Circulating von Willebrand Factor Levels in Patients with Prediabetes

Prediyabetli Olgularda von Willebrand Faktör Düzeyleri

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Geliş Tarihi/*Received:* 17.07.2008
Kabul Tarihi/*Accepted:* 12.09.2008

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ABSTRACT Objective: Impaired glucose tolerance (IGT) is a well established risk factor for cardiovascular diseases. However there is not clear evidence to show that the same is true for impaired fasting glucose (IFG). von Willebrand factor (vWF) is an important marker for endothelial dysfunction. The vWF levels are elevated in all well known risk factors for cardiovascular diseases. The aim of this study is to search for any elevation of vWF levels in subjects with IFG and IGT and to compare them with the levels of the healthy volunteers. **Material and Methods:** Ninety five prediabetics without any confounders; IFG (n= 47, mean age= 46.55 ± 6.6, M/F= 28/19), IGT (n= 48, mean age= 47.3 ± 6.9, M/F= 28/20) and 58 healthy volunteers (mean age= 44.5 ± 9.8; F/M= 25/33), matched for age, body mass index and gender were enrolled. Morning venous samples were collected after 12 hours of fasting. Fasting blood glucose, insulin, lipid and vWF levels were studied. Insulin resistance state was estimated by using HOMA test. **Results:** All groups were similar in terms of age, body mass index and gender. The FPG (p< 0.001), insulin (p= 0.003), Homeostasis Model Assesment (HOMA) (p< 0.001), triglycerides (p= 0.003) and vWF levels (p= 0.03) were significantly higher when compared to those of the control subjects. No significant difference was observed regarding the above parameters in subjects with IFG and IGT. **Conclusion:** The high vWF levels in subjects with IFG imply the presence of endothelial dysfunction even in the very early stage of prediabetes. Regarding that there was no significant difference between IFG and IGT, it can be concluded that endothelial dysfunction is present even in patients with IFG. In conclusion, IFG should not be undervalued as a cardiovascular risk factor.

Key Words: Prediabetic state; von Willebrand factor; endothelium; glucose intolerance

ÖZET Amaç: Bozulmuş glukoz toleransı (BGT) bulunan hastalarda kardiyovasküler olay riskinin arttığı bilinmektedir. Ancak bozulmuş açlık glukozu (BAG) ile kardiyovasküler olay ilişkisi net olarak gösterilememiştir. von Willebrand faktörü (vWF) endotel disfonksiyonunun çok iyi bir göstergesidir. Kardiyovasküler hastalıklar için risk faktörü olan durumlarda vWF düzeyleri yükselmektedir. Bu çalışmanın amacı, bir erken kardiyovasküler risk belirteci olan vWF düzeyinin BAG ve BGT olgularında yüksek olup olmadığını araştırmak ve sağlıklı gönüllülerle karşılaştırmaktır. **Gereç ve Yöntemler:** Çalışmaya, eşlik eden bir risk faktörü bulunmayan 95 prediyabetli olgu [BAG (n= 47, ortalama yaş= 46.55 ± 6.6, E/K= 28/19), BGT (n= 48, ortalama yaş= 47.3 ± 6.9, E/K= 28/20)] ile yaş, beden kitle indeksi ve cinsiyet olarak uyumlu 58 sağlıklı gönüllü (yaş= 44.5 ± 9.8; E/K= 25/33) dahil edildi. On iki saatlik açlık sonrası alınan venöz kan örneklerinde açlık kan şekeri (AKŞ), insülin ve lipid profilleri ile birlikte vWF düzeyleri çalışıldı. İnsülin direnci HOMA formülü ile hesaplandı. **Bulgular:** Tüm gruplarda yaş, cinsiyet ve arteryel kan basıncı düzeyleri benzerdi. BAG ve BGT olgularında AKŞ (p< 0.001), insülin (p= 0.003), HOMA (p< 0.001), trigliserit (p= 0.003) ve vWF düzeyleri (p= 0.03) kontrollere göre belirgin olarak yüksekti. BAG ve BGT olgularında yukarıdaki faktörler açısından farklılık saptanmadı. **Sonuç:** BAG olgularında da vWF düzeylerinin yüksek ölçülmüş olması bize prediyabetin bu erken döneminde bile endotel disfonksiyonunun bulunduğunu göstermektedir. BAG ve BGT olguları arasında vWF düzeyleri açısından fark olmaması BAG nun da en az BGT kadar önemli bir risk faktörü olarak üzerinde durulması gerektiğini düşündürmektedir.

