OCULAR MALIGNANCIES; LACTATE DEHYDROGENASE LEVELS IN AQUEOUS HUMOUR & SERUM OF PATIENTS

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ABSTRACT

A total of 50 cases with clinical diagnosis of ocular malignancy were studied for lactate dehydrogenase (LDH) levels in aqueous humour and serum. On histological examination, 40 cases were those of retinoblastoma, 7 had other types of ocular malignancies (squamous cell carcinoma 5, one each of malignant melanoma and sebaceous carcinoma), whereas three turned out to be non-malignant. Aqueous LDH levels were significantly raised (P<0.001) in cases of retinoblastoma when compared to other malignancies as well as controls. Mean aqueous humour to serum LDH ratios were also significantly raised (P<0.001) in cases of retinoblastoma.

INTRODUCTION

Intraocular tumors are important both clinically and pathologically. Their clinical importance arises from the fact that a correct diagnosis may save the patients' life. These tumours are the only ocular condition which, if left untreated, may kill the patient. Their pathological importance derives from the fact that they show many unique features in structure and behaviour.

Mahoney et al. have described the epidemiologic characteristics of primary eye cancers. Amongst these, melanoma, retinoblastoma and squamous cell carcinoma are the leading malignant tumours.

Retinoblastoma is one of the most frequent malignant ocular tumour of the childhood. In various studies conducted at different enters for the frequency of tumors in Pakistan, lymphoma and retinoblastoma were the two commonest malignancies in childhood.

A number of benign conditions may clinically resemble retinoblastoma by producing leukokoria, strabismus or fundal mass. Misdiagnosis and subsequent delay in therapy results in markedly increased mortality. It is recommended that the decision to enucleate an eye with a suspected lesion should not be made on the basis of any single test or procedure. Such a decision should be made on the basis of a clinical observation in conjunction with the diagnostic adjuncts available.
been several reports indicating that the aqueous humour to plasma lactate dehydrogenase (LDH) ratio is elevated in patients with retinoblastoma but not in patients with various pseudoretinoblastomas\textsuperscript{13,14}.

Lactate dehydrogenase is a glycolytic enzyme that catalyzes the interconversion of lactic and pyruvic acids\textsuperscript{14}. It is a cytoplasmic enzyme found in all tissues. It is found in serum, cerebrospinal fluid, urine, blood and aqueous humour\textsuperscript{15,16}. Normal concentrations of most of the substances in aqueous humour are approximately equal to their plasma levels\textsuperscript{17}.

Because of its widespread activity in numerous body tissues, LDH is elevated in a variety of non malignant as well as malignant disorders\textsuperscript{18-22}. Therefore, assay of LDH in serum or plasma may have a value in the diagnosis of early malignancies\textsuperscript{2}. The increased activity of LDH is attributable to the necrosis of proliferating neoplastic cells which contain this enzyme. This is a relationship between the growth rate of the tumour and the elevation of LDH\textsuperscript{2}. Moreover, it has been reported that malignant cells are capable of actively secreting lactate dehydrogenase into the surrounding body fluids and that the level of enzyme in the body fluid bathing the malignant neoplasm is more than that of the serum level\textsuperscript{19}.

The present study was carried out to reveal the diagnostic significance of LDH level in aqueous humour in cases of ocular malignancies particularly the retinoblastomas with doubtful clinical diagnosis. This procedure might be helpful as a diagnostic adjunct in order to select the mode of treatment for the patient.

MATERIALS & METHODS

The present study included fifty cases with a clinical diagnosis of malignant ocular tumour irrespective of age and sex. They were selected for enucleation of the affected eye. Thirty control cases were also included. They comprised of cases with a clinical diagnosis of a disease other than ocular malignancy selected for intraocular surgery. The specimens were collected from ophthalmological units of Ganga Ram Hospital, Lahore General Hospital, Datta Darbar Hospital Lahore, Allied Hospital, Punjab Medical College Faisalabad and Nishtar Medical College/Hospital Multan.

