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Plasma von Willebrand Factor (vWF) Levels Correlate with Soluble Interleukin-2 Receptor (SİL-2R) Levels in Type 2 Diabetes Mellitus Patients with Retinopathy

RETİNOPATİSİ OLAN TİP 2 DIABETES MELLİTUSLU HASTALARDA PLAZMA von WILLEBRAND FAKTÖR VE SOLUBLE İNTERLEUKİN-2 RESEPTÖR DÜZEYLERİ ARASINDAKİ İLİŞKİ

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Summary

The aim of this study was to investigate the possible relationship between plasma vfVF (endothelial injury) and sIL-2R (mononuclear cell activation) levels in type 2 diabetes mellitus patients with retinopathy.

Twenty-four type 2 diabetes mellitus patients [14 women, 10 men; age 52±1I years; duration of diabetes 7±5 years; 9 with background retinopathy (R+) and 15 without retinopathy (R-)J and 18 healthy subjects (7 women, 11 men; age 45±11 years) were included in the study. Both R+ and R- diabetic patients had poor but comparable metabolic control (HbAlc 12.1±2.0% vs 12.5±3.1%; p>0.05). Plasma levels ofvWFand sIL-2R were measured by EIA and pairwise comparisons of the groups and multivariate stepwise regression analysis with respect to these parameters were performed.

Both sIL-2R [49.3±27.0 pM vs 50±27.8 pM] and vWF levels [U9.6±16.8% vs 104.8±19.7%)] were comparable in R+ and R- diabetic patients. R+ patients had significantly higher plasma vWF levels than the control group [101±18% (p=0.027)J. No significant differences were found in plasma sIL-2R levels of diabetic patients and healthy controls [63±42.3 pMJ. vWF levels correlated with sIL-2R levels only in R+ diabetic patients.

Our preliminary findings suggest that mononuclear cells might be involved in the pathogenesis of microvascular injury in diabetic retinopathy.

Key Words: Diabetic retinopathy, Mononuclear cell activation, vWF, sIL-2R

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Özet

Çalışmamızın amacı retinopatisi olan tip 2 diabetes mellituslu hastalarda endotel hücre hasarının göstergesi olan Von Willebrand Faktör (vWF) ve mononükleer hücre aktivasyonunun göstergesi olan soluble interleukin-2 reseptör (sIL-2R) düzeyleri arasındaki ilişkiyi araştırmaktı. Bu amaçla yirmi dört tip 2 diabetes mellituslu hasta [14 kadın, 10 erkek; yaş 52±11, diabet süresi 7±5 yıl, 9 hastada background retinopati tespit edildi (R+), 15 hastada yoktu (R-)J ve 18 sağlıklı erişkin (7 kadın, 11 erkek; yaş 45±11) çalışmaya alındı. R+ ve R- grupların HbAlc düzeyleri arasında fark yoktu (12.1±2.0% vs 12.5±3.1%; p>0.05). Plasma vWF ve sIL-2R düzeyleri EİA ile ölçüldü.

R+ ve R- diabetik hasta gruplarında plasma sIL-2R [49.3±27.0 pM vs 50±27.8 pMJ ve vWF düzeyleri [119.6±16.8% vs 104.8±19.7%)j arasında anlamlı bir fark tespit edilmedi. R+ hasta grubunda plasma vWF düzeyleri kontrol grubuna göre anlamlı olarak yüksek bulundu [101±18% (p=0.027)j. Diabetik hastalarla kontrol grubunun sIL-2R düzeyleri arasında da fark bulunmadı [63±42.3 pMJ. R+ hasta grubunda vWF ve sIL-2R düzeyleri arasında korelasyon tespit edildi.

Bu bulgular diabetik retinopatide görülen mikrovasküler hasarın patogenezinde mononükleer hücrelerin rol oynayabileceğini düşündürmektedir.