Anahtar Kelimeler: Prediyabet: von willebrand faktör; endotel; glukoz intoleransı

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Turkiye Klinikleri J Med Sci 2008;28(6):822-7

The relationship between diabetes and cardiovascular disease begins earlier in the progression from normal glucose tolerance to type 2 diabetes mellitus (T2DM), and is associated with resistance to the biologic activity of insulin.[1-3] Prediabetes, the state of high blood glucose that has not yet reached to the levels for T2DM, is a prevalent condition of cardiovascular risk in Turkey.[4] It has two entities, IGT and IFG. Although both IGT and IFG are associated with insulin resistance and increased risk of developing T2DM, two measures do not seem to define the same population.[5] The increased risk of cardiovascular disease is well established in IGT while the same is not that clear for patients with IFG.[6-8] The predictive value of IFG for clinical events is reported to be lower than IGT.[9] Although the increased cardiovascular risk in prediabetic subjects is mainly attributed to hyperglycemia, insulin resistance and other associated cardiovascular risk factors, the mechanisms involved in the development of a proinflammatory and prothrombotic state in prediabetes are not well established.[3,5] Moreover, there is data indicating that the increased cardivoascular risk in prediabetes is independent from the inflamatory products of adipose tissue and the insulin resistance itself.[10] This suggests that increased CVD-risk associated with prediabetes is also mediated by factors other than the established markers of inflammation and insulin resistance.

vWF is a multimeric glycoprotein which mediates platelet aggregation and adhesion and is produced almost exclusively by endothelial cells.[11] Plasma levels of vWF are raised in different states of endothelial damage, therefore, it is a useful marker for endothelial dysfunction.[12] All established cardiovascular risk factors including ageing, smoking, hypertension and dyslipidemia are associated with high vWF levels.[8,13,14] Patients with T2DM also have increased vWF levels.[8] In addition, elevated plasma vWF levels of come before and is a significant determinant of new diabetes, independent of other major diabetes risk factors such as age, physical inactivity, obesity, lipid and blood pressure abnormalities and IFG or IGT.[15]

Despite the role of vWF in the development of T2DM and its vascular complications, there is hardly enough data about the circulating vWF levels in subjects with prediabetes. A study performed in the elderly population reported high vWF levels in patients with IGT while there is so far no data in patients with IFG.[16] Regarding the previous data about the probability of increased CVD risk in association with the vWF levels, we aimed to search for any relationship of vWF levels with the prediabetic states, IFG and IGT, in subjects free of confounders including medications, metabolic diseases and any other chronic disorder accompanied with inflammation.

MATERIAL AND METHODS

SUBJECTS

The subjects for this study were selected among the patients who were referred for routine annual screening to the Gülhane Outpatient Clinics of Internal Medicine and whose current medical situations necessitated oral glucose tolerance test (OGTT). According to the results of glucose tolerance test, a total number of 95 prediabetic patients (mean age= 47.1 ± 6.9 years; M/F= 56/39) were allocated as IFG (n= 47, mean age= 46.55 ± 6.6, M/F= 28/19) or IGT (n= 48, mean age= 47.3 ± 6.9, M/F= 28/20), consistent with the criteria of the American Diabetes Association.[17] All the patients had normal blood pressures without any evidence of renal, hepatic, endocrine or allergic diseases.[18] Also, they were not taking any medicine including over the counters. The subjects standing height and body weight were measured in light indoor clothes without shoes. Body mass index (BMI) was calculated as weight divided by squared height (kg/m^2).

Fifty eight healthy volunteers (mean age= 44.5 ± 9.8 years; M/F= 25/33) were enrolled as the control group. Age, BMI and sex distribution of the controls were similar to those of the patients. Routine physical and laboratory evaluations were done to ascertain that they had no acute or chronic systemic diseases. The purpose and design of the study were explained to all patients and controls and informed consents were obtained. The local ethic committee of Gülhane Medical School approved the study protocol.

