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Correlation of aqueous humour lactic acid dehydrogenase activity with intraocular pathology

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SUMMARY An analysis of the lactic acid dehydrogenase (LDH) activity in the aqueous humour of 24 enucleated eyes with retinoblastoma showed that though there was no relationship between the LDH levels and the age of the patient, there appeared to be an increase in the LDH activity with increase in the duration of the tumour. Undifferentiated tumour cells and tumour cell necrosis seemed to contribute to an increase of LDH levels in the aqueous humour, but there was no relationship between the occurrence of calcification and the LDH secretion into the aqueous humour. Massive cell necrosis caused by radiotherapy and central retinal artery occlusion significantly increased the LDH levels in the aqueous. It also appeared that recurrence was common after external cobalt therapy and that secondary extension of the tumour into the optic nerve and choroid was favoured by this procedure.

Although an increase in the lactic acid dehydrogenase (LDH) activity in the aqueous humour has been reported in non-malignant intraocular conditions associated with cell necrosis (Stone and Krupin, 1976) there appears to be no doubt that, together with other ancillary investigations, the estimation of LDH activity in the aqueous humour is an invaluable procedure in the diagnosis of retinoblastoma (Dias *et al.*, 1971; Kaneko and Suzuki, 1972; Swartz *et al.*, 1974; Kabak and Romano, 1975; Felberg *et al.*, 1977).

The purpose of this study was to correlate the aqueous humour LDH activity with intraocular pathology to determine whether factors like secondary spread of the tumour, duration of the tumour, age of onset, radiotherapy, and tumour pathology could influence the LDH activity.

Materials and methods

Aqueous humour was collected by aqueous puncture from 25 patients who presented at the Victoria Memorial Eye Hospital, Colombo, for enucleation of 1 of their eyes. These specimens were kept at 4°C and analysed the same day.

LDH activity was estimated by the colorimetric method of King (1959), in which the increase in optical density at 340 nm was measured. A unit of activity is the amount of LDH which reduces 1 μmol lactate to 1 μmol pyruvate in 15 minutes at 37°C. Activity is expressed in units/100 ml of aqueous humour (SI conversion: units/l = units/100 ml × 10). Care was taken to see that none of these specimens was contaminated with blood.

The enucleated eye was fixed in formol saline, and the paraffin sections were stained with haemotoxylin and eosin. Ten sections containing the tumour were examined from each eye.

Twenty-four of these patients presented with a white mass behind the pupil and were diagnosed preoperatively on clinical grounds as having a retinoblastoma. Subsequent histology showed a retinoblastoma in each case. One patient, however, had a non-malignant intraocular condition associated with a raised LDH level in the aqueous humour.

Results

DURATION OF THE TUMOUR

It was difficult to obtain an accurate assessment of the exact duration of the tumour. The only indication was the duration of time since it was first noticed by one or other parent. Both parents were closely questioned independently to obtain an idea of its duration. The relationship between the duration of the tumour as estimated in this manner and the LDH activity is presented (Table 1).

The 3 patients in whom the duration of the tumour was more than 6 months were clinically diagnosed as retinoblastoma and confirmed by LDH

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Table 1 *Relationship between the duration of the tumour and aqueous humour LDH activity*

Duration (months)	*Number of patients*	*LDH activity in aqueous humour (units/100 ml)*	
		Mean	*Range*
Less than 1	6	1927	1220–2660
1–3	11	2272	1890–2660
4–6	4	2700	2480–2840
7–12	2	3555	3460–3650
More than 12	1	3850	—

Table 2 *Relationship between radiotherapy and aqueous humour LDH activity*

Age of child when first seen	*Initial LDH activity (units/100 ml)*	*Duration since radiotherapy (months)*	*LDH activity (units/100 ml) on enculeation*
1 year 2 months	2120	9½	3460
2 years 7 months	2860	16½	3850
4 years 3 months	1800	10	3650

Table 3 *Relationship between age of child and aqueous humour LDH activity*

Age of child (years)	*Number of children*	*LDH activity in aqueous humour (units/100 ml)*	
		Mean	*Range*
Less than 1	4	2675	2580–2820
1–3	11	2280	1640–2860
4–5	8	2014	1220–2660
Over 5	1	2240	—

assay (Table 2). They were advised enucleation but the parents refused and prefererred to have the eyes irradiated. All 3 of them presented at a later date for enucleation and the LDH values prior to enucleation are shown in Table 1.

