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Correlation of DNA ploidy and cell cycle analysis with diffusion tensor and dynamic susceptibility contrast MRI metrics in meningiomas

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ABSTRACT

Purpose: To investigate the relationship between diffusion tensor imaging and dynamic susceptibility perfusion MRI metrics with cell cycle analysis findings in meningiomas.

Material and Methods: Fourteen patients (4 men, 10 women, aged 30-76 years; median 58 years) with meningiomas were included in the study. There were 9 grade I and 5 grade II meningiomas. The MRI protocol consisted of T2-weighted turbo spin echo, unenhanced and contrast enhanced T1-weighted 3D spoiled gradient echo sequences. Diffusion tensor imaging (DTI) metrics, such as apparent diffusion coefficient (ADC) and fractional anisotropy (FA), were assessed with a single shot, multi-slice, spin-echo planar sequence (max b-value: 700 sec/mm²) and dynamic susceptibility perfusion imaging (DSC) metrics, such as the relative cerebral blood volume (rCBV), with a T2* gradient-echo, multishot echo planar imaging sequence. DNA ploidy and cell cycle analysis was analysed by flow cytometry.

Results: There was a significant negative correlation between rCBV and G0/G1 phase fraction (r=-0.791, p=0.004) and a positive correlation with G2/M phase fraction (r=0.621, p=0.04). A significant correlation was also observed between FA ratio and G0/G1 phase fraction (r=0.721, p=0.018). No significant correlation was found between G0/G1, S-phase, G2/M and ADC values.

Conclusions: DSC MRI and DTI metrics are correlated to meningioma aggressiveness as assessed by cell cycle analysis.

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KEY WORDS
MRI; diffusion tensor imaging; perfusion imaging; meningioma; cell cycle

1. Introduction

Meningiomas constitute the most common primary intracranial tumour, accounting for 36.6% of all central nervous system tumours in adults [1]. Grade I meningiomas are the most common, accounting for 81.1%, grade II tumours account for 6.9% and grade III tumours account for 1.7%. Meningiomas are far more common in women and are exceedingly rare in children [2]. Although grade I meningiomas are usually associated with a favourable outcome, grade II and grade III tumours recur more frequently. For malignant meningiomas, 10-year relative survival has been reported to be 57.1% [1]. Meningiomas, even of low grade, may display diverse outcome and several genetic and epigenetic alterations have been reported to provide some clues for assessing prognosis [3]. Assessment of tumour proliferation with markers such as Ki-67 index and cell cycle analysis have also been suggested as an important predictor of tumour behaviour [4, 5].

Flow cytometry has been applied to analyse the distribution of DNA-content in tumours and recently its intraoperative usage was also reported [5, 6]. In meningiomas, a significant difference in G0/G1, S-phase and G2/M cell cycle fraction has been reported between benign and atypical/anaplastic tumours. All benign meningiomas are diploid, contrary to tumours of higher grade that are usually aneuploid, due to different DNA content [5]. In a previous study we have found that lesion/normal relative cerebral blood volume (rCBV) ratios and peritumoural/normal tissue rCBV ratios are useful for the differentiation between grade I from grade II/III menigiomas. Among benign meningiomas, meningiomas of meningothelial subtype showed higher lesion/normal tissue rCBV ratios from the other subtypes [7]. Nevertheless, it still remains unknown whether diffusion tensor imaging (DTI) metrics, such as apparent diffusion coefficient (ADC) and fractional anisotropy (FA) and dynamic susceptibility perfusion imaging (DSC) metrics, such as rCBV, correlate with the proliferative activity of meningiomas as assessed by flow cytometry.

The aim of this study was to investigate the relationship between DTI and DSC metrics with the cell cycle and DNA ploidy findings in meningiomas.

2. Material and Methods

2.1 Patients

The study included 14 patients (4 men, 10 women, aged 30-76 years; median 58 years) who underwent surgical resection and were pathologically diagnosed with meningiomas over a 3-year period. In these patients a tumour sample was received intraoperatively for analysis by flow cytometry. Preoperative magnetic resonance imaging (MRI) including DTI was performed in all patients. DSC was available in 11 patients. The institutional review board approved the study protocol, and informed consent was obtained from all patients. Preoperatively all patients underwent a brain MRI. Histopathological diagnosis and grading of meningiomas was performed based on the WHO 2007 classification for central nervous system tumours [8].

