Table 3 Multivariate-adjusted[a] hazard ratios for the development of ischemic stroke according to the presence or absence of obesity and each established risk factor in men, the Hisayama Study, 1988–2000

		Population at risk	*No. of events*	*HR*	*95% CI*	*P value*
Obesity[b]	*Hypertension*					
No	No	477	14	1.00	Referent	
No	Yes	308	17	1.59	0.76–3.34	0.22
Yes	No	111	7	3.79	1.44–10.00	0.007
Yes	Yes	141	9	2.95	1.19–7.30	0.02
Obesity[b]	*Diabetes*					
No	No	678	25	1.00	Referent	
No	Yes	107	6	1.60	0.65–3.97	0.31
Yes	No	200	8	1.83	0.77–4.38	0.17
Yes	Yes	52	8	7.91	3.08–20.28	<0.001
Obesity[b]	*Smoking*					
No	No	369	17	1.00	Referent	
No	Yes	416	14	1.18	0.56–2.48	0.67
Yes	No	148	8	2.13	0.83–5.46	0.11
Yes	Yes	104	8	3.62	1.39–9.43	0.008

Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval.
[a]Multivariate adjustment was made for age, systolic blood pressure, ECG abnormalities, diabetes, total and high-density lipoprotein-cholesterols, triglycerides, smoking, drinking and regular exercise, but the factor which was used for each grouping was excluded from the confounding factors.
[b]Obesity is defined as a body mass index $\geq 25\,kg\,m^{-2}$.

The strengths of our study include its longitudinal population-based design, the direct collection of height, weight and biological markers from all participants, long duration of follow-up, perfect follow-up of subjects and accuracy of diagnosis of stroke. One limitation of our study is that our findings are based on a one-time measurement of BMI, as was the case in most other epidemiological studies. During the follow-up, BMI and other risk factor levels were changed due to modifications in lifestyle or medication, and misclassification of BMI categories was possible. This could have weakened the association found in this study, biasing the results toward the null hypothesis. Therefore, the true association may be stronger than that shown here.

In conclusion, our data suggest that overweight and obesity are significant risk factors for the development of ischemic stroke in contemporary Japanese men. In Japan, BMI levels have increased steadily over the last several decades. For prevention of stroke, it is important to correct obesity while controlling other risk factors.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This study was supported in part by Grants-in-Aid for Scientific Research A (No. 18209024) and C (No. 20591063) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and a Health and Labour Sciences Research Grant of the Ministry of Health, Labour and Welfare of Japan (Comprehensive Research on Aging and Health: H20-Chouju-004). The authors thank the staff of the Division of Health and Welfare of Hisayama for their cooperation in this study.

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Graefes Arch Clin Exp Ophthalmol (2012) 250:71–78
DOI 10.1007/s00417-011-1777-7

RETINAL DISORDERS

Comparative study of vitrectomy versus intravitreous triamcinolone for diabetic macular edema on randomized paired-eyes

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Received: 4 February 2011 /Revised: 11 July 2011 /Accepted: 28 July 2011 /Published online: 19 August 2011

Abstract
Background The present study was performed to compare the effects of pars plana vitrectomy (PPV) and single intravitreaous triamcinolone acetonide (IVTA) on diabetic macular edema (DME) in paired eyes.
Methods Prospective comparative study on randomized paired-eyes was carried out at two hospitals. Forty eyes of 20 patients with bilateral DME were included. One randomly-selected eye was treated with PPV (PPV group), and the other eye was treated with IVTA (4 mg, IVTA group). The central macular thickness (CMT) measured by optical coherence tomography (OCT) and best-corrected visual acuity (BCVA) were monitored for 12 months after treatment. Changes from baseline and differences between groups were analyzed using a mixed model.
Results At 1 and 3 months, CMT decreased significantly in the IVTA group compared to baseline ($p<0.0001$ both), but CMT then increased gradually and no significant difference was found at 12 months ($p=0.90$). In the PPV group, CMT decreased continuously and reached a significant level at 12 months ($p<0.0001$). CMT of the IVTA group was significantly less than that of the PPV group at 1 month ($p=0.009$); however, there was no significant difference at 3 months. Conversely, CMT was significantly less in the PPV group than in the IVTA group at 12 months ($p=0.0003$). The changes of BCVA paralleled those of CMT, but no significant difference was detected between baseline BCVA and any time point.
Conclusions Despite the short-term improvement, DME recurred 6 months after IVTA, while it remained resolved after PPV. Although this study did not reveal a significant change of BCVA with either treatment, PPV resolved DME more effectively than IVTA at 1 year.

Keywords Diabetic retinopathy · Corticosteroid · Central macular thickness · Pars plana vitrectomy · Pharmacologic therapy

Registration The study was registered with (University Hospital Medical Information Network, Japan (UMIN) ID# 000000432; date of registration June 21 2006, beginning date of trial July 1 2006).

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Recently, pharmacologic treatments for diabetic macular edema (DME), such as treatment with corticosteroid or anti-vascular endothelial growth factor (VEGF), have attracted the attention of ophthalmologists. Compared to other treatments, pharmacologic treatment has an immediate effect, which is a great advantage for patients. Intravitreous steroid injection has been reported to resolve DME significantly [1–8]. A systematic review suggests that intravitreal steroid injections may have benefits in some cases of persistent or refractory DME when the current standard of care is insufficient [6]. Meanwhile, a randomized clinical trial shows that grid laser photocoagulation is more effective and less harmful to DME eyes over 3 years [9]. Anti-VEGF drugs are a new pharmacological option and are effective for resolving DME without serious

adverse events [5, 7, 8]. However, the superiority of this treatment over corticosteroid treatment has not yet been validated completely. Both treatments have comparatively short-term effects, and the need for repeated injections is a major drawback.

Vitrectomy is alternative approach for treating DME [10–30]. After the report by Lewis et al, subsequent reports also claim it is effective for reducing DME [10, 12, 16, 24, 28]. At present, the mechanism by which vitrectomy resolves DME is not fully understood, but it is speculated that vitrectomy not only removes the vitreous traction, but also improves the local environment [16, 17]. Indeed, some studies showed that PPV is more effective for reducing the macular volume of DME eyes than grid laser photocoagulation [22, 23], and our previous study found that DME continued to be resolved more than 4 years after a single vitrectomy [24]. However, to our knowledge, there has been no randomized comparative study of PPV and pharmacologic treatment for DME.

For the present study, we performed a randomized paired-eye investigation of these two treatments for DME. An advantage of paired-eye studies is the elimination of extra-ocular factors, that obscure the precise effects of the two forms of intervention. Consequently, the results provide important information to justify the use of either treatment before data from a large-scale study and/or wide-spread use become available.

Methods

This prospective controlled clinical study was carried out with the approval of the institutional review boards of Imamura Hospital and Kagoshima University Hospital, and in accordance with the ethical standards laid down by the 1989 Declaration of Helsinki. All treatments were performed at either Imamura Hospital or Kagoshima University Hospital, Kagoshima, Japan. The study was registered with University Hospital Medical Information Network, Japan (UMIN) ID# 000000432. Patients attending the two hospitals during the study period received explanations on the experimental nature of the study. Only those who gave informed written consent were enrolled in this study.

Primary outcome measures

The primary outcome measure was resolution of DME. This was first estimated by change of CMT at 12 months after intervention. A significant decrease of CMT was defined as a decrease of more than 20% from baseline. Our preliminary study shows that intravitreous triamcinolone (IVTA) led to a significant decrease in 30% to 50% of treated eyes (data not shown) at 12 months, while our previous studies showed that a significant decrease of CMT was observed in approximately 85% of DME eyes at 12 months after PPV [24, 25]. Therefore, the projected effectiveness of IVTA was hypothesized to be 40%. On this basis, the minimum number of patients to be included in the study to achieve statistical significance was calculated to be 17 for each group on the basis of a calculation using a binomial distribution with a 2-sided significance level of 0.05, and a power of 80%. Therefore, 20 patients were recruited to ensure that the projected number was achieved. Using the same patients, best-corrected visual acuity (BCVA) was also analyzed.

Eligibility criteria

The inclusion criteria were as follows (1) Those with bilateral diffuse DME (CMT; 300 μm <) determined by optical coherence tomography (OCT version 3, Zeiss, Dublin, CA, USA), without a history of retinal diseases except diabetic retinopathy (DR), (2) those with BCVA between 0.2 and 1.0 of a logarithm of the minimum angle of the resolution chart (logMAR), and (3) those aged 20 years or older.

The exclusion criteria were as follows (1) Eyes with signs of vitreo-macular traction on biomicroscopy or OCT, (2) eyes with apparent posterior vitreous detachment, (3) eyes with active proliferative DR, (4) eyes with known history of glaucoma, (5) eyes with optic nerve atrophy, (6) eyes with a history of photocoagulation within 3 months, (7) eyes with a history of vitrectomy, (8) eyes with a history of intravitreous or periocular injection of drugs, and (9) eyes with significant cataract that prevents preoperative OCT evaluation. Patients with HbA1c 10% or higher, a history of hemo-dialysis, or diastolic blood pressure of more than 100 mmHg were also excluded.

