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Rapid communication

Lens autofluorescence is increased in newly diagnosed patients with NIDDM

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Summary Lens and cornea autofluorescence has been shown to be increased in patients with insulin-dependent diabetes mellitus and to be positively correlated to glycaemic control and duration of diabetes. We have studied lens and cornea autofluorescence at the clinical onset of non-insulin-dependent diabetes mellitus (NIDDM), in comparison with age-matched subjects with normal glucose tolerance. Fourteen subjects with NIDDM diagnosed less than 6 months prior to the examination were characterised by ocular fluorometry, glycosylated hemoglobin A_{1c}, plasma lipid status, arterial blood pressure, and an oral glucose tolerance test (OGTT). Eleven age- and gender-matched healthy subjects without a family history of diabetes and with a normal glucose tolerance underwent the same examinations. In 11 of the 14 diabetic patients lens autofluorescence was increased to levels higher than the age-related mean + 2 SD of healthy subjects. For the entire study population, control and diabetic subjects, lens fluorescence was positively correlated with HbA_{1c} ($p < 0.0001$, $r = 0.73$), fasting plasma glucose ($p = 0.002$, $r = 0.60$) and the plasma glucose level 2 h after an OGTT ($p = 0.004$, $r = 0.55$). Cornea autofluorescence was also significantly increased in the group of newly diagnosed NIDDM patients, but only 9 patients had values above the mean + 2 SD of the healthy subjects. NIDDM could be detected by ocular fluorometry with a sensitivity of 79 % and a specificity of 100 %. We conclude that lens and cornea autofluorescence is abnormally increased in the majority of patients with newly diagnosed NIDDM. The sensitivity and specificity of the method indicate that lens fluorometry may potentially be useful for screening for undiagnosed NIDDM in the general population. Additionally, we propose that the method may be a clinically useful indicator of cumulative glycaemia and risk of development of secondary complications in patients with diabetes. [Diabetologia (1996) 39: 1524–1527]

Keywords Ocular fluorescence, screening, fluorometry, lens, cornea, NIDDM, hyperglycaemia, non-enzymatic glycosylation.

It is known from several cross-sectional studies of healthy subjects and insulin-dependent diabetic patients with varying duration of diabetes that lens autofluorescence increases as a function of age, duration of diabetes and cumulative glycaemia [1–4]. Thus, in diabetes, the rate of increase of autofluorescence appears to be two to three times higher than in healthy subjects [2, 4].

These findings, together with in vitro studies [5], indicate that the natural fluorescence of the cornea and the lens may be induced by non-enzymatic glycation of proteins with secondary, irreversible, formation of fluorescent fluorophores (advanced glycosylation endproducts). The formation of these fluorophores is initiated by the non-enzymatic binding of glucose to amino groups in the tissue proteins, similar to the formation of glycosylated hemoglobin A_{1c} from the non-enzymatic binding of glucose to haemoglobin.

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Abbreviations: NIDDM, Non-insulin-dependent diabetes mellitus; OGTT, oral glucose tolerance test.

Hyperglycaemia accelerates non-enzymatic glycation of tissue proteins in seemingly linear proportion to the concentration of glucose, including lens and cornea [5]. It has been shown that in diabetes collagen browning is increased [6], which may also explain the increased fluorescence of the cornea. Lens autofluorescence is considered to represent a cumulative index of life-long glycaemia as there is little or no turnover of lens proteins.

Non-insulin-dependent diabetic (NIDDM) patients have an increased risk of premature death due to cardiovascular events and at the time of diagnosis many patients have dyslipidaemia (40 %) and hypertension (50 %). Fewer have retinopathy (10 %) and nephropathy (5 %); about 80 % are obese [7].

Since NIDDM develops gradually the clinical onset is preceded by months or years of hyperglycaemia [8]. The harmful effects of the hyperglycemia seem obvious in patients who have developed long-term complications at the time of diagnosis. Thus, a marked increase in lens and corneal fluorescence is to be expected in a large proportion of the NIDDM patients at the time when diagnosis is made in clinical practice.

The aim of the present study was to investigate whether the autofluorescence of the lens and the cornea is increased in *newly* diagnosed NIDDM patients and on that assumption to consider the method as a screening procedure for unrecognized diabetes.

Subjects and methods

Study population. Fourteen patients with NIDDM, as defined by the National Diabetes Data Group [9], diagnosed less than 6 months prior to the examination were recruited from the outpatient clinic at Steno Diabetes Center. Eleven age-matched healthy normoglycaemic subjects were selected for the study. The control subjects had a normal oral glucose tolerance test (OGTT), normal blood pressure, and no family history of diabetes. Clinical data are presented in Table 1. The study protocol was approved by the local medical ethics committee. Informed consent was obtained from all study participants according to the Helsinki Declaration.

