silverstein J, Crockett S, She JX, Maclaren NK: Although DR3-DQBI* may be associated with multiple component diseases of the autoimmune polyglandular syndrome, the human leukocyte antigen DR4-DQB110302 haplotype is implicated only in beta cell autoimmunity. J ClinEndocrinolMetab 81:1-5,1996

19. Banerji M, Lebovitz H: Insulin sensitive and insulin resistant variants in IDDM. Diabetes 38:784-792,1989

20. Reaven GM, Bernstein R, Davis B, Olefsky JM: Nonketotic diabetes mellitus: insulin deficiency or insulin resistnce? Am J Med 60:80-88,1976

21. Olesfsky JM, Kolterman OG, Scarlett, JA: Insulin action and resistance in obesity and noninsulin-dependent tye 2 diabetes mellitus. Am J Physiol 243:E15-E30,1982

22. Defronzo R, Deibert D, Hendler R, Felig P: Insulin sensitivity and insulin binding to monocytes in maturity-onset diabetes. J Clin Invest 63:939-946, 1979

23. Turner RC, Holman, RR, Matthews D, Hockaday TDR, Peto J: Insulin deficiency and insulin resistance interaction in diabetes: estimation of their relative contribution by feedback analysis from basal plasma

insulin and glucose concentrations. Metabolism 28:1086-1096, 1979

24. Kolterman OG, Gray RS, Griffin J, Burstein P, Insel J, Scarlett JA, Olesfsky JM: Receptor and postreceptor defects contribute to the insulin resistance in non-insulin-dependent diabetes mellitus. J Clin Invest 68:957-969,1981

25. Bogardus C, Lillioja S, Mott DM, Hollenbeck C, Reaven G: Relationship between degree of obesity and in vivo insulin action in man. Am J Physiol 248:E286-E291, 1985

26. Butkiewicz EK, Leibson C, O'Brien PC, Palumbo PJ, Rizza RA: Insulin therapy for diabetic ketoacidosis. Diabetes Care 18:1187-1190,1995

27. Banerji MA, Chaiken RL, Huey H, Tuomi T, Norin AJ, Mackay IR, Rowley MJ, Zimmet P, Lebovitz H: GAD antibody negative NIDDM in adults black subjects with diabetic ketoacidosis and increased frequency of human leukocyte antigen DR3 and DR4. Diabetes 43:741-745, 1994

28. Umpierrez GE, Casals MMC, Gebhart SSP, Mizon PS, Clark WS, Phillips LS: Diabetic ketocidosis in obese African- American. Diabetes 44:79-85, 1995

29. Newman B, Selby JV, Slemenda C, Fabsitz R, Friedman GD: Concordance for type 2 (non-insulin-dependent)diabetes mellitus in male twins. Diabetologia 30: 763-738, 1987

30. Barnett AH, Eff C, Leslie RDG, Pyke DA: Diabetes in identical twins. Diabetologia 20:87-93, 1981

31. Herman WH, Fajans SS, Oritz FJ, Smith MJ, Sturis J, Bell GI, PolonskyKS, Halter JB: Abnormal insulin secretion, not insulin resistance, is the genetic or primary defect of MODY in the RW pedigree. Diabetes 43:40-46, 1994

32. Byrne MM, Sturis J, Menzel S, Yamagata K, Fajans SS, Drondfield MJ, BainSC, Hattersley AT, Velho G, Froguel P, Bell GI, PolonskyKS: Altered insulin secretory response to glucose in diabetic and nondiabetic subjects with mutations in the diabetes susceptibility gene MODY3 on chromosome 12. Diabetes 45:1503-1510, 1996

33. Clement K, Pueyo ME, Vaxillaire M, Raketoambinina B, Thuillier F, Passa P, Froguel P, Roberts J, Velho G: Assessment of insulin sensitivity in glucokinase-deficient subjects. Diabetologia 39: 82-90, 1996

34. Vaxillaire M, Boccio V, Philippi A, Vigouroux C, Terwilliger J, Passa P, Beckman JS, Velho G, Lathrop GM, Froguel P: A gene for maturity onset diabetes of the young (MODY) maps to chromosome 12q. nature Genet 9:418-423, 1995

35. Yamagata K, Oda N, Kaisaki PJ, Menzel S, Furuta H, Vaxillaire M,

Southarm L, Cox RD, Lathrop GM, Boriraj VV, Chen X, Cox NJ, OdaY, Yano H, Le Beau MM, Yamada S, Nishigori H, Takeda J, Fajans SS, Hattersley AT, Iwasaki N, Hansen T, Pedersen O, Polonsky KS, Bell GI: Mutations in the hepatocyte nuclear factor-1α gene in maturity-onset diabetes of the young (MODY 3). Nature 384:455-558, 1996

