N-acetyl-p-D glucosaminidase (NAG) enzyme activity in urine for early diagnosis of nephropathy in diabetes mellitus type II

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We studied urinary N-acetyl-p-D glucosaminidase enzyme (NAG) activities in 50 type II diabetic patients (27 M, 23 F) with negative albuminuria, well controlled blood sugar and blood pressure. Ten healthy subjects (7 M, 3 F) were taken as controls. The patients in the study group were classified according to the duration of diabetes (each 2, 3 and 4 years). In all cases, urinary NAG levels were determined. The mean urinary NAG levels in the control group were 7±0.3 limol/hour/mmol creatinine. Urinary NAG levels in the study group were found to be 14.5±0.6, 16.5±0.2, 20.9±0.9, 28.4±2 and 51 ±2.3 \xmol/hr/mmol creatinine in the 2-3, 4-5, 6-7, 8-9 and 10-14 year diabetics respectively. The mean urinary NAG levels in patients classified at intervals of 3 years consecutively (2-4, 5-7, 8-10 and >11 years) were found to be 13.5±0.29, 19.14±0.4, 27.78±1.75 and 60.7±3.29 \xmol/hr/mmol creatinine. The average urinary NAG levels of diabetic groups formed consecutively at intervals of 4 years (2-5, 6-9 and 10-14 years) were determined to be 15.5±0.2, 23.7+0.8 and 51.04+23 \imol/hr/mmol creatinine respectively. These results indicate that renal damage occurs early in type II diabetes mellitus and urinary NAG levels could be useful in diagnosis the onset of the disease. [Turk J Med Res 1995, 13(4): 141-146]

Key Words: Diabetic nephropathy, NAG, Diabetes Mellitus

Although the tendency to develop end stage kidney failure in type II noninsulin-dependent-diabetes mellitus (NIDDM) patients is 3-8%; because type II diabetic patients make up 80-90% of total diabetic patients, uremia cases due to type II NIDDM in the general population, are at least as many as those due to insulin dependent diabetes mellitus (IDDM) cases (1-4). The prevalence of nephropathy is dependent on the stage of diabetes (5). In a study conducted on vascular complications concerning diabetes mellitus, it was found that while the ratio of proteinuria was 0%-16% in diabetics of less than 7 years, the same value was 2-27% in diabetics of more than 17 years (6). Clinical nephropaty is observed after the 10th-15th year of type I IDDM where as it is observed in the first 10 years in type II NIDDM (1,7,8).

Changes related to diabetic nephropathy, can be determined during the early stages of diabetes by inspection of glomerular and tubular functions. Evaluation can be done by determination of total protein and albumin in urine for glomerular functions and by determination of tubular cell enzymes for tubular dysfunctions (9). Limited researches done in the last 10 years have shown that N-acetyl-p-D-glucosaminidase (NAG), which is a lysosomal enzyme, obtained from the renal proximal tubules can be a sensitive indicator to be used in the early diagnosis of diabetic nephropathy (10,11,12). N-acetyl-p-D-glucosaminidase is a 150 dalton lysosomal enzyme which can be found in different organs and tissues. NAG decreases the level of glycoproteins and mucopolysaccharides which cause the formation of diabetic angiopathy (13).

In this study we have investigated the importance of urinary NAG levels for the early diagnosis of diabetic nephropathy in patients who were diagnosed as type II diabetes mellitus and the correlation of these levels with the stage of diabetes.

MATERIALS AND METHODS

This study involves 50 patients with type II diabetes mellitus followed at the Black Sea Technical University Medical Faculty Department of Internal Medicine, and 10 control subjects.

The control group was formed from a total of 10 subjects (7 male, 3 female, between the ages of 23-76 years with an average age of 47.1 years)

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Table 1. Urinary NAG levels for two yearly intervals

	Control n:10	Group I (2-3 years) n:10	Group II (4-5 years) n:10	Group III (6-7 years) n:10	Group IV (8-9 years) n:6	Group V (>10 years) n:14
Mean age (year) (range)	47.1(23-76)	48.5(36-75)	55.2(34-76)	55.1(45-63)	61.8(47-78)	56.6(38-74)
Urinary NAG (mmol/hr/mmol-creatinine) (95% CI)	7.11 ±0.33 (2.1-11.9)	14.52±0.58 (6.7-24)	16.49±0.22 (13.8-20)	20.87+0.92 (9.8-39)	28.36±2.03 (11-54)	51.04±2.31 (11.6-95)

