The assessment of early microangiopathic complications in non-insulin dependent diabetes mellitus

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The aim of this study is to detect the diabetic complications in early reversible stages, to find out some parameters which point out the complications and their levels, and to show the effect of hypertension on these complications and parameters. We assessed glomerular functions with urinary albumin (Alb) excretion and Creatinine clearance, renal tubular functions with urinary beta-2 microglobulin (B2mGI) excretion and retinopathy by fundus fluorescein angiography (FFA). We observed that the retinal microaneurysms began before microalbuminuria and that the diabetics, whose "Overnight Alb Excretion Rate" exceeded 7 g/min, represented the high risk group for background retinopathy. Urinary B2mGI values were not directly affected by mild or moderate hypertension and there was no correlation with fundus findings. Our findings suggested that urinary B2mGI excretion increased with the decline of creatinine clearance. Obviously, high blood pressure effected daytime Alb excretion more than the overnight Alb excretion. This result supported the observations approving the dominance of glom rular capillary pressure in the pathogenesis of microalbuminuria. We concluded that in the assessment of early diabetic patients, "Overnight Alb Excretion Rate" could give an idea about diabetic fundus changes meanwhile minimizing the effect of blood pressure on albumin excretion, and the ratio "Overnight Alb Excretion Rate/Daily B2mGI Excretion" could inform us about the patients'blood pressure status.
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Key Words: NIDDM, Microalbuminuria, beta-2-microglobulin

In the last years, evidences suggested that preventing hyperglycemia can delay or even regress some of the microvascular complications of diabetes (1-4). Therefore the early diagnosis and determination of diabetic complications have become one of the most debated and important issues in diabetes. We know that hypertension aggravates diabetic retinopathy, nephropathy and peripheral vascular disease (3,5,6). It is difficult to make differential diagnosis between essential hypertension and hypertension secondary to diabetic glomerulopathy in non-insulin dependent diabetes mellitus (NIDDM) (3,5,7). While it has been proved that antihypertensive therapy can delay the progression of nephropathy, it will be logical to evaluate the possible effect of hypertension during determination of diabetic complications (3,4,5,8). Therefore we studied NIDDM patients, who were likely to have hypertension before diabetic retinopathy and nephropathy have developed.

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Increased glomerular filtration is the first finding of diabetic kidney. The last theory is that, the increased intraglomerular capillary pressure due to afferent vasodilatation, causes hyperfiltration, which is followed by the microalbuminuria period where the glomerular Alb excretion increases (8,9). Of the small quantity of Alb normally filtered at the glomerulus, 95-97% is reabsorbed nonselectively by the proximal tubule. The reabsorptive process is working at near maxima capacity, so that moderate increases in filtered proteins would be reflected in an elevation of its urinary excretion rate (10,11). Reports are indicating that there is no widening in the glomerular pore diameter, but the transglomerular pressure gradient is increased and the negative electrical charge of the basement membrane is lost in the early diabetic kidney (8,11,12,13). It has been demonstrated that, decreased density of Heparan Sulphate within the glomerular basement membrane leads to this charge loss (12). Initially, the electrical charge selectivity of the glomerular basement membrane decreases, then with the developing glomerulopathy the size selectivity also decreases. Albustix positive proteinuria (urinary proteins 500 mg/24 hrs) is termed as clinical

nephropathy and is considered as the irreversible stage. Many of these patients present with elevated blood pressure (7,8,11,14,15). The Alb excretion rate is normally less than 25 mg/24 hrs or 15 g/min, and hyperglycemia, hypertension, congestive heart failure, urinary tract infection or excessive exercise can effect this value (10,16). Certain levels of Alb excretion rate defined as "microalbuminuria" or "incipient diabetic nephropathy" range among 25 and 250 mg/24 hrs. Long term prospective studies have revealed that microalbuminuria is a high risk indicator for clinical nephropathy and proliferative retinopathy in insulin dependent diabetic (IDDM) patients and additionally for cardiovascular mortality in NIDDM patients (4,5,10-12,14,17,18). It has been shown that good blood glucose and blood pressure regulation, together with protein restriction can regress albuminuria during this period (2,10,13,16,19,20,21).