Treatment protocol

The following preoperative data were reviewed from the records: age, gender, and serum HbA_1c level within 2 weeks before intervention. The following baseline clinical features were also obtained by the examination within 1 week before surgery: BCVA, CMT, status of DR, duration of diabetes, duration of visual disturbance, and history of photocoagulation treatment. To determine CMT, OCT scanning was performed horizontally and vertically through the fovea. CMT was defined as the distance from the vitreoretinal interface to the retinal pigment epithelium. Measurements were taken manually according to our previously described method [19, 31].

Follow-up examinations were performed for the following BCVA, CMT, intraocular pressure (IOP), and postoperative complications. Complications defined as events that caused significant loss of vision were apparently related to primary intervention and required additional treatment.

IVTA (4 mg, Kenacort; Bristol-Myers Squibb, Tokyo, Japan) was performed according to our previously described method [32]. Surgery consisted of standard 20-gauge three-port PPV with endophotocoagulation according to our previously described method [26]. Triamcinlone acetonide (TA) was not used during or after surgery. No eyes underwent photocoagulation of the macular area during surgery. The posterior hyaloid was separated from the optic disc in eyes with no posterior vitreous detachment. No eyes underwent internal limiting membrane peeling. Surgery was performed by two qualified surgeons (ND, and KY).

Randomization and retreatment protocol

The eye of each patient treated with PPV was randomly selected by the sealed-envelope method. The other eye was treated with IVTA. IVTA and PPV treatments were performed on the same patient on the same day. As a rule, retreatment was considered in the following cases after at least 6 months according to the following criteria. (1) eyes in which CMT increased 30% or more from the previous CMT and BCVA dropped by more than 0.3 of logMAR from the previous examination. In such cases, the same treatment was repeated as a second intervention. An alternative treatment could be performed at the physician's discretion. However, even if the eye met the above criteria, retreatment could not be performed without the patient's agreement. (2) When the physicians judged it necessary to perform an additional PPV at any time (e.g., for recurrent hemorrhage, retinal detachment, or other reasons), PPV was performed. (3) When BCVA was impaired significantly due to cataract, cataract surgery was performed. If IOP increased to 25 mmHg or more for 3 days, antiglaucoma medication was administered. If the, IOP rise could not be controlled with medication, filtering surgery was considered.

Factors related to individuals

It is possible for a paired-eye study to give additional information on the influence of non-ocular factors upon analyzing data of individuals. For example, if bilateral CMT changes regardless of ocular intervention, it indicates that the difference between PPV and IVB is not great enough to change the course of DME, but that non-ocular systemic factors might be important.

To see this effect, the therapeutic results for each eye were first divided into three subgroups based upon CMT at 12 months: "improvement" was defined as CMT of 80% or less than baseline, "worsening" was defined as CMT of 20% more than baseline, and "stable" was any value between those of "improvement" and "worsening". Each individual was further categorized based on subgroup. For example, when bilateral eyes were categorized into the same group, one was categorized into "same" group. When the categorization of each eye differed, one was categorized into "unequal" group. When eyes were in opposing categorizations. such as "improvement" group and "worsening" group, one was categorized as "opposition."

Statistical analysis

For the statistical analysis, BCVA was converted into the logarithm of logMAR. To retain all the results of individuals with or without missing data, a mixed model analysis was performed. Changes from baseline CMT, BCVA or IOP at 1, 3, 6, and 12 months were analyzed by a mixed model using treatment, time, and treatment-by-time interaction as fixed effects and baseline value as covariate. Baseline adjusted mean values were calculated from this model. Differences of CMT, BCVA or IOP between each time point and baseline were also analyzed using a mixed model. Multiple comparisons between each time point and baseline were also evaluated by Bonferroni's method. Duration of disease was analyzed by the Wilcoxon rank sum test. The above analyses were performed using SAS version 9.2. A null hypothesis was rejected for p values of less than 0.05.

Results

Between July 2006 and December 2008, 40 eyes of 20 patients (13 males and seven females) were enrolled in this study. All patients had type-2 diabetes. During the follow-up period, examination at 1 month was completed for all 20 patients (100%), examination at 3 months was completed for 19 patients (95%), examination at 6 months was completed for 17 patients (85%), and 12-month follow-up was completed for 16 patients (80%). As a result, four patients had dropped out due to personal reasons unrelated to the disease at 1 year. Because the percentage of missing data was not negligible, the data were analyzed using a mixed model analysis. Therefore, all results for individuals with or without missing data could be retained in the analysis.

All eyes were phakic. PPV was performed for 13 right and seven left eyes (Table 1). Duration of diabetes was 14.4 years on average (10 to 20 years, median 14 years). Three patients used insulin daily, and three patients used

Table 1 Baseline characteristics

	PPV group (n=20)	IVTA group (n=20)	P between groups
Right eyes (%)	13 (68.8)	7 (31.3)	NS
Lens			P=1
Phakic	20	20	
Pseudophakic	0	0	
Duration of visual disturbance (months)			NS
Median	3	3	
(Range)	(2 to 24)	(1 to 24)	
CMT (μm)			P=0.83
Median	412	439	
(Range)	(301 to 788)	(331 to 834)	
BCVA			P=0.75
Median	0.4	0.4	
(Range)	(0.2 to 1.0)	(0.2 to 1.0)	
Stages of DR			P=1
Severe non PDR	16	16	
Moderate non PDR	4	4	
Previous Photocoagulation			P=1
Scattered	16	16	
No	4	4	

IVTA, intravitreous triamcinolone: PPV, pars plana vitreectomy: BCVA, best-corrected visual acuity (log MAR): CMT = central macular thickness: DR, diabetic retinopathy: PDR, proliferative diabetic retinopathy.

insulin occasionally when blood glucose was uncontrollable. Preoperative HbA1c ranged from 5.1% to 9.1% with a median of 7.1%. Thirty-two eyes of 16 cases were severe non-PDR, and all of them received scattered retinal photocoagulation 6 months or more before intervention. Eight eyes of four patients were moderate non-PDR without retinal photocoagulation (Table 1). Duration of visual disturbance did not differ between groups (Table 1). In 32 eyes that completed follow-up at 12 months, three eyes of the IVTA group met the criteria for re-treatment; however, no patients agreed to receive retreatment. As a result, no eye received a second intervention, such as change of method of intervention, second IVTA, or PPV. No progression of cataract that required surgery was found in either group during the observation period.

CMT (central macular thickness)

In terms of baseline CMT, there was no significant difference between IVTA and PPV groups [p=0.83, non-parametric Wilcoxon rank sum test: 448 ± 131 μm in IVTA group and 508 ± 197 μm in PPV group, mean (± SD), Table 1]. CMT decreased initially after IVTA. At 1 month and 3 months, CMT was significantly less than baseline (p<0.0001 both). However, CMT increased thereafter and no significant difference was found at 6 months or 12 months (p=0.021 and p=0.90 respectively). On the other hand, CMT decreased continuously after PPV and there was a significant difference at 12 months (p=0.28 at 1 month, p=0.016 at 3 months, p=0.15 at 6 months, and p<0.0001 at 12 months; significant difference was p<0.0125 after Bonferroni's correction, mixed model) (Fig. 1).

Comparing the two groups, IVTA reduced CMT more significantly than PPV at 1 month (p=0.0009). However, statistical significance was lost at 3 or 6 months (p=0.16 or p=0.54 respectively). Eyes that received PPV had significantly less CMT than those that received IVTA at 12 months

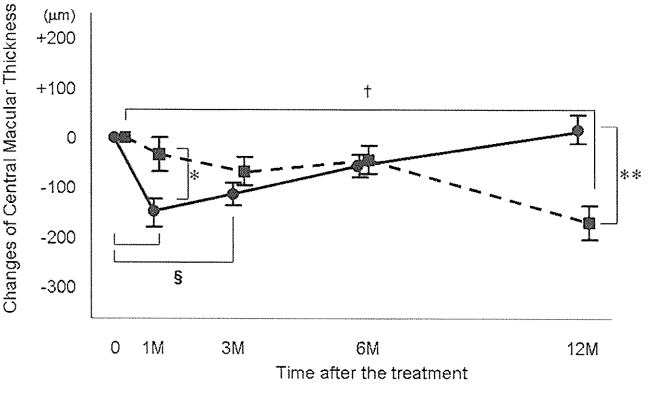

Fig. 1 Change of central macular thickness (CMT) over 12 months. At 1 month or 3 months after intravitreous triamcinolone (IVTA, *closed circle*), CMT was significantly less than that of baseline (§, p< 0.0001). However, CMT increased thereafter and no significant difference was found after 6 months. CMT decreased continuously after pars plana vitrectomy (PPV, *closed square*) and there was a significant difference at 12 months (†, p<0.0001). Comparing the two groups, IVTA reduced CMT more significantly than PPV at 1 (*, p= 0.0009). However, the difference was lost at 6 months. Eyes treated with PPV had significantly less CMT than those treated with IVTA at 12 months (**, p=0.0003). A statistical analysis was performed using a mixed model. (average ± SE)

(p=0.0003, significant difference was P<0.0125 after Bonferroni's correction, a mixed model).

