

Close Relationship between Serum Hyaluronan Levels and Vascular Function in Patients with Type 2 Diabetes

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ABSTRACT

OBJECTIVE: To clarify the relationship between serum hyaluronan levels and vascular function in type 2 diabetic patients.

METHODS: A cross-sectional cohort study.

RESULTS: In multivariate regression analysis, endothelium-dependent flow-mediated dilation was associated with age and duration of diabetes, but not with serum hyaluronan level, while endothelium-independent nitroglycerine-mediated dilation (NMD) was only associated with serum hyaluronan level (standardized estimate = -0.401, $P = 0.003$). NMD in the lowest tertile of hyaluronan level was significantly higher than the other tertiles (15.9% vs. 12.5% and 10.4%, $P = 0.047$ and $P = 0.002$, respectively).

CONCLUSIONS: Serum hyaluronan level may be useful as a surrogate marker for early changes in the vascular function, which often occurs in patients with diabetes mellitus.

Introduction

Cardiovascular disease is the major cause of mortality in patients with type 2 diabetes. Therefore, a surrogate marker for vascular function, which would allow the initiation of early intervention, is urgently needed. Currently, endothelium-dependent flow-mediated dilation (FMD) and endothelium-independent nitroglycerine-mediated dilation (NMD) are considered sensitive and reproducible indicators of vascular function. However, their clinical application is limited due to technical and methodological issues, such as the requirement for nitroglycerin use.

On the other hand, hyaluronan is produced during the tissue restoration process following inflammation. Previous reports have indicated that serum hyaluronan levels are higher in patients with diabetes, in association with metabolic markers such as HbA1c, BMI, and carotid intima-media thickness that indicate pathological change in arteries (Mine et al., 2006, Nieuwdorp et al., 2007). Other studies have reported a critical role for hyaluronan synthesis in diabetes-related arterial sclerosis (Heickendorff et al., 1994, Chajara et al., 2000). However, no study has yet examined the relationship between hyaluronan level and vascular function. Thus, we aimed at assessing the use of hyaluronan as a surrogate marker for vascular function in patients with diabetes.

Research Design and Methods

Patients

In this cohort cross-sectional study, we consecutively enrolled 79 patients with type 2 diabetes (age, 62 ± 13 years; duration, 10.7 ± 9.0 years) who had been treated as inpatients at the Shimane University Hospital. Major exclusion criteria included diagnosis with cancer or collagen disease. The study protocol and informed consent procedure were approved by the Ethics Committee of the Shimane University Faculty of Medicine.

Measurements

Serum samples were collected early in the morning, from patients in fasting state, and serum hyaluronan level in the fasting blood samples was measured using a latex immunoturbidimetric assay (Mitsubishi Chemical Medience Corporation, Tokyo, Japan). Intra-assay coefficients of variation for hyaluronan were 4.22%, 1.68%, and 1.41% with low, midrange, and high levels of hyaluronan, respectively. Interassay coefficients of variation for hyaluronan were 1.81%, 0.99%, and 0.58% with low, midrange, and high levels of hyaluronan, respectively. We measured N-terminal pro-peptides of type IV collagen 7S domain (type IV collagen · 7S) which is marker of liver fibrosis by Double-antibody radioimmunoassay.

HbA1c was measured using high-performance liquid chromatography, which was standardized by the Japan Diabetes Society (JDS), with a normal range of 4.3–5.8%. National Glycohemoglobin Standardization Program (NGSP)-equivalent values were obtained using the following equation (Kashiwagi et al., 2012):

$$\text{HbA1c (NGSP) (\%)} = 1.02 \times \text{HbA1c (JDS) (\%)} + 0.25\%.$$

Glomerular filtration rate (GFR) was estimated using the following equation, which was proposed by the Modification of Diet in Renal Disease study group (Levey et al., 2006) and modified for Japanese individuals (Imai et al., 2008):

$$\text{eGFR (mL/min/1.73 m}^2\text{)} = 194 \times \text{SCr (mg/dL)}^{-1.094} \times \text{Age (years)}^{-0.287} \times 0.739$$
 (if female), where eGFR stands for estimated GFR and SCr for serum creatinine level.