Urinary B2mGI excretion is a sensitive indicator of tubular reabsorptive capacity (10,11,18,22). Low molecular weight B2mGI is totally filtrated at the glomerulus and reabsorbed by the proximal tubule (11). "Tubular proteinuria" is characterized by large increases in B2mGI excretion with small changes in albumin (10,11). Normal urinary B2mGI excretion is less than 0.40 mg/24 hrs (11,15,21). It has been reported that B2mGI excretion is not affected by blood glucose levels unless extremely poor metabolic control and ketosis are present [11].

MATERIALS AND METHODS

The study group is consisted of patients with NIDDM who were between ages 35-55 and had duration of diabetes less than ten years. We excluded patients having manifest clinical proteinuria, urinary tract infection (WBC in urinary sediment), heart failure and age of diabetic onset under 30. Glucohaemoglobin levels, fasting and postprandial glucose measurements were used to exclude bad glycaemic control. These are known as factors effecting albuminuria (19). Blood pressures (BP) were measured in supine position after 30 minutes resting from each arm and mean values were calculated ("Mean BP"- diastolic BP +1/3 (systolic BP - diastolic BP)] (7). 24 hours urine and night-time urine (from bed time till morning) were collected. Urine Alb and B2mGI amounts were measured with Radio Immune Assay in duplicate (Pharmacia, Albumin RIA 100 and B2 MicroRIA). Parameters were calculated as:

"Daily*Alb Excretion"(mg/24hrs): Alb excreted during 24 hours

"Overnight Alb ER"(g/min): Alb amount excreted per minute during night-time (ER= excretion rate)

The values "Daily B2mGI Excretion" and "Overnight B2mGI ER" were calculated in the same way. We calculated the ratio "Overnight Alb ER / Daily B2mGI Excretion" by direct division of the parameters above. Creatinine clearance (ml/min) was calculated as - 24 hrs urine volume (ml) x Urine creatinine concentration (mg/dl) / 1440 (min) x Blood creatinine cocentration (mg/dl). The retinal assessment was performed by direct ophthalmoscopy and Fundus Fluorescein Angiography (FFA) by an ophthalmologist and classified in five grades [4]:

Grade	Ophthalmoscopic Findings:	FFA Findings:
0	Normal	Normal
1	Normal	3 or less microaneurysms
2	3 or less exudates	more than 3 microaneurysms or less than 3 ischemic lesions
3	more than 3 exudates or hemorrhagia	more than 3 ischemic lesions
4	Proliferative Retinopathy	

Data were analyzed by computer using the statistics program "Microstat" in the Statistics Department of Hacettepe University /Ankara.

RESULTS

Our study group consisting of five male and fifteen female had 47.9±7.5 years mean age and 4.2±3.1 years mean duration of diabetes. Mean GlucoHb was found 9.22±1.55%. Six patients were regulated with diet, nine with diet + oral hypoglycemic agent, three

Table 1. Comparison of patients having retinopathy with patients having none

	Fundus (+) n=9	Fundus (-) n=11	P
Duration of Diabetes (yrs)	5.0±3.1	3.7±3.1	>0.05
Mean BP (mmHg)	107.0+31.6	99.2±8.9	>0.05
Gluco Hb (%)	8.8+1.8	9.5±1.3	>0.05
Daily Alb Exc (mg/24hrs)	41.0±54.6	5.7±2.6	<0.05*
Overnight Alb ER (ng/min)	31.3±36.2	3.7±1.7	<0.02*
Daily B2mGI Exc (mg/24hrs)	0.317+0.147	0.265±0.103	>0.05
Overnight B2mGI ER (ng/min)	0.321+0.156	0.208±0.129	>0.05
Cr clearance (ml/min)	102.5+25.7	132.1 ±45.6	>0.05
Overnigt Alb ER/Daily B2mGI Exc	180.2+190.4	22.5İ21.5	<0.02*

