A Cross-Sectional Evaluation of Spontaneous Platelet Aggregation in Relation to Complications in Patients With Type II Diabetes Mellitus

Eiichi Iwase, Masato Tawata, Kaoru Aida, Yukio Ozaki, Shoji Kume, Kaneo Satoh, Ruomei Qi, and Toshimasa Onaya

To clarify the relationship between platelet function and diabetic complications, we investigated spontaneous platelet aggregation (SPA) and agonist-induced platelet aggregation by a particle counting method using light scattering (LS) and by a conventional light transmission method (LT) in 23 age- and sex-matched control subjects and 74 patients with type II diabetes mellitus. We also observed platelets using the FIC-2 (TOA Medical Electronics, Kobe, Japan) flow cytometer and imaging device. Observation by the FIC-2 device showed microaggregates of platelets in samples with increased SPA-LS. SPA-LS was significantly elevated in patients with type II diabetes mellitus as a whole compared with control subjects. SPA-LS also showed significant differences between control subjects and three diabetic patient subgroups with a varying severity of retinopathy, nephropathy, or neuropathy, and the mean values increased along with the increasing severity of complications. On the other hand, although SPA-LT also showed significant differences between these groups, the absolute values were all less than 10%, which we believe does not warrant quantitative analysis. Adenosine-5′-diphosphate (ADP)-induced platelet aggregation failed to show significant differences between controls and subjects with a varying severity of retinopathy by either LS or LT, which indicates that SPA is more sensitive than agonist-induced platelet aggregation in relation to diabetic complications. We observed significant correlations between SPA-LS and the patients' age, hemoglobin A_{1c} (HbA_{1c}) level, plasma fibrinogen level, or 6-keto-$PGF_1\alpha$ (6KF) to 11-dehydro-thromboxane B2 (TXB2) ratio. Our study demonstrated a close relationship between platelet hyperaggregability and diabetic complications, and a longitudinal prospective study of SPA-LS in diabetic patients is warranted to clarify cause-and-effect relationships.

ALTHOUGH THE FACTORS responsible for angiopathy in diabetic patients have not been fully elucidated, platelet hyperfunction is implicated as a risk factor for both microvascular and macrovascular disease.[1-4] Among the various platelet functions, platelet aggregation has been most extensively evaluated. Concerning the role of platelet aggregation in relation to complications, none of the data reported thus far are convincing. Most of the published studies used the conventional light transmission (LT) method[5,6] to assess platelet aggregation in the presence of various agonists. However, several lines of evidence suggest that changes in LT do not quantitatively reflect platelet activation, since they occur only after the formation of large aggregates from preexisting small aggregates.[7,8] In addition, platelets show different responses depending on the agonist.[9,10]

Ozaki et al[11] developed a novel method to detect platelet aggregation by a particle counting method using light scattering (LS). This method is unique in that it allows estimation of not only the formation of aggregates consisting of two or three platelets but also the size distribution of platelet aggregates depending on the intensity of LS as a function of time in platelet-rich plasma (PRP). We recently reported that spontaneous platelet aggregation (SPA) measured by this new method is increased in patients with type II diabetes mellitus compared with healthy control subjects, and this increase in SPA is well correlated with serine phosphorylation of the myosin light chain of platelets.[12] In the present study, we measured SPA and agonist-induced platelet aggregation in patients with type II diabetes mellitus and healthy control subjects and investigated the correlation of platelet aggregation with the increasing severity of complications.

SUBJECTS AND METHODS

Subjects, Diagnosis, and Classification of Diabetic Complications

The present study was performed according to the principles of the Declaration of Helsinki, and full informed consent was obtained from all subjects before the study. The study design was approved by the Ethical Committee of Yamanashi Medical University. We recruited consecutive patients with type II diabetes mellitus at the outpatient clinic of Yamanashi Medical University Hospital. All patients were diagnosed according to World Health Organization criteria.[13]

Diabetic retinopathy was diagnosed by expert ophthalmologists at Yamanashi Medical University Hospital by fundoscopic examination with occasional fluorescent retinal photography when necessary, and classified as no retinopathy, simple retinopathy, and proliferative retinopathy according to Fukuda.[14]

Diabetic nephropathy was diagnosed by measuring urinary albumin excretion (milligrams per day).[15] Urine was collected for 24 hours on 2 consecutive days, and the mean albumin excretion was used as the value for each patient. Those with albuminuria less than 28 mg/d, greater than 28 mg/d and less than 280 mg/d, and greater than 280 mg/d, were classified as normoalbuminuria, microalbuminuria, and overtly albuminuria, respectively. Subjects with the following conditions were excluded from the study[16]: (1) gross hematuria or persistent microscopic hematuria, (2) nephrotic syndrome within 10 years of the onset of diabetes mellitus or without evidence of retinopathy or (3) progressive renal dysfunction.