Anahtar Kelimeler: Diabetik retinopati, Mononükleer hücre aktivasyonu, vWF, sIL-2R

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Inflammatory cell-induced capillary closure may be an early event in diabetic retinopathy. Inflammatory cells, particularly macrophages, might play a central role in the development of pathological changes in this condition (1). It has re-

cently been shown that mononuclear cell activation and alteration of the adhesiveness between circulating monocytes and capillary endothelial cells may be associated with diabetic microvascular injury (2). Soluble interleukin-2 receptor (sIL-2R) is the circulating form of IL-2R and increased plasma levels reflect activation of mononuclear cells, particularly T-cells (3), but also B-cells (4), monocytes and macrophages (5). Elevated levels have been reported in hematological malignancies and a variety of diseases associated with aberrant immune activation (6).

von Willebrand factor (vWF) is currently considered to be one of the best marker of endothelial cell injury or dysfunction (7) and increased plasma levels have been reported in diabetic patients with retinopathy (8).

In the light of these, we decided to investigate the possible relationship between plasma sIL-2R and vWF levels in diabetic patients with retinopathy.

Materials and Methods

Study Population

Twenty-four type 2 diabetes mellitus patients [14 women and 10 men, age 52±11 years, duration of diabetes 7±5 years] with poor metabolic control [HbA1c 12.3±2.7%] were included in the study. The patients had no clinical evidence of recent infections and were not suffering from a neoplastic disease or an immunological disorder. Nine patients had background retinopathy (R+) and 15 had no retinopathy (R-) which was assessed by fundoscopic examination. The duration of diabetes was 12±5 years in R+ and 5±3 years in R- diabetic patients ($p<0.05$). Both R+ and R- diabetic patients had poor but comparable metabolic control as evidenced by high HbA1c levels [HbA1c 12.1±2.0% vs 12.5±3.1 %; $p>0.05$). Seventeen patients were on oral antidiabetics and 7 on insulin therapy. The control group was consisted of 18 age- and sex-matched healthy subjects [7 women and 11 men, age 45±11 years]. All subjects gave informed consent to participate in the study.

Blood Collection

Blood samples were withdrawn into plastic tubes containing 1 volume trisodium citrate and 9 volumes of blood, after an overnight fasting between 8 and 9 a.m. in order to overcome the confounding effect of circadian variation of plasma sIL-2R levels (9). All blood samples were immediately placed in ice bath, centrifuged at 2500 g at 4° C for 15 minutes and plasma aliquots were stored at - 70° C until analysis.

Laboratory Methods *

Plasma levels of sIL-2R (sIL-2R Immunoenzymometric Assay Kit, Immunotech S.A., France) and vWF (Asserachrom vWF, Diagnostica Stago, Asnieres, France) were determined quantitatively by enzyme immunoassay technique (10-12). [Monoclonal antibodies directed against IL-2R and vWF were used. Blood samples were incubated in the wells of a microtiter plate coated with anti-sIL-2R and anti-human vWF monoclonal antibody, in the presence of a second monoclonal antibody conjugated with alkaline phosphatase. The amount of bound enzyme-conjugate was measured by adding a chromogenic substrate. The intensity of the resulting colour was proportional to the concentration present in the samples].

All determinations were performed in duplicate. Plasma samples of R+ and R- diabetic patients and healthy subjects were run in parallel. The procedures outlined by the manufacturers were meticulously followed.

Statistical Analysis

Deviation from normal distribution was tested using the Kolmogrov-Smirnov one sample test. Since data were normally distributed, comparisons of groups were performed by using unpaired t test. Pearson's coefficient of correlation was used to study the correlation between vWF as dependent and age, duration of diabetes, HbA1c and sIL-2R as predictor variables. A p value below 0.05 was considered statistically significant. Data in the text are shown as mean ±SD.

