Serum Hyaluronan Concentration as a Marker of Angiopathy in Patients with Diabetes Mellitus

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Abstract. Accumulation of hyaluronan (HA) around smooth muscle cells in lesions of atherosclerosis in diabetic patients suggests that this protein plays an important role in diabetic angiopathy. The aim of this study was to determine the correlation between serum HA concentrations and diabetic angiopathy. Diabetic patients treated with or without an oral hypoglycemic agent and/or insulin for at least 1 year were recruited (n = 95). We also included 20 non-diabetic control subjects. We measured serum levels of HA, body mass index (BMI), fasting plasma glucose (FPG), HbA1c, total cholesterol, triglyceride, glycated albumin (GA), high sensitivity CRP (hs-CRP), monocyte chemoattractant protein (MCP)-1 and evaluated diabetes mellitus history, drug use and presence of related complications. Serum HA levels were significantly (P<0.05) higher in diabetic patients (83.6 ± 5.6 ng/ml, mean ± SEM) than in normal subjects (41.7 ± 12 ng/ml). In diabetic patients, serum HA concentration significantly correlated with FPG, HbA1c, GA, triglyceride and also significantly correlated with BMI, hs-CRP and MCP-1 and tended to be higher in diabetic patients with complications than in those without such complications. Our data suggest that serum HA level correlates with poor blood glucose control and diabetic angiopathy and that it could be used as a marker of diabetic angiopathy.

Key words: Diabetes mellitus, Serum hyaluronic acid, Angiopathy, Complication, Diabetes control

(Hyaluronan (HA), the principal CD44 ligand, is synthesized by resident cells of the arterial wall, including endothelial cells, smooth muscle cells, and adventitial fibroblasts and is also abundantly present in the intima and adventitia of all blood vessels. Physiologically, hyaluronan is a multifunctional protein, composed of repeating disaccharides of D-glucuronic acid and N-acetyl-D-glucosamine, and it involved in water and protein homeostasis, cell proliferation, cell locomotion and migration through interactions with its receptor, CD44 [1, 2]. Recently, we and others have reported that stimulation of CD44 with mAbs or HA transmits the signal into the cells, which leads to activation of T cells and cytokine or chemokine of release from monocytes/macrophages and synoviocytes [3–5]. Recent studies have reported HA production in diabetes-related arterial sclerosis and diabetic nephropathy [6–8]. It has been suggested that HA is possibly related to diabetic microangiopathy as well as macro-angiopathy. However, there is little information on the correlation between serum HA and diabetes-related vascular disorders.

The present study was designed to determine the correlation between serum HA concentrations and some of the components in the metabolic disorders of diabetes and examine the role of HA in predicting the presence of diabetes-related vasculopathies.

Methods

Study participants

We studied patients who visited our hospital be-
between April and July 2002. The inclusion criteria were the presence of type 2 diabetes mellitus, treated with or without an oral hypoglycemic agent and/or insulin for at least one year. We excluded patients who had a history of alcohol abuse, evidence of liver disease, or severe cardiac problems. We also included a control group consisting of 20 individuals referred to our department for admission who were free of subjective symptoms and showed no pathologic signs. In the present study, evaluation was based on the following criteria for diagnosis of diabetes complications: retinopathy as represented by more than stage 1a of Scott classification, regardless of the presence or absence of photocoagulation; neuropathy as represented by a decrease of the Achilles tendon reflex or \( CV_{R,R} < 2\% \) and nephropathy as represented by the presence of microalbuminuria, which showed albumin excretion of 30 to 300 mg/day [9], creatinine level <2 mg/ml. Patients on dialysis were also excluded.

The study was performed in accordance with the Declaration of Helsinki. The study protocol was approved by the Human Ethics Review Committee of the Nakama Municipal Hospital, Japan, and informed consent was obtained from each subject.

Sample analysis

HbA1c, fasting plasma glucose (FPG), high sensitivity c-reacting protein (hs-CRP), hematology, biochemistry and lipid (triglyceride, total cholesterol) levels were determined using standard laboratory tests (SRL, Tokyo, Japan). In this study, the subjects whose hs-CRP value was so high that inflammation was doubted (over 2 mg/ml) were excluded from examination. Glycated albumin (GA) was measured by an enzymatic method [10] (SRL, Tokyo, Japan). Serum MCP-1 levels were determined by enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Inc, Minneapolis, MN, USA).