Measurement of vascular function

FMD and NMD were measured in the morning in fasting patients (no eating, smoking, or drinking coffee) using UNEXEF18G (UNEX, Japan). The participants relaxed in

a supine position and instructed to remain quiet during the measurements. FMD was assessed as the dilation of the brachial artery in response to increased flow. B-mode scans of the right brachial artery were obtained in longitudinal sections 5 cm above the elbow, and the probe was held by a stereotactic clamp to ensure constant imaging. B-mode images were triggered to the electrocardiogram signal to obtain end-diastolic frames. A cuff was placed around the forearm just below the elbow, inflated for 5 min at 50 mm Hg above systolic BP, and then deflated to induce reactive hyperemia. FMD was expressed as the percentage change in brachial artery diameter from baseline value (before cuff occlusion) to peak value (after cuff release). NMD was assessed as the dilation of the brachial artery in response to increased flow due to sublingual administration of a low dose (75 µg) of nitroglycerin. NMD was expressed as the percentage change in brachial artery diameter from baseline value to peak value following nitroglycerin administration. We calculated FMD and NMD (%) using following equation. $FMD (\%) = [(Peak\ value - Baseline\ value) / Baseline\ value] \times 100$, peak value means the maximum value of the arterial diameter after cuff release or after nitroglycerin administration in each measurement.

When subject was tested twice on the same day with an interval of 30 minutes between test and a retest, interday coefficients of variation for FMD were 10% (Onkelinx et al., 2013). Intraday coefficients of variation for FMD were 11% (Onkelinx et al., 2013).

Statistical analysis

Data were expressed as percentages, or arithmetic mean \pm standard deviation as appropriate based on data distribution. Relationships between variables were assessed using Pearson correlational or multivariate linear regression analysis. Analysis of variance and covariance was performed for comparison between 3 groups. All analyses were performed using the PASW Statistics 18 software (SPSS Japan Inc., Tokyo, Japan). $P < 0.05$ was

considered statistically significant.

Results

Patient characteristics

Baseline clinical characteristics and laboratory data of patients are listed in Table 1. Subjects were normotensive and non-obese, but with poor glycemic control. Mean (\pm SD) FMD and NMD values were $4.4 \pm 3.0\%$ and $13.3 \pm 6.4\%$, respectively.

Association between FMD, NMD, and variables

In univariate regression analysis, FMD was significantly associated with age ($r = -0.490$, $P < 0.001$), duration of diabetes ($r = -0.421$, $P = 0.001$), BMI ($r = 0.244$, $P = 0.030$), systolic blood pressure ($r = -0.240$, $P = 0.036$), eGFR ($r = 0.344$, $P = 0.002$), and hyaluronan level ($r = -0.320$, $P = 0.004$; Table 2 and Figure 1). NMD was significantly associated with age ($r = -0.417$, $P < 0.001$), eGFR ($r = 0.248$, $P = 0.032$), and hyaluronan level ($r = -0.392$, $P < 0.001$; Table 2 and Figure 1). In multivariate regression analysis, FMD was associated with age (standardized estimate = -0.399 , $P = 0.002$) and duration of diabetes (standardized estimate = -0.276 , $P = 0.027$), whereas NMD was only associated with hyaluronan level (standardized estimate = -0.401 , $P = 0.003$; Table 2). In this multivariate regression analysis, we used conventional risk factors as covariates; age, duration of diabetes, BMI, systolic blood pressure, eGFR, as well as HbA1c, C-reactive protein, and LDL-cholesterol levels. Since both serum hyaluronan and type IV collagen levels are elevated in liver fibrosis, we excluded 5 patients with high levels of type 4 collagen (≥ 6.0 ng/mL), and still obtained similar results. In multivariate regression analysis, FMD was associated with age (standardized estimate = -0.409 , $P = 0.002$) and duration of diabetes (standardized estimate = -0.280 , $P = 0.028$), whereas NMD was only associated with hyaluronan level (standardized estimate = -0.409 , P

= 0.003).

Association between hyaluronan and variables

In univariate regression analysis, serum hyaluronan level was significantly associated with age ($r = 0.403$, $P < 0.001$) and duration of diabetes ($r = 0.316$, $P = 0.011$), but not with renal function and other metabolic markers (Table 3).

NMD according to hyaluronan level

We classified participants into 3 groups, according to hyaluronan level. NMD in patients with low hyaluronan levels was significantly higher than that in patients with intermediate and high hyaluronan levels (15.9% vs. 12.5% and 10.4%, $P = 0.047$ and $P = 0.002$, respectively). Similar results were obtained after adjusting for conventional risk factors. Adjusted NMD in patients with low hyaluronan levels was generally higher than that in patients with intermediate and high hyaluronan levels (16.5% vs. 12.2% and 11.5%, $P = 0.065$ and $P = 0.054$, respectively; Figure 2).

