Platelet Derived Growth Factor May Be A Diagnostic Marker in Patients with Diabetic Nephropathy

Platelet Kökenli Büyüme Faktörü Diyabetik Nefropatili Hastalarda Tanısal Bir Belirteç Olabilir

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ABSTRACT Objective: Microalbuminuria is still the most current diagnostic tools to detect diabetic nephropathy. However, this technique may not work in the very early stages of nephropathy. So detection of nephropathy in earlier stages will be more useful. This is why we need new diagnostic markers. Growth factors and cytokines are known to mediate the development of diabetic nephropathy. Existing study has been designed to investigate the usability of platelet derived growth factor (PDGF) as a diagnostic marker in the development of diabetic nephropathy in the earlier stages. **Material and Methods:** Twenty-three men, 40 women, total of 63 cases included in the study. Cases were diagnosed as type 2 diabetes mellitus (average duration: 6.4 ± 6 years, BMI: 27.17 ± 4:12 kg/m^2). The inclusion criteria included; less than 1 g/day of proteinuria, absence of hypertension and insulin treatment, creatinine level of less than 2.5 mg/ dL and absence of any cardiovascular disease. Cases divided into 4 groups based on urinary albumine levels; control (group 1, n= 10), normoalbuminuric (group 2, n= 19), microalbuminuric (group 3, n= 20) and macroalbuminuric (group 4, n= 14). Plasma levels of PDGF and serum biochemical profile were determined in all patients. **Resuls:** It is found that all diabetic groups have significantly higher PDGF levels ($p< 0.01$) compared with control groups. Between patient groups, PDGF-levels were similar, statisticaly. PDGF levels were found to be correlated with duration of the diabetes ($p< 0.023$), HbA1c ($p< 0.002$) and albuminuria ($p< 0.011$) in all groups. **Conclusion:** PDGF is correlated with diabetes duration, HbA1c levels and presence of albuminuria, so it can be used as a diagnostic marker in the prediction of diabetic complications.

Key Words: Platelet-derived growth factor, albuminuria, diabetic nephropathies, diabetic complications, proteinuria

ÖZET Amaç: Mikroalbuminüri, diyabetik nefropatinin saptanmasında halen en geçerli tanısal araçtır. Bununla beraber bu teknik çok erken evre nefropatilerde kullanışlı olmayabilir. Ancak daha erken evrede nefropatinin saptanması daha faydalı olacaktır. Bunu sağlayacak yeni tanısal belirteçlere ihtiyaç vardır. Sitokin ve büyüme faktörlerinin diyabetik nefropati gelişimine aracılık ettiği bilinmektedir. Bu çalışma diyabetik nefropati gelişiminde platelet kökenli büyüme faktörü (PDGF)'nün tanısal belirteç olarak kullanılabilirliğini araştırmak için tasarlandı. **Gereç ve Yöntemler:** Çalışmaya 23 erkek, 40 kadın olmak üzere toplan 63 olgu alındı. Hastalar Tip 2 diabet tanısı olan olgulardı (ortalama süre: 6.4 ± 6 yıl, BKİ: 27.17 ± 4.12 kg/m^2). Dahil edilme kriterleri olarak 1 g/gün'den az proteinüri, hipertansiyon ve insülin tedavisinin yokluğu, kreatinin seviyesinin 2.5 mg/ dl'den düşük olması ve bilinen bir kardiyovasküler hastalığın yokluğu kabul edildi. Olgular üriner albumin seviyelerine göre, kontrol (grup 1, n= 10), normoalbuminürik (grup 2, n= 19), mikroalbuminürik (grup 3; n= 20) ve makroalbuminürik (grup 4; n= 14) olmak üzere 4 gruba ayrıldı. Tüm olgularda plazma PDGF seviyesi ve serum biyokimyasal değerleri ölçüldü. **Bulgular:** Kontrol grubuyla kıyaslandığında tüm diyabetik gruplarda PDGF seviyesi anlamlı olarak yüksek bulunurken ($p< 0.01$) hasta grupları arasında PDGF seviyesi istatistiksel olarak benzer bulundu. PDGF seviyesi tüm gruplarda diyabetin süresi ($p< 0.023$), HbA1c ($p< 0.002$) ve albuminüri ($p< 0.011$) ile korele bulundu. **Sonuç:** PDGF, diyabetin süresi, HbA1c düzeyi ve albuminüri ile korele bulunduğu için diyabetik komplikasyon gelişiminde tanısal belirteç olarak kullanılabilir.