Diabetic neuropathy was diagnosed based on complaints of persistent numbness, cold sensation, or pain or diminished or absent deep tendon reflexes and classified by measuring the vibratory threshold (VT) at the right internal malleolus using an SMV-5 vibrometer (Teknologue, Tokyo, Japan) as we previously reported.[17,18] Those with VT values less than 29×10^{-2} G, greater than 30×10^{-2} G and less than 99×10^{-2} G, and greater than 100×10^{-2} G were classified as no neuropathy, mild neuropathy, and severe neuropathy, respectively. Patients with periph-

From the Third Department of Internal Medicine and the Department of Clinical Laboratory Medicine, Yamanashi Medical University, Yamanashi, Japan.

Submitted July 21, 1997; accepted December 1, 1997.

Address reprint requests to Masato Tawata, MD, Third Department of Internal Medicine, Yamanashi Medical University, Tamaho, Yamanashi 409-38, Japan.

0026-0495/98/4706-0014$03.00/0

eral neuropathy due to toxic or metabolic causes other than diabetes mellitus or cancer were excluded from the present study. Patients who were taking agents such as vitamin B_1, B_6, B_{12}, or E, prostanoids, aspirin, cilostazol, antidepressants, or tranquilizers were also excluded from the present study.

In total, we recruited 74 patients with type II diabetes mellitus. There are 46 men and 28 women with a mean age of 55.2 ± 1.6 years. Thirty were taking oral hypoglycemic agents, 20 were injecting insulin, and others were on the diet regimen only. As a normal control group, 23 age- and sex-matched healthy subjects (12 men and 11 women) with a mean age of 55.2 ± 2.8 years were also recruited.

Measurement of SPA and Agonist-Induced Platelet Aggregation

Blood was obtained in the presence of 1:10 vol 3.8% citric acid after an overnight fast, and PRP was prepared by centrifugation at 150 × *g* for 10 minutes at room temperature. Yamamoto et al[19] developed a platelet aggregometer that simultaneously measures platelet aggregation by two methods. One is the conventional method based on changes in the LT of a platelet suspension. The other is based on a particle counting method using LS.[11] An optical device focuses on a limited area of a platelet suspension and measures the intensity of light scattered by particles passing through the area, thus minimizing multiple LS. PRP was poured into a cuvette, and SPA was observed under constant stirring in the absence of agonists. The intensity of LS in an arbitrary unit (V) detected by this device provides information on the number and size of aggregates in a suspension.[11] The total LS intensity was recorded 10 minutes after the beginning of stirring, and is expressed as SPA-LS in this study. In case of agonist-induced platelet aggregation, adenosine-5′-diphosphate ([ADP] 0.1, 0.5, or 1 μmol/L) or epinephrine ([Epi] 0.01, 0.1, or 0.3 μmol/L) were added 1 minute after stirring the PRP and observed by LS and LT at 5 minutes or 10 minutes after addition of ADP or Epi, respectively.

Observation of Platelets by a Flow Cytometer and Imaging Device

After observation of SPA-LS, 10 μL of the sample was removed from the PRP and immediately mixed with 40 μL 2.6% auramine O in 95.9% ethyleneglycol solution (Ret-Search dye; TOA Medical Electronics, Kobe, Japan) and 1,950 μL diluent (Ret-Search diluent; TOA Medical Electronics).[20] This dye stains RNA and DNA in the cells. Then, the mixed sample was observed by an FIC-2 device (TOA Medical Electronics),[20] which provides a flow cytometric scattergram and images.

Other Parameters

Blood samples were also obtained at the same time for PRP, and blood glucose, hemoglobin A_{1c} (HbA_{1c}), serum lipids, plasma fibrinogen, and prostaglandin metabolites (11-dehydro-thromboxane B2 [TXB2] and 6-keto-$PGF_{1\alpha}$ [6KF]) were determined before the aggregation study. Blood glucose levels were measured by the glucose oxidation method using Dry Chem 2000 (Fuji Film, Tokyo, Japan). The HbA_{1c} level was measured by affinity column chromatography. Total cholesterol and triglycerides were determined by enzymatic methods, and high-density lipoprotein cholesterol was determined by the heparin Ca^{2+} precipitation method. Prostaglandin metabolites were determined by radioimmunoassay.