AGE OF ONSET

Though classically retinoblastoma is a tumour of infancy, in this series it tended to occur more frequently in a slightly older age group (Table 3).

One child in this series was a girl of 9 years, and doubt existed whether this could be an endophthalmitis or a case of Coats's disease. A diagnostic aqueous puncture revealed an LDH activity of 2240 units/100 ml, indicating that the mass was most likely to be a retinoblastoma. The eye was enucleated, and histological examination showed an undifferentiated retinoblastoma containing areas of necrosis and calcification with no invasion of the choroid or optic nerve. Retinoblastoma, when it occurs in the older age group, usually appears as a flat diffuse type of growth, being more like a thickened retina. The exophytum type of growth as was seen in this patient is not a common feature in the older age group.

TUMOUR PATHOLOGY

All 24 eyes examined showed necrotic areas within the tumour mass and all of them were poorly differentiated retinoblastoma. Areas of calcification, however, were seen only in 14 patients. None of the patients in whom the duration of the tumour was less than 1 month showed calcification.

There was no correlation either between the occurrence of calcification and the LDH activity of the aqueous humour or between the occurrence of calcification and the age of the patient.

EFFECT OF RADIOTHERAPY

In this series 3 patients had received radiotherapy prior to enucleation. They were all clinically diagnosed initially as cases of retinoblastoma and confirmed by aqueous humour LDH assay (Table 3). They were all given external cobalt therapy, 3500 to 4000 rad over 4 weeks (cobalt plaques are not available in Sri Lanka).

All 3 children returned to the Eye Hospital, Colombo, within 1½ years with a proptosed eye which needed enucleation. LDH activity at the time of enucleation was recorded (Table 2). Histology showed an undifferentiated retinoblastoma in each case with secondary extension into the optic nerve and choroid.

EXTRAOCULAR SPREAD

Four eyes in this series showed on histological examination an extraocular extension into the optic nerve and choroid. Three of these patients had previously received radiotherapy, and their LDH activity in the aqueous humour just prior to enucleation was 3850, 3650, and 3460 units/100 ml.

The other patient, who had not received radiotherapy but who had an extension into the optic nerve, showed an LDH activity of 2660 units/100 ml at the time of enucleation. In all patients the tumour had persisted for more than 5 months.

The case history of the patient who had a non-malignant intraocular condition with increased LDH activity in the aqueous humour is recorded below.

Case report

A 47-year-old man presented with blindness and pain of sudden onset in the left eye of 3 weeks'

duration. Visual acuity was RE, 6/6; LE, NPL. The left eye showed a chemosis of the bulbar conjunctiva and the pupil was dilated and fixed. The anterior chamber in the right eye was shallow. Slit-lamp examination revealed the presence of cells, and there was a visible flare. Keratic precipitates were also seen. Funduscopic examination showed optic atrophy in the left eye. This was a clear-cut case of left central retinal artery occlusion with acute uveitis. Further investigations revealed an absent right radial pulse and an absence of both carotid pulsations. Archangiogram revealed a complete occlusion of the innominate artery and the upper part of the right internal carotid artery. There was a large amount of atheromatous change in the aorta which was probably the source of the embolic changes in the left eye.

The case was diagnosed as one of aortic arch syndrome, and the left eye was enucleated. Just prior to enucleation aqueous humour was removed for LDH assay.

