Ocular changes in patients undergoing long-term desferrioxamine treatment

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SUMMARY In a group of young patients with thalassaemia and iron overload treated by subcutaneous infusions of desferrioxamine we have found a number of minor alterations in retinal function. The incidence of such changes is not related to drug dosage or to ferritin level but to abnormality of the extended glucose tolerance test.

Patients with beta thalassaemia major require regular blood transfusions and may thus develop iron overload, which is treatable by long-term subcutaneous administration of the chelator desferrioxamine (DFO).^{1,2} The ferrioxamine formed is excreted mainly in the urine³ and bile.⁴ Continuous iron chelation can reduce or even return to normal serum ferritin levels which provide an index of the iron burden in the body.⁵

To achieve adequate chelation DFO, 60 to 70 mg/kg body weight, is given as a slow, continuous, subcutaneous infusion five to six nights a week. Some patients find this treatment difficult to comply with, and as a result a cardiomyopathy may develop. DFO has then been given intravenously in a dose two to four times greater than is usual by the subcutaneous route. Although improvement of cardiac function occurs, sudden onset of retinopathy has been reported in two patients so treated,⁶ and other cases have been encountered⁷ (Arden GB, Carter R, paper in preparation).

This study investigates whether there is any evidence of a retinotoxic action of DFO when given by prolonged subcutaneous infusion in clinically effective doses, and, if so, whether the iron damage or an associated thalassaemic defect causes such changes. In order to clarify these points we have investigated a group of patients most of whom had beta thalassaemia major. They had a variety of signs related to their thalassaemia and iron overload, but none had any complaints about their eyes. All but two had been on long-term (four to five years) subcutaneous chelation.

These patients have been carefully followed up, and their initial and recent biochemistry and serum ferritin levels were available.

Patients and methods

We have studied 43 patients. Thirty-nine had beta thalassaemia major and were of Greek, Turkish, and Indian origin. Four patients had beta thalassaemia intermedia. Three of these started DFO treatment after the eye tests were completed, and another patient had a very small amount of DFO prior to eye testing. Twenty-six were male and 17 female (age range 9-27 years, mean 18 years). Twenty-six were born and treated exclusively in the United Kingdom, and their haemoglobin had always been maintained above 10-11 g/dl. Of the remainder none was anaemic at the time of testing, but details about the transfusions received were not available. The four children with thalassaemia intermedia received irregular transfusions. The remainder received blood transfusions from the first year of life at intervals of four to six weeks. Regular subcutaneous DFO iron chelation treatment was introduced four to five years before the present study to all those with beta thalassaemia major.

Serum ferritin,⁸ extended glucose tolerance tests (GTT), and insulin⁹ levels were available at the time of the study in addition to the approximate dose of DFO received.

ELECTROPHYSIOLOGICAL EXAMINATIONS

Electro-oculograms were recorded as described.¹⁰ This test measures the change in the current produced by the pigment epithelium in darkness and in light.

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The eyes make movements between fixed marks, and electrodes on the temples record the potentials produced. In the normal population the increase in light is 220%, and the lower limit of normal is taken as 180%. However, in rare cases in which there is no demonstrable eye disease lower values are encountered, and some common minor complaints (for example, myopia) are associated with low values. In cases of retinopathy there is scarcely any change of current during the test, so values close to 100% are encountered. The significance of 'borderline' subnormality is discussed below.

The electroretinogram (ERG) was evoked with flashes of light of different intensity and colour and repetition rate to distinguish rod and cone based responses. The protocol used has been described in detail.¹¹ The amplitude and response peak times were measured. In retinal disease, particularly that involving cones, the peak time of the responses may be delayed. Their amplitude may also be reduced, particularly when retinal sensitivity is reduced and weak flashes are employed. The normal ERG amplitude varies by as much as 50%, and a response is considered as suspiciously reduced if the value is decreased by more than 2 SD from the mean. In a test consisting of 22 independent trials the finding of an 'abnormal' result to a single trial cannot be considered as indicating absolutely that the retina is diseased—especially since artefacts such as blinks may affect the individual trace. In assessing the results of the experimental group we have included such samples in order to discover if there were any minor deviations from normal in the group as a whole.