2.2 MRI Protocol

All MR examinations were performed on the same 1.5 Tesla MR unit (Gyroscan Intera; Philips Medical Systems, Best, The Netherlands) using a head coil. The imaging protocol included: (a) a T1-weighted high resolution (1 mm×1 mm×1 mm) 3-dimensional spoiled gradient echo sequence (repetition time [TR]: 25 ms, echo time [TE]: 4.6 ms, acquisition matrix: 256×228, field of view [FOV]: 220 mm), which was utilised for structural imaging before and after intravenous (iv) injection of gadolinium-DTPA; (b) a single shot, multi-slice, spin-echo planar sequence (TR: 9,807 ms, TE: 131 ms, FOV: 230 mm, acquisition matrix: 128×128, slice thickness: 3 mm, max b-value: 700 sec/mm^2, 16 non-collinear diffusion directions), which was used for measurement of the ADC and fractional anisotropy (FA); (c) T2* gradient-echo, multishot echo planar imaging (EPI) sequence (TR: 702 ms, TE 30 ms, flip angle: 40°, FOV: 250 mm, slice thickness: 7 mm, gap: 0, EPI factor: 17, acquisition matrix: 128×51, dynamic scans: 50, imaging time per dynamic scan: 2.1 sec, 0.1 mmol/Kg gadolinium at 5 cc/sec, which was used for rCBV meas-

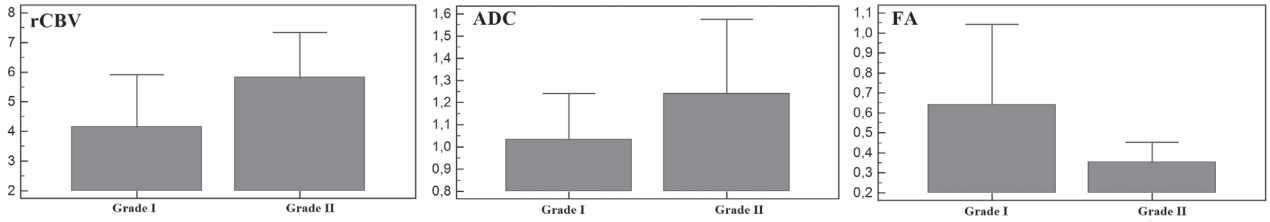

Fig. 1. *The L/N rCBV and L/N ADC were higher in atypical meningiomas. The L/N FA were higher in benign meningiomas. The data are represented as the mean +/- standard deviation (SD).*

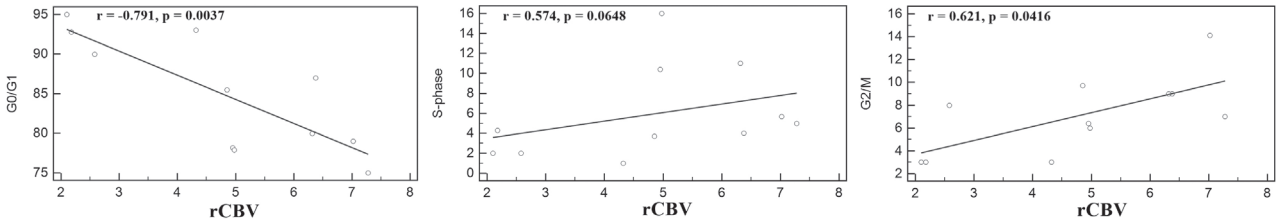

Fig. 2. *The rCBV values in meningiomas correlate with the level in the G0/G1, S-phase and G2/M-phase fractions.*

urements; (d) T2-weighted turbo spin echo, axial plane, TR: 3,000 ms, TE: 90 ms, acquisition matrix: 250×250, FOV: 230 mm, slice thickness 6 mm, gap 0.6 mm; (e) T2-weighted inversion recovery based sequence for cerebrospinal fluid (CSF) suppression, sagittal plane, TR: 6,300 ms, TE: 120 ms, inversion recovery (IR): 2,150 ms, FOV: 250 mm, slice thickness 6 mm, gap 0.6 mm, acquisition matrix: 250×250.

