Table 1 Comparison of risk factors in NAION patients and controls

Risk factor		NAION patients	Controls	Odds ratio	95% CI	p Value
Apo E4	Homo	0/19	0/593	31.2	0.60 to 1615.3	1
Factor II G20210A	Homo	0/19	0/593	31.2	0.60 to 1615.3	1
	Hetero	0/19	10/593	0	0.00 to 17.52	1
Factor V G1691A	Homo	0/19	0/200	10.5	0.20 to 545.6	1
	Hetero	0/19	5/200	0	0.00 to 13.10	1
MTHFR C677T	Homo	0/19	12/625	0	0.00 to 15.06	1
	Hetero	4/19	161/625	0.77	0.21 to 2.52	0.79
MTHFR A1298C	Homo	0/19	57/625	0	0.00 to 2.71	0.39
	Hetero	7/19	322/625	0.55	0.19 to 1.52	0.30
PI^{A2} allele	Homo	0/19	12/509	0	0.00 to 12.22	1
	Hetero	3/19	137/509	0.51	0.12–1.89	0.41
Age, mean (SD)	–	58.8 (8.5)	57.1 (4.2)	–	–	0.40
Sex (M:F)	–	14:5	351:160	1.28	0.42 to 4.13	0.64
Diabetes	–	13/19	256/511	2.16	0.75 to 6.47	0.18
Hypertension	–	10/19	209/511	1.61	0.59 to 4.38	0.43
Hyperlipidaemia	–	2/19	59/511	0.68	0.11 to 3.17	1
CAD	–	1/19	27/511	0.55	0.03 to 4.03	1
NS mtDNA changes	–	14/19	11/100	22.6	6.03 to 91.07	< 0.001

NAION, non-arteritic ischaemic optic neuropathy; CAD, coronary artery disease; NS mtDNA changes, non-synonymous (changing an amino acid in the resultant protein) mitochondrial DNA nucleotide change; Homo, homozygous; Hetero, heterozygous. Diabetes, hypertension, hyperlipidaemia, and CAD were assessed by patient report both from NAION patients and from controls. Controls previously reported for mtDNA changes and for atherosclerotic and prothrombotic risk factors (see text). Odds ratio and p values compare prevalence of different risk factors in NAION patients to controls.

medical or family history of a thrombotic or vascular event.

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Inflammatory cytokine of basal and reflex tears analysed by multicytokine assay

Tear cytokine has a major role in various pathophysiological conditions of the ocular surface. So far, studies on tear cytokines have shown significant progress in providing an understanding of ocular surface diseases.[1–3] The information that could be acquired from each subject, however, until recently has been severely hampered by limited sample volume and assay sensitivity. More importantly, it has become apparent that the relative balance between various cytokines and combinations of cytokines could be more important than absolute concentrations. Previous studies showed that the composition of basic and reflex tears was different, which made it more difficult to understand the ocular surface disorder correctly or to treat the patients suitably.[4 5] Cytometric bead array (CBA) is a microparticle based flow cytometric assay that allows us to quantify multiple molecules from a very small sample.[3 6 7] Using this method, we evaluated the inflammatory cytokines of basal and reflex tears from a single sample of individual eyes.

Methods

Twenty three normal volunteers (11 males and 12 females, 22–44 years of age, average 28 years) were recruited for this study. None of the subjects had signs of ocular diseases. The study was performed with the approval of the institutional review board. The basal tear samples of 10–15 µl were obtained from each eye by capillary flow, with no nasal stimulation or previous instillation of drugs or vital dyes. Each sample was collected at 5 pm. No anaesthetic drops were instilled. The samples were collected non-traumatically from the inferior meniscus. Successively, reflex tear samples were collected by inserting application sticks into a participant's nose. The amounts of six inflammatory molecules interleukin (IL)-1β, IL-6, IL-8, IL-10, IL-12p70, and tumour necrosis factor α (TNF-α), were measured by CBA (BD Biosciences, San Diego, CA, USA), according to the manufacturer's instructions. Briefly, for the tear sample and cytokine standard mixture, 10 µl of sample or standard were added to 40 µl sterile purified water, a mixture of 50 µl each of capture Ab-bead reagent and detector Ab-phycoerythrin (PE) reagent. The mixture was subsequently incubated for 3 hours at room temperature, and washed to remove any unbound detector Ab-PE reagent before data acquisition using flow cytometry. A two colour flow cytometric analysis was performed using a FACScan flow cytometer (Beckton Dickinson Immunocytometry Systems). Data were acquired and analysed using BD cytometric bead array software.

Results

The concentrations of IL-1β, IL-6, IL-10, IL-12p70, and TNF-α were not significantly different between basal and reflex tears. In contrast, the concentration of IL-8 was significantly decreased in reflex tears compared with basal tears in each eye (paired *t* test, $p<0.01$, fig 1). In order to illuminate the inter-relation of each cytokine, the ratio of two different cytokines is shown in table 1.