The cases were evaluated on the basis of the history, clinical examination, ophthalmoscopy, slit lamp biomicroscopy and radiological examination of the skull and orbit. The diagnosis was confirmed by histopathological examination of the enucleated eyes.

The aqueous humour (approximately 0.2 ml) was collected from the diseased eye in a dry tube under anaesthesia before enucleation, using a 1.0 ml tuberculin syringe with a 26 gauge needle. The venous blood sample (5 ml) was also collected from the patient at the same time. Care was taken not to use a haemolysed blood sample. Aqueous humour samples were free from blood contamination and were centrifuged before analysis. LDH assay was done using commercially available reagents (Randox Laboratories Ltd, Ireland) on Lab system instruments. The assay was performed on the same day and the samples were kept at 4°C when required. The results were expressed in IU/L (International Units per Litre). When required the concentrations of LDH were converted to log IU/L to give a gaussian curve for statistical analysis. Students 't' test and Chi square test with Yates correction were used to analyze the data.

RESULTS

Among the 50 cases with clinical diagnosis of an ocular malignancy, 40 were those of retinoblastoma, 7 had other types of ocular malignancies (squamous cell carcinoma 5 cases, malignant melanoma and sebaceous carcinoma one case each) whereas three turned out to be non malignant on histopathological examination.

In a total of 40 cases of retinoblastoma, 26 were males while 14 females with a mean age of 3.27 ± 1.50 and 3.48 ± 2.50 years, respectively. Control cases had mean age of 47.7 ± 28.5 years. Ten percent cases were bilateral. Aqueous humour in six cases of retinoblastoma was found to be either inadequate or
unsatisfactory for analysis. The aqueous humours LDH levels were significantly raised in patients with retinoblastoma when compared with controls and other malignant ocular tumours (Table I & II).

### Table I. Comparison of aqueous humors LDH levels in various ocular malignancies and controls

<table>
<thead>
<tr>
<th>Group</th>
<th>No of pts.</th>
<th>Range (IU/L)</th>
<th>Mean ±SD (Log IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinoblastoma</td>
<td>34</td>
<td>22 - 5589</td>
<td>2.829 ± 0.614*</td>
</tr>
<tr>
<td>Other malignancies</td>
<td>7</td>
<td>18 - 46</td>
<td>1.417 ± 0.131**</td>
</tr>
<tr>
<td>Controls</td>
<td>30</td>
<td>0.2 - 93</td>
<td>1.499 ± 0.344</td>
</tr>
</tbody>
</table>

*P<0.01 Highly significant. P**>0.05 Significant.

### Table II. Comparison of aqueous humor LDH level in retinoblastoma, other ocular malignancies and controls

<table>
<thead>
<tr>
<th>Group</th>
<th>No of pts.</th>
<th>Aqueous humor LDL level Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;100 IU/L</td>
</tr>
<tr>
<td>Retinoblastoma</td>
<td>5</td>
<td>29*</td>
</tr>
<tr>
<td>Other malignancies</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>Controls</td>
<td>30</td>
<td>-</td>
</tr>
</tbody>
</table>

*P<0.05 Significant.

### Table III. Comparison of aqueous humor's serum LDH ratios between retinoblastoma and control group

<table>
<thead>
<tr>
<th>Group</th>
<th>No of pts.</th>
<th>Range (IU/L)</th>
<th>Mean ±SD (Log IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinoblastoma</td>
<td>34</td>
<td>0.04 - 18.32</td>
<td>4.29 ± 4.55</td>
</tr>
<tr>
<td>Controls</td>
<td>30</td>
<td>0.007 - 0.30</td>
<td>0.127 ± 0.069</td>
</tr>
</tbody>
</table>

P<0.001. Significance

The difference between the mean values of aqueous humours LDH levels in malignancies other than retinoblastoma and the controls were insignificant (Table I). The comparison between mean aqueous humour to serum LDH ratios in cases of retinoblastoma and controls revealed a significant (P<0.001) difference (Table III).