A semiquantitative method of image analysis was accomplished by calculating the lesion-to-normal (L/N) ratio: a region-of-interest (ROI) was manually defined in the enhancing region of the lesion on a transverse slice that depicted maximal tumour size in T1-weighted imaging with contrast medium, and a second region was drawn in the contralateral normal appearing white matter. The L/N ratio was calculated by dividing the mean of values of the pixels in the tumour region ROI with the mean value of the ROI in the normal region. The ROIs were evaluated for eligibility independently by two experienced neuroradiologists blinded to the final diagnosis and possible disagreements were solved by consensus. Agreement between the two neuroradiologists was calculated for each parameter (ADC, FA and rCBV) by the use of the weighted kappa value and applying Landis and Koch's [9] categorisation of responses.

2.3 DNA analysis Protocol (Ioannina Protocol)

Ioannina protocol for cell cycle analysis has been reported previously [10]. Briefly, immediately after tumour resection, tumour samples were minced (Medimachine System, BD Bioscience) in Phosphate-buffered saline (PBS) buffer (Ca^{2+} and Mg^{2+} free, with 0.5 mg/ml RNase) for 1 min resulting in a cell suspension. The suspension was then filtered (Consult No 10, Medicons, BD Bioscience). Cells were then stained by adding propidium iodine (PI) (125 µg/ml) and, after 3 min of incubation, flow cytometric analysis was performed. All the stained samples were processed using a FACS Calibur flow cytometer, equipped with 2 lasers (488 nm, 635 nm) and 6 parameters [forward scattering (FSC), side scattering (SSC), FL1 channel-FL4 channel] and using CellQuest software. Normal cells obtained from the peripheral blood mononuclear cells (PBMCs) and chicken red blood cells (Ficoll-Paque separation med Pharmacia) were used as the standard to define the position of the diploid G0/G1 peak in the DNA histograms. The DNA index consisted of the ratio between the modal channel of the G0/G1 peak of the aneuploid cells (tumour cells) and that of the diploid cells.

2.4 Statistical analysis

The rCBV, FA ratio, ADC ratio, G0/G1, S-phase and G2/M between grade I and grade II meningiomas were compared using the two-sided, non-parametric Mann-Whitney U test. Correlations between FA ratio, ADC ratio, rCBV, and G0/G1, S-phase, G2/M and tumour ploidy were analysed using Spearman rho test. A two-sided p-value <0.05 was considered statistically significant.

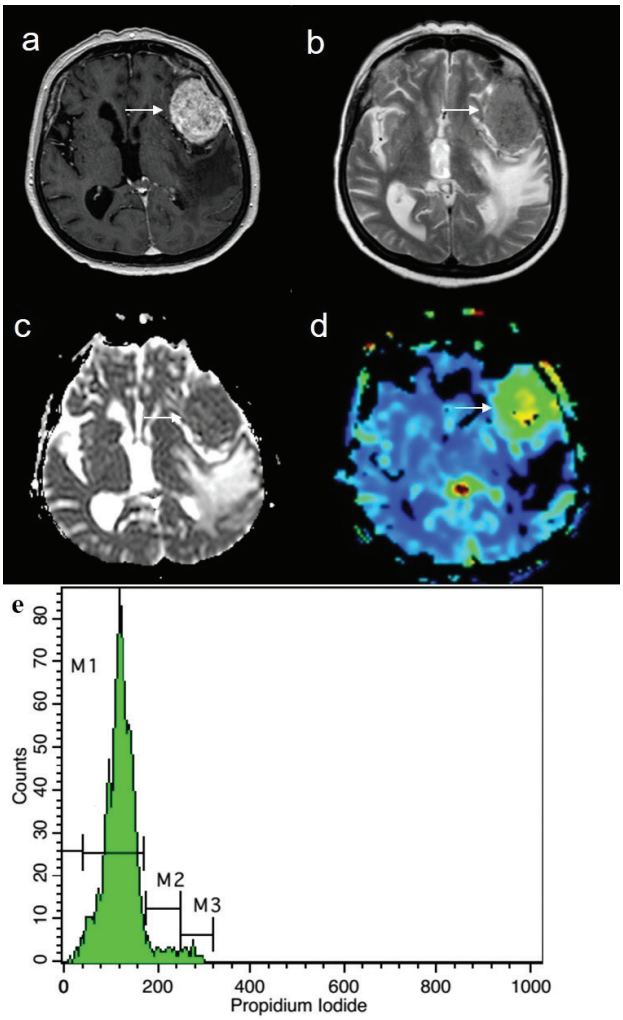

Fig. 3. *A patient with histologically confirmed grade I meningioma. Contrast-enhanced T1-weighted image shows a homogeneous enhancing mass lesion (a). T2 weighted image (b) and ADC map (c). In rCBV map (d) the yellow colour within tumour parenchyma indicates a higher rCBV. Ploidy histogram (e) from the patient shows G0/G1 = 94% (M1), S = 3% (M2), G2/M = 3% (M3). The rCBV value was 2.9.*