Statistical Analysis

All numerical variables are expressed as the mean ± SEM. The values for platelet aggregation in control subjects and diabetic patients as a whole were compared by the Kruskal-Wallis test, and in other cases the nonparametric Bonferroni test was used for statistical analysis.

RESULTS

As we previously reported,[12] there was no LS intensity in PRP freshly obtained from a control subject or diabetic patient, indicating no platelet aggregates. With constant stirring, an

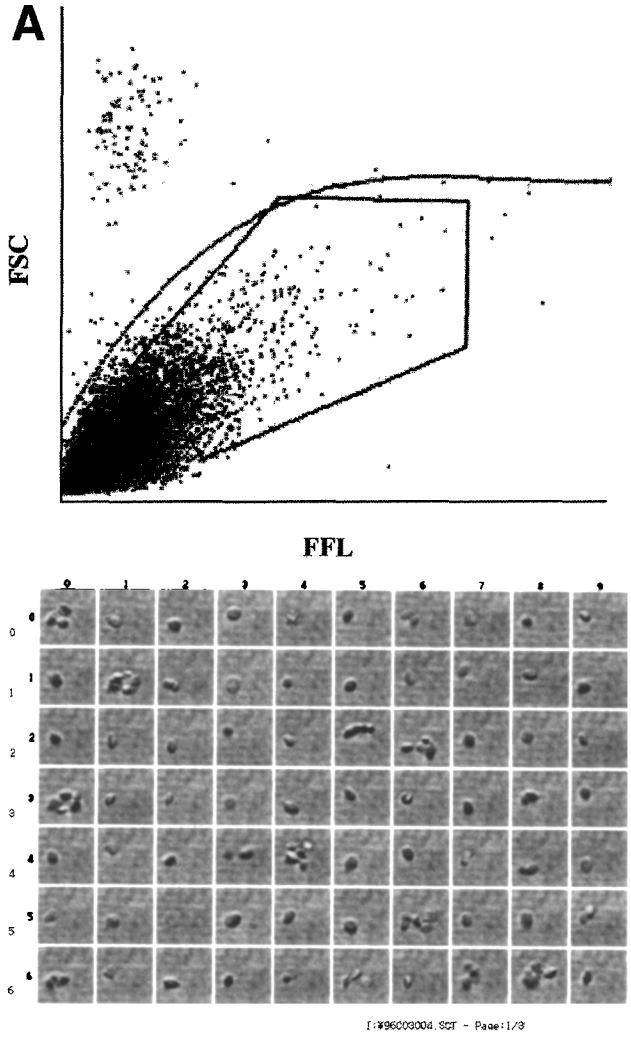

Fig 1. Flow cytometric scattergram and image of platelets observed by the FIC-2. PRP obtained from diabetic patients was first observed to measure SPA-LS. Ten minutes later, 10 μL of the sample was removed and treated for observation by the FIC-2, providing a flow cytometric scattergram (A) and an image (B). FSC, forward scatter intensity, which indicates the size of particles; FFL, forward fluorescence intensity, which indicates the RNA or DNA content in the particles. The clustering dots with high FSC and low FFL indicate red blood cells. The curving line and pentagon in (A) indicate the range of platelets and the range of image observation, respectively. (B) Representative image of platelets within the pentagon. The image revealed the clustering of 2 to several platelets, consistent with the prediction by the particle counting method. Samples with no increase in the SPA-LS signal did not show platelet clustering by this device (data not shown).