Pattern electroretinograms (PERGs) were obtained as described.¹² A series of normal values obtained in children and young adults has shown that the response mean amplitude is 2.7 μ V, SD 0.3. Any value under 2.0 μ V is taken as abnormal. The results are influenced by the refractive state of the eye, and our normal population does not include patients with the heavy pigmentation seen in patients with iron overload.

Visual fields were measured on a calibrated Goldmann perimeter using carefully measured stimulus intensities. The peripheral extent of the field was measured and compared with the population average results supplied with the perimeter. If there was a reduction of $>20^\circ$ in two or more quadrants of the field this was considered significant. Usually such defects would not be considered as definitely indicating an abnormality unless there was other evidence of retinal or optic nerve disease.

Dark adapted visual thresholds were determined on a computer driven modified perimeter.¹³ Visual acuity was measured with a standard test-type. Fundi were inspected and abnormalities noted. Fundus

colour photographs and fluorescein angiograms were obtained in a number of cases.

Results

Visual acuity. These ranged from 6/4 to 6/12. Only in one patient was the response abnormal, and the values in the group as a whole did not indicate any abnormality.

Visual fields. No isolated field defects (scotomata) or enlargements of the blind spot were found. However, the average field size (mean of extent of four quadrants) was 49° , SD 0.6, while the average field for this age group is 61° (maker's figures, no statistics given). Thus, as a whole, there appears to be field constriction. Analysis of the results in individual cases showed that in 15 patients there was apparent field loss (of 20° or more) in one quadrant of each eye, but in only eight of these was the loss found in at least two adjacent quadrants in one of the eyes. This was considered to represent a possible abnormality.

Dark adaptation. The average thresholds for rod and for cone vision were determined 10° nasally, and the results were (within the limit of discrimination of the apparatus) equal to those found in a series of young adults. However, four patients had rod thresholds raised by more than 0.8 log unit from the normal mean, and one had an elevation of the cone threshold by a similar amount. The raising of threshold by more than 0.6 log unit from the mean is usually considered to suggest an abnormality.¹¹

Fundal appearances. Funduscopy showed no focal areas of pigment disturbance in any of the patients, although, in keeping with their haemochromatosis, there was considerable hyperpigmentation of the retinal pigment epithelium. Perhaps because of this the retinal reflexes in many patients were unusually prominent. No focal lesions of the retina were seen, and no macular oedema. In one case the disc was noted as being unusually pale. In nine an unusual vascular pattern was seen, the retinal vessels being markedly tortuous ('corkscrewing'). While this is seen in otherwise normal persons, it is much less common than in our group. In 30 of the group adequate colour photographs and fluorescein angiograms of the fundus were also obtained, and later study of these also disclosed no abnormality.

ELECTROPHYSIOLOGICAL TESTS

Electro-oculogram. The average value of 205% was only slightly and insignificantly lower than that previously reported.¹⁰ There was a large variation in results. Twelve patients had EOGs in one or both eyes which were below the clinic normal level (180%), and in six of these the value was below 160%. Although subnormal values are suspicious in cases of

Fig. 1 Electrooculograms in patient group. The arrows mark the mean and the lower limit of normal

frank eye disease, values in the range 100–140% were encountered. However, the distribution of results (Fig. 1) was skewed.

Electroretinograms. The scotopic responses of the patient were nearly identical with those of the normal data for the clinic (Table 1). However, the responses to intense white flashes and to flicker were slightly smaller and the peak times slightly prolonged. The standard errors of these mean values were small, so for flashes which alter the state of adaptation of the eye these differences can be taken as significant. The results in individuals also show this. In four patients the time to peak of the flicker responses was prolonged beyond the normal limits (32 ms), in two more the responses to red light flashes were abnormally small (more than 2 SD below the mean) and in one the response to white light was abnormally reduced. In four cases the time to peak of the rod response was increased beyond the normal limits.