Anahtar Kelimeler: Platelet kökenli büyüme faktörü, albuminüri, diyabetik nefropati, diyabetik komplikasyonlar, proteinüri

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Diabetes mellitus (DM) is a systemic disease with high morbidity and mortality leading to multiorgan dysfunction. The clinical course and prognosis vary between severe morbidity in some patients and mild form in others. Inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interlokine (IL)-1 and IL-6; and growth factors such as PDGF, endothelium derived growth factor (EDGF), transforming growth factor- β (TGF-β), and insulin like growth factor (ILGF) are considered in the pathogenesis of diabetic complications.[1] Nevertheless the exact role of the growth factors in the pathophysiology of the disorder, whether they lead to or occur secondary to the disease process remains unknown.

PDGF could be secreted by different cells. But it usually secreted by vascular endothelial and smooth muscle cells and stored by platelets. Secretion of PDGF occur during platelet aggregation. It's half life is very short and it has autocrine and paracrine effect. Free active form of PDGF is not available in the serum.[2,3] So we think that circulating form of PDGF in the plasma has a clinical importance.

Clinical course of diabetic nephropathy is considerable in terms of prevention of the end stage renal failure. For the present, microalbuminuria is a common diagnostic factor in the evaluation of the nephropathy. But several histological alterations in the kidney proceed albuminuria among which increased glomerular filtration rate is known to be the earliest one. Renal size is increased depending on the thickness of the mesenchymal matrix. Negative electrical charge of the basal membrane is decreased due to glycosylation and mesenchymal thickening which leads to microalbuminuria. Interaction of growth factors and cytokines in the development of this process may have a diagnostic value with respect to detection of nephropathy earlier than detection of albuminuria.[4]

MATERIAL AND METHODS

The study was approved by the Committee for Ethics of Medical Experiment on Human Subjects, Faculty of Medicine. Submission of the individuals to the study was conditioned by an obtained written informed consent form. The study proceeded in agreement with the Helsinki Declaration approved on the World Medical Association meeting in Edinburgh. Patients with type II DM were included in the study based on the inclusion criteria including proteiuria less than 1g/day, having creatinine levels lower than 2.5 mg/dL, having DM as a sole factor underlying nephropathy, and absence of any known cardiovascular disorder. Patients were not receiving medication other than oral antidiabetics. Patients with severe atherosclerosis, atrophic kidney, and diabetic retinopathy were excluded.

Presence of acute and chronic diseases were excluded by physical examination, laboratory test and radiological examinations. To exclude reasons other than diabetic nephropathy (glomerulonephritis, IgA nephropathy, tubulo-interstitial nephritis, nephrotic syndrome); urine analysis and daily urinary protein excretion measurement were made. And also, renal size, contour and parenchimal echo were examined by ultrasonography and patients with chronic renal disease due to those investigations were exluded. Renal arterial resistance was evaluated in patients with doppler ultrasonography using 2.5-4 mHz convex probes to exclude the causes of secondary hypertension and nephropathy.

Controls were selected from otherwise healthy patients admitted to other departments for different reasons. A total of 63 subjects (mean age: 53.7 ± 8.4 years) composed of 23 males and 40 females were evaluated in the study. Average value for the body mass index (BMI) was 27.2 ± 4 kg/m^2. Patients were divided into four groups as control (group 1, n= 10), normoalbuminuric (group 2, albumin excretion <30 mg/day; n= 19), microalbuminuric (group 3, albumin excretion between 30-300 mg/day; n= 20), and macroalbuminuric (group 4, albumin excretion >300 mg/day; n= 14) patients according to urinary albumin levels.