Regarding the changing pattern of individuals, of 16 patients that completed 12 months of follow-up, five patients showed bilateral "improvement" (31.3%), three patients showed "improvement" for one eye and "stable" for the other eye (15.7%), three eyes showed the bilaterally "stable" CMT (15.7%), three patients had "stable" in one eye and "worsening" in the other (15.7%), one patient had the bilateral "worsening" (6.3%), and one patient had the improvement for one eye and "worsening" for the other (6.3%). Therefore, nine participants (56.3%) were categorized into the "same" group and five participants were categorized into the "unequal" group (31.3%). Only one case showed the "opposition" change pattern (6.3%) (Fig. 2).

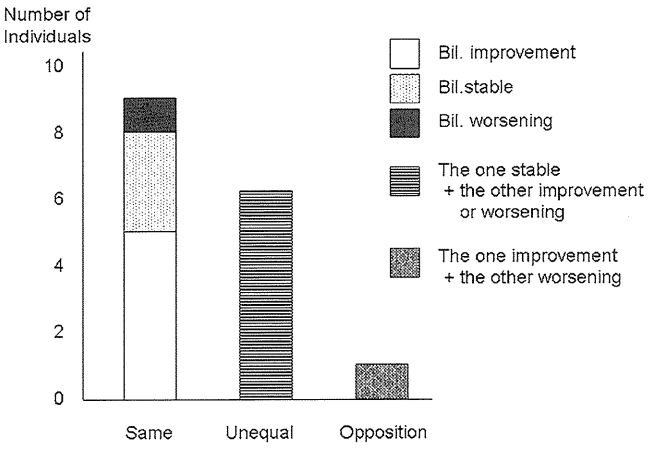

Fig.2 Central macular thickness changing pattern of each case at 12 months. CMT at 12 months was compared to the baseline. Each eye was categorized into one of three groups: "improvement", "stable," or "worsening" (See Methods). Then, each individual was further categorized into one three groups "same", "unequal" or "opposition" based upon the pattern of change. As a result, nine cases (56.3%) were categorized into the "same" group and five were categorized into the "unequal" group (31.3%). Only one case showed the "opposition" change pattern (6.3%). *Bil*, bilaterally

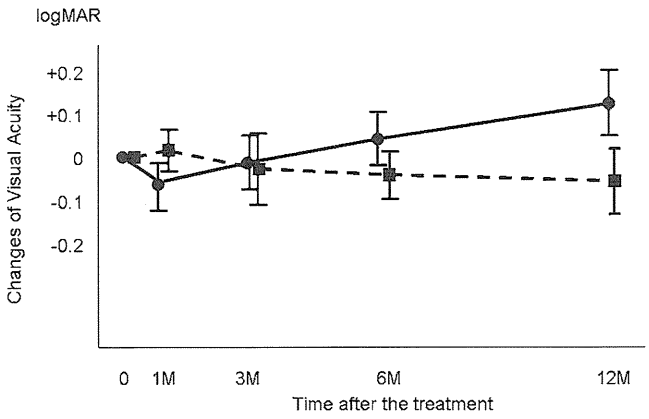

Fig. 3 Change of visual acuity over 12 months . Although the visual acuity of each group, PPV (*closed square*) or IVTA (*closed circle*), showed a changing pattern in parallel to CMT over 12 months, there was no significant difference between the groups or between the baseline at each time point within the group (mixed model). Values were expressed as the difference from the baseline; average ± SE

BCVA

Baseline BCVA was not significantly different (p=0.75, non-parametric Wilcoxon rank sum test: 0.46 ± 0.31 log MAR in IVTA group and 0.51 ± 0.28 log MAR in PPV group, mean ± SD).

The average BCVA of IVTA eyes improved after 1 month without reaching statistical significance. But it deteriorated continuously for 12 months (p=0.25 at 1 month, p=0.98 at 3 months, p=0.52 at 6 months, and p= 0.093 at 12 months; significant difference was p<0.0125 after Bonferroni's correction, mixed model, Fig. 3). In PPV-treated eyes, the average BCVA improved continuously for 12 months; however, no significant difference was found throughout the period (p=0.87 at 1 month, p=0.96 at 3 months, p= 0.44 at 6 months and p= 0.34 at 12 months; significant difference was p<0.0125 after Bonferroni's correction, mixed model, Fig. 3).

BCVA showed a changing pattern paralleling CMT over 12 months and did not reach significance at any time point (p= 0.37 at 1 month, p= 0.96 at 3 months, p= 0.36 at 6 months, and p= 0.082 at 12 months; significant difference was p<0.0125 after Bonferroni's correction, mixed model, Fig. 3).

Complications

Baseline IOP was not significantly different. The increase of IOP was significant at 1 month in the IVTA group (p= 0.0021), but there was no significance thereafter (p= 0.032 at 3 months, p= 0.042 at 6 months, and p= 0.14 at 12 months; significant difference was p<0.0125 after Bonferroni's correction, mixed model, Fig. 4). In the PPL group, no significance was detected at any time point (p= 0.58 at 1 month, p= 0.50 at 3 months, p= 0.042 at 6 months, and p= 0.13 at 12 months; significant difference was p< 0.0125 after Bonferroni's correction, mixed model, Fig. 4). There was no difference between the 2 groups (p= 0.028 at 1 month, p= 0.20 at 3 months, p= 0.068 at 6 months, and p= 0.89 at 12 months; significant difference was p<0.0125 after Bonferroni's correction, mixed model, Fig. 4).

In the IVTA group, two eyes received anti-glaucoma eye drops: 28 mmHg at 3 months and 32 mmHg at 6 months respectively. Both were controlled well, and IOP returned to less than 20 mmHg with no medication within 1 month. In the other patient, the eye treated with PPV had an uncontrollable IOP rise after 4 months, and was treated

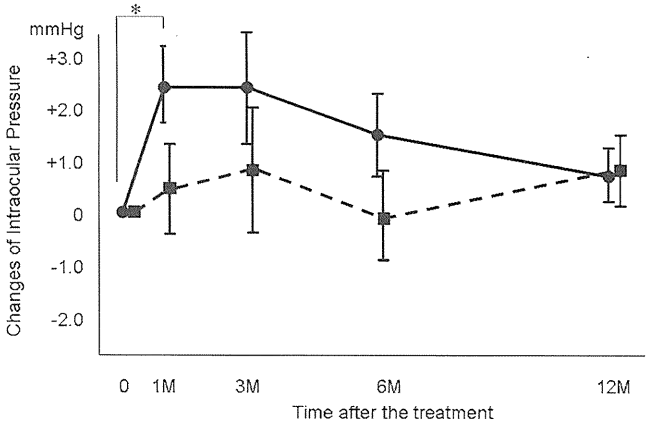

Fig. 4 Intraocular pressure change (IOP) over 12 months. IOP increased significantly at 1 month in the IVTA group (*: $p = 0.0021$), but not significant thereafter. In the PPV group; no significance was detected. Comparison showed no differences between the two groups (mixed model, the difference from baseline; average ± SE)

with filtering surgery. The other eye, which was treated with IVTA, had severe vitreous hemorrhage after 5 months, and then was treated with PPV. There were no complications related to intravitreous injection or PPV. No additional cataract surgery was done within 12 months for eyes treated with IVTA.

Discussion

In this randomized paired-eye study at 12 months, DME eyes treated with PPV had a more significant CMT reduction than those treated with IVTA. Although no statistically significant difference was detected, the changing pattern of BCVA paralleled that of CMT. These results indicate that PPV can resolve DME more effectively than single IVTA for 1 year.

Many previous studies have shown that PPV is effective for reducing CMT in DME [10–17, 19–21, 24, 28, 29]. However, as a general rule, these studies involved small numbers of patients, were non-comparative, and were not controlled. To our knowledge, there have been only limited numbers of randomized trials on PPV for DME, and these were also small [23, 33]. Although they had minimal selection bias, systemic factors related to each individual were likely to influence the results of these studies, which might have obscured an accurate evaluation of PPV for DME. Yanyali et al. reported the superiority of PPV over grid laser photocoagulation using the paired-eye study, although Thomas et al. did not find PPV to be superior in a randomized-controlled trial of unpaired eyes [22, 33]. The pathogenesis of DME is multi-factorial and influenced by duration of diabetes and systemic factors. So, the results of intervention for DME cannot be free from the influence of these factors. The discrepancies between these two reports might be related to the advantages of a paired-eye study, which can eliminate the influences of systemic factors. Therefore, it is possible that the present paired-eye study accurately reflect differences in therapeutic values between PPV and IVTA for DME.