PATTERN ERG

In contrast to these relatively mild changes the PERG abnormalities were much more pronounced. Of the 39 patients only four gave responses greater

Fig. 2 Pattern electroretinograms in patient group. The arrows mark the population mean value and the lower limit of normal.

than the average normal, and no fewer than 25 gave responses below the clinic normal (Fig. 2).

CORRELATIONS BETWEEN TESTS

In the ERG recordings 2.2% of all the traces were abnormal. The definition of abnormality is a response which deviates more than 2 SD from the mean, and statistically one would expect a response to be reduced by this amount in about 2% of trials. Again it might be that the normal subjects who provided results for our statistics were better observers than the patients investigated in this series (we have tried to avoid such a trap). One way of investigating this possibility is to inquire whether the defects found in the different tests are related. Of the 10 patients with abnormal ERG results of any sort five had abnormal EOGs. Most of the patients with abnormal pattern ERGs had abnormal tests in other senses, but this is to be expected because there were so many abnormalities in the PERG. A summary of the results is given in Table 2.

Of the patients with one defect only one had not got an abnormal PERG. In that patient the field appeared slightly constricted. In the 18 patients with more than one defect and an abnormal PERG the

Table 1 ERGs in patient group compared with normals*

Stimulus	Patients		Normals	
	Amplitude (μV)	Peak time (ms)	Amplitude (μV)	Peak time (ms)
Blue light (12)	322	61	319	57
Red light (18)	143	46	153	48
White (19)	348†	46	383	46
Flicker (22)	40	30.5†	48	29

* Each test consisted of 22 trials. Results of only four are shown here for simplicity. The exact stimulus conditions—coded by the numbers in the first column—are described by Arden *et al.*¹¹
† Mean values significantly different from normal, *t* test: *p* < 0.02.

Table 2 Results of tests showing abnormal PERG, ERG, EOG, fields, dark adaptation, and VA

No of tests abnormal	No of patients
0	14
1	10
2	11
3	7
4	1

Table 3 Relationship between ocular abnormalities and drug dosage

	Total dosage			
	0-0.2 kg	2-3 kg	3-4 kg	>4 kg
Patients with no ocular abnormality	2	8	3	2
Patients with ocular abnormalities	2	8	4	1

average PERG amplitude was 1.53 μ V, SEM 0.16. In the remainder the average is 2.33 μ V, SEM. Thus, smaller PERGs are associated with other noted defects. Such correlations strongly suggest that the minor changes in electrodiagnostic tests are not 'noise' but have a significance. Accordingly we split the patients into two groups—the 18 with two or more abnormalities and the remainder. In this way we had a basis for determining if the abnormalities detected were related to clinical aspects of the patients or to their treatment.

Relationship to drug dosage. This analysis was confined to a subgroup of patients on whom the most detailed records had been kept. Table 3 shows the relationship between the presence of ocular abnormalities (defined in the way indicated above) and the total dose.

It is evident that no relationship can be established. In addition the dosage was calculated on the basis of body weight. The range was from 0.093 g/kg/day to 0.037 g/kg/day. The patients were ranked according to dosage and the presence or absence of ocular abnormalities. The result of the Mann-Whitney U test was that the rank sums were nearly identical, and the level of significance was 0.85 (significance is taken as 0.05 or lower).

Serum ferritin levels. These varied widely, and to attempt more precise correlations the analysis was confined to the subgroup of 30 patients whose tests had been done in a single laboratory. The range was between 9000 and 400 μ g/l for patients with no ocular abnormalities, and 7430 and 370 μ g/l in patients with abnormal tests. The Mann-Whitney U test showed that there was no significant relationship.

Total blood transfused. In patients with ocular abnormalities the total blood transfused ranged from 0 (one patient who suffered from thalassaemia intermedia) to 430 units. The mean was 204, SD 106. In those with no abnormalities the corresponding figures are 34-880, and the mean value is 332, SD 223. The Mann-Whitney U test showed that there was no difference between the two groups. There was a significant correlation between the total blood transfused and the serum ferritin levels.