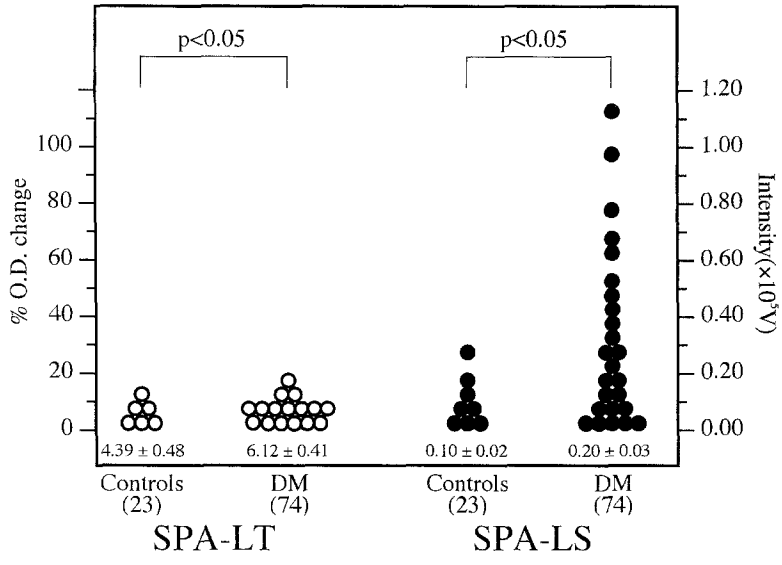

Fig 2. SPA-LS and SPA-LT values in age- and sex-matched healthy control subjects and diabetic (DM) patients. (○) SPA-LT; (●) SPA-LS. Each dot represents values for ≤5 individuals in both groups. Values are the mean ± SEM in each group (number of subjects indicated in parentheses).

increase in LS intensity was observed in PRP obtained from a diabetic patient, which indicates aggregates consisting of less than 100 platelets, and plateaued after several minutes of stirring. So, the LS intensity in each individual was recorded after 10 minutes of stirring and expressed as SPA-LS.

Figure 1 shows the scattergram (A) and image (B) of platelets obtained by the FIC-2. The device revealed that platelets obtained from patients with type II diabetes mellitus with increased SPA-LS showed microaggregates of several platelets. Platelets obtained from healthy controls without increased SPA-LS did not show the clustering of platelets (data not shown).

Figure 2 shows the distribution of SPA-LS and SPA-LT values in each of the age- and sex-matched control subjects and diabetic patients. SPA-LS values in diabetic patients are distributed at higher levels than the control values. On the other hand, SPA-LT values are distributed within a narrow range in control subjects and diabetic patients. Although both SPA-LS and SPA-LT showed a significant increase ($P < .05$) in diabetic patients as a whole compared with control subjects, the absolute value for SPA-LT was less than 10% and the difference between the two groups was small.

When the control subjects and three diabetic subgroups with a varying severity of retinopathy were compared as a whole by the Kruskal-Wallis test, there was a significant difference ($P < .0005$) in SPA-LS among the groups (Table 1). In addition, the mean values for SPA-LS increased along with the increasing severity of retinopathy, and a nonparametric Bonferroni test showed a significant increase in patients with proliferative retinopathy compared with controls or patients with no retinopathy. Although SPA-LT also showed a significant difference ($P < .05$) among these groups, the absolute values were all less than 10%. There was no significant difference in age or in gender distribution among the groups (Table 1). On the other hand, ADP-induced platelet aggregation failed to show significant difference among the groups by either LS or LT (Table 2). Other values for ADP-induced or Epi-induced platelet aggregation also showed similar results (data not shown).

Concerning SPA-LS in relation to diabetic nephropathy (Table 3) or neuropathy (Table 4), similar results were observed as in diabetic retinopathy. The mean values for SPA-LS also increased as the severity of complications increased (Tables 3 and 4). Although SPA-LT also showed significant differences

Table 1. SPA in Relation to Retinopathy

Parameter	Controls	DM Patients			P
		No Retinopathy	Simple Retinopathy	Proliferative Retinopathy	
No. of subjects	23	33	18	23	
M/F	12/11	22/11	12/6	12/11	NS
Age (yr)	55.2 ± 2.8	52.2 ± 2.2	59.3 ± 3.9	56.5 ± 2.4	NS
LT (% OD)	4.59 ± 0.40	5.39 ± 0.42	5.11 ± 0.66	8.26 ± 0.95*	<.05
LS (×10⁵ V)	0.06 ± 0.01	0.11 ± 0.02	0.18 ± 0.04	0.35 ± 0.07*†	<.0005

NOTE. The Kruskal-Wallis test was applied to compare values for the 4 groups as a whole, shown as the *P* value. The nonparametric Bonferroni test was used to compare between-group values.

Abbreviations: DM, diabetes mellitus; M/F, male to female ratio; NS, not significant.

*$P < .01$ *v* controls.

†$P < .05$ *v* no retinopathy.

