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Comparison of Gliclazide Treatment with Diet Therapy on Acute Phase Protein Levels in Patients with Type 2 Diabetes*

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Aims: It is known that there is a relationship between diabetic complications and chronic inflammation characterized by alterations in circulating acute phase proteins. It has been emphasized that inflammation contributes to diabetic complications and that gliclazide -an antidiabetic sulfonylurea - decreases the development of such complications. In this study, we aimed to investigate whether gliclazide or diet treatment has an effect on serum levels of acute phase reactants, markers of inflammation.

Materials and Methods: Fifty-six newly diagnosed patients with type 2 diabetes and 30 clinically healthy subjects were evaluated prospectively. Twenty-six patients were treated with proper diet and daily gliclazide (80 mg/daily) and 30 patients were randomized to only diet therapy for 6 months. After the 6-month therapy, blood was taken from patients and controls and acute phase protein levels were determined.

Results: Serum alpha-1 acid glycoprotein, alpha-2 macroglobulin, alpha-1 antitrypsin, and albumin levels did not differ between the gliclazide and control groups. In the diet group, mean serum alpha-1 acid glycoprotein, alpha-2 macroglobulin, and alpha-1 antitrypsin were significantly higher and mean albumin level was significantly lower than the other groups. Glucose, hemoglobin A1C and haptoglobulin levels were significantly higher, whereas transferrin and prealbumin levels were significantly lower in both diet therapy and gliclazide therapy when compared with the control group. Mean C-reactive protein level did not differ between the groups.

Conclusions: Levels of acute phase proteins significantly differ in type 2 diabetes when compared with healthy subjects. Gliclazide may provide better control on acute phase proteins.

Key Words: Diabetes mellitus, inflammation, gliclazide, acute phase proteins

Tip 2 Diyabetli Hastalarda Gliklazid Tedavisinin Akut Faz Protein Düzeyleri Üzerine Etkisinin Karşılaştırılması

Amaç: Dolaşımdaki akut faz proteinlerinin değişimi ile karakterize olan kronik inflamasyon ve diyabetik komplikasyonlar arasındaki ilişki bilinmektedir. İnflamasyon diyabetik komplikasyonlara katkıda bulunduğu ve antidiyabetik bir sülfanilüre olan gliklazid komplikasyonların gelişimini azalttığı belirtilmektedir. Bu çalışmada gliklazidin veya diet tedavisinin inflamasyon göstergeleri olan serum akut faz reaktanlarının düzeyleri üzerine bir etkisi olup olmadığını araştırmayı amaçladık.

Yöntem ve Gereç: Tip 2 diyabetli yeni tanı konulmuş 56 hasta ve 30 klinik olarak sağlıklı birey prospektif olarak değerlendirildi. 6 aylık bir süre 26 hasta uygun diyetle birlikte Gliklazid tedavisine (80 mg/gün) ve 30 hasta sadece diyet tedavisine alındı. Altı aylık tedavi sonrası hastalardan ve kontrol grubundakilerden kan alınarak akut faz proteinlerinin düzeyleri ölçüldü.

Bulgular: Serum alfa-1 asid glikoprotein, alfa-2 makroglobulin, alfa-1 antitripsin ve albumin düzeylerinde gliklazid grubu ile kontrol grubu arasında anlamlı fark yoktu. Diyet grubunda ise serum alfa-1 asid glikoprotein, alfa-2 makroglobulin, haptoglobulin, alfa-1 antitripsin kontrol grubundan anlamlı şekilde yüksekken, albumin düzeyleri ise anlamlı şekilde daha düşüktü. Hem diyet ve hem de gliklazid grubunda glukoz, hemoglobin A1C ve haptoglobulin düzeyleri anlamlı şekilde yüksekken, transferrin ve prealbumin düzeyleri ise anlamlı şekilde daha düşüktü. Ortalama C-reaktif protein düzeylerinde ise gruplar arasında fark yoktu.

Sonuç: Tip 2 diyabette akut faz protein düzeyleri sağlıklı bireylerle karşılaştırıldığında anlamlı şekilde farklıdır. Gliklazid akut faz proteinlerini daha iyi düzenleyebilir.

Anahtar Sözcükler: Diyabet, inflamasyon, gliklazid, akut faz proteinleri

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Introduction

Type 2 diabetes mellitus is a major worldwide health problem predisposing to markedly increased cardiovascular mortality and morbidity related to development of

nephropathy, neuropathy and retinopathy (1). The underlying mechanisms that cause complications in patients with type 2 diabetes are still not completely understood. Many recent studies point to the important role of free radical activity, changes in serum protein composition and consequent endothelial dysfunction. Some recent observations have related complications to chronic low-degree inflammation characterized by alterations in circulating acute phase proteins produced by the liver (1-3).