Results

Plasma vWF and sIL-2R levels of diabetic patients and the control group are presented in figure 1. Both sIL-2R [49.3±27.0 pM vs 50±27.8 pM] and vWF levels [119.6±16.8% vs 104.8±19.7%] were comparable in R+ and R- diabetic patients ($p>0.005$). R+ patients had significantly higher plasma vWF levels than the control group

[101±18% (p=0.027)]. No significant differences were found in plasma sIL-2R levels of diabetic patients and healthy controls [49.3±27 pM]. vWF levels correlated with sIL-2R levels only in R+ diabetic patients and multiple stepwise regression analysis of vWF on age, duration of diabetes, HbA1c, and sIL-2R revealed that the sIL-2R was the sole predictor of vWF level (R=0.82, p=0.007) (Figure 2).

Discussion

Although a gold standard marker for endothelial cell injury is currently not available (7), in vitro and in vivo data suggest that vWF, a high molecular weight procoagulant product of the endothelium (13), is released into the circulation when endothelial cells are damaged (14). Increased plasma vWF levels have been reported in many clinical situations where endothelial cell damage is suspected, such as inflammatory and atherosclerotic vascular disease (15). Likewise, elevated plasma vWF levels in R+ diabetic patients have been reported (8), reflecting probably actual endothelial cell membrane damage rather than activation of the endothelium by cytokines (7).

Concordant with previous reports, we found significantly higher plasma vWF levels in R+ diabetic patients than the control group, suggesting the release of vWF from the endothelial cell. Furthermore, there was a significant positive correlation between vWF and sIL-2R levels only in R+ diabetic patients, which suggests an association between mononuclear cells and endothelial cell injury. Other factors such as HbA1c, duration of diabetes and age of the patients that may all affect vWF levels, were not found to have significant influence on this correlation. Elevated sIL-2R levels have been reported to reflect mononuclear cell activation including T-cells, B-cells, monocytes and macrophages (3-5). Direct evidence for the role of macrophages in the pathogenesis of diabetic retinopathy has recently been described in alloxan-induced diabetic rats (2). In that study, retinal capillary closure was shown to be the result of small vessel occlusion by monocytic and macrophage-like cells. Our finding may be a further evidence for such an association.

There could be several explanations for a positive correlation of plasma sIL-2R with vWF, but