Discussion

In this study, we have shown that elevated serum hyaluronan levels are associated with vascular dysfunction in patients with type 2 diabetes. Furthermore, we observed that hyaluronan level is independently associated with NMD, but not with FMD. These results suggest that serum hyaluronan level may be used as a surrogate marker for functional changes in vascular smooth muscle rather than in vascular endothelium.

FMD has become the most widely used technique to assess vascular endothelial function (Flammer et al., 2012), and it has been shown to correlate with coronary artery endothelial function (Teragawa et al., 2005). On the other hand, NMD is used to assess

vascular smooth muscle function, and patients with arteriosclerosis have lower NMD than healthy control subjects (Raitakari et al., 2001). Furthermore, a previous study has indicated that diabetes is significantly correlated with reduced NMD, independently of FMD (Adams et al., 1998). Impaired FMD theoretically results from endothelial and/or smooth muscle dysfunction. However, impaired FMD with normal NMD specifically indicates endothelial dysfunction. Therefore, NMD should be examined, despite the technical and methodological difficulties associated with its measurement, particularly pertaining to nitroglycerin use.

The endothelium is covered by a so-called glycocalyx that has been shown to orchestrate vascular homeostasis (Nieuwdorp et al., 2005). Hyaluronan glycosaminoglycans, which are major constituents of the glycocalyx, are crucial for maintaining the endothelial barrier properties for plasma macromolecules (Henry et al., 1999). A previous study demonstrated that hyperglycemia reduced glycocalyx volume, leading to endothelial dysfunction, and an increase in plasma hyaluronan levels and coagulation activation markers (Nieuwdorp et al., 2006a). Another study showed that serum hyaluronan levels of patients with diabetes were higher than those of healthy control subjects (Nieuwdorp et al., 2006b). Our univariate regression analysis showed an inverse association between hyaluronan level and FMD, while in multivariate regression analysis, hyaluronan did not associate with FMD. Since our subjects were relatively older patients with type 2 diabetes and no healthy controls, several factors such as age, complication, and medication might affect the association between FMD and hyaluronan.

Hyaluronan is synthesized by resident cells of the arterial wall that include endothelial cells (Vigetti et al., 2010), smooth muscle cells (Sainio et al., 2010), and adventitial fibroblasts; it is also found abundantly in the intima and adventitia of all blood vessels. Interestingly, fibroblasts are activated during the repair process in inflammatory diseases, including vascular disorders such as atherosclerosis. Therefore, the production of

hyaluronan increases at lesion sites during the repair process. Serum hyaluronan concentration reportedly correlates with the severity and pathological stage of inflammatory disease (Mine et al., 2006, Ceriello. 1998). In our study, hyaluronan level was not correlated with HbA1c, C-reactive protein, and LDL-cholesterol levels. This may be due to the homogeneity of our patients regarding these indices and to the superiority of vascular indices.

The main principal hyaluronic acid receptors are receptor for hyaluronan-mediated motility and CD44 (Slevin et al., 2007) and hyaluronan binding proteoglycan, versican (Lennon et al., 2011). Hyaluronan has been shown to regulate arterial smooth muscle cell function, including proliferation and migration, through these receptors (Lennon et al., 2011). In fact, a previous study demonstrated that increased accumulation of hyaluronan is associated with smooth muscle cell proliferation in experimentally injured arteries (Riessen et al., 1996). Furthermore, hyaluronan receptors are upregulated in atherosclerotic lesions and CD44 knockout pro-atherosclerotic mice has a 50-70% reduction in aortic lesions (Lennon et al., 2011). This suggests that hyaluronan may not only be involved in physiological vascular remodeling but also in the development of arteriosclerosis.

Our study has some limitations. First, our study sample was biased toward patients with poor glycemic control, due to characteristics of the university hospital. Second, we did not measure the molecular size of hyaluronan. Some reports have shown that hyaluronan biological function depends on the size of hyaluronan fragments (Pardue et al., 2008, Stern et al., 2006).

Conclusion

In conclusion, the present study indicates that hyaluronan level and vascular smooth muscle function are closely correlated in patients with type 2 diabetes. Therefore, at least for this group of patients, hyaluronan can be used as a potential surrogate marker for vascular

function, independently of other metabolic markers. Future studies are required for further validation.

Abbreviations

FMD: Endothelium-dependent flow-mediated dilation; NMD: endothelium-independent nitroglycerine-mediated dilation; JDS: Japan Diabetes Society; NGSP: National Glycohemoglobin Standardization Program; GFR: Glomerular filtration rate

Competing interests:

The authors declare that they have no competing interests.