In this study, we chose IVTA as the pharmacologic treatment because it was widely used for treating DME at the time the study was initiated. Furthermore, IVTA showed a more significant benefit than intravitreous bevacizumab at 6 months in our previous study [27]. Indeed, Cochrane Systemic Review described that intraocular steroid therapy improved the visual acuity of DME eyes [6]. However, it was disclosed that grid laser photocoagulation is more effective than IVTA for improving vision over 3 years [7]. Therefore, one might criticize the use of IVTA as a control pharmacologic intervention for DME in this study. Nonetheless, IVTA is used as a standalone or adjuvant of combined treatment for DME even now, and long-term benefits have been confirmed in selected cases [34]. The earlier report of DRCR found no significant difference in visual acuity or CMT between IVTA and grid laser photocoagulation at 1 year [31]. Moreover, a recent report of DRCR suggested IVTA might reduce the risk of progression of diabetic retinopathy for a long period, although a final conclusion yet to be obtained [32]. Thus, there should be a value in applying IVTA as a pharmacologic treatment for DME and in comparing PPV to IVTA over 12 months.

Kumar et al. reported that PPV with internal limiting membrane peeling could significantly reduce the macular volume of DME eyes over 6 months [23]. Although PPV was more effective than grid laser photocoagulation, no significant improvement of BCVA was found, similarly to the present results [23]. In contrast, Yanyali et al. showed that PPV improved both CMT and BCVA of DME over 6 months [22]. We cannot ascertain exactly why the present results differ from Yanyali's results. It is possible that baseline visual acuity was much better in the present study than in Yanyali's study; the average baseline BCVA was 0.51 logMAR in the present study and 0.75 logMAR in Yanyali's study. Eyes with worse baseline visual acuity are likely to have vision improved by intervention [35].

The mechanism whereby PPV reduces CMT of DME is still unclear, but long-term benefits have been reported. The beneficial effects of PPV on diffuse DME appear slowly, but are maintained steadily for years [24, 29]. This is probably because PPV can remove local exacerbation factors such as taut hyaloid traction and also improve local oxygen concentration or vitreous clearance permanently [30]. By contrast, IVTA reduces the CMT of DME very rapidly, but recurrence is frequent. TA can inhibit the synthesis of molecules including VEGF through which IVTA might

have resolved DME [36]. However, in general, the drug works only when it is present. Conversely, a rebound phenomenon associated with glucocorticoid withdrawal is widely reported [37, 38]. Continuous stimulation with corticosteroids is reported to cause steroid resistance by increasing nonfunctional nuclear receptors. These might have caused the rebound-like progression of DME from 3 months after IVTA found in this study. Therefore, PPV is considered to be more beneficial than IVTA over a long period, and it should be considered more positively for the treatment of DME; however, it should be remembered that there is always a risk of complication related to surgery [26]. Furthermore, intravitreous injection of microplasmin is known to cause posterior vitreous detachment [39]. If the present effect of PPV was caused by induction of posterior vitreous detachment, microplasmin injection can also be a treatment for DME.

It is known that both of PPV and IVTA are cataractogenic. However, a vision-impairing cataract was not observed in either of interventions of this study. Our previous studies of PPV or IVTA for Japanese patients showed that cataract was not evident until 1 year , then appeared thereafter [24–26, 40, 41]. This might be something to do with ethnicity. At this moment, there are various methods of treating DME, and this issue will be followed up in the next report.

This study obtained another important result. The factors related to individuals influenced the progression of DME regardless of form of ocular intervention. Of 16 patients, nine patients (56.3%) had the same progression pattern of bilateral eyes (five "improvement," three "stable," and one "worsening"). In only one case did eyes have opposite results; that is, one eye had "improvement " with PPV and the other eye had "worsening" with IVTA. It is impossible to identify the non-ocular factors that specifically affect the course of DME from the present limited cases. This is probably one of the most difficult issues when evaluating interventions for DME.

Because the primary objective of this study was to identify differences in the value of PPV and IVTA for reducing CMT, the number of patients enrolled it is a material factor. Naturally, the goal of treating DME should be to improve vision. Although changes of BCVA paralleled those of CMT in each group, there might be insufficient power to detect differences of BCVA. Second, IVTA is usually repeated in the treatment of DME. However, a single IVTA was performed in this series because the patients did not consent to further intervention. We did not intend to evaluate the effects of a single IVTA, but they showed subsequently in the results of single IVTA. If we had used a higher dose of IVTA which was expected to be effective for longer, the results might have been different. In addition, the present study was carried out on patients who had comparatively well-controlled diabetes with bilateral DME. There are several other limitations of the present study. Central visual field damage can be caused by vitrectomy or grid laser photocoaguation. Because visual field test was not done in the present study, this adverse event must have been overlooked. This issue should be considered carefully when determining the treatment. CMT reduction can be caused by retinal atrophic degeneration and/or reduction of retinal edema. If CMT reduction was a result of atrophic change, the value of treatment would not be great. We did not differentiate between these changes in the present study. In addition, it is necessary to know if the power is insufficient to detect adverse events related to each intervention in this study. Therefore, interpretation and generalization of the results should be done with care.

In summary, the present study showed that PPV is more effective for resolving DME than single IVTA at 12 months. Because the adverse events were limited and controllable, another controlled comparative study of PPV could be justified.

Acknowledgements We appreciate the technical support by Drs Hiroki Otsuka, MD, and Toshifumi Yamashita, MD.

Grant support Supported in part by a Grant from the Research Committee on Chorioretinal Degeneration and Optic Atrophy, Ministry of Health, Labor, and Welfare; by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of the Japanese Government.

Conflict of interest None.

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High Serum Bilirubin Levels and Diabetic Retinopathy

The Hisayama Study

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Purpose: To assess the association between serum total bilirubin levels and diabetic retinopathy prevalence in participants of the Hisayama Study who had diabetes and impaired glucose metabolism.

Design: Population-based, cross-sectional study.

Participants: Of 3119 participants of the Hisayama Study Eye Examinations in 2007, Japan, 1672 aged ≥40 years with either diabetes or impaired glucose metabolism (defined by a 75-g oral glucose tolerance test) were enrolled in the present study.

Methods: Diabetic retinopathy was assessed via ophthalmic examination after pupil dilatation. The presence and the severity of diabetic retinopathy were determined by grading of color fundus photographs using the modified Airlie House classification system. Association of diabetic retinopathy with serum bilirubin quartiles was assessed using logistic regression model adjusting for age and known risk factors for diabetic retinopathy.

Main Outcome Measures: Prevalent diabetic retinopathy.

Results: Diabetic retinopathy was present in 70 of 1672 (4.2%) participants. The prevalence of diabetic retinopathy in persons with the highest bilirubin quartile (≥0.9 mg/dL) was 2.7%, compared with the prevalence of 3.4%, 5.1%, and 5.1% in those with the first (<0.6 mg/dL), second (0.6–0.69 mg/dL), and third quartiles (0.7–0.89 mg/dL). After adjusting for factors known to be associated with diabetic retinopathy, the prevalence was significantly lower among persons with the highest bilirubin quartile compared with those with the lowest quartile (odds ratio [OR], 0.25; 95% confidence interval [CI], 0.09–0.72) or compared with those in the 3 lower quartiles (OR, 0.25; 95% CI, 0.11–0.58).

Conclusions: Elevated serum bilirubin levels may be protective against diabetic retinopathy among persons with either diabetes or impaired glucose metabolism, independent of known risk factors for diabetic retinopathy.

Financial Disclosure(s): The authors have no proprietary or commercial interest in any of the materials discussed in this article. *Ophthalmology 2011;118:1423–1428 © 2011 by the American Academy of Ophthalmology.*

Diabetic retinopathy (DR) is a common complication of diabetes and is among the leading causes of blindness and visual impairment among working age persons in developed countries.[1] A number of population-based studies have reported retinopathy lesions not only present in persons with diabetes but also in persons with impaired glucose tolerance or impaired fasting glucose.[2]

Bilirubin has been recognized as an important endogenous antioxidant.[3] In several prospective studies, an inverse relationship has been reported between high bilirubin levels and cardiovascular disease[4] as well as coronary heart disease.[5–7] Cross-sectional studies reported similar protective associations of bilirubin levels with coronary artery disease,[8] peripheral vascular disease,[9] carotid intimal medial thickness,[10] and stroke.[11] This inverse relationship of bilirubin levels to cardiovascular disease was confirmed by a meta-analysis,[12] and bilirubin has now been discussed as a therapeutic target for cardiovascular disease.[13] However, several clinical studies have examined the associations between serum bilirubin levels and retinopathy of prematurity and concluded that there is no protective effect of bilirubin on the development of this retinopathy.[14,15]

Although bilirubin has been recognized as an endogenous inhibitor of cardiovascular disease,[4–12] the relationship between bilirubin and diabetic vascular complications has not been fully understood, with limited relevant reports available.[16–18] There has been no population-based study about the association between serum bilirubin levels and DR. We therefore aimed to examine the association between serum bilirubin levels and DR in patients with diabetes and impaired glucose metabolism in a general Japanese population.