•Significant correlations

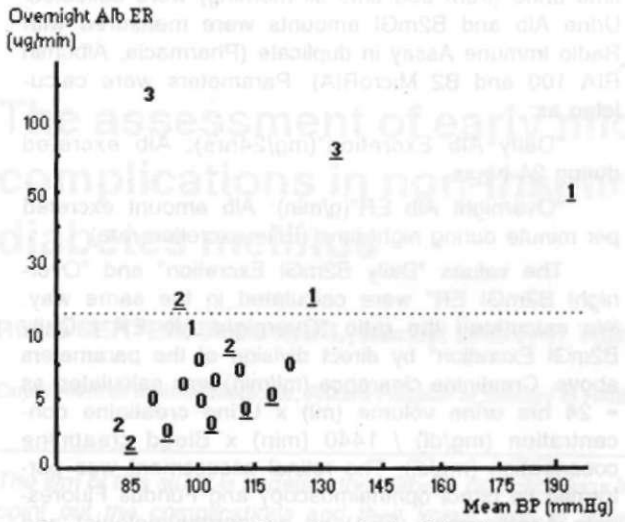

Figure 1. The dispersion of patients related to their mean blood pressures. Fundus findings and "Overnight Alb ER"

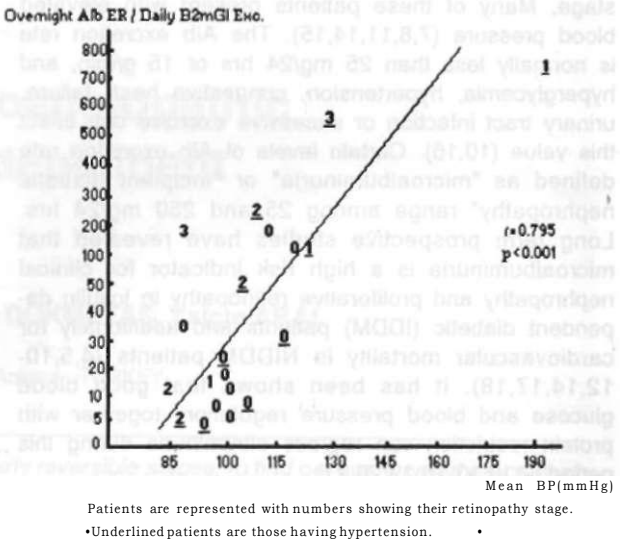

Figure 2. The relations between blood pressure. Fundus findings and the ratio "Overnight Alb ER / Daily B2mGl Exeration"

with diet + insulin, one with diet + oral hypoglycemic agent + insulin while one patient was out of treatment. Ten of our twenty patients were hypertensive with mean hypertension age 3.4±4.7 years, treated with various anti-hypertensive agents. The rest did not have any history of high blood pressure (BP). Four of the hypertensive group had high BP measurements at the application examination; The rest were regulated with anti-hypertensive treatment. Nine patients had retinopathy findings, three of them having grade 1, four of them grade 2 and two of them grade 3 fundus changes. None of our patients had proliferative retinopathy (Table 1 and Fig 1).

Five patients had increased Alb excretion and all of them had also retinopathy. Four of them were hypertensive (Fig 1). Six patients had increased B2mGl excretion. One of them had hypertension + retinopathy, two of them only retinopathy, another one only hypertension. The other two had neither hypertension nor retinopathy (Fig 3).

The increase in "Overnight Alb ER" was more significant than "Daily Alb Excretion" in the group having retinopathy. There was not any difference in B2mGl excretion between two groups. Patients with retinopathy had a significant, increase in the ratio "Overnight Alb ER / Daily B2mGl Exc". Although it was not statistically significant a clear decrease in creatinine clearance was noticed in the group having retinopathy (Table 1).