CI: Confidence intervals

CXGI(p<0.05) CXGII(p<0.05) CXGIII(p<0.05) CXGIV(p<0.05) CXGV(p<0.05)

GIXGII(p>0.05) GIXGIII(p<0.05) GIXGIV(p>0.05) GIXGV(p<0.05)

GIIXGIII(p>0.05) GIIXGIV(p>0.05) GIIXGV(p<0.05)

GIIIXGIV(p>0.05) GIIIXGV(p<0.05)

GIVXGV(p<0.05)

who had normal physical examination findings, arterial blood pressure, routine urine analysis and albustix (-), and had fasting blood glucose levels, BUN, creatinine levels as normal, and who did not use any medicine.

The patients group was formed from 50 NIDDM patients who were followed for at least 2 years with diagnosed type II diabetes mellitus. Of these patients 23 were females and 27 were males with an average age of 55.6 years (34-74 years). Their fasting blood glucose levels, BUN and creatinine levels were determined, routine urine analysis done and arterial blood pressure taken. The cases who had high arterial blood pressure, unregulated blood glucose, urinary infection, urine albustix (+), renal transplantation, cardiac bypass, polycystic renal disease, glomerulonephritis, rheumatoid arthritis, liver cirrhosis, nephrocalcinosis, and those using aminoglycosides, acetaminophen, and analgesics were not taken into the study. At the end of physical examination 44 normotensive diabetic patients with regulated blood glucose, having negative albustix at the end of urine and blood analysis and 5 patients who were followed for more than 12 years and who had no other pathological findings apart from having positive albustix in urine analysis were taken into the study. The cases included In the study were grouped according to the duration of diabetes at 2-3-4 year intervals. *

In the study group 35 patients were regulated with diet and/or oral diabetic agents. In the remaining patients, glycemia was regulated with subcutaneous insulin, as diet and oral diabetic agents did not suffice to regulate it.

Morning urine samples in the study and control groups were taken and investigated with albustix (Ames Division, Miles Laboratories Limited, Slough SLZ, 4LY, England) and stored at -20°C. NAG measurements were made afterwards. Blood creatinine measurements were made in the blood samples collected at the same time. To find albumin in urine with the test based on indicator usage shows that there is 150-200 ug/ml albumin in urine (14). Tucker method was used to determine blood and urine NAG enzyme levels and the measurements were made with fluorometre (Locarte moc j|) (15). The buffers and 4-methyl - umbelliferyl- 2 - acetamido - 2 - deoxy - p - D-glucocophyranoside substrate used in determination of NAG levels is urine were obtained from Sigma Chemical Company (St Louis USA) and the methyl-lumbelliferrone standard was obtained from Koch-Light Laboratories (Colnbrock, UK), Mann-Whitney U test was applied for statistical analysis.

RESULTS

In the control group the urine NAß levels were between 2.1-11.9 p:mol/hour/mml creatinine with an average of 7.11 ±0.33 umol/hour/mmol creatinine.

In the diabetes group the cases who had positive albustix were taken into the study as a separate group as they had diabetes for more than 10 years, and were at the stage of clinical diabetic nephropathy. The urine NAG levels were determined simultaneously with urine creatinine levels and urine NAG levels of diabetic

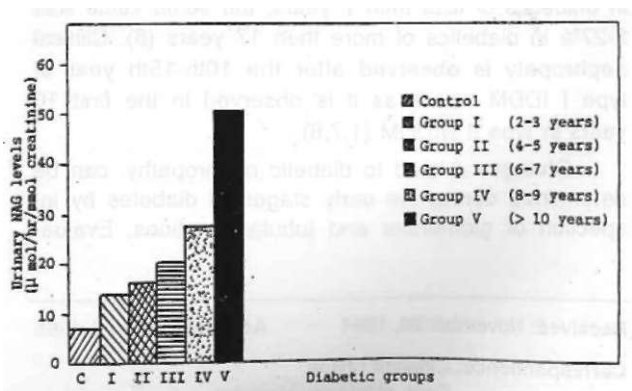

Figure 1. Demonstration of urinary NAG levels graphically based on the data on Table 1.