Materials and Methods

Study Population

The Hisayama Study is an ongoing, long-term, cohort study on cardiovascular disease and its risk factors in the town of Hisayama adjoining Fukuoka City, a metropolitan area in southern Ja-

ISSN 0161-6420/11/$–see front matter
doi:10.1016/j.ophtha.2010.12.009

pan.[19,20] As a part of the study, an epidemiologic study of eye disease among residents of the town has been underway since 1998.[20] In 2007, of the 4298 residents aged ≥40 years, 3119 (79.8%) consented to participate and underwent an ophthalmic examination for the present study; of these, 2880 (92.3%) underwent a 75-g oral glucose tolerance test. Of the 2880 subjects examined, 1672 (58.1%; 466 with diabetes, 583 with impaired glucose tolerance, and 623 with impaired fasting glucose) were included in this study.

This study was approved by the Human Ethics Review Committee of Kyushu University Graduate School of Medical Sciences and was carried out in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants.

Ophthalmic Examination and Definition of Diabetic Retinopathy

The methods used for the ophthalmic examination have been described in detail previously.[21] Briefly, each participant underwent comprehensive ophthalmic examination, including stereoscopic fundus examination using indirect ophthalmoscopy, and examination with a slit lamp biomicroscope with a "superfield lens" (Volk, Mentor, OH) after pupil dilatation with 1.0% tropicamide and 10% phenylephrine. Fundus photographs (45°) were taken from both eyes of each participant using a Topcon digital TRC NW-6SF fundus camera (Topcon Corporation, Tokyo, Japan). The photographs were taken in 1-field per eye, centered on the macula. The presence of DR was determined based on both fundus examinations using indirect ophthalmoscopy and slit lamp, and grading of color fundus photographs. The photographs were assessed by photographic graders who were masked to clinical information, following the modified Airlie House Diabetic Retinopathy Classification System, and classified as (i) no retinopathy, (ii) mild retinopathy, (iii) moderate retinopathy, or (iv) proliferative retinopathy.[22,23] The presence of any DR was defined as the presence of mild or moderate or proliferative retinopathy in either eye.

Data Collection

Blood samples were collected from an antecubital vein after an overnight fast for the determination of the serum bilirubin, lipid, gamma-glutamyl transpeptidase, plasma glucose, and hemoglobin A_{1c} levels. After the fasting blood specimen had been taken, the 75-g oral glucose tolerance test was performed between 08.00 and 10.30 hours. At 120 minutes after ingestion of the solution, a blood sample was obtained to determine postloading plasma glucose levels. These specimens were analyzed within 24 hours. The serum bilirubin concentration was measured enzymatically using an autoanalyzer (TBA-80S; Toshiba Inc., Tokyo, Japan). The normal range of serum total bilirubin levels as measured used in the study was 0.3 to 1.2 mg/dL. The plasma glucose concentration was determined using the glucose-oxidase method, and the hemoglobin A_{1c} levels were measured by the high-pressure lipid chromatographic assay. Serum total cholesterol and high-density lipoprotein cholesterol were determined enzymatically using the same autoanalyzer, and gamma-glutamyl transpeptidase was measured using Orlowsky's method.

Diabetes classification was based on plasma glucose results, using the 2003 American Diabetes Association criteria.[24] Diabetes was diagnosed on the basis of fasting plasma glucose (FPG) of ≥126 mg/dL (7.0 mmol/L), 2-hour postload plasma glucose (2-hour PG) of ≥ 200 mg/dL (11.1 mmol/L), or current treatment with insulin or oral hypoglycemic medication, impaired glucose tolerance was defined if FPG <126 mg/dL (7.0 mmol/L) and 2-hour PG ≥140 mg/dL (7.8 mmol/L) but <200 mg/dL (11.1 mmol/L), and impaired fasting glucose was defined if FPG ≥100 mg/dL (5.6 mmol/L) but <126 mg/dL (7.0 mmol/L) and 2-hour PG <140 mg/dL (7.8 mmol/L). Blood pressure was measured 3 times after the subject had rested for ≥5 minutes in the sitting position. The average of the three measurements was used for the analysis. Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or current use of antihypertensive medication. Body height and weight were measured in light clothing without shoes, and the body mass index was calculated as the weight in kilograms divided by the height in meters squared. Information on smoking habits, alcohol intake, and physical activity during leisure time was obtained using a standard questionnaire, and smoking habits and alcohol intake were classified into either current habitual use or not, and those subjects who engaged in sports or other forms of exertion ≥3 times per week during their leisure time were designated the regular exercise group. The questionnaire also covered questions about histories of cardiovascular disease, including stroke and coronary heart disease.

Statistical Methods

Age-adjusted prevalence of DR was calculated via direct standardization to the whole Hisayama Study population. A linear pattern of the association was assessed initially for per unit change in bilirubin levels associated with DR prevalence. We further divided bilirubin levels into quartiles (<0.60, 0.60–0.69, 0.70–0.89, and ≥0.90 mg/dL), and considered the lowest quartile or the 3 lower quartiles as reference. Test for trend across quartiles was performed in the logistic regression model. The age- and gender-adjusted or multivariable-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. In the multivariable-adjusted analysis, we included possible associated factors of either DR or serum bilirubin level that were available in our study, namely, age, gender, 2-hour PG, systolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, gamma-glutamyl transpeptidase, smoking habits, alcohol intake, and history of cardiovascular disease. We also performed additional analysis restricted to subjects with diabetes. In the multivariable-adjusted analysis of this subsample, we included risk factors for DR, namely, age, gender, duration of diabetes, hemoglobin A_1c, insulin treatment, and history of cardiovascular disease. The SAS software package (SAS Inc., Cary, NC) was used to perform all statistical analyses. A 2-sided $P<0.05$ was considered significant.

Results

Of the study participants, 70 (4.2%) were found to have DR. Mild, nonproliferative retinopathy (category ii), moderate retinopathy (category iii), and proliferative retinopathy (category iv) were found in 40 (2.4%), 29 (1.7%), and 1 (0.1%) participants, respectively.

Participants with DR were more likely to be men (Table 1). The mean age and mean levels of FPG, 2-hour PG, hemoglobin A_{1c} and systolic blood pressure, the frequency of hypertension and having history of cardiovascular disease were significantly higher among subjects with DR, whereas the mean level of total cholesterol and the frequency of smoking habits were significantly lower in those with DR (Table 1). Furthermore, we compared the mean values or frequencies of risk factors between subjects having diabetes with DR and those without DR. The mean duration of diabetes and mean hemoglobin A_{1c}, and the frequency of insulin treatment and history of cardiovascular disease were significantly higher among subjects with DR (Table 1).

Table 1. Characteristics of Subjects by Status of Diabetic Retinopathy

Variable	Without Diabetic Retinopathy	With Diabetic Retinopathy
All subjects (n)	1602	70
Age (y)	64±11	68±10**
Men (%)	52.5	72.9**
Bilirubin level (mg/dL)	0.78±0.32	0.76±0.30
Fasting plasma glucose (mmol/L)	6.1±1.2	8.7±2.5**
2-hour post-load plasma glucose (mmol/L)	9.1±3.9	18.0±5.1**
Hemoglobin A_1c (%)	5.3±0.8	7.0±1.4**
Systolic blood pressure (mmHg)	135±18	142±17**
Diastolic blood pressure (mmHg)	82±10	81±11
Hypertension (%)	57.7	77.1**
Total cholesterol (mmol/L)	5.5±0.9	5.1±0.8**
High-density lipoprotein cholesterol (mmol/L)	1.7±0.4	1.7±0.4
Gamma-glutamyl transpeptidase (IU/L)	3.5±0.8	3.7±0.9
Body mass index (kg/m^2)	23.9±3.5	24.5±3.6
History of cardiovascular disease (%)	4.9	22.9**
Smoking habits (%)	21.3	11.4*
Alcohol intake (%)	51.9	51.4
Regular exercise (%)	12.7	10.0
Subjects with diabetes (n)	398	68
Duration of diabetes (year)	5.7±4.9	16.2±8.8**
Hemoglobin A_1c (%)	6.1±1.1	7.0±1.4**
Insulin treatment (%)	1.3	17.7**
History of cardiovascular disease (%)	9.5	23.5**
Duration of diabetes (y)	5.7±4.9	16.2±8.8**

Values are expressed as means ± standard deviation or percentages. Serum gamma-glutamyl transpeptidase was transformed to logarithm.
*P<0.05, **P<0.01 versus without diabetic retinopathy.

Table 2 compares the mean values or frequencies of potential factors associated with DR by bilirubin quartiles. Subjects with higher bilirubin levels were more likely to be men. Among subjects with the highest quartile of bilirubin levels, the mean values of 2-hour PG and high-density lipoprotein cholesterol were significantly higher, although the mean values of total cholesterol, the frequencies of history of cardiovascular disease or smoking were significantly lower, compared with subjects in other 3 lower quartiles. The prevalence of DR in persons with the highest bilirubin quartile (≥0.9 mg/dL) was 2.7%, compared with the prevalence of 3.4%, 5.1% and 5.1%, respectively, in those within the first (<0.6 mg/dL), second (0.6–0.69 mg/dL), and third (0.7–0.89 mg/dL) quartiles (Table 2).