Blood was drawn for the measurement of fasting blood glucose (FBG) (average of two distinct samples collected in the different times.), glycosylated hemoglobin HbA1c, PDGF, and high sensitivity C reactive protein (hsCRP) levels. Samples (5

mL) were centrifuged at 3000 rpm for 5 min to obtain plasma samples and were kept at -40°C for the measurement of PDGF levels using human PDGF-AA kit (Quantakine R&D Systems USA) with micro- ELISA method. Plasma of blood samples were separated when blood samples were taken in heparinised tubes. The measured amount reflects free and secreted amount of PDGF during procedure.

Statistical analysis of the data was made using SPSS (Statistical Package for Social Sciences) by Biostatistics Department at University Faculty of Medicine. Groups were compared using Mann-Whitney U test. Non parametric Spearman's correlation test was used to determine the correlation between groups. Data are shown as mean ± SD.

RESULTS

Age, height, weight and BMI scores were similar between four different groups (Table 1). Fasting blood glucose and HbA1c levels in the diabetic groups were also similar statistically. Serum creatinine levels in group 4 was found to be significantly higher compared to corresponding levels of other groups (p< 0.01, Table 1). Group 2 and group 3 were similar to control group in terms of serum creatinine levels. There was a marked increase in daily urinary albumin excretion in all diabetic groups, especially in group 3 and group 4 patients when compared to control group (p< 0.045, p< 0.009 and p< 0.008, respectively, Table 1). In addition to this, albuminuria was found to be significantly higher in group 3 and 4 when compared to levels detected in group 2 patients (p< 0.004, p< 0.005, respectively, Table 1). Urinary albumin levels in group 4 were markedly higher than those in group 3 patients (p< 0.003). hsCRP level in group 4 was significantly higher than both control and group 2 levels (p< 0.006 and p< 0.004, respectively, Table 1).

TABLE 1: Comparision of PDGF and other findings between diabetic group and control groups.

	Control (n= 10)	Group 2 (n= 19)	Group 3 (n= 20)	Group 4 (n= 14)
Age (years)	51.0 ± 3.8	52.8 ± 8.7	53.9 ± 9.0	56.5 ± 9.2
BMI (kg/m²)	27.3 ± 2.8	27.8 ± 5.1	26.6 ± 3.4	26.8 ± 3.8
Duration of DM (y)	0.0	5.8 ± 4.9	7.0 ± 5.4	10.9 ± 6.5[b]
FBG (mg/dL)	99.9 ± 12.2	212.9 ± 79.6 [aa]	213.8 ± 97.1[aa]	167.7 ± 65.2[aa]
HbA1c (%)	4.7 ± 0.9	8.6 ± 2.4 [aa]	9.9 ± 3.6 [aa]	9.0 ± 2.2[aa]
Cr (mg/dL)	0.6 ± 0.3	0.9 ± 0.7	0.8 ± 0.4	1.4 ± 0.6 [aa,bb]
Uri Alb(mg/day)	5.0 ± 4.8	9.1 ± 4.6 [aa]	48.0 ± 11.9 [aa,bb]	474.3 ± 245.6[aa,bb]
hsCRP (mg/dL)	5.6 ± 3.3	6.6 ± 4.9	12.2 ± 14.5	45.7 ± 55.2 [aa,bb]
PDGF (pg/mL)	293.2 ± 187.4	521.8 ± 165.9[aa]	711.1 ± 331.5[aa]	632.6 ± 276.4[aa]

a= p< 0.05 , aa= p< 0.01, b= p< 0.05, bb= p< 0.01, FBG: Fasting blood glucose, Uri Alb: Urinary albumin, Cr: Creatinine (a: significance level between control cases and diabetic groups. b: significance level between normoalbuminic group, and group 3 and 4).

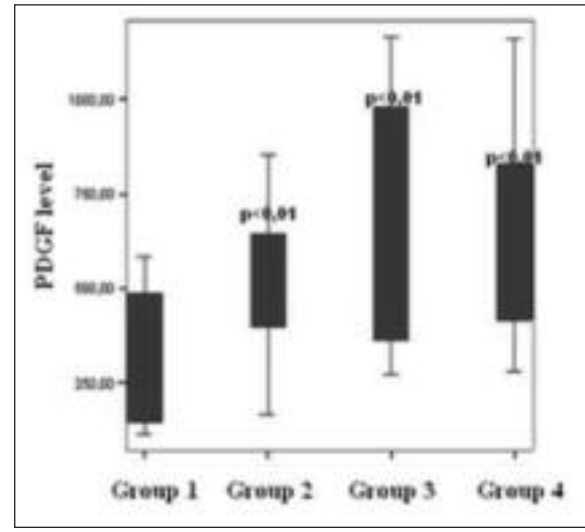

FIGURE 1: The increase of PDGF leves in all diabetic groups due to control groups.

