ORIGINAL ARTICLES – Laboratory science

Quantification of melanin and iron content in uveal malignant melanomas and correlation with magnetic resonance image

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Abstract

Eleven patients with uveal malignant melanomas (MM) were studied by magnetic resonance (MR) imaging before enucleation. The MR appearances varied, but often were different from those previously reported to be characteristic of these tumours. Using an image analyser to assess quantitatively the melanin and iron content of each tumour, a wide range of tumour melanin concentrations was found, but universally low tumour iron concentrations. These values were compared with MR appearances that were quantified and expressed as contrast to noise ratios. The correlation between T1 and T2 shortening and increasing melanin content did not reach statistical significance. There was no correlation between MR appearances and iron content. The theories postulated to explain the diverse MR appearances of uveal MMs are discussed and variations in tumour melanin content and differences in scanner strengths are suggested as the most likely explanations.

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Uveal malignant melanoma (MM) is the most common intraocular malignant neoplasm in adults.¹ Initial studies of malignant melanomas with magnetic resonance (MR) imaging described the appearances of T1 and T2 shortening (that is, T1 hyperintensity and T2 hypointensity) in between 72% and 93% of cases.^{2,3} These images were thought to be produced by the supposedly unique paramagnetic properties of melanin. However, recent studies have reported a spectrum of appearances with as few as 7% of tumours exhibiting the above 'typical' pattern.^{4,5}

There have been several explanations put forward to account for these differences including variable melanin content and the presence of iron in the form of methaemoglobin or haemosiderin.^{2,3,6} To our knowledge no previous quantitative analysis of melanin or iron content

in malignant melanomas has been reported. The aim of our study was to assess quantitatively the melanin and iron content in a series of malignant melanomas and to investigate the correlation of these concentrations with MR appearances.

Materials and methods

Patients who presented to the Bristol Eye Hospital with suspected malignant melanomas were identified prospectively over a 3 year period from September 1987. All patients underwent full ocular and systemic examination in addition to ocular ultrasound (US) scanning and were subsequently investigated by MR at the Bristol MRI Centre. Histological confirmation of the diagnosis was available in 11 patients, nine of whom were treated by enucleation and two by local resection (four men, seven women; age range 23-88 years; mean 65 (SD 19) years).

Patients were scanned with a 0.5 Tesla (T) Picker MR imager using a standardised technique. Spin echo (SE) sequences were performed to produce T1 and T2 weighted images in axial cuts of 5 mm. Time constant values for the various pulse sequences are shown in Table 1. The MR images were graded by expressing intensities as contrast to noise ratios (CNR), the formula for which is:

$$\text{CNR} = [(\text{SI}_{\text{tumour}} - \text{SI}_{\text{vitreous}}) / \text{SD}_{\text{noise}}]$$

where SI = signal intensity and SD_{noise} = standard deviation of background noise.⁷ Each signal was measured on the console of the imager with a region of interest cursor whose size was 3 mm² on the console, by a single investigator masked with respect to the histology results. This cursor was placed in a region of maximal tumour or vitreous intensity and 10 results were averaged for each final value of SI. For $\text{SI}_{\text{vitreous}}$, the contralateral vitreous was used. SD_{noise} was calculated from 10 cursor readings within the field of view but outside the patient.

Fresh paraffin slide preparations were made for each tumour and stained with haematoxylin and eosin and with Pearl's stain. A single investigator with normal colour vision (as tested by the Farnsworth-Munsell 100-hue test), and who was masked with respect to the MRI results, assessed all the tumours.

The tumours were assessed with computerised colour image analysis, using MicroScale TM/TC

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Table 1 Time constants for MR image pulse sequences

Pulse sequence	TR (ms)	TE (ms)	Plane of cuts
T1	500	26	Axial
T2	1800	80	Axial

TR = time to repetition; TE = time to echo

Figure 1 Photomicrograph (magnification $\times 20$) of malignant melanoma from patient G showing melanin stained brown with haematoxylin and eosin (A). After image analysis these brown areas are shaded yellow (B).

Fig 1A

Fig 1B

Figure 2 Photomicrograph (magnification $\times 20$) of malignant melanoma from patient B showing iron stained blue with Pearl's stain (A). After image analysis these blue areas are shaded red (B).

Fig 2A

Fig 2B

software. This is a performance PC based image analysis system that operates with Digihursts' proprietary MicroEye TM/TC transputer based framestore image processing cards.

In this study a MicroEye TC 24 bit colour framestore was used. The system can measure up to 16.8 million colour intensities, by combinations of 256 shades each of red, green, and blue. A colour threshold was set by viewing a microscope slide on a visual display unit and marking regions that stained for the material being studied. The MicroScale system then scanned the high power field, shading all areas that matched the measured colour threshold, and calculated the proportion of field area that was shaded as a percentage of the entire high power field. Colour thresholds were set for intracellular melanin which stains brown with haematoxylin and eosin (Fig 1), and for intracellular iron which

stains blue with Pearl's stain (Fig 2). Figures 1B and 2B show the superimposed shading produced by the image analyser.

