Figure 2 The electroretinograms (ERGs) of (A) case 1, (B) her mother, (C) case 2 and (D) case 3 were diagnostic for enhanced S-cone syndrome (ESCS)—a similar, simplified and delayed waveform response to flash under photopic and scotopic conditions; and a delayed 30-Hz flicker amplitude lower than that of the photopic a-wave. The left tracings of each column are for the left eye and right tracings for the right eye. Stimulus intensities are described in logarithmic (log) units relative to the standard stimulus (1.5–3 Candelas/m² at the surface of the Ganzfeld bowl). For each ERG in the first column (Rod Response), top tracings are for a minimal (0.25 log units) blue (430 nm) stimulus, middle tracings are for a standard (1.0 log unit) red (644 nm) stimulus, and bottom tracings are for a maximal (1.5 log units) white flash. Repeat recordings are seen in (A). In the second column (cone response), for the tracings in (A) and (C), the top tracings are for maximal flash with low background illumination (1 foot Lamberts), the middle tracings for standard flash with high background illumination (30 foot Lamberts), the second to last tracings are for maximal intensity flicker (30 Hz) with low background illumination, and the bottom tracings are for standard intensity flicker with low background illumination. Repeat recordings are seen for these tracings in (B) and (D), the top tracings are for maximal flash with low background illumination and the bottom tracings are for maximal intensity flicker with low background illumination; no repeats were done.

preservation of blue cone function. *Proc Natl Acad Sci USA* 2004;**101**:17819–24.

- 8 Haider NB, Naggert JK, Nishina PM. Excess cone cell proliferation due to lack of a functional NR2E3 causes retinal dysplasia and degeneration in rd7/rd7 mice. *Hum Mol Genet* 2001;**10**:1619–26.

Retinal and subdural haemorrhages from minor falls?

Retinal and subdural haemorrhages with or without occult bone fractures in children aged <3 years is a characteristic presentation of the shaken baby syndrome (SBS) with or without blunt head impact.^{1–3} The signs may pose diagnostic dilemmas resulting from an inconsistent history given by the child's caretakers and the frequent absence of evidence of

external injury. Accidental blunt impact that leads to subdural haemorrhage is usually associated with significant trauma, skull fractures and only rare cases of retinal haemorrhage. There is abundant evidence that minor head trauma, in the absence of underlying medical conditions such as osteogenesis imperfecta type I,⁴ is only very rarely associated with severe intracranial injury or retinal haemorrhage, particularly the extensive multi-layered haemorrhages extending to the ora serrata, as seen in two thirds of the victims with SBS.^{1–3, 5}

A letter in the *British Journal of Ophthalmology* considers prior reports regarding Japanese children who sustained subdural and retinal haemorrhage after short falls.⁶ We believe that the statistical analysis and discussion

presented in the letter have inaccuracies that could lead to the incorrect conclusion that biological factors may explain the reported clinical findings which otherwise disagree with the findings in the world's literature on this topic.

Gardner suggests statistically significant discrepancies in age distribution between two Japanese cohorts with allegedly accidental head trauma^{7, 8} and Kivlin's American series of victims with shaken baby syndrome³ as one possible biological explanation for the unique nature of the Japanese cohorts, which he claims have statistically similar ages.⁶ One of the Japanese publications reports on 26 infants with retinal and subdural haemorrhages after fall backwards from sitting or standing on tatami mats.⁶ The other does not include the mechanism of injury or eye examinations.⁷

The degree of freedom (df) for two-sample independent t tests, assuming equality of variance, is $(n_1 - 1) + (n_2 - 1)$, where n_1 and n_2 are sample sizes of the two groups. When variances are unequal, Satterwaite adjustment is recommended.⁹ For data presented by Aoki, Ikeda and Kivlin, the sample sizes are 26, 14 and 102, respectively. When comparing Kivlin's cohort with Aoki's, df is 126 for equal variances and 65.3 for unequal variances. For t tests conducted by Gardner, df values were 13, 13, 25 and 39, which should have been 38, 114, 126 and 140 for equal variances, and 22.2, 20.2, 65.3 and 103 for unequal variances, for testing Aoki versus Ikeda, Ikeda versus Kivlin, Aoki versus Kivlin, and the combined Japanese studies versus Kivlin, respectively. As p values depend on df, the correct p values are 0.0247, <0.001, <0.001 and <0.001, respectively. Gardner's conclusion of similarity in age distribution between the two Japanese groups is therefore incorrect.