## DISCUSSION

Impressive advances have been made in the chemotherapeutical, radiological and surgical treatment of malignancies. However, the prognosis depends upon how early the malignancy is detected. Unlike many malignancies, the diagnosis of retinoblastoma is usually made on purely visual grounds and unlike most malignancies, treatment is carried out even without histopathological confirmation. Hence the basic approach to the diagnosis of retinoblastoma has been through different ancillary procedures.

Biochemical investigations, particularly enzyme assays are believed to provide an early indication of malignancy. However these assays are more convenient and safe than histochemical techniques. A large number of serum enzymes have been screened for this purpose.

Since the introduction of lactate dehydrogenase analysis into clinical medicine, the elevation of serum LDH activity has been observed in association with a variety of diseases including certain malignancies.

Determination of LDH level in aqueous humour can serve as a useful adjunct to the clinical diagnosis of retinoblastoma. High levels of LDH in aqueous humour in malignant eye tumours were reported for the first time in 1969. Subsequently in 1971 it was shown that cells in retinoblastoma secreted various enzymes which by forward diffusion pass into the aqueous and reach significantly higher concentrations in aqueous than in the plasma.

In the present study mean aqueous humours LDH level was 1161.32 IU/L. In different earlier studies the mean aqueous LDH level reported ranged between 1031 and 1232 IU/L. There is a remarkable similarity between these studies and the present study.
The study by Piro et al on 23 cases of retinoblastoma reported very high mean aqueous LDH level (1886 IU/L) when compared with the presently study. This relatively higher mean aqueous LDH level may be ascribed to extraordinary high value in one case (15920 IU/L), otherwise the remainder values were almost the same. Similarly very high values have been reported on much smaller studies comprising of 4 to 11 cases. Such selected cases may be too advanced.

In another study significantly very high LDH levels in aqueous humour in the retinoblastoma group as compared with the control group was reported. In the present study we also found that the difference between these two groups was statistically highly significant (P<0.001). A consistent finding of raised LDH level in aqueous humour in retinoblastoma leads to the conclusion that determination of aqueous humour LDH level can serve as a useful adjunct to the clinical diagnosis of retinoblastoma.

The mean aqueous to serum LDH ratio in the present study was 4.29 while in the control group it was 0.12 (Table-III). These findings match considerably to the study conducted by Ying qi et al who mentioned mean aqueous to serum LDH ratio in retinoblastoma as 4.32. Similar data has been reported by other workers. Thus aqueous to serum LDH ratio is of diagnostic importance in retinoblastoma.

Studying the individual cases in the present work, it was noted that there was a wide range of aqueous humour LDH levels in cases of retinoblastoma. It ranged from 22 to 5589 IU/L. In fact 10% of the cases had level lower than the serum LDH level. Similar lower levels have also been reported earlier. Therefore it is evident that rise in aqueous humour LDH level is not a constant feature and cannot be applied for every patient. However it is clear that raised levels are highly suggestive of retinoblastoma whereas normal or lower levels do not exclude it.

However, very low results in any reports may be due to substrate depletion where very high levels of enzyme consume substrate during pre-incubation before monitoring kinetic change (AA/min) during estimation. In such cases a compulsory estimation with high dilutions may give high values.

Other primary intraocular malignancies are apparently not associated with the elevation. LDH ratios, since there was little difference in patients with primary uveal tract melanoma and non-malignant controls. In the present study the only case of a uveal malignant melanoma had aqueous and serum LDH level of 26 IU/L and 278 IU/L respectively. This finding is in accordance with the above mentioned studies. Although Machowa and Grochocki reported 3 out of 16 patients with intraocular melanoma in which the LDH level in aqueous humour was higher than the highest level in the serum but the difference was statistically insignificant. Combining all the malignancies other than retinoblastoma, this study showed no statistically significant difference in mean aqueous humour LDH level from the control group. This observation further supports the importance of raised aqueous humour LDH levels in the diagnosis of retinoblastoma.

It is therefore concluded that estimation of aqueous humour LDH levels is useful adjunct in cases of retinoblastoma, especially in cases of doubtful clinical diagnosis. For example three cases in the present study which turned out to be non malignant did not have raised LDH levels.

REFERENCES


13. Richterich R, Colombino JF. Clinical chemistry.