While PDGF was found to be elevated significantly in all diabetic patients when compared to control levels (p< 0.01 for each; Table 1, Figure 1), patient groups were similar statistically in terms of PDGF levels. Duration of the diabetes mellitus was found to be correlated positively with PDGF (p< 0.01; Figure 2), albuminuria (p< 0.01), creatinine (p< 0.01), (Table 2). PDGF was also correlated significantly with albuminuria (p< 0.05), and HbA1c (p<0.01; Figure 3), FBG (p< 0.01), (Table 2). Albuminuria was positively correlated with levels of hsCRP (p< 0.01), Cr (p< 0.01), HbA1c (p< 0.05). Creatinine levels on the other hand was found to be positively correlated with hsCRP (p< 0.05, Table 2).

DISCUSSION

Glomerular mesenchymal, parietal epithelial, cortical and medullary interstitial cells include recep-

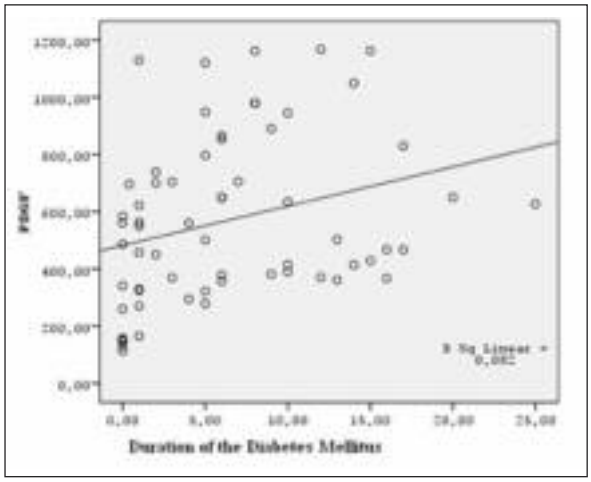

FIGURE 2: The correlation between PDGF level and duration of diabetes mellitus.

TABLE 2: Significant correlation of PDGF, and urinary albumin, duration of diabetes, HbA1c, FBG.

	PDGF (pg/dL)	Urinary albumin (mg/day)	Creatinine (mg/dL)
DM duration (years)	0.388**	0.548**	0.518**
Uri Alb (mg/day)	0.322*	-	0.443**
hsCRP (mg/dL)	0.139	0.424**	0.250*
PDGF (pg/mL)	-	0.322*	0.029
HbA1c (%)	0.408**	0.315*	0.228
FBG (mg/dL)	0.385**	0.126	0.222

*. p< 0.05, **p< 0.01

tors for PDGF which is one of the growth factors synthesized in the kidney. Also PDGF secreted by vascular endothelial and smooth muscle cells and stored by platelets.[2] PDGF and PDGF receptor mRNA gene expression was shown to be increased in immune and non-immune disorders such as diabetic nephropathy.[5-12] This factor is responsible for mesenchymal proliferation and extracellular matrix accumulation by means of mitogenic and chemotactic effects on the fibroblasts. It also causes renal vasoconstriction secondary to inhibitory effects on nitric oxide synthesis. In addition to this, PDGF increases TGF-α secretion from proximal tubular cells, initiates tubulointerstitial inflammation via triggering neutrophil and mononuclear chemotaxis.[6,12] Hyperglycemia and shear stress were shown to be responsible for increased release of PDGF from glomerular matrix and renal tubular cells.[13] Besides being a multifunctional peptide, PDGF is known to be stimulated by various factors. Increased release of PDGF secondary to stimulation by angiotensin II was reported by Amono et al. in experimental animal models.[14] In another experimental model by Fraser et al. prolonged exposure of proximal tubule epithelial cells to glucose was shown to be related with increased PDGF levels.[15] Finding of significant correlation between PDGF and HbA1c levels in the present study seems to indicate the stimulatory effect of hyperglycemia on the PDGF release.