Classical p values represent the probability of making an incorrect rejection of the null hypothesis: the probability of saying that the mean ages of two groups are different when they are equivalent. p Value is not the probability of ruling in or out differences in group means or proportions (not being able to rule out a difference with a 20% probability).⁸

The two Japanese cohorts actually have significantly different ages, and both are different from Kivlin's sample. It is therefore unlikely that age is an explanatory factor for their unusual clinical findings. In addition, the mere act of falling over from a sitting or standing position is not developmentally possible in most victims of SBS, the incidence of which is highest in the first 6 months. Crying is a major factor in inciting SBS. The normal crying curve is similar to the SBS incidence curve.¹⁰

Thus, it is not surprising that Kivlin's patients are different from the Japanese samples. But why are the Japanese reports of subdural and retinal haemorrhage after minor trauma so very different from the bulk of the world's literature? Some of the Japanese patients were recruited before SBS had even been described or widely recognised in the world's literature. Gardner notes the admission in Aoki's paper that cases of abuse might have been included. At the time of Aoki's study, there were no child abuse teams or reporting laws in Japanese society.² Abuse could not have been adequately ruled out.

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References

- 1 Duhaime AC, Christian CW, Rorke LB, et al. Non-accidental head injury in infants – the “shaken-baby syndrome”. *N Engl J Med* 1998;**338**:1822–9.
- 2 Levin AV. Retinal haemorrhages and child abuse. In: David TJ, eds. *Recent advances in paediatrics*. London: Churchill Livingstone, 2000;**18**:151–219.
- 3 Kivlin JD, Simons KB, Lazowitz S, et al. Shaken baby syndrome. *Ophthalmology* 2000;**107**:1246–54.
- 4 Ganesh A, Jenny C, Geyer J, et al. Retinal hemorrhages in Type I osteogenesis imperfecta after minor trauma. *Ophthalmology* 2004;**111**:1428–31.
- 5 Morad Y, Kim YM, Armstrong DC, et al. Correlation between retinal abnormalities and intracranial abnormalities in the Shaken Baby Syndrome. *Am J Ophthalmol* 2002;**134**:354–9.
- 6 Aoki N, Masuzawa H. Infantile acute subdural hematoma. Clinical analysis of 26 cases. *J Neurosurg* 1984;**61**:273–80.
- 7 Ikeda A, Sato O, Tsugane R, et al. Infantile acute subdural hematoma. *Childs Nerv Syst* 1987;**3**:19–22.
- 8 Gardner HB. Retinal and subdural haemorrhages: Aoki revisited. *Br J Ophthalmol* 2003;**87**:919–20.
- 9 Armitage P, Berry G. *Statistical methods in medical research*, 3rd edn. Oxford, UK: Blackwell, 1994:111–13.
- 10 Barr RG, Trent RB, Cross J. Age-related incidence curve of hospitalized Shaken Baby Syndrome cases: convergent evidence for crying as a trigger to shaking. *Child Abuse Negl* 2006;**30**:7–16.

Positive correlation between vitreous levels of advanced glycation end products and vascular endothelial growth factor in patients with diabetic retinopathy sufficiently treated with photocoagulation

We investigated whether vitreous levels of advanced glycation end products (AGEs) were positively correlated with vascular endothelial growth factor (VEGF) in patients with diabetic retinopathy patients sufficiently treated with retinal photocoagulation. Vitreous AGE and VEGF levels were significantly higher in patients with diabetes than in controls. Positive correlation between AGE and VEGF was found in patients with diabetic retinopathy sufficiently treated with retinal photocoagulation ($r = 0.44$, $p < 0.05$), but not in those who were insufficiently treated ($r = 0.26$, $p = 0.18$). The present observations

suggest that AGE may induce VEGF expression in an ischaemia-independent mechanism. AGE could be one of the important determinants of VEGF in diabetic retinopathy without obvious ischaemic regions.

