DTI Derived Metrics Correlate with Immunohistochemistry obtained Matrix Metalloproteinase (MMP-9) Expression in Cellular Fraction of Brain Tuberculoma

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Introduction: Tuberculosis kills over 2 million people a year and infects one third of the world's population[1]. Central nervous system (CNS) tuberculosis is the most dangerous form of the tubercular disease and is responsible for very high morbidity and mortality[2]. In CNS, where the function of neurons is protected by the maintenance of an anti-inflammatory environment, infection with *Mycobacterium tuberculosis* (*Mtb*) leads to catastrophic, inflammatory tissue destruction[3]. Matrix metalloproteinases (MMPs) have been implicated in inflammatory tissue destruction in a range of CNS pathologies[3-5]. Elevated MMP-9 activity in cerebrospinal fluid from patients with CNS tuberculosis is associated with signs of local tissue destruction[6]. Diffusion tensor imaging (DTI) derived indices are known to provide information about the tissue microstructural integrity in various disease conditions[7]. The most commonly used DTI indices are fractional anisotropy (FA) and mean diffusivity (MD). Besides FA and MD, other DTI indices i.e., linear case (CL), planar case (CP) and spherical case (CS) may provide additional information with respect to tissue microstructural integrity[8]. In the current study, for the first time we have correlated DTI indices from the cellular fractions of brain tuberculoma (BT) (n=10) with MMP-9. The study was driven by the hypothesis that MMP-9 causes tissue destruction which is likely to cause a reduction in FA, CL, CP and increase in MD and CS. Once the tissue repair is done as a result of anti-tubercular therapy (n=18), these parameters reversal would be the indicator of therapeutic response.

Materials and Methods:

***Patients*:** A total of 28 patients with BT were included in this study. Initial diagnosis of BT was based on the characteristic imaging features on magnetization transfer (MT) T_1 magnetic resonance (MR) imaging and/or proton MR spectroscopy (PMRS)[9]. The final diagnosis in these patients was based on the response to the anti-tubercular treatment (ATT) (n=18) and histopathology (n=10). The 18 patients were examined upto 12 months at 4 monthly intervals on ATT.

***MR Imaging*:** With informed consent all these patients underwent both conventional MR and DT imaging on a 1.5 T GE scanner, using quadrature birdcage head coil. The base line T_2 [repetition time (TR)/echo time (TE)/number of excitations (NEX)=4900 ms/85 ms/2)], T_1 (TR/TE/NEX=1000 ms/14 ms/1) and T_1 W MT imaging (TR/TE/NEX=1000 ms/14 ms/1, flip angle-65^o, off-resonance pulse with frequency offset-1200 KHz) were performed in the axial plane with 5mm slice thickness, 256×256 matrix, 240×240 mm field of view (FOV), with no slice gap. In vivo single voxel PMRS was done using point resolved spectroscopy with TE/TR/NEX=135/1500/8. DTI data were acquired using a single-shot echo-planar dual spin-echo sequence with ramp sampling. The acquisition parameters were: TR=8sec/TE=100ms/slice thickness=3mm/interslice gap=0/FOV=240mm/image matrix=256×256 (following zero-filling)/NEX=8/ diffusion weighting b-factor=1000 smm^{-2}. The DTI data were processed as described in detail elsewhere[10]. For calculation of various DTI indices (FA, MD, CL, CP and CS) a total of ten each ROIs were placed separately on the cellular fraction of excised BT. In case of follow-up BT the DTI indices from cellular fractions were calculated by encircling this fraction on all images showing the lesion at each study time point.

***Histopathology*:** The excised BTs were immuno-stained for monoclonal antibody against human MMP-9 (2C3, sc-21733, CA) antigen. Each MMP-9 immunostained slide was digitized with 10X objective using Canon Power Shot G5 camera and the captured images were subjected to morphometry analysis. The percentage of ten areas with maximal positive staining for MMP-9 was calculated at 10X resolution.

***Statistical Analysis*:** Pearson correlation analysis between DTI indices (both cellular and necrotic fractions) and quantitative MMP-9 was performed in case of excised tuberculoma. This correlation was performed based on the assumption that high MMP-9 expression in the cellular component will be associated with the low FA. A repeated measure of analysis of variance (ANOVA) was performed to see the time dependent changes in the various DTI metrics in case of follow-up tuberculoma.