Six of the ten hypertensive patients had regulated blood pressure with various medical treatments (systolic 140, diastolic 90 mmHg). Although it was not statistically significant, "Daily Alb Excretion" and the ratio "Overnight Alb ER / Daily B2mGl Excretion" were found higher and creatinine clearance lower in the hypertensive group (Table 2).

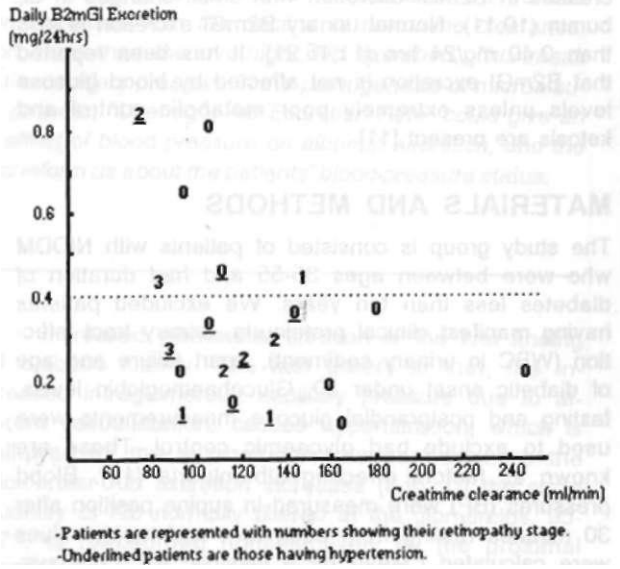

Figure 3. The dispersion of patients related to their fundus findings. Creatinine clearances and "Daily B2mGl excretion

The complicated group having hypertension + retinopathy is compared with the group uncomplicated in Table 3. The values, mean BP, "Daily Alb Excretion", "Overnight Alb ER" and "Overnight Alb ER / Daily B2mGl Excretion" were found significantly higher in the complicated group. The uncomplicated group had apparently higher creatinine clearance suggesting that they were in the hyperfiltration period. B2mGl amounts didn't show any difference between the two groups.

The correlation of the parameters to blood pressure and fundus findings are analyzed in Table 4. "Overnight Alb ER" showed the best correlation to fun-

dus findings having not significant correlation to BP. B2mGI had no correlation to both complications. Creatinine clearance values of patients having normal fundus findings were at the upper normal range while the clearance values declined with progression of the retinopathy (Table 1 and 4). The ratio "Overnight Alb ER / Daily B2mGI Excretion" correlated strongly to BP while the correlation was weak to fundus findings.

DISCUSSION

In our study, we included twenty patients having duration of diabetes less than 10 years to get them In the early stages of diabetic complications. Therefore only nine of them had retinopathy, and proliferative retinopathy wasn't encountered. It has been shown that in most diabetics proliferative retinopathy develops before clinical nephropathy, and microalbuminuria is a risk indicator for proliferative retinopathy (4,6,12). Most of these studies were performed with IDDM patients. It has been reported that, patients with normal Alb excretion (ug/min) have little risk for developing proliferative retinopathy in the following 10 years, and proposed to observe the patients having microalbuminuria closely for proliferative retinopathy [4]. "Overnight Alb ER" has shown the best correlation to retinopathy and we determined retinopathy in all the patients having "Overnight Alb ER" more than 7 g/min (Table 1 and 4, Fig 1). These findings reveal that retinal microaneurysms appear before microalbuminuria develops. Two patients with grade 2 retinopathy had little amounts of Alb excretion (Fig 1). We concluded that although being in the normal range (<l5ug/min), the patients having "Overnight Alb ER" over 7 ug/min are certain candidates for background retinopathy and retinopathy develops independently before initial glomerulopathy (Microalbuminuria). Vigstrup and Mogensen determined some patients having, microalbuminuria but not retinopathy, but they didn't use FFA to assess the fundus in their study (19). These patients might have microaneurysms not detected by ophtalmoscopic examination. As a conclusion we propose examination with FFA.