Background

Vascular endothelial growth factor (VEGF) elicits retinal vascular hyperpermeability, thrombosis and angiogenesis, having a central role in the pathogenesis of diabetic retinopathy.¹ Furthermore, vitreous VEGF levels are increased in proliferative diabetic retinopathy, whereas the levels are decreased after treatment with panretinal photocoagulation (PRP).² These observations suggest that retinal ischaemia and resultant hypoxia could mainly contribute to VEGF induction in diabetic retinopathy.

Advanced glycation end products (AGEs), senescent macroprotein derivatives formed at an accelerated rate under diabetes, also stimulate VEGF expression in cell cultures and animal models.^{3,4} In addition, vitreous AGE levels are positively correlated with VEGF in patients with diabetic retinopathy, suggesting that AGEs may be a stimulant of VEGF *in vivo*.⁵ However, as AGEs predispose the retinal vessels to thrombogenesis,³ whether AGEs could induce VEGF expression in an ischaemia-independent manner remains unknown. Therefore, in this study, we determined the relationship between vitreous levels of AGEs and VEGF in patients with diabetic retinopathy who were sufficiently treated with PRP for controlling retinal ischaemia.

Figure 1 Vitreous levels of advanced glycation end products (AGEs; A) and vascular endothelial growth factor (VEGF; B) in controls without diabetes and in patients with diabetes, including the sufficiently treated group with panretinal photocoagulation (S-PRP) and the insufficiently treated group with no or focal photocoagulation (IS-RP). Data analysed by Mann-Whitney U test.

Figure 2 Correlation between the vitreous levels of advanced glycation end products (AGEs) and vascular endothelial growth factor (VEGF) in the sufficiently treated group with panretinal photocoagulation (S-PRP; A) and in the insufficiently treated group with no or focal photocoagulation (IS-RP; B). Spearman's correlation coefficient calculated by rank test.

Patients and methods

The study protocol was approved by our institutional ethics committee, and informed consent was obtained from all patients. Undiluted vitreous samples were collected during vitrectomy from patients with diabetes with a mean (standard deviation (SD)) age of 53 (12.6) years (45 samples from patients with proliferative diabetic retinopathy and 6 from those with diabetic macular oedema). Twenty eight samples from patients without diabetes with a mean (SD) age of 65.3 (6.7) years, having idiopathic macular hole or epiretinal membrane, served as controls. Vitreous levels of AGEs and VEGF were measured as described previously.² We classified patients with diabetes into two groups: a sufficiently treated group with PRP (S-PRP; $n = 23$) and an insufficiently treated group (no or focal retinal coagulation; IS-RP; $n = 28$), on the basis of the extent of retinal photocoagulation before vitrectomy as described previously.⁶ The data were analysed by the Mann-Whitney U test and Spearman's correlation coefficient by rank test.

Results

Vitreous levels of AGEs and VEGF were significantly higher in patients with diabetes than in controls (mean (SD) 0.13 (0.07) v 0.04 (0.03) U and 2.15 (2) v 0.12 (0.23) ng/ml, respectively; $p < 0.01$). As fig 1A and B shows, vitreous VEGF levels were higher in IS-RP than those in S-PRP (2.75 (2.3) v 1.4 (1.3) ng/ml, respectively; $p < 0.05$), whereas there was no significant difference of vitreous levels of AGEs between IS-RP and S-PRP (0.13 (0.07) v 0.13 (0.06) U, respectively). A positive correlation was found between AGEs and VEGF in S-PRP ($r = 0.44$, $p < 0.05$), but not in IS-RP ($r = 0.26$, $p = 0.18$; fig 2 A,B).

Comment

Our observations suggest that AGEs may induce VEGF expression in an ischaemia-independent mechanism. In this study, the