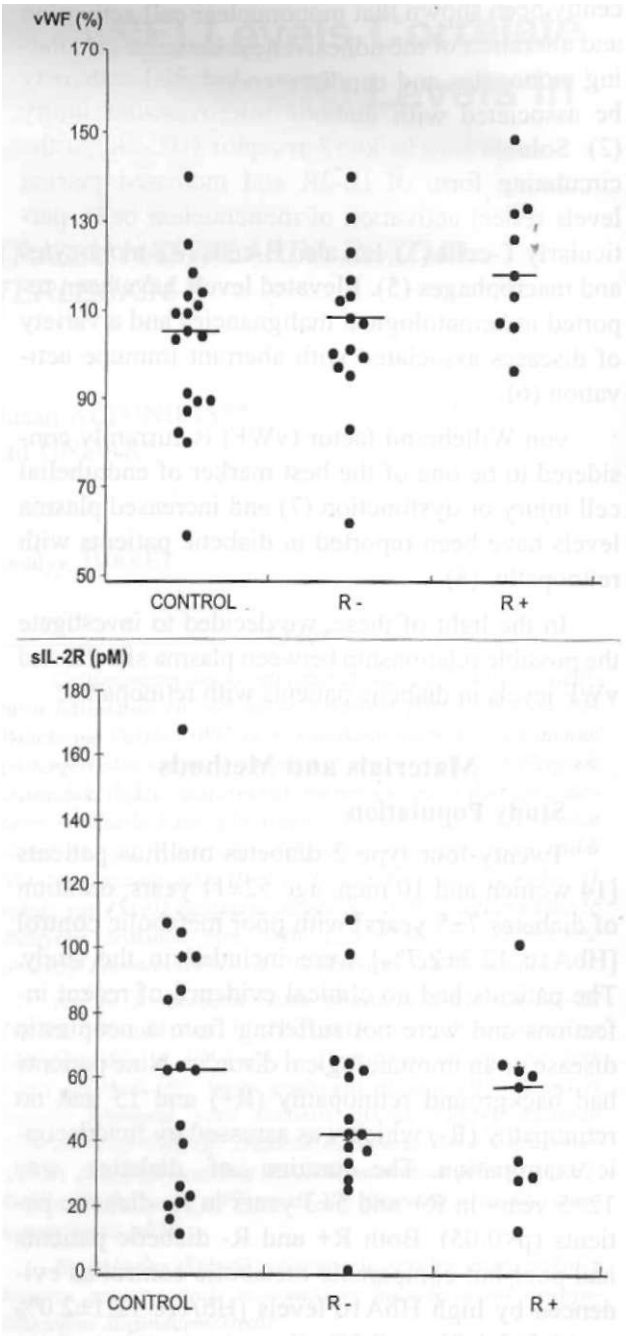

Figure 1. Plasma vWF and sIL-2R levels in the control group and R-, R+ diabetic patients.

no increase in sIL-2R in R+ diabetic patients. Since plasma sIL-2R is elevated predominantly as a result of activation of T-cells, activation of monocytes and macrophages alone may not be sufficient to increase this parameter. Furthermore, activated monocytes and macrophages may lose the IL-2R after a few days (no increase in plasma sIL-2R lev-

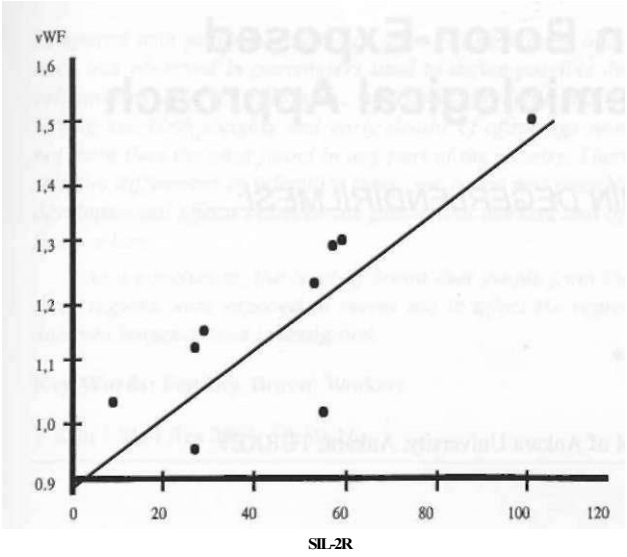

Figure 2. Correlation of plasma vWF and sIL-2R levels in R+ diabetic patients.

els), but retain other signs of activation and secrete growth factors and cytokines that may be responsible for endothelial cell activation and/or injury (increase in vWF).

In our study, we found comparable plasma vWF levels in R- diabetic patients and the control group. Moreover, there was no correlation between plasma vWF and sIL-2R levels in R- diabetic patients. Therefore, we suggest that fundoscopically undetectable retinopathy is not associated with mononuclear cell activation as detected by sIL-2R levels. On the other hand, it has been reported that hyperglycemia might cause activation of mononuclear cells per se (2). However, our results do not confirm this, because we found comparable plasma sIL-2R levels in diabetic patients with poor metabolic control and healthy subjects. Since our R+ and R- diabetic patients had similar poor metabolic control, it can be speculated that any effect of hyperglycemia on plasma sIL-2R should be comparable in both groups.

In conclusion, our preliminary findings suggest that mononuclear cells might be involved in the pathogenesis of microvascular injury in diabetic retinopathy. We think that further studies are needed to clarify the nature of the relation between plasma sIL-2R and vWF levels and the clinical implications of such an association.

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