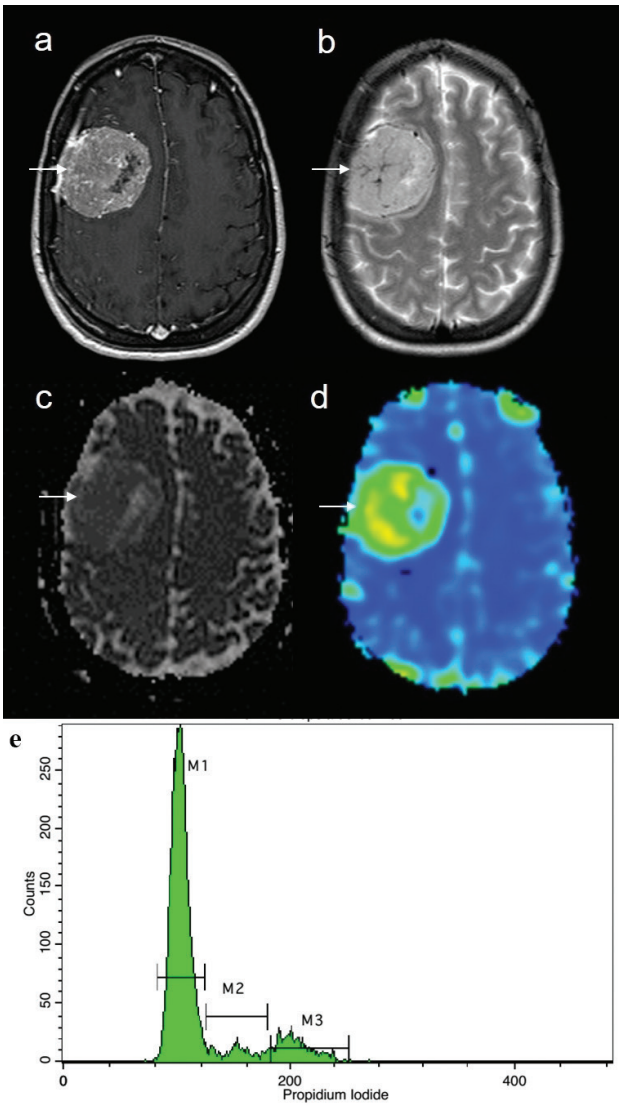

Fig. 4. *A patient with histologically confirmed grade II meningioma. Contrast enhanced T1-weighted image (a) and T2 weighted image (b). ADC map (c). The rCBV (d) value was 6.38. Ploidy histogram (e) from the patient shows G0/G1 = 80.5% (M1), S = 7.5% (M2), G2/M = 12% (M3).*

3. Results

Fourteen patients met the inclusion criteria and were included in the study. There were 9 grade I and 5 grade II meningiomas. Seven meningiomas were located in the convexity, 3 were parasagittal, 2 tentorial, one in the olfactory groove and one located in the sphenoidal ridge. All grade I meningiomas were diploid and 3/5 grade II meningiomas were aneuploid. Grade I meningiomas had lower L/N rCBV ratio than grade II meningiomas, however the difference was not statistically significant (median 4.9 *vs* 6.3, p=0.26). Grade I meningiomas had non-significant higher FA ratio and lower ADC ratio than grade II meningiomas (median 0.5 *vs* 0.35, p=0.12 and median 1 *vs* 1.16, p=0.12 respectively) (**Fig. 1**). To assess the suitability of rCBV, ADC and FA for the assessment of meningiomas aggressiveness, we correlated the above metrics with the flow cytometric markers of aggressiveness. There was a significant negative correlation between rCBV and G0/G1 phase fraction (r=–0.791, p=0.0037) and a positive correlation with G2/M (r=0.621, p=0.0416). A statistical trend

was found when we correlated rCBV to S-phase fraction (r=0.574, p=0.0648) **(Figs. 2-4)**. Significant correlation was also observed between FA ratio and G0/G1 phase fraction (r=0.721, p=0.018). No significant correlation was found between G0/G1, S-phase, G2/M and ADC values. No significant difference was found between diploid and aneuploid tumours with respect to rCBV, ADC and FA values.