Study protocol. The patients were examined by slitlamp biomicroscopy of the anterior segment of the eye, direct ophthalmoscopy, fundus photography with a Canon 60° fundus camera and ocular fluorometry. These examinations were carried out on the dilated eye (one drop of 0.5 % tropicamide and one drop of 10 % phenylephrine hydrochloride).

Analytical procedure. After a 10-h overnight fast, at 08.00 hours, a venous blood sample was drawn. Glucose in plasma and serum concentrations of insulin and C-peptide were measured as described previously [10]. Hemoglobin-A_{1c} was measured by HPLC (Bio Rad DIAMAT, Richmond, Calif., USA) (normal range 4.1 to 6.4 %). All patients underwent a 75-g OGTT in accordance with the recommendation of the World Health Organisation.

Table 1. Clinical and biochemical characteristics of the study population

	NIDDM patients	Control subjects	*p*-value
No of patients (male/female)	14 (8/6)	11 (5/6)	
Age (years)/range	52.9 (36–64)	54.7 (40–69)	NS
HbA_{1c} (%)	7.5 ± 1.9	5.5 ± 0.3	< 0.001
Cornea fluorescence (ng/ml)	20 ± 4	15 ± 2	0.006
Lens fluorescence (ng/ml)	594 ± 207	376 ± 101	< 0.001
Fasting plasma glucose (mmol/l)	8.3 ± 1.5	4.9 ± 0.4	< 0.001
2 h plasma glucose (mmol/l)	14.6 ± 3.3	5.3 ± 1.5	< 0.001
Fasting serum C-peptide (pmol/l)	680 ± 342	398 ± 104	0.006
Fasting serum insulin (pmol/l)	52 ± 31	38 ± 18	NS
Systolic blood pressure (mm Hg)	133 ± 22	122 ± 18	NS
Diastolic blood pressure (mm Hg)	81 ± 10	68 ± 10	0.009

Values are mean ± SD

Methods

Fluorometry: Autofluorescence was measured by an ocular fluorometer (Fluorotron, Ocumetrics, San Jose, Calif., USA) mounted with an adapter for measurement on the anterior segment of the eye. The instrument makes 149 fluorescence measurements (excitation 430–490 nm, fluorescence 530–630 nm) along the optical axis of the eye with an axial resolution of approximately 0.06 mm.

The term *lens fluorescence* refers to the intensity peak measured at the anterior part of the lens. Lens fluorescence was expressed for each scan as the mean of the five highest fluorescence readings of the lens peak. As for the corneal fluorescence the mean of the five highest readings around the corneal peak was used. For each subject the mean value of six scans, three on each eye, was used in the statistical analysis.

For every scan an internal fluorescence reference is measured automatically allowing for correction for lamp intensity fluctuations and photomultiplier sensitivity. The measurement is non-invasive with a duration of 20 s. The excitation intensity is approximately 5 μW of blue light, which is well below safety and discomfort levels.

The fluorometric method was validated by examining the day-to-day coefficient of variation, which was found to be below 0.07 for both the anterior lens peak and for the corneal peak. In two healthy control and two diabetic subjects fluorometry was performed every 30 min during a 3 h glucose tolerance test. No significant fluctuation was seen, thus excluding osmotic effects or other errors due to transient homeostatic changes during the test. Eleven subjects were examined before and after dilatation of the pupils. Corneal fluorescence before dilatation was 19 ± 7 ng/ml (mean ± SD) and after dilatation 18 ± 7 ng/ml. The mean lens fluorescence before dilatation was 374 ± 139 ng/ml and after dilatation 404 ± 166 ng/ml. The effect was nearly uniform, in that 10 out of 11 lens values increased after dilatation and 10 out of 11 cornea values decreased after dilatation.

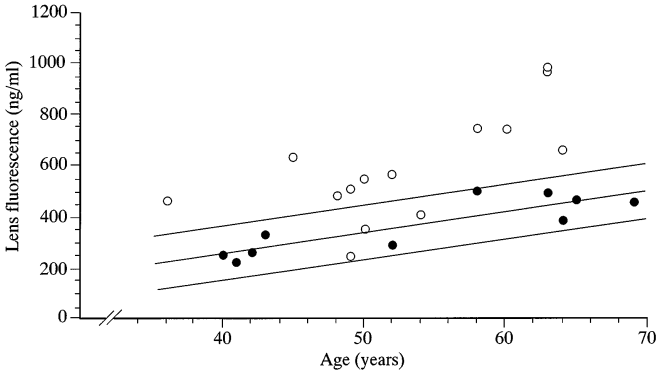

Fig. 1. Lens fluorescence in relation to age for healthy subjects with normal response to an OGTT (●) and patients with newly diagnosed NIDDM (○). Linear regression in healthy subjects demonstrated a significant increase in fluorescence with age (F = 8.2 * A–74), SD = 54 ng/ml. Mean regression line ± 2 SD (obtained by the age-related analysis) indicated on graph. Fluorescence is expressed as equivalent fluorescein concentration (nanograms per milliliter)