36. Froguel P, Vaxillaire M, Sun F, Velho G, Zouali H, ButelMO, Lesage S, Vionnet N, Clement K, Fougerousse F, et al.: Close linkage of glucokinase locus on chromosome 7p to early-onset non-insulin-dependent diabetes mellitus. Nature 356:162-164, 1992

37. Vionnet N, Stoffel M, Takeda J, Yasuda K, Bell GI, Zouali H, Lesage S, Velho G, Iris F, Passa P, et al,: Nonsense mutation in the glucokinase gene cause early-onset non-insulin-dependent diabetes mellitus. Nature 356:721-722 1992

38. Bell GI, Xiang K, Newman MV, Wu S, Wright LG, Fajans SS, Spielman RS, CoxNJ: Gene for non-insulin-dependent diabetes mellitus (maturity-onset diabetes of the young subtype) is linked to DNA polymorphism on human chromosome 20q. procNatlAcadSci 88:1484-1488, 1991

39. Yamagata K, Furuta H, Oda N, Kaisaki PJ, Menzel S, Cox NJ, Fajans SS, Signorini S, Stoffel M, Bell GI: Mutations in the hepatocyte factor-4α gene in maturity-onset diabetes of the young (MODY). Nature 384:458-460. 1996

40. Reardon W, Ross RJM, Sweeney MG, Luxon LM, Pembrey ME, Harding AE, Trembath RC: Diabetes mellitus associated with a pathogenic point mutation in mitochondrial DNA. Lancet 340:1376-1379, 1992

41. Van den Ouwenland JMW, Lemkes HHPJ, Ruitenbeek W, Sandkuijl LA, de Vijlder MF, Struyvenberg PAA, van de Kamp, Maassen JA: Mutation in mitochondrial tRNA (Leu(URR) gene in a large pedigree with maternally transmitted type 2 diabetes mellitus and deafness. Nature Genet 1:368-371, 1992

42. Kadowaki T, Kadowaki H, Mori Y, Tobe K, Sakuta R, Suzuki Y, Tanabe Y, Sakura H, Awata T, Goto Y, et al.: A subtype of diabetes mellitus associated with a mutation of mitochondrial DNA. N Engl J Med 330:962-968, 1994

43. Kahn CR, Flier JS, Bar RS, Archer JA, Gorden P, Martin MM, Roth J: The syndromes of insulin resistance and acanthosisnigricans. N Engl J Med 294:739-745, 1976

44. Taylor SI: Lilly Lecture: molecular mechanisms of insulin resistance: lessons from patients with mutations in the insulin-receptor gene. Diabetes 41:1473-1490, 1992

45. Schwartz SS, Zeidler A, MoossaAR, Kuku SF, Rubenstein AH: A

prospective study of glucose tolerance, insulin, C-peptide, and glucagon response in patients with pancreatic carcinoma. Digestive Dis 23:1107-1114, 1978

46. Cersosimo E, Pister PWT, Pesola G, McDermott K, Bajorunas D, Brennan MF: Insulin secretion and action in patients with pancreatic cancer. Cancer 67:486-493, 1991

47. Larsen S, Hilsted J, Tronier B, Worning H: Metabolic control and B cell function in patients with insulin-dependent diabetes mellitus secondary to chronic pancreatitis. Metabolism 36:964-967, 1987

48. Soffer LJ, Iannaccone A, Gabrilove JL: Cushing's syndrome. Am J Med 30:129-146, 1961

49. Jadresic A, Banks LM, Child DF, Diamant L, Doyle FH, Fraser TR, Joplin GF: The acromegaly syndrome. J Med202:189-204, 1982

50. Stenstrom G, Ernest I, Tisell L: Long term results in 64 patients operated upon for pheochromocytoma. Acta Med Scan 223:345-352, 1988

51. Berelowitz M, Eugene HG: Non-insulin dependent diabetes mellitus secondary to other endocrine disorders. In Diabetes Mellitus. LeRoith D, Taylor SI, Olefsky JM, Eds. New York, Lippincott-Raven, 1996, p.496-502

52. Conn JW: Hypertension, the potassium ion and impaired carbohydrate tolerance. N Engl J Med 273:1135-1143, 1965

53. Pandit MK, Burke J, GustafsonAB, Minocha A, Peiris AN: Drug-induced disorders of glucose tolerance. Ann Int Med 118:529-540,1993

54. O'Byrne S, Feely J: Effects of drugs on glucose tolerance in non-insulin-dependent diabetes (part 1 and 2). Drug 40: 203-219, 1990

55. Forrest, JA, Menser MA, Burgess JA: High frequency of diabetes mellitus in young patients with congenital rubella; Lancet ii:332-334, 1971