The LDH level was abnormally high—1075 units/100 ml—and was in the range of intraocular malignancy (aqueous humour LDH levels of over 1000 units/100 ml are regarded as being suggestive of intraocular malignancy (Dias *et al.*, 1971)).

Histological examination of the enucleated eye showed a haemorrhage at the disc and in the posterior part of the vitreous, with no abnormality in the anterior segment, but there was no tumour tissue in the eye.

Discussion

The correlation of the LDH activity in the aqueous humour with the duration of the tumour as assessed by the time since it was first noticed by the parents appears to indicate that the longer the duration of the tumour, the higher was the LDH activity in the aqueous humour. This could probably be attributed to the growth of the tumour and the resultant increase in the number of LDH-secreting malignant cells. Further, the growth of the tumour promotes necrosis of cells within the tumour mass, which increases the release of intracellular enzymes into the aqueous humour.

The 3 eyes in which the tumour had persisted for more than 6 months were from patients who had received radiotherapy, but the effect of radiotherapy as being the cause of an increase in LDH activity could be eliminated, because they had all received radiotherapy at least 9 months previously.

The age of onset of the tumour had little or no bearing on the LDH activity in the aqueous humour.

Of the 4 patients who showed retro-ocular spread of the tumour 3 had received radiotherapy, and they had very high LDH levels in the aqueous. In fact the highest LDH levels recorded in this series were in 3 patients who presented with a recurrence of the tumour after radiotherapy. The other patient who had not received radiotherapy but who also showed retro-ocular spread had a relatively lower LDH level in the aqueous. It appears that retro-ocular extension by itself is unlikely to cause a gross elevation of the LDH activity. It would have been interesting to estimate the LDH activity in the cerebrospinal fluid of these patients who showed a secondary spread into the optic nerve, but unfortunately this could not be done in any of them.

As cobalt discs are not available in Sri Lanka, external cobalt therapy is the method of radiotherapy for retinoblastoma. Though retinoblastoma is far more radiosensitive than melanoma, statistics are not available in Sri Lanka for the 5-year survival rate after irradiation by this method.

In the present series 3 patients were given the full dose of radiotherapy, but in all 3 the tumour recurred within $1\frac{1}{2}$ years, resulting in enucleation, and all 3 patients showed very high LDH levels in the aqueous with retro-ocular extension into the optic nerve and choroid. Radiotherapy appears to promote the retro-ocular spread of the tumour, probably on account of damage to the tissues in the posterior part of the eye, with resulting spread of the tumour.

Regarding recurrence of the tumour and retro-ocular spread following radiotherapy, a larger series and a long-term follow-up are essential to determine whether external cobalt therapy as it is administered today in Sri Lanka is of any significant value in the treatment of retinoblastoma, because the possibility exists that radiotherapy could in some way favour the spread of the tumour into the choroid and optic nerve.

Since all specimens examined showed areas of tumour necrosis, it was difficult to assess to what extent tumour necrosis contributed to the elevated LDH activity. Similarly, it was difficult to assess to what extent the degree of differentiation of the tumour affected the LDH activity in the aqueous humour. It seems that, though the bulk of the enzyme is actively secreted by poorly differentiated malignant cells (Wroblewski, 1957), intracellular enzyme release due to lysis of cells resulting from tumour cell necrosis may also contribute to the total LDH activity in the aqueous humour.

There was no relationship between the occurrence of calcification in the tumour mass and the LDH activity in the aqeous humour.

The fact that LDH levels could be elevated in the aqueous humour even in non-malignant intraocular conditions which cause cell necrosis is illustrated by

the patient with a central retinal artery occlusion.

As enzymes and metabolites from the retinal network could pass forwards by diffusion through the vitreous into the aqueous (Dias and Amarasiri, 1978) infarction of the retina could increase the LDH activity of the aqueous humour in the absence of intraocular malignancy.

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