Table 2. Urinary NAG levels for three yearly intervals

	Control n:10	Group A (2-4 years) n:12	Group B (5-7 years) n:18	Group C (8-10 years) n:10	Group D (>11 years) n:10
Mean age (year)	47.1(23-76)	49.75(36-76)	55.05(34-72)	58.9(47-78)	57.5(38-74)
Urinary NaG (mmol/hr/mmol-**creatinine)**	7.11 ±0.33 SD:1.07 (2.1-11.9)	13.5±0.29 SD:1.0 (6.7-20)	19.14±0.4 SD:1.66 (9.8-39)	27.78±1.75 SD:5.56 (11-54)	60.7±3.29 SD:10.4 (15-95)
	CXG.A(p<0.05) CXG.B(p<0.05) CXG.C(p<0.05) CXG.D(p<0.05)	G.AXG.B(p<0.05) G.AXG.C(p<0.05) G.AXG.D(p<0.05)	G.BXG.C(p>0.05) G.BXG.D(p<0.05)	G.CXG.D(p<0.05)	

cases were found to be between 6.7-95 umol/hour/ mmol creatininen.

The urinary NAG levels of the control group were compared with the urinary NAG levels of the study group formed at intervals of two years who had diabetes for more than 10 years (Table 1 and Figure **1).** Although the urinary NAG levels increased in the first four groups formed at intervals of two years, it was observed that only urinary NAG levels in group 3 was higher than that of group 1 (p<0.05). Also, there was a statistically significant difference between groups 1,2,3,4 and **5** for urinary NAG levels (p<0.05). There was also an important difference between the control group and every study group for urinary NAG levels (p<0.05).

The average urinary NAG levels showed an increase according to the duration of diabetes in the diabetes groups formed at intervals of three (Table 2 and Figure 2). Although the increase in the urinary NAG levels in the diabetes groups was found to be statistically insignificant between the 5-7 years and 8-10 years diabetes group, there was a statistically Important difference between the levels of A and B Qroups and C and D groups (p<0.05). Urinary NAG levels of the control group were significantly lower in comparison to the other 4 groups (p<0.05).

The average urinary NAG levels of diabetic Qroups formed consecutively at intervals of four years were found to be significantly higher than the control Qroup levels (p<0.05) and as the stage of diabetes advances the difference between the diabetic groups was 'ound to be statistically significant (p<0.05) (Table 3 *nd Figure 3).

DISCUSSION

Kidneys are the primary target organs in diabetes 0©.17). At the beginning, diabetes causes renal hypertrophic changes which over time leads to structural deformities of the renal arteries, interstitium, tubules and 9'omerulles.

In the study, we investigated the diagnostic significance of urinary NAG activity which could indicate the structural changes caused by diabetic nephropathy which develops in the first 10 years of type II NIDDM cases (1,7,8). According to epidemiological studies the diagnostic significance of albuminuria, in detecting the development of nephropathy in the first 5-10 years of diabetes in type II diabetics, was found to be low (4) while the diagnostic value of microalbuminuria was 75% in type I, IDDM, cases and 25% for type II, NIDDM, cases (4,18). Apart from this, changes due to nephropathy cannot be detected in the first 10 years in type II NIDDM cases and clinical proteinuria can only be detected after clinical diagnosis is made (4). In type I diabetic nephropathy low molecular weight protein molecules like albumin increase in the urine at the early stages of the disease, whereas in type II diabetic nephropathy macromolecules like IgG is excreted in urine, and albustix can be negative (19). Therefore, to evaluate the clinical prognosis of diabetic nephropathy, during the first stages of type II NIDDM nephropathy, prior to the detection of clinical proteinuria, the excretion of certain enzymes in urine was investigated. In recent years the studies done on this subject have intensified with regard to lysosomal

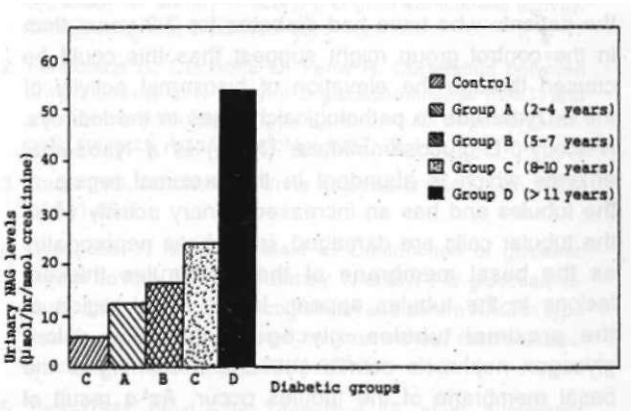

Figure 2. Demonstration of urinary NAG levels graphycally based on the data on Table 2.