Age and sex. The average age of the 18 patients

Table 4 Relation of ocular abnormalities to changes in GTT

	Abnormal ocular tests*	
	Present	Absent
GTT normal, or raised to <7 mmol/l at 60 min	5	13
Diabetic, or GTT >7 mmol/l	6	2

Probability of this result (Fisher's exact test)=0.022.

*In two cases GTTs were not done, and in two the results were clearly erroneous and have been excluded.

with ocular abnormalities was 16.4 years (SD 4.9) and of the remainder 18.4 (SD 4.9). Eight of the 18 patients with abnormalities were female as were nine of the remaining 25. This difference is not significant.

Extended glucose tolerance tests. In a number of patients the GTTs were abnormal. In some cases the fasting levels were raised, and in others the levels were raised at 60, 120, or 180 minutes. Using a single criterion, the raising of glucose levels above 7 mmol/l, we obtained the results shown in Table 4.

The groups were established by other criteria (greater elevations at 60 min, combined with elevation at 120 min) and Fisher's exact probability test remained below 0.05. Thus there appeared to be a significant correlation between the presence of abnormal eye tests and a prediabetic state.

Correlation between PERG and GTT. The pattern ERG was the test which showed the most frequent abnormality in our group of patients, and the result was quantitative. Table 5 shows the results obtained.

Abnormal funduscopy and other ocular tests. There was a correlation between these variables, but Fisher's exact probability test was not significant ($p=0.11$).

Discussion

Fundus examination and a number of special tests have indicated that while there are no cases of frank retinopathy in this series the values obtained are

Table 5 Results showing correlation between GTT and PERG

GTT	No of patients	PERG amplitude $\pm$ SEM
Glucose ≤ 7 g/l	5	2.2 $\pm$ 0.38
Glucose >7 <10 at 60 min	13	1.8 $\pm$ 0.17
Glucose raised at 60 and 120 min	6	1.4 $\pm$ 0.25
Diabetic	2	1.1 $\pm$ 0.35

There is a consistent relationship between the degree of reduction in the PERG and the severity of the diabetic abnormality. Those patients with normal GTTs have PERGs within the normal range.

not those which would be expected in a normal population.

In some cases (visual acuity, perimetry) the normal values are established on a world-wide basis. For other tests our clinic standards have been established for some years. Most of our tests require patient co-operation, and normal values have been established on people (often patients or their relatives) who are highly motivated. The children, adolescents, and young adults in the present series did not regard themselves as patients of the eye hospital, and some submitted grudgingly to our tests. It is possible that some of the low values recorded are due to this cause. However, the relatively simple tests, which demanded little co-operation, are abnormal, while there is a great difference in the pattern and flash ERG results which, from the patients' point of view, are very similar. Those patients who seemed recalcitrant were not those who necessarily had poor results.

In the case of funduscopy it is most likely that the incidence of tortuous vessels is related to changes which occur early in life¹⁴ before treatment, and there is only a poor correlation between the presence of such tortuosities and other functional changes. We can establish no correlation between the dosage of DFO and the presence or absence of 'eyesigns'. We can find no correlation between serum ferritin levels and the presence of the abnormal eye tests, as might be expected if the eye signs were simply due to the iron overload. There is no relationship between variables such as the total iron transfused or age and the presence of eye signs.

We also attempted more complex correlations. Thus, if the dosage of DFO was important in producing the lowered test results, the total iron transfused divided by the ferritin levels would form an index which compensated for non-compliance. However, such analyses did not yield any positive result.

The only significant correlations are between the presence of abnormal ocular tests and the diabetic status. It is already known that the pattern ERG is abnormal in early diabetic retinopathy^{15 16} (Arden GB, Hamilton P, paper in preparation). The abnormalities detected may be due to damage to the B cells in the pancreas caused by iron deposition secondarily causing retinal changes. However, the prediabetic state may alter the blood retina barrier and permit DFO to cross to the retina, where it is toxic. Although large doses of DFO administered by intramuscular or subcutaneous injection have appeared to cause no

disturbance in toxicity trials,^{17 18} it has recently been found possible to cause a loss of ERG sensitivity in rabbits by infusing DFO after anaesthetising with urethane, which causes leakiness in the blood retina barrier (paper in preparation).

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