Results: The values of various DTI indices form cellular component of both excised and follow-up tuberculoma are reported in Table 1. All the excised tuberculomas showed strong MMP-9 expression in granulomatous region (fig). MMP-9 showed significant inverse correlation with FA (r=-0.827, p<0.001), CL (r=-0.491, p<0.001) and CP (r=-0.582, p<0.001) while significant positive correlation with CS (r=0.641, p<0.001) in cellular fraction of tuberculoma. We did not find any correlation between MMP-9 and MD values in the cellular fraction. In case of tuberculomas on medical therapy the FA, CL and CP was significantly increased (p<0.001) while CS was significantly decreased (p<0.001) with no significant change in MD values in cellular fraction of tuberculoma in response to treatment over time.

Discussion: The proteolysis of the extracellular matrix (ECM) by MMP-9 has been well reported in infective pathologies[11]. MMP-9 also disrupts blood brain barrier (BBB) by degrading the type-IV collagen in the basal lamina[12]. The degradation of ECM along with BBB disruption provides more interstitial space for water molecule diffusivity. In the current study, increased MD and CS values was found in cellular fraction of BT compared to normal brain parenchyma suggest increased interstitial fluid due to the degradation of the ECM by high MMP-9 expression in granulomatous region (cellular fraction) of the BT. The decreased FA, CL and CS values in cellular fraction of excised BT can be explained on the basis of the dilution effect caused by the increased interstitial fluid rather than the microstructural integrity. In our study, the decreased CS in response to treatment over time in patients who were followed with antitubercular therapy suggests the repair of ECM and BBB due to the down regulation of MMP-9, which is also associated with the improvement in clinical outcome. Our findings are consistent with the previous study which showed decreased serum MMP-9 level over a period of 12 months in response to treatment in case of multiple sclerosis and this was associated with the gradual repair of BBB[13]. The repair of ECM and BBB result in reduction of interstitial fluid in follow-up of BT and improvement in the DTI indices (FA, CL, CP) over time. We conclude that DTI metrics correlate significantly with MMP-9 and may be used as a surrogate marker in vivo.

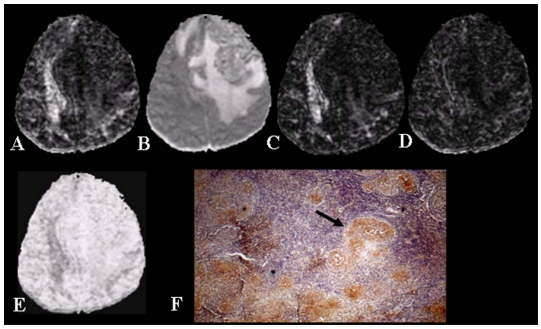

Figure: *showing the map of various DTI indices i.e. FA (A), MD (B), CL (C), CP (D) and CS (E) from a 32 years old female patient with frontal brain tuberculoma. The granulomatous region of this tuberculoma shows high MMP-9 expression (F).*

Parameters	Normal brain parenchyma	Excised BT(n=10)	Follow-up BT (n=18)			
			Baseline	4-months	8-months	12months
FA	0.43±0.12	0.12±0.01	0.13±0.02	0.16±0.03	0.20±0.04	0.25±0.04
MD (× 10^{-3} mm^2s^{-1})	0.72±0.09	0.97±0.05	0.96±0.10	0.96±0.14	0.95±0.14	0.98±0.16
CL	0.23±0.10	0.04±0.01	0.04±0.01	0.05±0.01	0.06±0.01	0.09±0.02
CP	0.13±0.04	0.08±0.02	0.09±0.02	0.10±0.03	0.14±0.04	0.16±0.04
CS	0.62±0.09	0.88±0.02	0.86±0.03	0.83±0.03	0.78±0.05	0.75±0.04
MMP-9 (% area)	-	17.7±2.47	-			

Table 1: *showing the quantitative values (mean±SD) of various DTI indices both in excised and follow-up BT patients. (BT= brain tuberculoma)*

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