LABORATORY ANALYSES

OGTT was performed after 10–12 h of overnight fasting by ingesting 75 g of oral glucose load over a 2-min period, and obtaining blood samples at baseline and 2 h after glucose load for plasma glucose measurements. The patients were instructed not to restrict carbohydrate intake in the week before the test. No patient had any acute infection or stress during or before the procedure. The test was performed onetime to all the patients and the ones who were eligible for the study and who gave consent were enrolled for further procedures.

The blood samples were collected between 08:00 and 08:30 A.M. after a 12 h fasting. The tubes were promptly centrifuged, and the plasma was separated and stored at -80°C. All plasma samples were run in the same assay. Glucose, total cholesterol, high density lipoprotein (HDL)-cholesterol and triglyceride (TG) levels were measured by the enzymatic colorimetric method with Olympus AU 600 auto analyzer using reagents from Olympus Diagnostics, (GmbH, Hamburg, Germany). Low density lipoprotein (LDL)-cholesterol was calculated by Fridewald's formula.[19] Serum basal insulin level was determined in duplicate by the coated tube method (DPC-USA). vWF level was measured using a commercially available enzyme-linked immunosorbent assay kit (Technoclone, Surrey UK). All assays were performed in duplicate. Insulin sensitivity was determined by Homeostasis Model Assessment Model (HOMA) index with formula: HOMA-IR= Fasting insulin (μU/mL) x Fasting glucose (mg/dL)/405.[20] Low HOMA-IR values indicate high insulin sensitivity, whereas high HOMA values indicate low insulin sensitivity (insulin resistance).

Statistical Analysis

The distribution characteristics of the variables were evaluated by Kolmogorov-Smirnov Test and then Levene's test was used to determine the homogeneity of variance. The differences between the parameters were measured by Chi-Square and independent samples T test where necessary. The association between vWF and HOMA or the lipid parameters were investigated by Pearson's correlation test. All values were expressed as mean ± SD. $p< 0.05$ was accepted as statistically significant.

RESULTS

The characteristics of the patients and the controls are given in Table 1. No difference between the subjects with IFG and IGT were established as regards to age, gender, BMI, fasting glucose, insulin, HOMA, lipid parameters and the vWF levels. Patients with IFG or IGT had significantly higher levels of vWF, FPG, insulin, HOMA, total cholesterol

TABLE 1: The comparison of the parameters of the IFG, IGT and the control subjects.

	IGT (n= 48)	IFG (n= 47)	Control (n= 58)	p1	p2
Age (years)	47.3± 6.9	46.55 ± 6.6	44.55 ± 9.8	0.1	0.2
BMI (kg/m^2)	28.1 ± 2.8	28.0 ± 2.76	28.2 ± 4.1	0.8	0.7
Gender (m/f)	28/20	28/19	25/33	0.1†	0.1†
FPG (mg/dL)	113.1±7.9	111.2 ± 9.7	90.9 ± 6.4	**< 0.001**	**< 0.001**
Insulin (μU/mL)	7.4 ± 4.2	7.7 ± 5.7	5.1 ± 3.6	**0.003**	**0.008**
TC (mg/dL)	212.3 ± 39.1	211.5 ± 40.6	195.3 ± 37.6	**0.02**	**0.03**
LDL Chol. (mg/dL)	127.9 ± 34.1	130.2 ± 34.3	117.1 ± 34.2	0.1	0.05
TG (mg(dL)	153.4 ± 76.5	162.1 ± 93.8	113.8 ± 55.9	**0.003**	**0.002**
HDL (mg/dL)	50.4 ± 8.3	50.3 ± 16.2	50.4 ± 10.3	0.9	0.9
HOMA-IR	2.1 ± 1.2	2.1 ± 1.7	1.1 ± 0.8	**< 0.001**	**< 0.001**
vWF (IU/dL)	117.3 ± 36.5	117.2 ± 35.5	100.1 ± 41.9	**0.03**	**0.03**

p1: IGT vs. control, p2: IFG vs. control (Independent samples t-test), † Chi-Square (mean ± SD).

BMI: Body Mass Index, FPG: Fasting Blood Glucose, TC: Total cholesterol, HOMA-IR: Homeostasis model assessment of insulin resistance.

and TGs when compared to those of the control subjects. There were no correlations between the vWF levels and the other parameters in either group.