Measurement of HA concentration

Serum HA concentrations were measured by a sandwich binding protein assay kit (Hyaluronan Plate Chugai, Chugai Pharmaceuticals, Tokyo). Samples were incubated with HA binding protein coated on microplates for one hour at room temperature. After incubation, the microplates were again washed three times with wash buffer, and further incubated with 100 µl of peroxidase labelled liquid phase HA binding protein for 30 minutes at room temperature. After incubation, the microplates were again washed three times with wash buffer, and further incubated with 100 µl of peroxidase reactant substrate for 30 minutes at room temperature in a dark room. The reaction was stopped by the addition of 0.18 M sulphuric acid. Development of the color was monitored at 450 nm with a plate reader. HA level in each sample was calculated using the standard curve obtained with the purified HA solutions, which were included in the kit as references. According to the manufacturer’s instructions, the detection range of HA by the kit was 10–800 ng/ml [11, 12].

Statistical analysis

Data were expressed as mean ± SEM. Differences between groups were evaluated for statistical significance using the Student’s \( t \)-test. Comparisons among several groups were performed by ANOVA. For multiple comparisons, Bonferroni’s correction was applied. Correlation of serum hyaluronan level and other measurement items was determined by Pearson’s correlation coefficient. A \( p \) value of <0.05 was regarded as statistically significant.

Results

Patients characteristics

A total of 95 diabetic patients were enrolled in this study. Peripheral blood HA levels were significantly (P<0.05) higher in diabetic patients (83.6 ± 5.6 ng/ml, mean ± SEM) than in the normal control (41.7 ± 12 ng/ml). No significant difference in serum HA levels was found based on the type of medications (data not shown). Of the 95 diabetic patients, 57 had diabetic microangiopathy including nephropathy and neuropathy (Table 1). Patients with diabetic complications had significantly higher levels of HA concentrations than those without complications (\( p = 0.0236 \)) (Table 1, Fig. 3A).

Serum HA levels correlate with HbA1c, FPG, glycated albumin and triglyceride

Next, we examined the correlations between peripheral blood HA levels and various parameters in 95 dia-
HA concentrations correlated significantly with short-term and long-term diabetic control markers such as FPG (r = 0.769, p<0.0001), HbA1c (r = 0.781, p<0.0001) and GA (r = 0.784, p<0.0001) (Fig. 1A, 1B, 1C). Hyaluronan levels also correlated significantly with triglyceride (r = 0.699, p<0.0001) (Fig. 1D).

We also examined the correlations between peripheral blood HA levels and various atherosclerotic parameters in 95 diabetic patients. HA concentrations correlated significantly with markers of insulin resistance and inflammation, including BMI, hs-CRP, and MCP-1.

**Table 1.** Patients characteristics

|                     | Non-diabetic control (n = 20) | Diabetic patients With DC (n = 57) | Diabetic patients Without DC (n = 38) | P value 
|---------------------|-------------------------------|-----------------------------------|--------------------------------------|-------
| Age (years)         | 57.4 ± 2.4                    | 68.1 ± 1.5                        | 64.7 ± 1.7                           |       
| Sex (M/F)           | 8/12                          | 26/31                             | 19/19                                |       
| BMI (kg/m²)         | 24.1 ± 0.6                    | 23.3 ± 0.4                        | 23.4 ± 0.5                           |       
| FPG (mg/dl)         | 105.4 ± 3.1                   | 169.8 ± 9.3                       | 154 ± 9.3                            |       
| HbA1c (%)           | 5.0 ± 0.1                     | 6.8 ± 0.2                         | 6.5 ± 0.2                            |       
| Hyaluronan (ng/ml)  | 41.7 ± 12                     | 88 ± 10.6                          | 54.7 ± 5                             |       
| T-cho (mg/dl)       | 211 ± 7.7                     | 212 ± 5                            | 213 ± 6.8                            |       
| hs-CRP (mg/dl)      | 0.63 ± 0.1                    | 0.55 ± 0.1                         | 0.67 ± 0.1                           |       
| Glycated albumin (%)| 14.3 ± 0.3                    | 22.1 ± 0.8                         | 19.8 ± 0.7                           |       

DC: diabetic complications

Fig. 1. Correlation between serum concentrations of HA and FPG, HbA1c, GA and triglyceride. Correlations between serum concentrations of HA and fasting plasma glucose (FPG), HbA1c, GA and triglyceride were evaluated in 95 diabetic patients. FPG, HbA1c, GA and triglyceride correlated significantly with serum HA concentrations.