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Table legends

Table 1

Data are represented as mean \pm standard deviation.

Abbreviations: FMD, endothelium-dependent flow-mediated dilation; NMD, endothelium-independent nitroglycerine-mediated dilation; LDL-C, LDL-cholesterol; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein.

Table 2

Abbreviations: FMD, endothelium-dependent flow-mediated dilation; NMD, endothelium-independent nitroglycerine-mediated dilation; LDL-C, LDL-cholesterol; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein.

Table 3

Abbreviations: LDL-C, LDL-cholesterol; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein.

Figure legends

Figure 1

Correlation between serum hyaluronan level, FMD, and NMD. A) Endothelium-dependent flow-mediated dilation (FMD) is significantly associated with hyaluronan level ($r = -0.320$, $R^2 = 0.102$, $P = 0.004$). B) Endothelium-independent nitroglycerine-mediated dilation (NMD) is significantly associated with hyaluronan level ($r = -0.392$, $R^2 = 0.154$, $P < 0.001$).

Figure 2

NMD analysis according to hyaluronan level. Participants were classified into 3 groups on the basis of serum hyaluronan level: low ($n = 28$), 17 ± 6 ng/mL; intermediate ($n = 26$), 40 ± 12 ng/mL; high ($n = 25$), 110 ± 74 ng/mL. In analysis of covariance, NMD was adjusted for age, duration of diabetes, BMI, systolic blood pressure, eGFR, as well as HbA1c, CRP, and LDL-C levels. Values shown indicate means; error bars indicate standard error.

Abbreviations: LDL-C, LDL-cholesterol; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein.

TABLES

Table 1. Baseline clinical and biochemical characteristics of patients

	n = 79	Reference range
Age (years)	61.7 ± 12.6	
Male (%)	62	
Duration of diabetes (years)	10.7 ± 9.0	
BMI (kg/m ²)	24.2 ± 5.4	
Systolic blood pressure (mm Hg)	123 ± 18	
Diastolic blood pressure (mm Hg)	75 ± 12	
FMD (%)	4.4 ± 3.0	
NMD (%)	13.3 ± 6.4	
HbA1c (%)	8.9 ± 2.3	4.7 - 6.2
(mmol/mol)	73.6 ± 25.0	
LDL-C (mmol/L)	115 ± 39	70 - 139
eGFR (mL/min/1.73 m ²)	75.0 ± 24.5	
CRP (mg/dL)	0.11 ± 0.17	< 0.2
Hyaluronan (ng/mL)	54 ± 57	< 50

Table 2. Association between FMD, NMD, and variables according to univariate and multivariate regression analyses

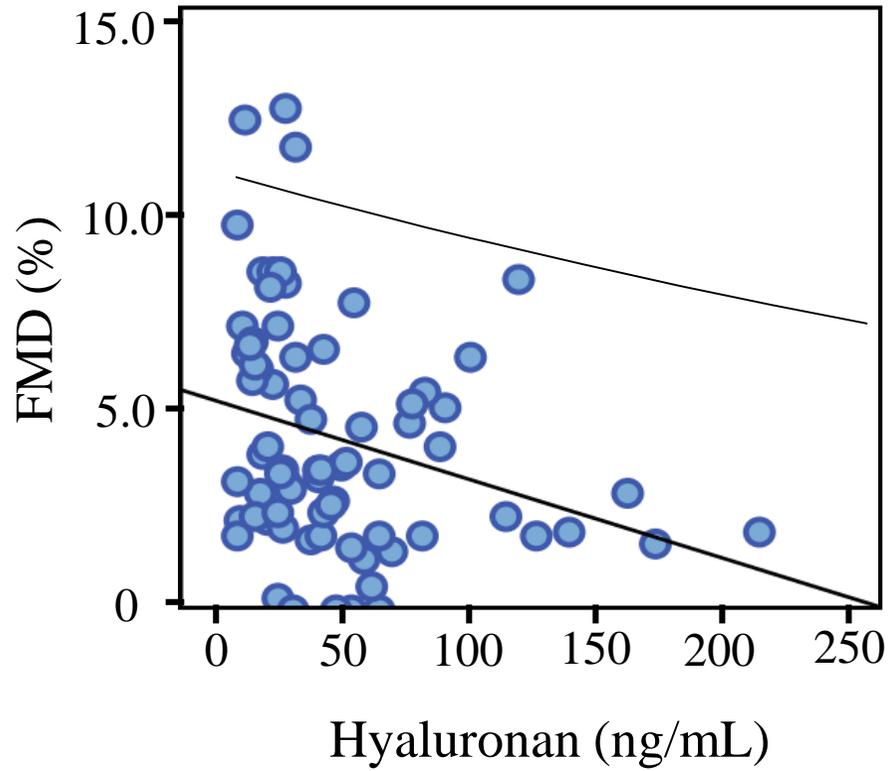
	<u>Univariate</u>				<u>Multivariate</u>			
	FMD		NMD		FMD		NMD	
	r	P	r	P	β	P	β	P
Age (years)	-0.490	< 0.001	-0.417	< 0.001	-0.399	0.002		
Duration of diabetes (years)	-0.421	0.001	-0.215	0.090	-0.276	0.027		
BMI (kg/m ²)	0.244	0.030	0.174	0.130				
Systolic blood pressure (mm Hg)	-0.240	0.036	-0.200	0.085				
HbA1c (%) (mmol/mol)	-0.048	0.691	-0.089	0.461				
LDL-C (mg/dL)	0.167	0.145	0.042	0.717				
eGFR (mL/min/1.73 m ²)	0.344	0.002	0.248	0.032				
CRP (mg/dL)	-0.019	0.869	-0.050	0.672				
Hyaluronan (ng/mL)	-0.320	0.004	-0.392	< 0.001			-0.401	0.003