Alb excretion has more correlation to fundus findings than to BP (Table 1,2,4 and Fig 1). BP regulation can regress albuminuria in hypertensive patients (5,20,21). The smallness of the difference in albuminuria between groups in Table 2 can be explained by the six regulated patients included in the hypertensive group. Four of the five patients having microalbuminuria were hypertensive, three of them having high BP currently (Fig 1). That means three of the four unregulated patients had accelerated Alb excretion. The good correlation between albuminuria and diabetic retinopathy and the knowledge that high BP

Table 2. Comparison of patients having hypertension with normotensive patients

	Hypertensive n-10	Normotensive n-10	P
Duration of Diabetes (yrs)	3.1 ±2.1	5.5±3.4	>0.05
Mean BP (mmHg)	111.8±27.1	93.6±9.5	<0.05*
Gluco Hb (%)	9.1 ±1.5	9.3±1.6	>0.05
Daily Alb Exc (mg/24hrs)	33.6±54.0	9.8±1.6	>0.05
Overnight Alb ER (ug/min)	17.4±24.7	14.8±31.8	>0.05
Daily B2mGI Exc (mg/24hrs)	0.299±0.167	0.376±0.216	>0.05
Overnight B2mGI ER (fig/min)	0.240±0.169	0.278±0.134	>0.05
Cr clearance (ml/min)	104.4±22.0	132.9±49.5	>0.05
Overnight Alb ER/Daily B2mGI Exc	126.6± 180.8	60.3± 106.2	>0.05

'Significant correlations

Table 3. Comparison of hypertensive diabetics having retinopathy with normotensive diabetics having normal fundus findings

	Fundus (+) Hypertensive n-6	Fundus (-) Normotensive n-7	
Duration of Diabetes (yrs)	3.5±2.4	.4.4±3.5	>0.05
Mean BP (mmHg)	117.0±34.1	97.1 ±8.4	<0.05
Gluco Hb (%)	8.8±1.6	9.4±1.3	>0.05
Daily Alb Exc (mg/24hrs)	52.8±64.1	6.6±2.6	<0.05*
Overnight Alb ER (ug/min)	27.5±28.1	4.5±1.4	<0.02*
Daily B2mGI Exc (mg/24hrs)	0.285±0.193	0.380±0.253	>0.05
Overnight B2mGI ER (pg/pmin)	0.261 ±0.145	0.208±0.065	>0.05
Cr clearance (ml/min)	100.0±242	143.6±53.4	O.05
Overnight Alb ER/Daily B2mGI Exc	203.0±203.3	28.5±25.2	<0.02*

•Significant correlations

Table 4. The correlation of Parameters to retinopathy and blood pressure

	Fundus findings		Blood pressure	
	r	P	r	p
Daily Alb Exc (mg/24hrs)	0.622	<0.01	0.586	<0.01
Overnight Alb ER (ng/min)	0.700	<0.001		
Daity B2mGl Exc (mg/24hrs)		NS		NS
Overnight B2mGl ER fog/min)		NS		NS
Cr clearance (ml/min)	-0.454	<0.05		NS
Overnight Alb ER/Daily B2mGl Exc	0.389	<0.05	0.795	<0.001

(NS: not significant)

aggravates retinopathy (6) together support the observation that albuminuria is an important indicator for early diabetic microangiopathy also affected by BP.

Urinary B2mGI excretion, the indicator of renal tubular functions did not show any difference between groups in Table 1. This is parallel to the concept that tubular functions are prevented in early stages of diabetic nephropathy (9,13,15,23). Our findings as B2mGI excretion did not change in the hypertensive group supported the authors' opinion that mild or moderate hypertension does not effect renal tubular functions (5,20). Our findings do not confirm Scarpelli and colleagues' report claiming that every stage hypertension does accelerate B2mGI excretion (21). B2mGI excretions were found accelerated in six of our patients having no correlation to fundus findings or BP. Four of these six patients had declining glomerular filtration rates (Fig 3) which suggested similarity with the results of Viberti and colleagues (24). They reported that the urinary clearance of B2mGI increased during the decline of glomerular filtration rate. It is accepted that, when creatinine clearance falls under 40 ml/min, serum B2mGI levels increase leading to saturation of tubular reabsorption and resulting in accelerated urinary excretion (18). Further investigation is needed to clarify whether this status is the beginning of an increase in B2mGI excretion that will become overt with the decline in creatinine clearance or it is a result of some unknown factors.