While proteinuria is accepted to be compatible with the early stage of the nephropathy, glomerular structural alterations have been evident in the biopsies. By the time structural alterations develop in the kidney, an inflammatory process including the increase of cytokines and growth factors also begins. Detection of elevated PDGF levels even in the normoalbuminuric patients is considerable as it may indicate the future microalbuminuria. In our study serum PDGF levels were found to be increased in all diabetic patients when compared to control subjects. Uehara et al showed that level of PDGF receptors in laboratory animals is higher in early diabetic nephropathy group than advanced diabetic nephropathy group.[16] Nakagawa et al also indicated that PDGF and PDGFR-proteins were

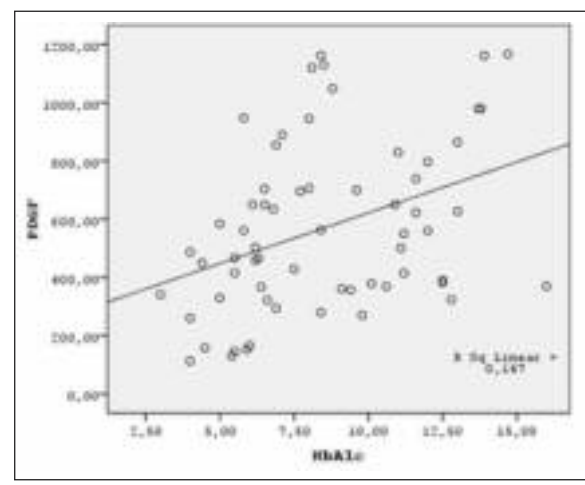

FIGURE 3: Increase of HbA1c and PDGF levels has significantly correlation.

expressed in early glomerular lesions of streptozotocin-induced diabetes in rats.[17]

Prominent increase of PDGF correlated with albuminuria in diabetic patients may indicate the presence of structural alterations in the kidney at the earlier stages of the disease and the role of PDGF in the maintenance of alterations during the progression of the disease. Compatible with the idea considering the role of hyperglycemia and glycosylated proteins in the elevation of PDGF levels, HbA1c levels were found to be higher in all patients when compared to control subjects.

The finding of the correlations between albuminuria and PDGF and hsCRP levels demonstrates the inflammation accompanying albuminuria. In a large scale study by Schalkwijk et al, diabetic patients with high hsCRP and fibrinogen levels were reported to have higher incidence of the microalbuminuria.[18] These findings demonstrate the influence of growth factors and cytokines not only on development but also maintenance of albuminuria.[19]

Hyperglycemia is considered to be responsible for the maintenance of ongoing inflammatory response in the diabetic patients. In a study by Saraheimo et al hsCRP levels were reported to be higher in micro and macroalbuminuria when compared to normoalbuminuria cases. Moreover, the significant positive correlation between albumin excretion rate and hsCRP levels was also demonstrated reflecting the presence of low response inflammatory process underlying the diabetic nephropathy.[20,21] In our study, although detected to have a tendency towards an increase in all diabetic groups, hsCRP was found to be increased significantly only in patients with microalbuminuria. Another correlation determined by us between HbA1c and hsCRP displays the role of hyperglycemia in the maintenance of the underlying inflammatory process. Furthermore our finding of positive correlation between albuminuria and serum creatinine renders microalbuminuria as an important tool in the determination of nephropathy progression. However it is not helpful in determination of the future risk for the nephropathy development. Therefore, it can not be used as an early marker.

In conclusion, PDGF and hsCRP seem to be elevated in case of hyperglycemia which at the same time has a significant contribution to development of diabetic nephropathy. Determination of high levels of these markers in diabetic patients having no microalbuminuria displays the close follow up requirement of them. PDGF is important markers of the diabetic complications especially in the case of diabetic nephropathy. This study is a pilot study which focuses upon interaction of plasma PDGF levels and diabetic nephropathy. We think that it is helpful to confirm this study with larger patients series.

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