Table 2. ADP-Induced Platelet Aggregation in Relation to Retinopathy

Parameter	Controls	DM Patients			P
		No Retinopathy	Simple Retinopathy	Proliferative Retinopathy	
No. of subjects	23	33	18	23	
M/F	12/11	22/11	12/6	12/11	NS
Age (yr)	55.2 ± 2.8	52.2 ± 2.2	59.3 ± 3.9	56.5 ± 2.4	NS
LT (% OD)	10.6 ± 3.9	8.4 ± 0.8	8.7 ± 5.7	13.7 ± 10.1	NS
LS ($\times 10^5$ V)	0.32 ± 0.10	0.37 ± 0.05	0.49 ± 0.10	0.58 ± 0.09	NS

NOTE. Platelets were stimulated by 0.5 μmol/L ADP. The Kruskal-Wallis test was applied to compare values for the 4 groups as a whole, shown as the *P* value.

Abbreviations: DM, diabetes mellitus; M/F, male to female ratio; NS, not significant.

among the groups, the absolute values were again all less than 10% (Tables 3 and 4). There was no significant difference in age or in gender distribution between these groups, except for the age in neuropathy. Again, agonist-induced platelet aggregation failed to show significant differences by either LS or LT in nephropathy or neuropathy, as in retinopathy (data not shown).

SPA-LS showed a significant correlation with the patients' age, HbA_{1c} level, plasma fibrinogen level, or 6KF/TXB2 ratio (Table 5).

DISCUSSION

Diabetic complications not only reduce the quality of life but also are life-threatening in diabetic patients. Thus, elucidation of the etiology of complications is a prerequisite for improving the quality of life and life expectancy in diabetic patients. It has been postulated that platelet hyperfunction may contribute to diabetic complications.[3,4] Although there are a few conflicting reports,[9,21] most investigators agree that platelet hyperfunction does exist in either type I or type II diabetic patients compared with normal control subjects.[10,22-24]

On the other hand, platelet hyperfunction in relation to the progression of complications is still controversial. Agardh et al[25] reported increased platelet aggregation in type I diabetic patients with proliferative retinopathy compared against those without it. However, they concluded that their study did not support the postulation that abnormal platelet function can be a primary cause of diabetic retinopathy, because they also observed a significant correlation between platelet aggregation and the duration of diabetes mellitus. Chitre and Velaskar[26] reported increased platelet aggregation in patients with retinopathy compared with normal control subjects. However, there was no comparison in their report between diabetic patients with and without retinopathy. On the contrary, Fritschi et al[27] observed no difference in platelet aggregation between diabetic patients with and without nephropathy. Szenasi et al[28] reported that platelet aggregation was decreased in diabetic patients with nephropathy compared with normal control subjects. Even a 3-year longitudinal study by Plu et al[29] did not reveal a correlation between deterioration of retinopathy and platelet aggregation. Thus, the findings reported thus far are conflicting and far from convincing. The conflicting results may be attributed to the type of platelet aggregometry used in the studies. Conventional aggregometry using changes in LT can only detect the formation of large platelet aggregates, which reflects rigorous platelet activation. However, platelet activation in vivo, ie, in diabetic patients, is expected to be subtle.

To overcome the drawback of the conventional LT method, Cho et al[30] performed a study measuring spontaneous whole blood platelet aggregation (SWBPA) in 563 type I diabetic patients. They observed a significant increase in SWBPA only in patients with neuropathy. However, there was no comparison of SWBPA in relation to the severity of neuropathy. Since SWBPA estimates platelet activation by measuring the decrease in the platelet count after shaking, it does not necessarily reflect platelet aggregation, because platelet counts can also be affected by the interaction between platelets and neutrophils or erythrocytes. So, none of the data currently available are sufficient to verify the postulation that platelet hyperfunction may contribute to complications in diabetic patients.

Table 3. SPA in Relation to Nephropathy

Parameter	Controls	DM Patients			P
		Normoalbuminuria	Microalbuminuria	Overt Albuminuria	
No. of subjects	23	27	29	18	
M/F	12/11	15/12	21/8	10/8	NS
Age (yr)	55.2 ± 2.8	56.3 ± 2.9	54.2 ± 2.5	55.2 ± 2.6	NS
LT (% OD)	4.59 ± 0.40	4.96 ± 0.49	6.03 ± 0.57	8.39 ± 1.08*†	<.01
LS ($\times 10^5$ V)	0.06 ± 0.01	0.13 ± 0.02	0.16 ± 0.04	0.37 ± 0.08*	<.001

NOTE. The Kruskal-Wallis test was applied to compare values for the 4 groups as a whole, shown as the *P* value. The nonparametric Bonferroni test was used to compare between each group.