Table 3. Urinary NAG levels for four yearly intervals

	Control n:10	Group AA (2-5 years) n:20	Group BB (6-9 years) n:16	Group CC (>10 years) n:14
Mean age (year)	47.1(23-76)	51.85(36-76)	58.45(45-78)	56.6(38-74)
Urinary NAG (mmol/hr/mmol-creatinine)	7.11 ±0.33 SDM.07 (2.1-11.9)	15.5±0.21 SD:0.96 (6.7-24)	23.68±0.87 SD:3.49 (9.8-54)	51,04*2.31 SD:8.66 (11.6-95)
	CXG.AA(p<0.05) CXG.BB(p<0.05) CXG.CC(p<0.05)	G.AAXG.BB(p<0.05) G.AAXG.CC(p<0.05)	G.BBXG.CC(p<0.05)	

enzyme NAG secreted by the cells of proximal tubules (9).

In this study we have found that urinary NAG enzyme activity increases in type II diabetics who have no proteinuria and have albustix (-). They were grouped starting from the second year and then formed in groups at intervals of 2 years. In all the groups the urinary NAG activity was found to be significantly, statistically higher than the control group ($p<0.05$). In a study Shimajo et al has grouped 165 diabetes patients according to te duration of diabetes and found urinary NAG activity to be higher in patients who had diabetes for 2-8 years than in patients who had diabetes for less than 2 years (20). In all studies investigating the relationship of urinary NAG values with diabetic nephropathy in type I and type II diabetes cases, who had no clinical proteinuria, urinary NAG values were found to be higher than the control groups, and the difference was found to be statistically significant (9,12,14,21-23). In the histopathological course of diabetic nephropathy, thickening of the glomerular basal membrane, development of the mesenchymal matrix develops during the first 1.5-2.5 years (24). In time, the diffuse and nodular changes of diabetic nephropathy develop (24). In this study, the fact that the urinary NAG enzyme activity is higher in the patients who have had diabetes for 2-3 years than in the control group might suggest thas this could be caused through the elevation of lysosomal activity of the enzyme due to pathologicalchanges in the kidneys. N-acetly-p-D-glucosaminidase (NAG) is a lysosomal enzyme which is abundant in the proximal region of the tubules and has an increased urinary activity when the tubular cells are damaged. In diabetic nephropathy as the basal membrane of the glomerules thicken, lesions in the tubules appear. In the distal region of the proximal tubules, glycogen infiltration called glycogen nephrosis comes out, and thickening of the basal membrane of the tubules occur. As a result of the structural deformity in the tubular cells, the NAG enzyme present in the lysosomes of the proximal tubules comes out in urine. In a study by Watanabe et al, it has been found that urinary NAG levels of patients who had diabetes for 2.5 and 5 years without clinical proteinuria were higher than the control group, and it has been claimed that the urinary NAG activity increase at the beginning of diabetes is the result of nonenzymair glycolyzation of NAG molecules (14). Also, it has been reported that the settling of glycolized albumin in the glomerul-basal membrane causes damages in the basal membrane directly or by immune complexes by glycolization of the collagen tissue and by antibodies formed against the glycolysed collagen.

In our study, although urinary NAG values in the study group were higher than that of the control group, in the diabetic groups formed at consecutive intervals of two years, in the first 4 groups, it was observed that only urinary NAG activity in group 3 was higher than of group 1 ($p<0.05$). Also, the mean urinary NAG value in the group 5 was higher than those of the other groups ($p<0.05$) (Table 1). Apart from this, in the diabetic groups formed at consecutive intervals of three years, although no any significant difference in urinary NAG values was found between group B and C, the differences among other all groups were significant ($p<0.05$) (Table 2).