Statistical analysis. Linear regression analysis was used to describe the correlation between variables, with lens and cornea autofluorescence being the dependent variables and diabetic status, HbA_{1c}, fasting plasma glucose, OGTT, fasting serum C-peptide, fasting serum insulin and arterial blood pressure being the independent variables. Non-parametric statistical testing (Mann-Whitney-Wilcoxon) was used to compare the two groups. Lens fluorescence, F, is assumed to be linearly proportional to age and for the healthy subjects the equation

$$F = a*age + k \quad (1)$$

is used, where a is the coefficient for age and k is a constant. From the regression coefficients obtained in the analysis it was possible to calculate an estimate of HbA_{1c} (HbA_{1c}') from the lens fluorescence measurements

$$HbA_{1c}' = b*F + k' \quad (2)$$

Where b is the coefficient for lens fluorescence, and k' a constant.

Results

Clinical and biochemical data are presented in Table 1. In the NIDDM group one patient had macular drusen in one eye. Two NIDDM patients had background retinopathy, one with less than five microaneurysms in either eye and one with more than five microaneurysms, less than five small haemorrhages and less than five hard exudates in the eye with most pronounced diabetic retinopathy. None of the participants had cataract or lens opacities of subclinical grade within 3 mm of the optical axis of the lens.

Lens. In both healthy control subjects and NIDDM patients lens fluorescence increased significantly with age (Fig. 1). The lens fluorescence in the diabetic group was 37 % higher than in the age-matched group of healthy subjects (Table 1). Of fourteen subjects with NIDDM 11 had lens fluorescence intensities higher than the age-related mean + 2 SD of healthy subjects. The remaining three NIDDM patients had lens fluorescence values within the age-related mean ± 2 SD interval of the healthy subjects.

In the total group of study participants lens fluorescence correlated positively with HbA_{1c} ($p < 0.0001$, $r = 0.73$), as well as with fasting plasma glucose ($p = 0.0019$, $r = 0.60$) and the 2 h plasma glucose levels of OGTT ($p = 0.0042$, $r = 0.55$). From the linear regression coefficients, it was possible to calculate an estimated HbA_{1c} with a root mean square deviation of 13 % from the actual HbA_{1c}.

When using lens autofluorescence as a screening method NIDDM was detected with a sensitivity of 79 % and a specificity of 100 %. The sensitivity of a diagnostic test is defined as the percentage of positive diagnosis in all eyes that do have the disease, the specificity as the percentage of diagnoses of absence of disease in all eyes that do not have the disease.

Cornea. In the total group of study participants corneal fluorescence was also positively correlated to HbA_{1c} ($p = 0.031$, $r = 0.43$). Corneal fluorescence was positively related to age in either group but increased by 25 % in the diabetic patients to 20 ± 4 ng/ml (mean ± SD) as compared to 15 ± 2 ng/ml in the control group (Table 1). The overlap between the two groups is larger, however, for the corneal fluorescence than for the lens fluorescence. Thus, only 9 out of 14 patients with NIDDM had cornea fluorescence values higher than the mean + 2 SD of the healthy subjects.

Discussion

The present study shows that 11 out of 14 patients with newly diagnosed NIDDM had higher lens fluorescence than was found in an age-matched group of healthy subjects. Clinically and biochemically the three undetected patients with normal lens fluorescence were in the least diabetic half of the NIDDM group; all three had a near normal HbA_{1c}.

The strong positive correlation between hyperglycaemia and hyperfluorescence corroborates the theory that non-enzymatic glycosylation of lens proteins and collagen in the cornea is one of the main mechanisms for the formation of fluorophores at this wavelength in the tissue. Both lens and cornea autofluorescence was significantly increased in the diabetic group. The overlap, however, was larger with cornea measurements, possibly because the turn-over of corneal proteins is faster than in the lens, which may completely lack a protein replacement process. These observations and the high sensitivity and specificity indicate that the method can, non-invasively, detect

unrecognized NIDDM with efficiency and at a low cost, and this could lead to earlier initiation of anti-glycaemic treatment.

Also on the basis of relatively simple measurements of lens fluorescence it appears to be possible to provide an estimate of the patient's cumulative glycaemia, which may be clinically useful. Theoretically, a quantitative estimate of cumulative glycaemia, expressed as lens fluorescence, in any NIDDM patient, may provide a useful estimate of the risk of developing long-term diabetic complications. This may be useful especially in patients for whom no record of prior glycaemia or precise duration of diabetes is available. The explanation of the deviation between the estimated HbA_{1c} and the standard HbA_{1c}, is that the standard HbA_{1c} covers a period of 2 to 3 months, whereas lens fluorescence represents a lifelong cumulated glycaemic exposure.

In conclusion, we have found solid evidence that the intrinsic fluorescence of the ocular lens harbours valuable information about the glycaemic history, which may be utilized for screening purposes and potentially for the clinical evaluation of newly recognized NIDDM cases.

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