When bilirubin levels were assessed continuously, we found that each 0.1 mg/dL increase in bilirubin levels was associated with a 16% reduction of the likelihood of having DR (OR, 0.84; 95% CI, 0.76–0.93), after multivariable adjustment. Compared with persons in the lowest quartile of bilirubin levels, those with the highest quartile had a significantly lower odds of having DR, after adjustment for age, gender, 2-hour PG, systolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, gamma-glutamyl transpeptidase, history of cardiovascular disease, smoking habits, and alcohol intake (OR, 0.25; 95% CI, 0.09–0.72; Table 3). When the lower 3 quartiles were combined to form a reference group, persons in the highest quartile also had reduced prevalence of DR (OR, 0.25; 95% CI, 0.11–0.56). We also examined the age- and gender-adjusted OR of having DR by quartiles of serum total bilirubin levels among subjects with diabetes. The OR of DR decreased as quartiles of bilirubin levels increased, but the trend did not reach significance ($P = .07$), probably because of the small number of subjects. This association did not change even after adjustment for age, gender, duration of diabetes, hemoglobin A_1c level, insulin treatment, and history of cardiovascular disease (Table 3).

Discussion

We investigated the association of serum bilirubin levels with DR among participants of Hisayama Study who had either diabetes or impaired glucose metabolism. After adjusting for age, gender, and known risk factors for DR, serum bilirubin level was found to be independently and inversely associated with the prevalence of DR. Persons with diabetes or impaired glucose metabolism who were also in the highest quartile of bilirubin levels were 75% less likely to have DR, compared with those in the lowest quartile. Although this observed protective association of bilirubin with DR is in keeping with the documented protective associations of bilirubin with cardiovascular disease and the antioxidant property of bilirubin,[4–12] our findings need to be confirmed in future studies.

Several clinical studies have examined the association between serum bilirubin and diabetic vascular complications.[16–18] Among these, 2 case-control studies reported the association between serum bilirubin level and DR, and the findings were inconsistent.[16,17] One study showed that although serum bilirubin concentrations were significantly higher among normal subjects compared with patients with diabetes, there was no significant difference in mean serum bilirubin levels between patients having diabetes with DR and those without DR.[16] The other study reported a lower prevalence of diabetic vascular complications (retinopathy, macroalbuminuria, coronary artery disease, and cerebrovascular disease) in patients with both diabetes and Gilbert's syndrome, a congenital hyperbilirubinemia defined as serum bilirubin level >1.2 mg/dL.[17] Our findings are consistent with those of the latter report.

Mechanisms underlying the protective association of bilirubin with DR are not yet fully understood, and possible explanations have been proposed. Bilirubin has been recognized as an endogenous antioxidant[1] and suppresses inflammation in the vasculature.[5] The microvasculature of the retina responds to hyperglycemic milieu through a number of biochemical changes, including increased oxidative stress, polyol pathway, protein kinase C activation, and advanced glycation end product formation.[25] Oxidative stress and inflammation are considered crucial contributors in the pathogenesis of DR.[25,26] Oxidative stress-induced biochemical changes contribute to both functional and structural changes in the retina microvasculature, including basement membrane thickening, microvascular cell loss, capillary closure, and acellular capillary formation.[27] Structural changes may contribute to, and also result from, functional changes such as altered blood flow, loss of intercellular junctions, and increased vessel permeability. Animal models of DR have shown beneficial effects of antioxidants on the development of retinopathy in diabetic rats.[25] An-

Table 2. Mean Values or Frequencies of Relevant Factors by Quartiles of Serum Total Bilirubin Levels

Variable	Quartile of Serum Total Bilirubin Level (mg/dL)				P-Value for Trend
	<0.6	0.6–0.69	0.7–0.89	≥0.9	
n	358	548	396	370	
Age (y)	63±11	64±11	64±10	64±11	0.22
Men (%)	54.2	47.8	51.5	62.7	<0.001
Diabetic retinopathy (%)	3.4	5.1	5.1	2.7	0.65
Fasting plasma glucose (mmol/L)	6.2±1.4	6.2±1.3	6.3±1.5	6.2±1.3	0.45
2-hour post-load plasma glucose (mmol/L)	8.6±3.8	9.6±4.4	9.8±4.7	9.9±4.4	<0.001
Hemoglobin A_1c (%)	5.4±0.8	5.4±0.8	5.5±1.0	5.3±0.9	0.09
Systolic blood pressure (mmHg)	135±17	135±18	136±19	137±18	0.34
Diastolic blood pressure (mmHg)	81±10	81±10	82±10	83±10	0.06
Hypertension (%)	55.6	57.1	61.9	59.7	0.29
Total cholesterol (mmol/L)	5.4±0.9	5.5±0.9	5.5±0.9	5.3±0.9	0.005
High-density lipoprotein cholesterol (mmol/L)	1.5±0.4	1.7±0.4	1.7±0.4	1.7±0.5	<0.001
Gamma-glutamyl transpeptidase (IU/L)	3.5±0.8	3.5±0.7	3.5±0.8	3.6±0.8	0.51
Body mass index (kg/m^2)	24.1±3.4	23.9±3.2	24.1±3.8	23.7±3.5	0.43
History of cardiovascular disease (%)	9.2	5.1	4.5	4.5	0.002
Smoking habits (%)	33.5	20.6	17.7	12.4	<0.001
Alcohol intake (%)	51.7	49.3	50.0	58.1	0.05
Regular exercise (%)	12.3	12.1	10.7	15.7	0.19

Values are expressed as the means ± standard deviation or percentages. Serum gamma-glutamyl transpeptidase was transformed to logarithm.

other experimental study of animals has shown that inhibition of the inflammatory cascade at any stage of disease course could inhibit the progression of early stage DR.[25] Therefore, it is possible that an increase in serum bilirubin level inhibits oxidative stress and inflammation processes and thus slows or interrupts the pathways to the development of DR.

Before adjustment for other known DR risk factors, subjects with the highest quartile of bilirubin levels had a significantly higher mean value of 2-hour PG levels than subjects in other quartiles. The findings were carefully rendered to ensure that there was no mistake in the findings presented in this report. Our data seem to indicate a countereffect of elevated bilirubin levels against the effect of elevated 2-hour PG levels on DR prevalence. We also documented that the protective effect of elevated bilirubin level on DR prevalence was independent of other DR risk factors, suggesting that the underlying mechanisms for the

Table 3. Odds Ratios (OR) and 95% Confidence Intervals (CI) of Diabetic Retinopathy by Quartiles of Serum Total Bilirubin Levels*

	Quartile of Serum Total Bilirubin Level (mg/dL)				P Value for Trend
	<0.6	0.6–0.69	0.7–0.89	≥0.9	
All subjects					
Population at risk (n)	358	548	396	370	0.35
Case of diabetic retinopathy (n)	12	28	20	10	
Age- and gender-adjusted OR (95% CI)	1.0	1.59 (0.79–3.18)	1.55 (0.74–3.23)	0.70 (0.30–1.66)	
Multivariable-adjusted OR (95% CI)	1.0	1.11 (0.48–2.57)	0.86 (0.35–2.11)	0.25 (0.09–0.72)*	0.004
Subjects with diabetes					
Population at risk (n)	83	151	116	116	0.09
Case of diabetic retinopathy (n)	11	28	19	10	
Age- and gender-adjusted OR (95% CI)	1.0	1.41 (0.65–3.03)	1.27 (0.56–2.87)	0.52 (0.21–1.31)	
Multivariable-adjusted OR (95% CI)	1.0	1.41 (0.56–3.54)	1.12 (0.41–3.01)	0.39 (0.12–1.30)	0.07

Multivariable adjustment was made for age, gender, 2-hour post-load plasma glucose, systolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, gamma-glutamyl transpeptidase, history of cardiovascular disease, smoking habits, and alcohol intake.
*$P<0.01$versus first quartile.

association with bilirubin levels are likely different from the common pathway via elevated serum blood glucose levels. If confirmed, this may provide a new therapeutic approach to complement current available therapies for patients with diabetes (e.g., lowering serum glucose, lipid levels, and blood pressure levels).

In our data, there also seemed to be a threshold of bilirubin levels at the highest quartile (≥0.9 mg/dL) for the significant protective effect on DR (Table 2). However, because of the relatively small numbers of DR cases in this group, caution should be taken and confirmation of our findings in studies with large sample size is necessary.

Several limitations of our study should be discussed. Our findings were based on a single serum bilirubin level measurement, which might not capture various ranges of bilirubin levels over times in particular participants. However, if such a variation is random and nondifferentiated between cases and controls, it would only dilute the association and bias the results toward the null. A cross-sectional association has no implication of causal relationship. Because the numbers of DR cases were relatively small in our sample, particularly in the highest quartile of bilirubin group, we cannot exclude the possibility of a chance finding.