Hypertension effected overnight Alb excretion relatively lesser than daily excretion (Table 2) and "Daily Alb excretion" was the only albuminuric parameter having positive correlation to BP (Table 4). These results suggest that high BP effects day-time Alb excretion apparently more than night-time. It is accepted that an increase in glomerular capillary pressure is taking place in the pathogenesis of increased albuminuria; therefore it is logical that high BP will effect Alb excretion during effort full day-time more than the resting period, as shown in our results (5). "Overnight Alb ER" is considered as the specific indicator for glomerular functions because it is not affected by factors like effort that accelerate albuminuria (5,7,11,21). Our findings confirm this consideration adding the point that the effect of BP on albuminuria which is due to the increase in glomerular capillary pressure can be partially excluded by using the measurement of "Overnight Alb ER".

The mean glomerular filtration rate was found increased in the uncomplicated group while the complicated group had declining values (Table 3 and Fig 3). In a research this value was found as 140 ml/min in early diabetics that is very similar to ours (16).

Teppo and Groop studied the ratio "urinary Alb / B2mGI" to differentiate diabetic proteinuria from non-diabetic, and found it elevated in diabetic proteinuria (15). We investigated this ratio for early diabetic complications and hypertension. The ratio "Overnight Alb ER / Daily B2mGI Excretion" had good positive correlation to BP while relatively weak correlation to fundus findings (Table 4). The increase of this parameter in the hypertensive group was small (Table 2). This can be explained by the six patients included in this group having regulated BP. As a conclusion, during assessment of early diabetic patients the parameters "Overnight Alb ER" informed us of fundus findings and "Overnight Alb ER / Daily B2mGI Excretion" of the patients' BP status.

insuline bağımlı olmayan diabètes mellitus'da erken mikroanjiopatik komplikasyonların değerlendirilmesi

Çalışmamızda diabetik komplikasyonları erken reverslbil dönemlerdeyken tespit edebilmeyi, bu komplikasyonları ve düzeylerini belirleyebilecek birtakım parametreleri ve hipertansiyonun bu komplikasyonlar ve parametrelere etkisini ortaya koymayı amaçladık. Renal glomerüler fonksiyonları idrar albumin atılımı ve kreatinin klirensiile, tübüler fonksiyonları idrarda beta-2 mikroglobulin atılımı ile, retinopatiyi ise fundus flurasein anjiografi ile değerlendirdik. Retinada mikroanevrizmaların, mikroalbuminüriden önce başladığını ve "Gece boyunca albumin atılım hızı" değeri 7 pg/dk'yı geçen diabetiklerin başlangıç retinopati için yüksek risk grubunu oluşturduğunu gözledik. Üriner beta-2- mikroglobulin atılımının arttığını düşündürmekteydi. Yüksek kan basıncının, gece süresince albumin atılımından ziyade gündüz süresince atılımı daha fazla etkilediğini belirledik. 3u bulgular mi-

roalbuminürinin patogenezinde glomerüler kapiller basıncın önemini savunan verileri destek-lemektedir. Erken diabetik hastaların değer-lendirilmesinde, "Gece boyunca albumin atılım hı-zı" ölçümünün kan basıncının albumin atılımına olan etkisini en aza indirgeyerek diabetik retina değişiklikleri hakkında fikir verebileceği, ayrıca "Gece boyunca albumin atılım hızı/Günlük beta-2 mikroglobulin atılımı" oranının hastaların kan ba-sıncı durumu hakkında bize bilgi verebileceği ka-naatine vardık.

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