DISCUSSION

Diabetes is a well known risk factor for atherosclerotic cardiovascular diseases.[1,2,21,22] Epidemiologic evidence suggests that the relationship between diabetes and cardiovascular disease begins earlier in the progression from normal glucose tolerance to T2DM and is associated with insulin resistance.[8] Hence, there is a relationship between duration of the prediabetic state and cardiovascular risk, which increases during progression to overt diabetes.[21,22] Insulin resistance and chronic subclinical inflammation are well-defined risk factors for atherosclerosis and cardiovascular disease.[23-26] However these factors alone can not totally elucidate the mechanism of the increased cardiovascular events in prediabetic subjects.

Endothelial dysfunction and subclinical inflammation is the prominent mechanism which is thought to have role in the pathogenesis of cardiovascular events in the disrease states associated with insulin resistance.[27] The vascular endothelium is involved in the production of many important substances which are important in the cardiovascular pathophysiology. One such substance is vWF which is synthesized by and stored in endothelial cells.[11] When released vWF appears to mediate platelet aggregation and adhesion. The primary physiologic function of vWF is to maintain homeostatic balance in the vasculature.[11] Because the endothelium is a primary source of vWF, elevated levels are representative for endothelial dysfunction.[12] High vWF levels have been shown in patients with risk factors for ischemic heart disease and peripheral vascular disease.[13,14] Elevated vWF levels were shown in patients with T2DM as well.[8] It has been shown that vWF elevation is a determinant of the development of T2DM independent of other risk factors.[15] The results of the present study show higher plasma vWF levels in patients with prediabetes when compared to healthy controls. The vWF levels were higher both in patients with IGT and in patients with IFG. Elevated vWF levels in patients with IGT were reported previously.[16] However, high vWF in IFG is a novel finding that may implicate that the endothelial functions are impaired in the very early stages before the occurrence of T2DM. This data is also suggestive of another likely mechanism that plays role in the development of atherosclerotic events in patients with prediabetes.

Both IFG and IGT represent intermediate metabolic states between normal and diabetic glucose homeostasis. These two categories not only identify individuals at risk for diabetes, but also (especially IGT) predict cardiovascular disease and mortality.[6-9] These two measures are reported to define different populations.[5] The data is supportive for IGT to become a risk factor for cardiovascular disease.[6,28] However, the reports for IFG are not clear.[7-9] Also, there has been some disagreement about which one is the best means of identifying at-risk populations, whether they characterize the same degree of risk, and whether IFG and IGT represent manifestations of the same process or fundamentally different mechanisms.[26,27] Also, it must be stated that the implication about the presence of any risk in a prediabetic case depends on the parameter under investigation. Recently we reported that we did not establish any alteration in the plasma P-selectin levels and platelet agregation studies[31] in patients with prediabetes.[30] According to the data of the present study, both isolated IFG and isolated IGT have similar features. The subjects in both categories did not differ with regard to age, BMI, sex, HbA1c, blood lipids, smoking habits, blood pressure, insulin sensitivity and vWF levels. The presence of similar vWF levels in both groups implicates that elevated vWF may be an early finding of prediabetes which is seen both in IFG and IGT.

This study, however, has several limitations. The sample size was small because of the narrow selection criteria. Hence, our data may not be representative for all subjects with prediabetes. However, the abnormalities in glucose metabolism are frequently accompanied by some other disorders which can potentially affect endothelial responses

as well as platelet functions. Therefore, including only the subjects free from any associate disorder for increased vWF is a strong feature of the present study. It is also imperative to mention that the HOMA formula used for the measurement of insulin sensitivity is only an estimate and may not be as accurate as glucose clamp test.

In conclusion, the results of the present study show that the vWF levels are elevated in patients with IGT and IFG. No significant difference between the parameters of these two states of prediabetes, including the HOMA and vWF levels, was established. The elevated vWF levels in IFG may be an early finding of endothelial dysfunction in patients with prediabetes. Future prospective studies may have the potential to highlight the importance of IFG as a potential cardiovascular risk factor.

Acknowledgement

The study was supported by the Gülhane Research Center Grant (GATA: 2006/05). We are grateful to Miss Suna Firer for her cooperation and intense efforts during the selection of the study population.

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