Abbreviations: DM, diabetes mellitus; M/F, male to female ratio; NS, not significant.

*$P < .01$ *v* control.

†$P < .05$ *v* normoalbuminuria.

Table 4. SPA in Relation to Neuropathy

Parameter	Controls	DM Patients			P
		No Neuropathy	Mild Neuropathy	Severe Neuropathy	
No. of subjects	23	25	20	29	
M/F	12/11	14/11	13/7	19/10	NS
Age (yr)	55.2 ± 2.8	46.7 ± 1.8	53.4 ± 3.3	63.9 ± 1.9*	<.0001
LT (% OD)	4.59 ± 0.40	4.48 ± 0.46	6.75 ± 0.74	7.35 ± 0.75†‡	<.005
LS ($\times 10^5$ V)	0.06 ± 0.01	0.11 ± 0.02	0.18 ± 0.04†	0.30 ± 0.06†‡	<.0005

NOTE. The Kruskal-Wallis test was applied to compare values for the 4 groups as a whole, shown as the *P* value. The nonparametric Bonferroni test was used to compare between each group.

Abbreviations: DM, diabetes mellitus; M/F, male to female ratio; NS, not significant.

*$P < .01$ *v* control.

†$P < .05$ *v* controls.

‡$P < .05$ *v* no neuropathy.

Therefore, we measured SPA by the method developed by Ozaki et al,[11] which allows estimation of the formation of microaggregates of platelets. In fact, the presence of microaggregates of platelets was confirmed by the FIC-2 flow cytometer and imaging device. By the particle counting method using LS, we were able to confirm that SPA-LS is increased in type II diabetic patients compared with control subjects, and to demonstrate for the first time that SPA-LS is increased along with the increasing severity of complications. On the other hand, since agonist-induced platelet aggregation failed to show significant differences among these groups, we believe that SPA is more sensitive than agonist-induced platelet aggregation for distinguishing the varying severity of complications. Because SPA represents small platelet aggregates without agonists, these findings suggest that platelets obtained from diabetic patients can be easily activated and the myosin light chain can be easily phosphorylated.[12] We think that the positive correlation between SPA-LS and the age of the patients does not devaluate the above-mentioned interpretation, because we observed no significant differences in age between control subjects and diabetic patients as a whole or between control subjects and the three subgroups for severity of complications, except neuropathy. However, we cannot exclude the effect of age on SPA-LS in relation to diabetic neuropathy.

Although we also observed significant differences in SPA-LT between control subjects and diabetic patients as a whole or between control subjects and the three subgroups of increasing severity of complications, we do not believe that the values for SPA-LT warrant quantitative analysis, because the absolute values were all less than 10% and the differences between groups were small. Thus, we believe SPA-LS is a better and more sensitive indicator than SPA-LT[7,11,31] to evaluate the correlation of platelet aggregation with the increasing severity of diabetic complications.

Table 5. Correlation of SPA-LS With Other Parameters

Parameter	r	P
Age	.307	<.01
Diabetes duration	.252	NS
HbA_{1c}	.437	<.005
Fibrinogen	.326	<.05
6KF	−.288	NS
TXB2	.043	NS
6KF/TXB2	−.380	<.05
TG	.18	NS
TC	.13	NS
HDL-C	.106	NS

Abbreviations: TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; NS, not significant.

Since the present study is a cross-sectional evaluation of SPA, these results alone do not provide definite information as to whether platelet hyperaggregability is a cause or result of diabetic complications. A longitudinal prospective study of SPA by this new method and/or intervention with antiplatelet agents may provide more information to clarify cause-and-effect relationships between platelet function and diabetic complications.

For the relation of platelet aggregation and the age of the patients, no significant correlation was reported.[32,33] For platelet aggregation and the duration of diabetes mellitus, a positive correlation was observed in type I[25,34] but not in type II[32] diabetic patients. For the relation of platelet aggregation and glycemic control, a positive correlation was observed in both type I[34,35] and type II[24,35] diabetic patients, as well as no correlation in type I[36] and type II[21,32,37] diabetic patients. For other parameters, Cho et al[30] reported fibrinogen as a risk factor for microangiopathy. Fuller[38] also reported increased fibrinogen in subjects with diabetic nephropathy compared against those without it. On the other hand, Dallinger et al[39] reported no significant increase in fibrinogen in patients with type I diabetes. The available data are also conflicting in these aspects. The increase in the TXB2 concentration or decrease in the 6KF/TXB2 ratio is reported to be associated with increased platelet aggregation.[40] By the particle counting method, we observed significant correlations between SPA-LS and the patients' age, HbA_{1c} level, plasma fibrinogen level, or 6KF/TXB2 ratio. We speculate that the platelet hyperaggregability may be a consequence of a hypercoagulable state. However, we think it is also possible that platelet abnormalities play a role in platelet hyperaggregability. These points should also be clarified in future studies.