Gliclazide, a second-generation sulfonylurea that possesses a unique azabicyclo-octyl ring, is an antidiabetic agent. The main goal of antidiabetic therapy is to prevent the complications of diabetes (4,5). There are views on changes of serum acute phase protein levels and a few reports suggesting a relation between gliclazide and inflammation in diabetes (6,7). Gliclazide decreases the development of diabetic complications. This effect may involve decrease in inflammation. We sought to investigate the effect of gliclazide or diet therapy on acute phase reactants in patients with type 2 diabetes without any complications.

Materials and Methods

Patients

In this study, 56 newly diagnosed type 2 diabetic patients (29 M, 27 F; mean age: 46 years) and 30 clinically healthy subjects (15 M, 15 F; mean age: 45 years) were evaluated. There were no smokers or alcohol consumers among the patients or control subjects. Twenty-six patients were prospectively randomized to take gliclazide (Betanorm® 80 mg) daily for 6 months in addition to the proper diet therapy. Thirty patients were randomized to diet therapy alone.

The diets of all patients were composed as an energy intake of 30 kcal/kg of body weight per day. Subjects utilized a diet consisting of 55% carbohydrate, 25% fat, and 20% protein. Less than 10% of energy intake was derived from saturated fats, and dietary cholesterol intake was <300 mg/day. Fiber content of the diet was organized as ~15 g/1000 kcal.

Thirty clinically healthy subjects were recruited as the control group. Patients and controls with active inflammatory and infectious diseases were excluded. Patients and control cases that use anti-hyperlipidemic, anti-hypertensive or anti-inflammatory drugs were also excluded.

Biochemical Measurements

Venous blood was collected in vacutainers without additive at 09.00 a.m. after overnight fasting, allowed to clot for 30 min at room temperature, and centrifuged at 3000 X g for 5 min to obtain serum. Serum aliquots were stored at -80°C until biochemical analyses. Hemolyzed samples were excluded. Serum levels of C-reactive protein (CRP), alpha-1 acid glycoprotein (AAG), alpha-2 macroglobulin (AMG), alpha-1 antitrypsin (AAT), and haptoglobulin (HPT) as positive acute phase reactants and transferrin (TRF), prealbumin (PAB) and albumin (ALB) as negative acute phase reactants were measured by nephelometric method (Array 360 Protein System, Beckman Coulter Instruments Corporation, Brea, CA, USA) by using Beckman specific calibrators. Serum glucose (GLU) levels were measured with an autoanalyzer (Hitachi 717 Analyser, Boehringer-Mannheim, Germany) using commercial kits. Hemoglobin A_{1C} (HbA_{1C}) levels were measured by high-performance liquid chromatographic assay (Hi-Auto A1C HA 8121; Kyoto Dai-ichi Kagaku, Japan).

Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS, version 10.0, Chicago, IL, USA). The results were expressed as *mean ± SD.* Statistical analysis was performed using the one-way ANOVA, followed by Tukey's post hoc test to compare the means. The accepted level of significance was $P<0.05$.

Results

The comparison of acute phase reactants between the groups is shown in Table. Serum AAG, AMG, AAT, and ALB levels did not differ between the gliclazide and control groups. In the diet group, mean serum AAG, AMG, and AAT were significantly higher and mean ALB level was significantly lower than in the other groups. GLU, HbA_{1C} and HPT levels were significantly higher, whereas TRF and PAB levels were significantly lower in both diet therapy and gliclazide therapy when compared with the control group. Mean CRP did not differ between the groups.

Table. Levels of acute phase proteins and characteristics of study groups.

	Normal Values	Gliclazide Group	Diet Group	Controls
n		26	30	30
TRF (mg/dl)	200-360	218.1 ± 55.6[a]	218.7 ± 53.1[a]	261.6 ± 67.9
PAB (mg/dl)	16-35	21.1 ± 9.0[b]	24.4 ± 7.6[a]	30.6 ± 9.7
ALB (mg/dl)	3500-5500	4097 ± 883	3585 ± 920[a]	4200 ± 992
AAG (mg/dl)	55-140	116.7 ± 40.4	131.7 ± 51.6[a]	103.5 ± 42.7
AMG (mg/dl)	130-300	196.2 ± 33.9	220.9 ± 70.5[a]	183.9 ± 42.2
HPT (mg/dl)	30-200	180.8 ± 81.7[c]	204.7 ± 76.2[b]	117.3 ± 57.2
AAT (mg/dl)	124-348	174.1 ± 40.5	192.2 ± 51.9[a]	160.1 ± 34.5
CRP (mg/dl)	0.5-1.5	2.0 ± 1.4	2.1 ± 1.3	1.3 ± 0.7
GLU (mg/dl)	70-115	142.3 ± 46.2[b]	158.3 ± 55.9[b]	92.0 ± 22.2
HbA_{1C} (%)	4.5-5.7	7.4 ± 1.5[b]	8.0 ± 1.1[b]	5.1 ± 0.6
Age (years)		45.2 ± 10.6	46.8 ± 9.1	45.4 ± 7.0
BMI (kg/m^2)		29.6 ± 4.7	27.1 ± 4.4	24.3 ± 5.2

TRF: transferrin; PAB: prealbumin; ALB: albumin; AAG: alpha-1 acid glycoprotein; AMG: alpha-2 macroglobulin; HPT: haptoglobulin, AAT: alpha-1 antitrypsin, CRP: C-reactive protein, GLU: glucose; HbA_{1C}: hemoglobin A_{1C}; BMI: body mass index.