56. King ML, Bidwell D, Shaikh A, Voller A, Banatvala JE: Coxsackie-B-virus-specific IgM responses in children with insulin-dependent (juvenile-onset; type 1) diabetes mellitus. Lancet i:1397-1399, 1983

57. Karjalainen J, Knip M, Hyoty H, Linikki P, Ilonen J, Kaar M-L, Akerblom HK: Relationship between serum insulin antibodies, islet cell antibodies and Coxsackie-B4 and mumps virus-specific antibodies at the clinical manifestation of type 1 (insulin-dependent) dibetes. Diabetologia 31:146-152, 1988

58. Pak CY, Eun H, McArthur RG, Yoon J: Association of cytomegalovirus-infection with autoimmune type 1 diabetes. Lancet ii:1-4, 1988

59. Solimena M, Folli, Aparisi R, Pozza G, De Camilli P: Autoantibodies to GABA-nergic neurons and pancreatic beta cells in stiffman syndrome. N Engl J Med 41: 347-353, 1992

60. Rimoin DL: Genetic syndromes associated with glucose intolerance. In The Genetics of Diabetes Mellitus. Berlin Springer-Verlag, 1976

61. Barrett TG, Bundey SE, Macleod AF: Neurodegeneration and dibetes: UK nation wide study of Wolfrm (DIDMOAD) syndrome. Lancet 346:1458-1463, 1995

62. Langer O, Rodriguez DA, Xenakis EMJ, McFarlandMB, Berkus MD, Arrendondo F: Intensified versus conventional management of gesttional diabetes. Am J ObstetGynecol 170:1036-1047, 1994

63. Magee MS, Walden CE, Benedetti TJ: Influence of diagnostic criteria on the incidence of gestational diabetes and perinatl morbidity. JAMA 269:609-615, 1993

64. Cousins L: Obstetric complications. In Diabetes Mellitus and Pregnancy: Principles and Practice. 2nd ed. New York, Churchill Livingstone, 1995, p. 455-468

65. O'Sullivan JB, Mahan CM: Criteria for the orl glucose tolerance test in pregnancy. Diabetes 13:278, 1964

66. Brown IR, McBain AM, Chalmers J, Campbell IW, Brown ER, Lewis MJ. Sex difference in the relationship of calcium and magnesium excretion to glycaemic control in type-1 dibetes mellitus. ClinChimActa 1999;283:119-28

67. Cunningham JJ, Fu A, Mearkle PL, Brown RG. Hyperzincure in individuals with insulin-dependent dibetes mellitus: concurrent zinc status and effect of high dosa zinc supplementation. Metabolism 1994;43(12):1558-62.

68. Gallery P. Advancedglyction end products (AGEs), free radicals nddibetes. J SocBiol 2001;195(4):387-90.

69. Abou-Seif MAM, Youssef H. Oxidative stress and male IGFI, gonadotropin and related hormones in diabetic patients. ClinChem Lab Med 2001;39(7):618-23.

70. Paterson J, Pettegrew A, Dominiczak MH, Small M. Screening for hyperlipidaemia in diabetes mellitus. Relation to glycemic control. NnClinBiochem 1991;28:254-8.

71. Lopes-Virella MF, Virella G. Lipoproteins and immune response in the vascular wall and their contribution to atherosclerosis in diabetes. Metabolism 1992;4(5):11-5.

72. Velazquez E, Winocour PH, Kesteven P, Alberti KG, Laker MF. Relation of lipid peroxides to

macrovascular disease in type 2 diabetes. Diabet Med 1991;8:752-8.

73. Moncdo S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology and pharmacology. Pharmcol Rev 1991;43:109-42.

74. Hardt PD, Killinger A, Nalop J, Schnell-Kretschmer H, Zekorn T, Klor HU. Chronic pncreatitis and diabetes mellitus. A retrospective analysis of 156 ERCP investigations in patients with insulin-dependent and non-insulin-dependent diabetes mellitus. Pancreatology 2002;2(1):30-3

75. Zhou, H., Yuen, P.S., Pisitkun, T., Gonzales, P. A., Yasuda, H., Dear, J. W., Gross, P., Knepper, M. A., ans Star, R. A. (2006) Collection, storage, preservation, an normalization of human urinary exosomes for biomarker discovey. Kidney Int. 69, 1471-1476

76. Christensen, E. I., and Birn, H. (2001) Megalin and cubilin: synergistic endocytic receptors in renal proximal tubule. Am. J. Physiol. 280, F562-F573

77. Christensen, E. I. (2002) Pathophysiology of protein and vitamin handling in the proximal tubule. Nephrol. Dial. Transplant. 17, Suppl. 9, 57-58

78. Pisitkun, T., Shen, R. F., and Knepper, M. A. (2004) Identification and proteomic profiling of exosomesin human urine. Proc. Natl. Acad. Sci. U. S. A. 101, 13368-13373

79. American diabetes association diabetic nephropathy diabetic care 2002; 25 (supplement)

80. Group TMCS (the Microalbuminuria Collaborative Study Group). Predictors of the development of microalbuminuria in patients with Type 1 diabetes mellitus: a seven-year prospective study. Diabet Med (1999) 16:918–925.