In conclusion, we believe our study is the first to provide convincing data to demonstrate a close relationship between platelet hyperaggregability and the increasing severity of diabetic complications, which supports the postulation that platelet hyperfunction may contribute to complications. To clarify cause-and-effect relationships between platelet hyperaggregability and diabetic complications, a longitudinal prospective study of SPA-LS is warranted.

ACKNOWLEDGMENT

The authors are grateful to Mayumi Orii, Yukiko Sato, and Mari Shimegi for preparing the manuscript.

REFERENCES

1. Bern MM: Platelet functions in diabetes mellitus. Diabetes 27:342-350, 1978

2. Colwell JA, Winocour PD, Halushka PV: Do platelets have anything to do with diabetic microvascular disease? Diabetes 32:14-19, 1983 (suppl 2)

3. Breddin HK, Krzwanek HJ, Althoff P, et al: Haemostasiological parameters as risk factor for new arterial occlusions in diabetics. Klin Wochenschr 64:1042-1046, 1986

4. Barnett AH: Pathogenesis of diabetic microangiopathy. An overview. Am J Med 90:675-735, 1991

5. Born GVR: Aggregation of blood platelets by adenosine diphosphate and its reversal. Nature 194:927-929, 1962

6. Born GVR: Observations on the change in shape of blood platelets brought about by adenosine diphosphate. J Physiol 209:487-511, 1970

7. Kitek A, Breddin K: Optical density variations and microscopic observations in the evaluation of platelet shape change and microaggregate formation. Thromb Haemost 44:154-158, 1980

8. Gear AR, Lambrecht JK: Reduction in single platelets during primary and secondary aggregation. Thromb Haemost 45:298, 1981 (letter)

9. Kobbah AM, Ewald U, Tuvemo T: Platelet aggregability during the first two years of type 1 (insulin-dependent) diabetes mellitus in children. Diabetologia 32:729-735, 1989

10. Falcon CR, Cattaneo M, Ghidoni A, et al: The in vitro production of thromboxane B_2 by platelets of diabetic patients is normal at physiological concentrations of ionized calcium. Thromb Haemost 70:389-392, 1993

11. Ozaki Y, Satoh K, Yatomi Y, et al: Detection of platelet aggregates with a particle counting method using light scattering. Anal Biochem 218:284-294, 1994

12. Fukuda K, Ozaki Y, Satoh K, et al: Phosphorylation of myosin light chain in resting platelets from NIDDM patients is enhanced: Correlation with spontaneous aggregation. Diabetes 46:488-493, 1997

13. World Health Organization: WHO Expert Committee on Diabetes Mellitus. Second Report. World Health Organ Tech Rep Ser 646: 1-80, 1980

14. Fukuda M: Clinical arrangement of classification of diabetic retinopathy. Tohoku J Exp Med 141:331-335, 1983 (suppl)

15. Viberti GC, Pickup JC, Jarrett RJ, et al: Effect of control of blood glucose on urinary excretion of albumin and β2 microglobulin in insulin-dependent diabetes. N Engl J Med 300:638-641, 1979

16. Yum M, Maxwell DR, Hamberger R, et al: Primary glomerulonephritis complicating diabetic nephropathy: Report of seven cases and review of the literature. Hum Pathol 15:921-927, 1984

17. Shindo H, Tawata M, Inoue M, et al: The effect of prostaglandin on vibratory threshold determined with the SMV-5 vibrometer in patients with diabetic neuropathy. Diabetes Res Clin Pract 24:173-180, 1994

18. Tawata M, Nitta K, Kurihara A, et al: Effects of a single drip infusion of lipo-prostaglandin E_1 on vibratory threshold in patients with diabetic neuropathy. Prostaglandins 49:27-39, 1995

19. Yamamoto T, Egawa Y, Shirasawa Y: A laser light scattering in situ system for counting aggregates in blood platelet aggregation. Meas Sci Technol 6:174-180, 1995