**Serum HA levels correlate with BMI, hs-CRP and MCP-1**

We also examined the correlations between peripheral blood HA levels and various atherosclerotic parameters in 95 diabetic patients. HA concentrations correlated significantly with markers of insulin resistance and inflammation, including BMI, hs-CRP, and MCP-1.
tance such as BMI ($r = 0.793, p<0.0001$). HA levels also correlated significantly with markers of atherosclerosis such as hs-CRP ($r = 0.77, p<0.0001$), MCP-1 ($r = 0.793, p<0.0001$) (Fig. 2A, 2B, 2C).

Diabetic complications and HA levels

Serum HA concentrations were significantly (p<0.05) higher in the group with complications (diabetic patients: with complications; 88 ± 10.6 ng/ml; without complications, 54.7 ± 5 ng/ml, mean ± SEM) (Fig. 3A). The numbers of cases with the three major types of complications were nephropathy 13, nephropathy + retinopathy 11, and triopathy 11 cases. Peripheral blood HA levels in patients with retinopathy alone were not significantly different from the normal control. All other cases had higher HA levels compared with the normal control (Fig. 3B). Serum HA levels tended to be higher in patients with nephropathy alone and patients with more than two complications including nephropathy, than other patients (Fig. 3B).

Fig. 2. Correlation between serum concentrations of hyaluronan and BMI, hs-CRP and MCP-1. Correlations between serum concentrations of HA and BMI, high sensitivity CRP (hs-CRP) and MCP-1 were evaluated in 95 diabetic patients. BMI, hs-CRP and MCP-1 correlated significantly with serum HA concentrations.

Fig. 3. Correlations between serum HA concentrations and diabetic complications. (A) Serum HA concentrations tended to be significantly higher in patients with complications than in those without such complications. (B) Serum HA concentrations tended to be higher in patients with nephropathy alone and those with more than two complications including nephropathy. Data are mean ± SEM. * p<0.05; vs control.
Discussion

Serum HA concentrations are reported to correlate with the activity and pathological stage of inflammatory diseases including rheumatoid arthritis and liver cirrhosis [13]. That is, fibroblasts are activated in the repair process of inflammation in inflammatory diseases including vascular disorders such as atherosclerosis, and the production of HA increases during the repair process at the site of the lesion. In the present study, serum HA levels were significantly high in diabetic patients compared with non-diabetic subjects, and fasting plasma glucose, HbA1c and GA correlated positively with HA levels. Therefore, it is possible that serum HA concentrations reflect general impairment of blood vessels caused by hyperglycemia due to inappropriate plasma glucose control in diabetes and insulin resistance. This conclusion may be supported by the result that serum HA levels significantly correlated with BMI values and tended to be high in obese patients in our study.

One of the key proinflammatory mediators whose expression is stimulated by AGE and/or high glucose signaling pathway is MCP-1 [14]. Several investigators have reported that plasma levels of MCP-1 were associated with cardiovascular risk factors and may be an important biomarker during the preclinical phase of atherosclerosis [15, 16]. In addition, several studies have demonstrated that obesity, type 2 diabetes mellitus and presence of a metabolic syndrome are associated with increased plasma levels of CRP [17, 18]. It is possible that increased MCP-1 and CRP levels reflect atherosclerotic angiopathy in large blood vessels. In our study, MCP-1 and hs-CRP values correlated significantly with serum HA concentrations. On the other hand, HA concentrations were also significantly high in patients with diabetic microvascular complications, particularly those with nephropathy and neuropathy. These results suggest that serum HA concentrations reflect diabetic angiopathy.

In summary, we have demonstrated in the present study the presence of high serum HA concentrations in diabetic patients and that these levels reflect the control of blood glucose, suggesting that serum HA concentration could be used as a marker of diabetic angiopathy.

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References