81. Caramori ML, Fioretto P, Mauer M. The need for early predictors of diabetic nephropathy risk: is albumin excretion rate sufficient? Diabetes (2000) 49:1399–1408.

82. Tomino Y, Suzuki S, Azushima C, et al. Asian multicenter trials on urinary type IV **collagen** in patients with **diabeticnephropathy**. J Clin Lab Anal (2001) 15:188–192.

83. Koshimura J, Fujita H, Narita T, et al. Urinary adiponectin excretion is increased in patients with overt diabetic nephropathy. BiochemBiophys Res Commun (2004) 316:165–169.

84. Fujita H, Morii T, Koshimura J, et al. Possible relationship between adiponectin and renal tubular injury in diabetic nephropathy. Endocr J (2006) 53:745–752.

85. DallaVestra M, Masiero A, Roiter AM, et al. Is podocyte injury

relevant in diabetic nephropathy? Studies in patients with type 2 diabetes. Diabetes (2003) 52:1031–1035.

86. Nakamura T, Ushiyama C, Suzuki S, et al. Urinary excretion of podocytes in patients with diabetic nephropathy. Nephrol Dial Transplant (2000) 15:1379–1383.

87. Turk N, Mornar A, Mrzljak V, et al. Urinary excretion of advanced glycationendproducts in patients with type 2 diabetes and various stages of **protein**uria. Diabetes Metab (200**4**) 30:187–192.

88. Ha SW, Kim HJ, Bae JS, et al. Elevation of urinary betaig-h3, transforming growth factor-beta-induced protein in patients with type 2 diabetes and nephropathy. Diabetes Res ClinPract (2004) 65:167–173.

89. Cha DR, Kim IS, Kang YS, et al. Urinary concentration of transforming growth factor-beta-inducible gene-h3(beta ig-h3) in patients with Type 2 diabetes mellitus. Diabet Med (2005) 22:14–20.

90. D.Cvoriscec, A.Stavljenic and M.Radonic, JClin. Chem. Biochem., 23(1985)177

91. Hypertension in Diabetes Study (HDS): increased risk of cardiovascular complications in hypertensive type 2 diabetic patients. J Hypertens 11:319-325, 1993

92. Hansson L, Zanchetti A, Carruthers SG, Dahlof B, Elmfeldt D, Julius S, Menard J, Rahn KH, Wedel H, Westerling S: Effects of intensive blood-pressure lowering and low-dose aspirin in patients with hypertension: principal results of the Hypertension Optimal Treatment (HOT) randomized trial. Lancet 351:1755-1762, 1998

93. KrolewskiAS, Warram JH, Chrislieb A, Busick RJ, Kahn CR: The changing natural history of nephropathy in type 1 diabetes. Am J Med 78:785-794, 1985

94. Mehler PS, Jeffers BW, Estacio R, Schrier RW: Association of hypertension and complications in non-insulin dependent diabetes mellitus. Am J Hypertens 10:152-161, 1997

95. Parving HH, AndersenAR, Smidt UM, Hommel E, Mathiesen ER, Svendsen PA: Effect of antihypertensive treatment on kidney function in diabetic nephropathy. BMJ 294:1443-1452, 1987

96. Parving H, Hommel E: Prognosis in diabetic nephropathy. BMJ 299:230-233, 1989

97. Niaura R, Banks SM, Ward KD, et al. hostility and metabolic syndrome in older males. The normative aging study. Psych Som Med 2000; 62:7-16

98. Molitch ME, DeFronzo RA, Franz MJ, Keane WF, Mogensen CE,

Parving HH, Steffes MW: Nephropathy in diabetes (position statement). Diabetes Care 27 (Suppl 1):S79-S83, 2004

99. Donnelly R. Microalbuminuria: a therapeutic goal in patients with type 2 diabetes. Presse Med 2002; 31:S9-S12.

100. Augustine J, Donald G. diabetic nephropathy (2003) [online article], The Cleveland Clinic, Department of nephrology and Hypertension, The Cleveland Health Foundation, United States.

101. (U.S.Renal Data system, US RDS 2001 Annual Data report (2002).