20. Kubota F, Kusuzawa H, Kosaka T, et al: Flow cytometer and imaging device used in combination. Cytometry 21:129-132, 1995

21. Rodier M, Colette C, Crastes de Paulet P, et al: Relationships between serum lipids, platelet membrane fatty acid composition and platelet aggregation in type 2 diabetes mellitus. Diabete Metab 19:560-565, 1993

22. Cho NH, Becker D, Dorman JS, et al: Spontaneous whole blood platelet aggregation in insulin-dependent diabetes mellitus: An evaluation in an epidemiologic study. Thromb Haemost 61:127-130, 1989

23. Mandal S, Sarode R, Dash S, et al: Hyperaggregation of platelets detected by whole blood platelet aggregometry in newly diagnosed non–insulin-dependent diabetes mellitus. Am J Clin Pathol 100:103-107, 1993

24. Srivastava S, Joshi CS, Sethi PP, et al: Altered platelet functions in non–insulin-dependent diabetes mellitus (NIDDM). Thromb Res 76:451-461, 1994

25. Agardh CD, Agardh E, Bauer B: Platelet aggregation in type I diabetics with and without proliferative retinopathy. Acta Ophthalmol 65:358-362, 1987

26. Chitre AP, Velaskar DP: Role of platelets in diabetic microangiopathy. An additional factor. Angiology 39:458-465, 1988

27. Fritschi J, Christe M, Lammle B, et al: Platelet aggregation, β-thromboglobulin and platelet factor 4 in diabetes mellitus and in patients with vasculopathy. Thromb Haemost 52:236-239, 1984

28. Szenasi P, Toth L, Kammerer L, et al: Differences in platelet aggregation in various microangiopathic complications of diabetes mellitus. Orv Hetil 130:617-620, 1989

29. Plu G, Lecrubier C, Scarabin PY, et al: Does increased platelet aggregation have a prognostic value in the deterioration of background diabetic retinopathy? The DAMAD Study Group. Thromb Haemost 64:202-205, 1990

30. Cho NH, Becker DJ, Ellis D, et al: Spontaneous whole blood platelet aggregation, hematological variables and complications in insulin-dependent diabetes mellitus: The Pittsburgh Epidemiology of Diabetes Complications Study. J Diabetic Complications 6:12-18, 1992

31. Qi R, Ozaki Y, Satoh K, et al: Quantitative measurement of various 5-HT receptor antagonists on platelet activation induced by serotonin. Thromb Res 81:43-54, 1996

32. Altomonte L, Zoli A, Mangia A, et al: Thromboxane production in diabetes mellitus. Ric Clin Lab 16:539-542, 1986

33. Menys VC, Bhatnagar D, Mackness MI, et al: Spontaneous platelet aggregation in whole blood is increased in non–insulin-dependent diabetes mellitus and in female but not male patients with primary dyslipidemia. Atherosclerosis 112:115-122, 1995

34. Nowak-Gottl U, Kreuz WD, Besier E, et al: Thrombocyte function in children with type I diabetes mellitus. Cross-sectional study. Monatsschr Kinderheilkd 139:821-825, 1991

35. Jones DB, Davis TM, Bown E, et al: Determinants of ADP-induced platelet aggregation in diabetes mellitus. Diabetologia 29:291-294, 1986

36. Christiansen JS, Ingerslev J, Bernvil SS, et al: Near normoglycemia for 1 year has no effect on platelet reactivity, factor VIII, and von Willebrand factor in insulin-dependent diabetes mellitus: A controlled trial. J Diabetic Complications 3:100-106, 1987

37. Ratzmann KP, Schimke E, Beitz A, et al: Thromboxane production and platelet aggregation in type 2 diabetes mellitus without vascular complications. Klin Wochenschr 69:652-656, 1991

38. Fuller JH: Hemostatic function tests associated with diabetic microangiopathy. Horm Metab Res Suppl 11:59-62, 1981

39. Dallinger KJ, Jennings PE, Toop MJ, et al: Platelet aggregation and coagulation factors in insulin-dependent diabetes mellitus with and without microangiopathy. Diabet Med 4:44-48, 1987

40. Axelrod L, Camuso J, Williams E, et al: Effects of a small quantity of omega-3 fatty acids on cardiovascular risk factors in NIDDM. A randomized, prospective, double-blind, controlled study. Diabetes Care 17:37-44, 1994