In conclusion, we demonstrated that elevated serum bilirubin levels were significantly associated with low prevalence of DR in persons with diabetes or impaired glucose metabolism, independent of known DR risk factors. Further studies with a larger sample size, either cross-sectional or prospective, are needed to confirm these findings. If confirmed, our finding may have important implications to clinical management of diabetes and to the prevention of diabetic complications.

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Footnotes and Financial Disclosures

Originally received: June 17, 2010.
Final revision: October 30, 2010.
Accepted: December 9, 2010.
Available online: May 20, 2011. Manuscript no. 2010-839.

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Financial Disclosure(s):
The authors have no proprietary or commercial interest in any of the materials discussed in this article.

Partially supported by the Strategic Study of Sensory Organ founded by the Ministry of Health, Labor and Welfare, Japan.

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Original Report: Patient-Oriented, Translational Research

American Journal of Nephrology

Am J Nephrol 2012;35:1–6
DOI: 10.1159/000334357

Received: August 1, 2011
Accepted: October 6, 2011
Published online: November 29, 2011

A New Vasculitis Activity Score for Predicting Death in Myeloperoxidase-Antineutrophil Cytoplasmic Antibody-Associated Vasculitis Patients

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Key Words

Myeloperoxidase-antineutrophil cytoplasmic antibody · Birmingham Vasculitis Activity Score · Japanese Vasculitis Activity Score · Odds ratio · Receiver operating characteristic · Mortality rate

Abstract

Background/Aims: Myeloperoxidase-antineutrophil cytoplasmic antibody (MPO-ANCA)-positive microscopic polyangiitis patients with renal involvement have been shown to have a progressive clinical course. In this study, we compared the clinical utility of the Japanese Vasculitis Activity Score (JVAS) with the Birmingham Vasculitis Activity Score (BVAS) for predicting death in patients with MPO-ANCA-associated renal involvement. ***Methods:*** Sixty-nine patients with MPO-ANCA-associated vasculitis with renal involvement (22 males and 47 females, age 69.8 ± 8.7 years) were enrolled in this study. We retrospectively investigated which score was better for predicting the poor prognosis of patients. ***Results:*** The mortality rate of the patients within 2 years after disease onset was 33% (23/69). JVAS was not correlated with BVAS. Univariate logistic regression analysis for death showed that the odds ratio (OR) of JVAS was statistically significant (OR 1.76, 95% confidence interval, CI, 1.29–2.41, $p < 0.001$), while that of BVAS was not (OR 1.07, 95% CI 0.98–1.16, $p = 0.14$). Moreover, a multivariate model showed that JVAS was an independent determinant of death (OR 1.59, 95% CI 1.12–2.25, $p = 0.009$). The area under the receiver operating characteristic curve for JVAS was 0.778, which was significantly larger ($p = 0.02$) than that for BVAS (0.586). The estimated optimal cut-off point of JVAS for the prediction of death was 5. At this point, the sensitivity was 82.6% and the specificity was 60.9%. ***Conclusion:*** We demonstrated that compared with BVAS, JVAS was a simpler and more reliable measure for predicting death in patients with MPO-ANCA-associated vasculitis with renal involvement.

Introduction

Antineutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (AAV) is characterized by necrotizing inflammation of small vessels, which comprise three different diseases entities: Churg-Strauss syndrome, mi-

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Table 1. Scoring of JVAS

Parameter score	Serum creatinine mg/dl	Age years	Pulmonary lesion	Serum CRP mg/dl
0	<3.0	<60	–	<2.6
1	3.0–6.0	60–69		2.6–10
2	≥6.0	≥70	+	≥10.0
3	Dialysis therapy			

CRP = C-reactive protein.

croscopic polyangiitis, and Wegener's granulomatosis [1, 2]. ANCA specific for myeloperoxidase (MPO-ANCA) or proteinase 3 (PR3-ANCA) is implicated in the pathogenesis of AAV [3]. In vitro, ANCA can activate primed neutrophils to release inflammatory mediators and, in conjunction with the alternative pathway of the complement system, damage and lyse endothelial cells [4, 5]. In vivo, transfer of splenocytes from MPO-deficient mice immunized with mouse MPO into wild-type mice resulted in intrinsic pauci-immune renal vasculitis in these animals [5]. In addition, Wegener's granulomatosis is closely associated with PR3-ANCA, while MPO-ANCA is clinically involved in necrotizing small vessel vasculitis such as microscopic polyangiitis and Churg-Strauss syndrome [6, 7].

Although the estimated annual incidence of AAV in Japan [8] is similar to that in Europe [9, 10] and the USA [11], there are some regional differences in ANCA subsets and renal histology; in Japan, MPO-ANCA-positive microscopic polyangiitis is more common than PR3-ANCA-associated glomerulonephritis. Glomerulonephritis in relation to microscopic polyangiitis has more characteristics of chronic injury than that in PR3-ANCA-associated Wegener's granulomatosis [12]. Further, MPO-ANCA-positive microscopic polyangiitis patients with renal involvement have been shown to have a progressive clinical course, which can often lead to renal death [13, 14].

There are a couple of scores that could evaluate disease activity of AAV [13, 15, 16]. Among them, the Birmingham Vasculitis Activity Score (BVAS) [15] is one of the most commonly used and standard assessments for disease activity in AAV. Indeed, several clinical reports have shown that BVAS is useful for evaluating disease activity and therapeutic response or relapse in patients with AAV [15–17]. Further, a higher BVAS value was reported to be one of the independent poor prognostic factors of patient survival in AAV [18]. However, as far as we know, there are no studies examining the clinical utility of the BVAS value for predicting prognosis of MPO-ANCA-positive microscopic polyangiitis patients with renal involvement. Further, it is difficult to assess disease activity and prognosis by BVAS because its weighted score is based on clinical symptoms and signs in 9 separate organ systems. As MPO-ANCA-positive microscopic polyangiitis is more common in rapidly progressive glomerulonephritis in Japan than in Europe [8], and the Japanese Vasculitis Activity Score (JVAS), proposed by the Research Committee of Intractable Vasculitis Syndrome of the Ministry of Health, Labor and Welfare of Japan, could evaluate disease activity with a sum score of 4 clinical parameters, including age, serum creatinine and C-reactive protein (CRP) levels, and pulmonary lesion [19] (table 1), and is simpler than BVAS, we compared the utility of BVAS with JVAS for predicting prognosis of MPO-ANCA-associated microscopic vasculitis with renal involvement in Japan.

Patients and Methods

From 1995 to 2009, 69 patients (22 males and 47 females, age 69.8 ± 8.7 years) were diagnosed as new-onset MPO-ANCA-associated microscopic vasculitis with renal involvement in our hospitals and then followed up. All patients were found to be positive for MPO-ANCA, negative for PR3-ANCA or anti-glomerular basement membrane antibody. Fifty-five patients (79.7%) were classified as microscopic polyangiitis and 14 patients (20.3%) as renal limited vasculitis, according to the definition of the Chapel Hill Consensus Conference [20, 21] and/or the criteria of the European Systemic Vasculitis Study Group [22].

Medical records at the time of admission were obtained. The following values were evaluated: hemoglobin, serum creatinine, blood urea nitrogen (BUN), CRP, JVAS, BVAS, proteinuria, and hematuria. Almost all patients (97%) received standard immunosuppressive treatments (intravenous injection of methylprednisolone, oral corticosteroid or immunosuppressants). All patients provided written informed consent to undergo renal biopsy and to participate in the study, and were followed up until death or 2 years after the first admission. The current study received approval from the Ethical Committee of Kurume University Hospital.

Statistical Analysis

Data are appropriately shown as mean ± standard deviation (SD) or median (minimum–maximum). Differences between characteristics of survivors and non-survivors were tested by using χ^2 test, Fisher's exact test, Student's t test or Welch's t test. The association between JVAS and BVAS was assessed by Spearman rank correlation coefficient. The odds ratio (OR) of these two parameters for death was estimated by logistic regression analysis. The outcome event was all-cause mortality within 2 years after the initial diagnosis of MPO-ANCA-associated vasculitis with renal in-

volvement. To evaluate the clinical utility of JVAS and BVAS values for predicting the death in our patients, we calculated the sensitivity and specificity of these scores in receiver operating characteristic (ROC) analysis. A two-sided value of $p < 0.05$ was considered to be statistically significant. All statistical analyses were performed using the SAS version 9.2 (SAS Institute, Cary, N.C., USA).

Results

Demographic and Clinical Data

The clinical characteristics of the patients are shown in table 2. Serum creatinine, CRP, JVAS and BVAS were: 2.9 (0.47–15.7) mg/dl, 6.9 (0.09–27.4) mg/dl, 5.0 ± 2.1, and 17 (12–36), respectively. Systemic symptoms such as malaise, myalgia, arthralgia/arthritis, high fever and weight loss were observed in the majority of patients (89.1%), and 1.7% of the patients had pulmonary involvement, 74.5% nervous symptoms, 36.4% eyes and ear nose tract symptoms, 21.8% abdominal symptoms, 18.2% skin lesions, and 9.1% cardiovascular symptoms.