Table 3. Association between hyaluronan level and variables according to univariate regression analysis

	Hyaluronan (ng/mL)	
	r	P
Age (years)	0.403	< 0.001
Duration of diabetes (years)	0.316	0.011
BMI (kg/m ²)	-0.144	0.205
Systolic blood pressure (mm Hg)	0.107	0.354
Diastolic blood pressure (mm Hg)	-0.165	0.151
HbA1c (%)	-0.040	0.742
LDL-C (mg/dL)	-0.083	0.470
eGFR (mL/min/1.73 m ²)	-0.184	0.108
CRP (mg/dL)	0.087	0.459

Figure 1.

A



B

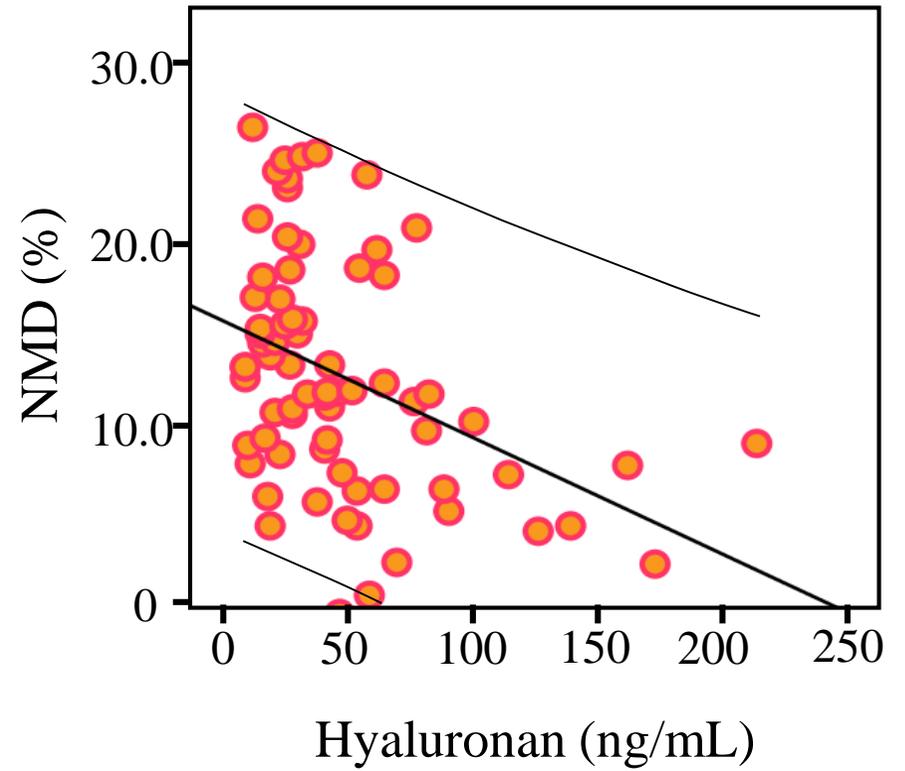


Figure 2.