To obtain a representative figure for melanin and iron content, 50 high power fields were studied using slides taken from four different levels within the tumour. For each slide, three adjacent high powered fields were randomly selected and assessed. To prevent operator bias, after the initial assessment of each of these three fields the slide mount was repeatedly moved

Table 3 Numbers of patients with different signal combinations

	T1; Hyper-	T1; Iso-	T1; Hypo
T2; Hyper-	8	1	0
T2; Iso-	0	0	0
T2; Hypo-	2	0	0

Table 2 Patient details

Patient index	A	B	C	D	E	F	G	H	I	J	K
Age	78	67	23	67	34	88	77	75	76	69	62
T1 CNR	54.7	44.2	49.1	50.7	0	78.1	86	47.5	47.5	55.1	73.5
T2 CNR	16.7	40.1	20.1	34.6	14.8	25.4	39.2	37.7	10.3	-54.3	-95.6
Melanin content (%)	0.29	0.35	0.81	1.06	1.43	1.71	3.2	4.7	5	13.44	23.19
Iron content (%)	0	0.14	0	0	0.03	0.06	0.01	0	0	0	0.01

CNR=contrast to noise ratio

Figure 3 MR image of patient D. The T1 weighted image (A) shows hyperintensity (CNR=50.7, see text) of the lesion in the left eye. Hyperintensity (CNR=34.6) is also shown on the T2 weighted image (B).

Fig 3A

Fig 3B

60 μ m vertically upward until the correct proportion of the 50 total field assessments had been made. The melanin and iron content calculated was an average of the 50 fields assessed.

Correlation between tumour signal intensity and melanin or iron content was calculated using the Spearman rank correlation test.

Results

The results for each patient are shown in Table 2.

MAGNETIC RESONANCE IMAGES

T1 weighted images

Ten of the 11 tumours were hyperintense (that is, CNR>0) on T1 weighted images. One tumour was not contrasted against vitreous and was therefore not visible on the T1 weighted image (CNR=0).

T2 weighted images

Nine of the 11 tumours were hyperintense (CNR>0) on T2 weighted images and two were hypointense (CNR<0).

The combinations of T1 and T2 weighted images found for each tumour are shown in Table 3. Eight of 11 tumours (73%) were hyperintense on both T1 and T2 weighted images (Fig 3). Two of the 11 (18%) showed hyperintensity on T1 and hypointensity on T2 weighted images (Fig 4). The remaining tumour was isointense on T1 and hyperintense on T2 weighted images.

MELANIN AND IRON CONTENT

Melanin content ranged from 0.29–23.19%

(mean melanin content 5 (SD 6.77)%). The T1 and T2 weighted CNR for each tumour is plotted against melanin content in Figure 5, which appears to show some inverse correlation between melanin content and T2 weighted CNR. However, this correlation did not reach statistical significance using Spearman rank correlation ($r_s = -0.518$, $p = 0.1$). There was no correlation between melanin content and T1 weighted CNR.

The iron, which may have been in the form of methaemoglobin or haemosiderin, was universally low or absent. The iron content varied from 0–0.14% (mean iron content 0.02 (SD 0.04)%). There was no correlation between iron content and T1 or T2 weighted CNR.

Discussion

Most uveal MMs can now be reliably diagnosed using ophthalmoscopy, transillumination, fluorescein angiography (FA), and ultrasound scanning (US). However, there are many well-documented cases of misdiagnosis,^{8–13} especially in the presence of opaque media. The reported rate of misdiagnosis has varied considerably between 2.7%¹² and 39.7%.¹³ More recently, an even lower misdiagnosis rate of 0.48%¹⁴ was reported by the Collaborative Ocular Melanoma Study (COMS), without the use of MR.

In the COMS study the diagnosis of MM was made by clinical examination, US, and FA. The report concluded: 'The major challenge with . . . uveal melanoma is no longer that of correct diagnosis but rather determination of the optimal treatment.' While this may be overstating the case, it does appear true that misdiagnosis leading to unnecessary enucleation is not the problem that it once was. However, false positive and false negative results both have adverse

Figure 4 MR image of patient K. The T1 weighted image (A) shows hyperintensity (CNR=73.5, see text) of the lesion in the left eye. The T2 weighted image (B) shows hypointensity (CNR=-95.2).