Comment

Previously published studies have demonstrated that CBA correlates well with enzyme linked immunosorbent assay (ELISA), but the absolute concentrations obtained from each assay were differed for kits of different manufacturers.[7] Indeed, the concentrations of tear cytokines in the present results were almost equal to the previous report using the same kit.[3] Nakamura *et al* performed ELISA for multiple cytokines measuring pooled tears.[1] The pooled tears enable measurement of multiple cytokines; however the results

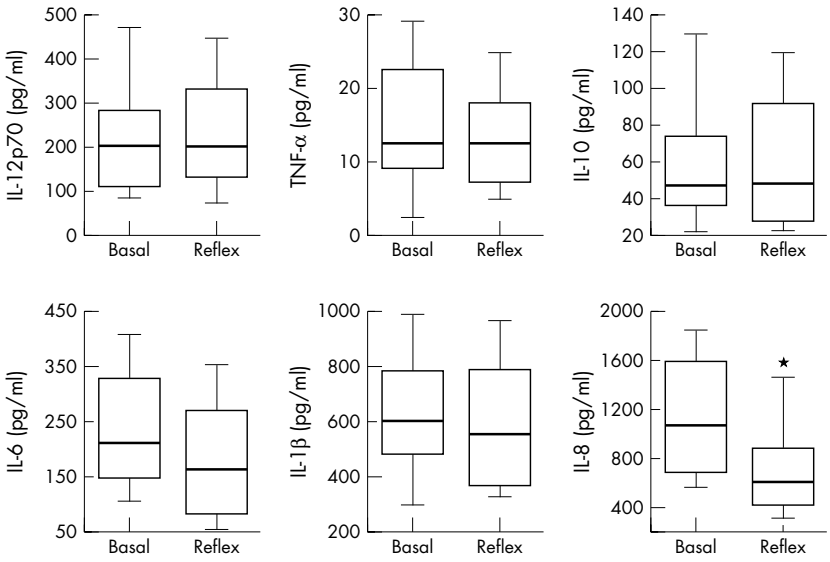

Figure 1 Change of concentrations of cytokines in basal and reflex tears. The concentration of IL-1β, IL-6, IL-10, IL-12p70 and TNF-α are not significantly different between basal and reflex tears. In contrast, IL-8 is significantly less in reflex tears compared with basal tears in each eye (paired *t* test, *p<0.01).

can be strongly influenced by samples with high concentrations. Because the absolute concentrations of tear cytokines varied widely, this can have a strong bias. In this study, to our knowledge, we measured the multiple cytokines of basal and reflex tears from a single sample for the first time, which can provide concentration ranges for these cytokines in normal subjects that may prove important for studies of ocular inflammation. Of note, only the concentration of IL-8 was decreased more significantly in reflex tears than in basal tears. Maitchouk *et al* showed that there is no distinctive role of major and accessory glands in secreting basal or reflex tears, thus these tears might be produced primarily by the same tissue and differences might be only the result of the secretory rate of reflex tears.[8] It was reported that a neuropeptide released from corneal sensory nerves stimulated conjunctival epithelium to secrete IL-8.[9] Because sensory nerves are present in the cornea so abundantly, IL-8 can be produced constantly on the ocular surface. IL-8 is a potent pro-inflammatory cytokine, and has a pivotal role in the host defence system.[10] But excessive IL-8 might be so harmful that constant washout might be helpful for homeostasis of the ocular surface. Indeed, a large amount of IL-8 was found in the tears of dry eyes.[11] Thus, basal tears might be composed of products of the ocular surface including IL-8 and small amounts of reflex tears that are induced by mild stimulation such as blinks.

In summary, the present study showed that pro-inflammatory and anti-inflammatory cytokines/chemokines are present in the ocular surface even in the absence of inflammation and this was detectable from a small sample of single eyes. Stimulating tears, with the exception of IL-8, has minimal effect on cytokine concentration. We believe the CBA technique can make a valuable contribution in understanding the specific immunopathological mechanisms underlying cytokine interaction with the ocular surface.

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Table 1 Ratios of cytokine concentration