Correlates of Mortality with Clinical Variables

Almost all patients received immunosuppressive therapy for AAV such as intravenous injection of methylprednisolone and oral administration of corticosteroid and cyclophosphamide. As shown in table 3, among our patients there were no significant differences in immunosuppressive treatments between survivors and non-survivors. Of the 69 patients enrolled, 23 (33.3%) died within 2 years after the initial diagnosis of MPO-ANCA-associated vasculitis with renal involvement. The number of deaths within 3, 3–6, 6–12, and 12–24 months after the initial diagnosis were 13, 2, 5 and 3, respectively. Ten patients died from active vasculitis, 7 from severe interstitial pneumonia or pulmonary hemorrhage, and 3 from enteritis. Ten patients died from infection. One died from stroke, and 2 patients from unknown causes. Twenty patients underwent hemodialysis (HD), of which 12 patients died and 4 needed maintenance HD, while 4 did not require HD therapy during the follow-up periods.

As shown in figure 1, the mortality rate increased with age, and age was a significant risk factor of death [OR per 5-year increment 1.50, 95% confidence interval (CI) 1.07–2.13, $p = 0.018$]. Fifty percent of the patients older than 76 years died within 2 years after the initial diagnosis.

We next examined the correlation of JVAS with BVAS in our patients. As shown in figure 2, JVAS was not correlated with BVAS ($\rho = 0.26$, $p = 0.02$). The OR of JVAS for death (1.76, 95% CI 1.29–2.41) was statistically significant ($p < 0.001$), while that of BVAS was not (1.07, 95% CI 0.98–1.16, $p = 0.14$; table 4). Bivariate logistic regression analysis revealed that JVAS (OR 1.74, 95% CI 1.26–2.40, $p < 0.001$), but not BVAS (OR 1.05, 95% CI 0.95–1.16, $p = 0.37$) was significantly associated with death. Further, in age- and BVAS-adjusted multivariate logistic regression analysis, JVAS was found to be an independent determinant of death in our AAV subjects (OR 1.59, 95% CI 1.12–2.25, $p = 0.009$). The area under the curve (AUC) of ROC for JVAS was 0.78 (95% CI 0.66–0.89), which was significantly larger ($p = 0.02$) than that for BVAS (0.59, 95% CI 0.44–0.73; fig. 3). The estimated optimal cutoff point of JVAS for the prediction of death was 5. At this point, the sensitivity was 82.6% and the specificity was 60.9%. Using this cutoff point, patients were categorized into high- and low-risk groups. Multivariate logistic re-

Table 2. Clinical characteristics of the patients

Number (% of female)	69 (68%)
Age, years	69.8 ± 8.7
Hemoglobin, g/dl	9.0 ± 1.6
Creatinine, mg/dl	2.9 (0.47–15.7)
Serum urea nitrogen, mg/dl	36.8 (9.0–140.0)
C-reactive protein, mg/dl	6.9 (0.09–27.4)
JVAS	5.0 ± 2.1
BVAS	17 (12–36)
Proteinuria, g/day	0.85 (0.0–9.0)
Hematuria, %	100

Results are appropriately given as the mean ± SD or the median (range, minimum–maximum).

Table 3. Treatment substances between survivor and non-survivors

	Survivors	Non-survivors	p value
Age, years	67.91 ± 8.34	73.39 ± 8.55	0.015
Males/females, n	14/32	8/15	0.72
Methylprednisolone intravenous injection for 3 days, mg/kg/day	14.49 ± 5.78	14.27 ± 5.90	0.90
Corticosteroid (oral administration), mg/kg/day	0.79 ± 0.20	0.81 ± 0.27	0.72
Cyclophosphamide (oral administration), mg/kg/day	0.96 ± 0.20	0.98 ± 0.49	0.87

Table 4. OR of mortality in MPO-ANCA-associated vasculitis patients with renal involvement

	Crude model		Bivariate model[1]		Multivariate model[2]	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
JVAS	1.76 (1.29–2.41)	<0.001	1.74 (1.26–2.40)	<0.001	1.59 (1.12–2.25)	0.009
BVAS	1.07 (0.98–1.16)	0.14	1.05 (0.95–1.16)	0.37		

[1] Bivariate model includes JVAS and BVAS simultaneously.
[2] Multivariate model includes JVAS, BVAS and age.

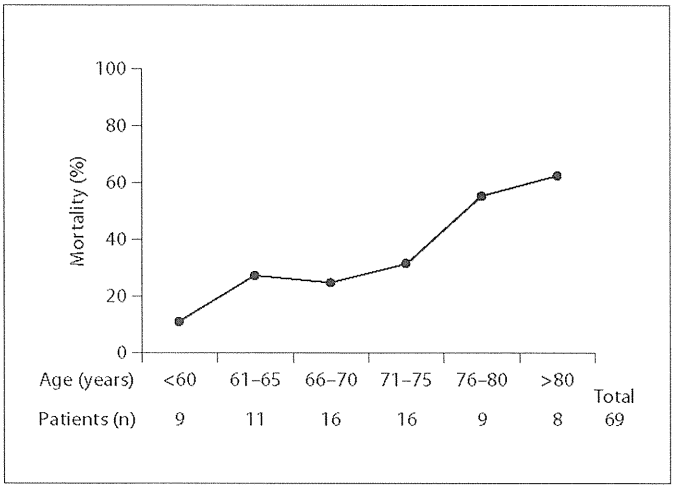

Fig. 1. Mortality rates of each age range patients. The mortality rate (%) was calculated as a percentage of death in each age range patient.

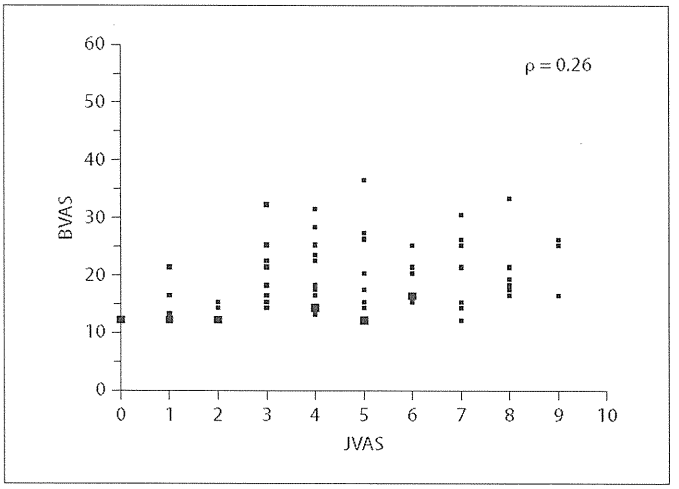

Fig. 2. Correlation between the JVAS and BVAS values. JVAS was not correlated with BVAS values (ρ = 0.26).

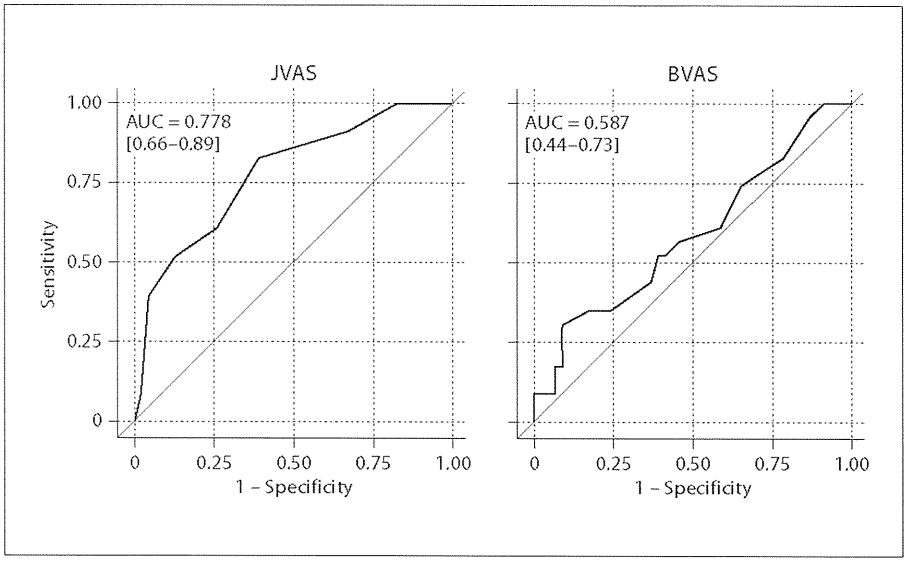

Fig. 3. Receiver operating characteristic curve for JVAS and BVAS to predict death in our patients. The area under the receiver operating characteristic curves for JVAS and BVAS was 0.778 and 0.586, respectively. The area under curve for JVAS was significantly higher than that for the BVAS (p = 0.02).