Fig 4A

Fig 4B

Figure 5 Graph showing T1 and T2 weighted contrast to noise ratios (CNR) plotted against percentage melanin content for each patient. For reference purposes, patients were arranged in order of increasing melanin content and then allocated an index letter (A-K).

consequences, because of the natural history of uveal MMs and the radical nature of the surgery that may be used in their treatment. Any investigations used in the management of suspected uveal MM should therefore be highly specific and highly sensitive.

Melanin causes enhancement of proton relaxation on MR that leads to a shortening of T1 and T2 relaxation times,¹⁵ the degree of which can be shown to be proportional to melanin concentration¹⁶⁻¹⁸ in vitro. It was originally hoped that the supposedly unique paramagnetic properties of melanin would enable MR to produce images that were both sensitive and specific for uveal MMs. This initial optimism was seemingly warranted because early studies showed that between 72% and 93% of MMs displayed T1 and T2 shortening^{2,3,6,19-21} and few other uveal lesions were seen to exhibit this pattern. However, more recent studies have shown a range of MR appearances with as few as 7% of tumours demonstrating T1 and T2 shortening.⁴ There have also been numerous reported cases of lesions other than MMs producing T1 and T2 shortening, such as retinoblastoma,^{3,6,22} suprachoroidal haemorrhages,^{6,23} metastatic adenocarcinoma,²⁴ haemangioma,²⁰ medulloepithelioma,²⁰ and retinal gliosis.²³ These findings obviously cast doubt upon the sensitivity and specificity of MR imaging as an investigation to diagnose MMs, and underline the fact that a diagnosis of uveal MM should not be abandoned merely because of 'atypical' MR appearances.

There have been various explanations proposed to account for this. The most widely supported of these explanations suggests that variations in melanin or iron content (the latter in the form of either haemosiderin or methaemoglobin) lead to a spectrum of MR scan appearances.^{2,5,6} An in vitro relationship has been demonstrated using melanin solutions,¹⁶ but we are aware of no studies to date that have quantitatively assessed tumour melanin or iron content to test the hypothesis. However, studies using crude, subjective assessment of MM melanin content have found anecdotal correlations between increasing melanin content and hypointensity on T2 weighted images, and between increasing melanin content and hyperintensity on T1 weighted images.¹⁹

Image analysis is a tool that has been widely used by histopathologists,²⁵ and is particularly helpful in the assessment of intracellular pigment

and of tissue stained with fluorescein labelled monoclonal antibodies. The technique is a simple one that allows a large number of fields to be assessed rapidly. To minimise bias from different staining techniques, and from degradation of colour stain with time, a single colour threshold was set in this study for melanin and iron to assess slides freshly cut and stained in the same laboratory.

With image analysis, it has been possible to quantify the melanin and iron content of the uveal MMs in this study. The melanin content of these tumours was shown to be variable (0.29–23.19%), and appeared subjectively to correlate inversely with hypointensity on T2 weighted images (Fig 5). However, this correlation did not reach statistical significance, perhaps because of inadequate numbers. Such a correlation would not be surprising in view of the paramagnetic properties of melanin and would confirm the findings of previous in vitro studies.^{16,19} It could also account for the high percentage of tumours in our study (73%) exhibiting hyperintensity on T2 weighted images as the melanin content in this group of tumours was low (0.29–5%).

By the same token, however, it might be expected that T1 weighted images of tumours with low melanin content would be less hyperintense than those of more melanotic tumours. However this study demonstrated no differences between the T1 CNR of tumours with varying melanin content.

Another important factor contributing to the findings of this study may have been that the scanner used was a 0.5 T machine. Such low field strength scanners may not demonstrate T2 shortening in tumours of low or intermediate melanin content because the enhancement of proton relaxation (and therefore of the effect on MR signal intensity) caused by melanin is proportional to the square of the magnetic field strength.^{5,19} This may also explain why the T1 shortening of high melanin content tumours was not more marked than that of tumours with lower melanin content.

In six of 11 tumours (55%) there was no detectable iron and the maximum iron content of any MM was 0.14%. This, along with the lack of correlation between MM iron content and T2 image intensity, suggests that variations in iron content are unlikely to account for the observed range of MR appearances.

This study has shown that the diversity of MR appearances reported in the literature may be explained by variations in MM melanin content, and we suggest that scanner strength may also be relevant. A further study on a larger group of melanomas using this image analysis technique may be able to prove statistically the suspected inverse correlation between hypointensity on T2 weighted images and increasing melanin concentration.

To prevent errors in diagnosis, the pattern of hyperintensity on T1 and hypointensity on T2 weighted images should not be considered to be pathognomonic for uveal MM; in our experience this combination is only found in tumours with a relatively high melanin content. Moreover, in view of the proved diagnostic success of funduscopy and simple investigations,¹⁴ it would seem

unwise to repudiate an otherwise firm diagnosis of uveal MM on the basis of 'atypical' MR appearances.

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