4. Discussion

The present study evaluated the correlation between DTI, DSC MRI metrics and flow cytometry findings in meningiomas. A strong negative correlation was observed between rCBV and G0/G1 phase fraction and a significant positive correlation with G2/M phase fraction. Significant correlation was also observed between FA ratio and G0/G1 phase fraction. Grade II meningiomas had higher rCBV and ADC values and lower FA values compared to grade I, though the differences were not significant.

Meningiomas constitute the most common primary central nervous tumour [11]. Previous studies have reported that perfusion MRI and DTI may provide important information for meningioma grading based on both tumoural and peritumoural rCBV values [7]. Non-invasive assessment of the grade of meningiomas is of paramount importance for preoperative planning, treatment decisions for incidental tumours and for gamma-knife surgery [12]. Additionally, among benign meningiomas, meningothelial subtype exhibited higher rCBV values. However, no correlation was found between diffusion tensor and perfusion metrics with Ki-67, a widely used immunohistochemical marker of tumour aggressiveness [7]. The present study showed a strong significant correlation between G0/G1 and G2/M phase fraction with rCBV and of G0/G1 with FA.

Analysis of cell cycle by flow cytometry for solid tumours has recently received a lot of interest [10, 13]. This technique can be easily performed and recently an intraoperative role has also been proposed given that analysis of cell cycle can been performed within 6 minutes [10]. Malignant tumours demonstrate higher S-phase, G2/M fraction and abnormal DNA ploidy compared to benign lesions. A G0/G1 cut-off value of 85.5% (87.5% sensitivity, 100% specificity), S-phase fraction of 7.1% (100% sensitivity, 100% specificity) and G2/M fraction of 6% (81.2% sensitivity, 100% specificity) were reported as optimal for the discrimination between benign and atypical/anaplastic meningiomas [5]. The G2/M by flow cytometry corresponds to cells at mitosis. Increased mitotic activity has been previously correlated with increased endothelial proliferation and thus increased rCBV values in gliomas [14]. The G0 phase corresponds to a resting phase. Thus benign tumours have higher G0/G1 values. We have previously reported higher FA values in benign meningiomas and lower FA values in grade II/III meningiomas. Regarding other imaging techniques, a significant positive correlation has been also reported between 99mTc-Tetrofosmin SPECT uptake and S-phase fraction in meningiomas [13].

In the present study, we have found that grade I meningiomas had non-significantly lower ADC values than grade II meningiomas. Malignancy is usually associated with decreased signal intensity on ADC images related to restricted diffusion, due to increased cellular density. In a study that included 135 benign, 37 atypical and 5 malignant lesions, ADC values and ratios were not found useful for determining histological behaviour and differentiating histopathological subtypes of meningiomas [14]. Nevertheless, Surov et al. found that the ADC mean values were higher in grade I meningiomas in comparison to grade II/III tumours. An ADC mean value of less than 0.85×10(-3) mm(2)s(-1) was found as the optimal cut-off for the differentiation between grade I and grade II/III meningiomas (sensitivity 72.9%, specificity 73.1%, accuracy 73.0%) in this study [15].

The present study has several limitations. Firstly, the results are based on a small series of 14 meningioma patients and should therefore be considered preliminary. Larger numbers of high-grade meningiomas are needed to further evaluate the associations between DWI features and meningioma grade. Furthermore, the limited number of cases included could not permit a safe analysis for establishing cut-off values for MRI and flow cytometry metrics. Finally, flow cytometry was performed in an excised tumour sample that might not always correspond to the area with the highest malignancy.

5. Conclusion

In conclusion, the present study showed that rCBV and FA metrics correlate with meningioma aggressiveness as assessed by cell cycle analysis. Future studies are needed, with larger number of patients that may also correlate perfusion/diffusion metrics with cell cycle fractions and meningioma recurrence as well as survival rates. R

Conflict of interest

The authors declared no conflicts of interest.

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