		B					
		IL-1b	**IL-6**	**TNF-α**	**IL-12p70**	**IL-10**	**IL-8**
(A) Basal tear							
	IL-1b	1	3.022 (0.246)	54.162 (6.549)	3.322 (0.316)	12.556 (0.889)	0.716 (0.086)
	IL-6	0.386 (0.037)	1	23.568 (5.952)	1.238 (0.184)	4.705 (0.553)	0.246 (0.03)
A	**TNF-α**	0.022 (0.002)	0.07 (0.008)	1	0.074 (0.011)	0.275 (0.028)	0.017 (0.003)
	IL-12p70	0.349 (0.026)	0.989 (0.071)	18.269 (2.086)	1	3.981 (0.166)	0.261 (0.042)
	IL-10	0.087 (0.005)	0.253 (0.02)	4.518 (0.47)	0.261 (0.012)	1	0.067 (0.011)
	IL-8	2.14 (0.34)	5.576 (0.694)	144.913 (45.971)	7.447 (1.546)	28.257 (5.078)	1
		B					
		IL-1b	**IL-6**	**TNF-α**	**IL-12p70**	**IL-10**	**IL-8**
(B) Reflex tear							
	IL-1b	1	3.71 (0.252)	53.061 (5.46)	2.918 (0.331)	12.012 (1.123)	0.972 (0.094)
	IL-6	0.304 (0.027)	1	15.807 (1.976)	0.808 (0.061)	3.381 (0.274)	0.276 (0.029)
A	**TNF-α**	0.021 (0.001)	0.078 (0.007)	1	0.062 (0.007)	0.254 (0.026)	0.02 (0.002)
	IL-12p70	0.390 (0.023)	1.361 (0.082)	21.357 (3.427)	1	4.231 (0.147)	0.382 (0.046)
	IL-10	0.093 (0.005)	0.324 (0.018)	4.837 (0.524)	0.241 (0.007)	1	0.093 (0.012)
	IL-8	1.043 (0.231)	4.779 (0.665)	73.465 (13.897)	4.02 (0.727)	17.235 (3.251)	1

Ratio of cytokine concentration was calculated as A/B. Each cell shows the mean (SEM).

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Outcome of implementing the national services framework guidelines for diabetic retinopathy screening: results of an audit in a primary care trust

Systematic screening for diabetic retinopathy is the key to achieving the targets set out in the recently published national service framework (NSF) guidelines.[1 2] One of the two priorities and planning framework (PPF) targets is that by March 2006 a minimum of 80% of people with diabetes are to be offered screening, rising to 100% by December 2007.[3 4] Digital photography is the modality for screening.[5–8] The British Diabetic Association has established standards for any diabetic retinopathy screening programme of at least 80% sensitivity and 95% specificity.

Screening programme

Our screening programme was set up to evaluate the existing nationally recommended diabetic retinopathy screening service.

In all, 2165 patients were invited over a period of 12 months; 909 participated. At the screening mydriatric drops (1% tropicamide and 2.5% phenylephrine) were instilled. The patients were photographed with a Topcon fundus camera and each photograph was screened and graded by consultant ophthalmologists (table 1).

Of the total 909; there were 198 patients with retinopathy (grades R1, M, R2, and R3), 644 patients without retinopathy (grade 0), 63 patients with OL (other lesions), and one was (grade U) ungradeable (tables 2 and 3). The sensitivity was 98% and specificity 97%. The retinopathy present was 21% and the referral rate for retinopathy (STDR) was 7%; 59% did not take up the offer to be screened. Some of the reasons cited: 956 (44%) declined or did not respond, 251 (11%) are being screened elsewhere, 11 (0.5%) are not diabetic, five (0.18%) are dead, six (0.27%) are registered blind, and 21 (1%) have moved.

Comment

Our screening methods surpassed the standards set by the NICE guidelines. However 59% of patients did not take up the offer. The achievable standard to which strategic health authorities and primary care trusts are working is 90% uptake of those offered screening with the minimum standards of 70%–80%, but even though quality assurance systems are in place uptake is still very poor.

In the intercollegiate audit, led by the Royal College of Ophthalmologists, data were analysed from 9827 patients with diabetes from 129 general practices in 25 health authorities. The lowest level of coverage in a district was 38% and the highest 85%. In general practice the coverage ranged from 14% to 97%. The likelihood of having an eye examination was marginally higher in districts with a systematic examination rather than opportunistic or without recognised schemes at all.

In the Hounslow Primary Care Trust our hospital episode statistics (HES) are the only existing screening programme. Patients registered with a GP are referred to the HES for the screening. The reasons for the 44% who did not respond were that some did not have the time, some were elderly living on their own with nobody to take them, some did not understand the screening leaflet, and some just forgot.

Problems about coverage could be tackled if we had a central electronic database linked to all screening programmes. Although software providers have been agreed for such a data collection exercise, primary care trusts have yet to implement this. People unable to attend during the week could be accommodated in weekend or evening clinics. These clinics could also be reserved, with bilingual support workers to explain the importance of screening for the ethnic patients. Community networks like the rotary, patient groups, ethnic resource centres, and senior citizens groups can be used. The National Service Framework targets will be achieved only if the diabetic population is convinced of the importance of screening.

Table 1 Referred patients

Diabetic patients type 1			
Invited	93	Appointment made	41
Diabetic patients type 2			
Invited	1442	Appointment made	609
Diabetic patients type not stated			
Invited	630	Appointment made	259

Table 2 Outcome by grade

Grade	Sex	Total
O	M	363
	F	261
	T	624
R1	M	74
	F	54
	T	128
M	M	23
	F	21
	T	44
R2	M	17
	F	9
	T	26
R3	M	1
	F	0
	T	1
OL	M	33
	F	30
	T	63
U	M	0
	F	1
	T	1

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Table 3 Final data

Total patients screened	Sample size	Ungradable	True positives	False negatives	True negatives	False positive	Sensitivity	Specificity	Retinopathy present
909	909	1	198	4	644	8	98%	97%	21.78%