[a]: $P < 0.05$ when compared with the control group.

[b]: $P < 0.001$ when compared with the control group.

[c]: $P < 0.005$ when compared with the control group.

Discussion

In the current study, we showed that positive acute phase reactants are increased and negative acute phase reactants are decreased in type 2 diabetes. In the gliclazide group, AAG, AMG, and AAT among the positive acute phase reactants and ALB among the negative acute phase reactants did not change significantly when compared with healthy subjects. In the diet group, all the positive acute phase reactants were increased and negative acute phase reactants were decreased, except CRP, when compared with healthy subjects.

Diabetes is a chronic metabolic disorder that continues to present a major worldwide health problem. Patients with diabetes have an increased risk of vascular complications. Recently, an interaction between inflammation and type 2 diabetes has been recognized. It was reported that markers of inflammation were associated with the development of diabetes mellitus in middle-aged adults (2,3).

The immune system consists of phagocytic cells such as monocytes and macrophages. These cells release mediators (i.e., cytokines, complement, and acute phase reactants). The acute phase response is a nonspecific inflammatory reaction of the host that occurs during any inflammation. The response includes changes in the concentration of plasma proteins called acute phase proteins, some of which decrease in concentration (negative acute phase proteins), such as ALB, PAB or TRF, and others of which increase in concentration (positive acute phase proteins), such as AAG, AMG, HPT, AAT, and CRP. Most positive acute phase proteins are glycoproteins synthesized mainly by hepatocytes upon stimulation by proinflammatory cytokines and released into the bloodstream (8). The change may be as little as a 50% increase, as in the case of complement components, or as large as a 1,000-fold increase, as with CRP.

AAG is a protein with a molecular weight of 41–43 kDa and is heavily glycosylated (45%). The serum concentration of AAG increases several fold during an acute phase response, the systemic answer to a local inflammatory stimulus. The biological function of this protein is not clear (9). AAT is the main serum inhibitor of serine proteases, controlling tissue degradation. The proteinase inhibitor in serum exhibiting the greatest concentration is AAT, and the proteinase inhibitor encompassing the broadest spectrum is AMG (10). HPT, known as acute phase protein, can capture Hb by forming a high affinity HPT-Hb complex. Therefore, Hb binding by HPT is essential for rapid clearance of Hb from plasma. For this reason, HPT plays a crucial role against Hb-induced oxidative stress by a mechanism thought to

involve its high-affinity binding with Hb and prevention of iron release from Hb (11).

Recent evidence suggests abnormalities in the inflammatory factors in type 2 diabetes mellitus. Increased concentrations of HPT, AAG, CRP, serum amyloid A and interleukin-6 have been reported in type 2 diabetes (12,13). Rema et al. (14) also reported that the serum levels of HPT were elevated in diabetics with retinopathy. The levels of serum ALB, AAG, and AAT were not significantly different between the diabetics with retinopathy and controls. Diabetes is not likely to generate tissue damage in the way that trauma, acute infection, and neoplasm do. But hormonal changes (such as insulin and glucagons) present in diabetes may be important in stimulating acute phase proteins (2). Studies of the isolated perfused rat liver have shown that glucagons can act as a strong stimulus for acute phase protein production in the presence of corticosteroids and insulin (2). Mc Millan (2) found that HPT, AAG, CRP and ceruloplasmin levels were significantly increased in diabetics. In the present study, serum levels of HPT, AMG, AAG, and AAT were elevated in diabetics not administered gliclazide. Although acute phase reactants were lower in the gliclazide group compared with the non-gliclazide group, no statistically significant difference was determined. On the other hand, in the gliclazide group, AAG, AMG, and AAT levels were not higher and ALB level was not lower when compared with the healthy group. That is to say, ALB, AAG, AMG, and AAT levels of patients administered gliclazide were similar to values in the healthy group as if they were not diabetics. An additional possibility is that the clearance rates of acute phase proteins vary widely (2). Gliclazide may have shown its anti-inflammatory effect by correcting disturbed hormonal balance or improving renal function. Further studies with larger cohorts are needed before reaching a final decision.

In conclusion, levels of acute phase proteins changed in type 2 diabetes. Patients on diet therapy combined with gliclazide showed partial benefit from the anti-inflammatory effect of this treatment regimen.

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