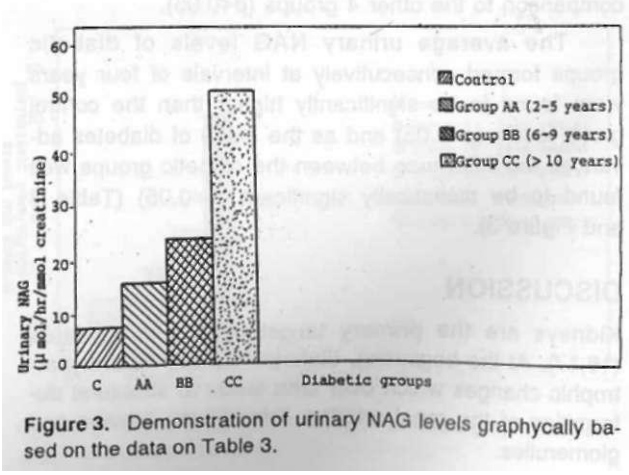

Figure 3. Demonstration of urinary NAG levels graphycally based on the data on Table 3.

These findings were found to be in accordance with the significant increase in urinary NAG values in diabeti[cs] who had the disease for 2-8 years described by shjrnajo et al (20). In the same study the urinary NAG values of patients who had the disease for 2-8 years with no proteinuria were compared with those who had the disease for 8-12 years and no increase was found. Again in the same study the results obtained for the increase in urinary NAG levels in 12±1 year patients with no proteinuria and 16.2±1 year patients with proteinuria show similarities to our results. It is not possible to explain the fact that there is no statistical significance for the increase of urinary NAG levels after the eighth year of diabetes while there is a statistical significance in the increase after j[n 0] 11-12th year of diabetes. But it was thought that after the eighth years of diabetes lysosomal activity in the proximal tubules of the kidneys may decrease or the impaired stability of the NAG activity may be improved temporarily.

In this study it was found that when the patients were formed in groups at consecutive intervals of our years, urinary NAG values increased significantly (Table 3). Therefore, it is thought that it would be beneficial to measure urinary NAG levels at 3-4 yearly intervals for the evaluation of diabetic nephropathy which starts developing from the onset of the disease. As a result, the determination of urinary NAG activity for the early diagnosis of type II NIDDM nephropathy is a more important indicator than albuminuria, and that the increase in urinary NAG activity is related to the duration of diabetes.

Tip II Diabetes Mellitusa Bağlı Nefropatinin Erken Tanısında İdrarda N-asetil-p-D-glukozaminidaz (NAG) Aktivitesi

Albüminürisi olmayan ve kan basıncı regüle diabetes mellitus tip II'li 50 hastada (27 erkek, 23 kadın) idrar N-asetil-p-D glukozaminidaz (NAG) enzim aktivitesi çalışıldı. Kontrol grubu olarak sağlıklı 10 birey (7 erkek, 3 kadın) çalışmaya alındı. Hastalar diyabet sürelerine göre 2, 3 ve 4'er yıllık sürelerle ardışık olarak gruplara ayrıldı. Tüm vakalarda idrar NAG seviyeleri ölçüldü. Kontrol grubunda ortalama idrar NAG seviyesi 7.1±0.33 wnol/saat/mmol kreatinin idi. Çalışma grubunda idrar NAG seviyelerinin 2-3, 4-5, 6-7, 8-9 ve 10-14 yıllık diyabetlilerde sırasıyla 14.5±0.6, 16.5±0.2, 20.9+0.9, 28.4+2 ve 51+2.3 \xmol/saat/mmolkreatinin olduğu saptandı. 3'er yıllık aralıklarla (2-4, 5-7, 8-10, >11 yıl) sınıflandırılan hastalarda idrar NAG seviyeleri sırasıyla 13.5±0.29, 19.14+0.4, 27.78+1.75 ve 60.7+3.29 vmol/saat/mmol kreatinin, 4 yıllık aralıklarla (2-5, 6-9 ve 10-14 yıl) sınıflandırılan hastalarda ise idrar NAG seviyeleri sırasıyla 15.5±0.2, 23.7+0.8 ve 51.04+2.3 \unol/saat/mmol kreatinin olarak ölçüldü.

Bu sonuçlar tip II diabetes mellitusda renal hasarın erken başladığını ve idrar NAG seviyelerinin hastalığın başlangıcını tayinde yararlı olabildiğini göstermektedir. [Turk J Med Res 1995; 13(4): 141-146]

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