Role of DTI in Understanding Pathophysiology and Assessing Therapeutic Response in Patients with Tuberculous Meningitis (TBM)

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Introduction: Tuberculosis meningitis (TBM) is the most devastating forms of tuberculosis associated with high morbidity and mortality worldwide (1). Its prevalence is increasing globally and becoming a major problem in the western world due to the increased number of immunocompromised patients (AIDS), and to multidrug-resistant patients with tuberculosis (2). In experimental models of TBM, lysis of bacteria in the subarachnoid space stimulates release of pro-inflammatory molecules (PMs) (TNF-α, IL-1β) and also increases the concentration of white blood cells (WBCs) in cerebrospinal fluid (CSF) (3). The production of TNF- α has been associated with the development of TBM pathology in vivo, including tissue necrosis and cachexia. TNF-α is produced in response to the bacterial pathogen and mediates the up-regulation of adhesion molecules like selectins, intercellular adhesion molecules (ICAMs) and vascular cell adhesion molecules (VCAMs) (4). A recent diffusion tensor imaging (DTI) study in neonatal meningitis has shown a correlation between FA in cortical regions, as measured by in-vivo DTI, and various PMs in the CSF of neonatal meningitis (5). The aim of this study was to demonstrate the changes in DTI metrics of the brain of TBM patients following anti-tubercular treatment and to correlate them with PMs measured from CSF. To the best of our knowledge, this is the first such study in TBM patients.

Materials and Methods: Twenty five patients with TBM including 18 males aged from 10-50 years (median age 25 years) and 10 age/sex matched healthy controls formed the study group. The diagnosis of TBM was based on demonstration of M tuberculosis, polymerase chain reaction (PCR) for tuberculosis, IgM and biochemistry and cytology of CSF. Conventional MRI (magnetic resonance imaging) and DTI were acquired on a 1.5 Tesla MR scanner using standard quadrature birdcage head coil. 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/number of slice=34-36/with contiguous 3 mm slice thickness/FOV=240mm/image matrix=256×256 (following zero- filling)/NEX=8/diffusion weighting b-factor=1000 s mm^{-2}. The DTI data were processed as described elsewhere (6). Post-contrast T1-weighted images were also obtained in patients, after injecting Gadodiamide (Gd-DTPA-BMA, Omniscan, Amersham Health, Oslo, Norway) intravenously at a dose of 0.1 mmol/ kg- body weight. Segmentation was implemented by using JAVA based Fuzzy C-means (FCM) clustering software (7). It makes unsupervised classification of data in a number of clusters, by identifying different tissues in an image without the use of an explicit threshold. In this study post contrast T1 weighted image was classified in four clusters, four masks were extracted form every image, each related to tissue distribution. The enhanced cortical as well as basal cistern regions are obtained. The implemented software automatically quantifies the signal intensity related to mask of enhanced meninges (enhanced meningeal mask was taken as ROI).
Enzyme linked immunosorbent assay kit (R&D Systems, Minneapolis, USA) was used to quantify human soluble intra-cellular adhesion molecules (sICAM), tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) cytokines in CSF collected on lumbar puncture of TBM patients. For the quantitation of FA and MD values in patient group and controls elliptical ROIs were placed on the cerebral cortical and brain stem regions. The ROIs were positioned on the diffusion weighted imaging (DWI) images (b=700) to ensure the absence of CSF contamination. A student's independent t-test was performed to evaluate the regional differences in the DTI metrics between meningitis patients and healthy controls.

Results: Abnormal meningeal enhancement especially in basal cisterns on post contrast T1 images was noted in all the patients with clinically diagnosed tubercular meningitis (n=25). On follow-up MRI studies in patients, after 3 months of anti-tubercular treatment relatively less intense meningeal enhancement than pretreatment imaging was observed. At the time of initial study, cortical FA values (0.15±.03) were significantly higher in patients with TBM compared to healthy controls (0.10±.02). Significant decreased FA values (0.13±.02) were observed in the patients after 3 months of anti-tubercular treatment compared to the initial study in the entire cerebral cortical region as well as basal meninges. No significant changes were observed in MD values in the entire cerebral cortical region and basal meninges [$(0.75\pm0.05) \times 10^{-3}$] as compared to control [$(0.75\pm0.04) \times 10^{-3}$]. A significant change in signal intensity was observed between baseline (740.09±53.62) and follow-up (657.26±91.88) study. Significant direct correlation was observed between PMs (sICAM, TNF-α, and IL-1β) quantitated from CSF of TBM patients and FA values from cerebral cortical as well as basal meninges. Significant correlation was also observed between sICAM and TNF- α and signal intensity but IL-1β did not show correlation with signal intensity.

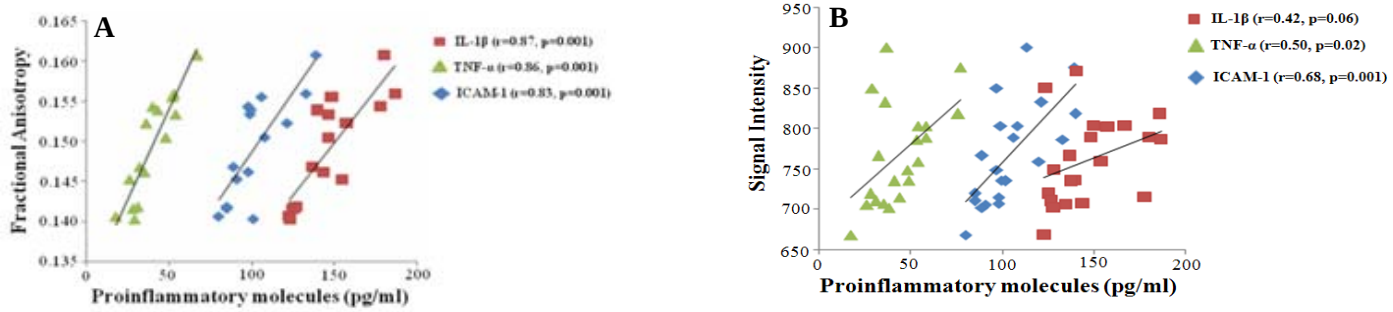

Fig.1: **(A)** Plot showing relationship between Fractional Anisotropy (FA) values and **(B)** showing relationship between signal intensity from Cerebral cortical regions as well as basal meninges and pro-inflammatory cytokines [interleukin1- β (IL1-β, red), tumor necrosis factor- α (TNF- α, green) and soluble intercellular cell adhesion molecules-1(sICAM-1, blue)] quantified from CSF of TBM patients.

Discussion: This study demonstrates significant direct correlation between PMs (sICAM, TNF-α, and IL-1β) quantified from CSF of TBM patients and FA values from cerebral cortical and basal meningeal regions. TNF-α and other PMs are the mediators of inflammatory changes in the meninges resulting in injury to blood-brain barrier. The CSF and blood levels of TNF-α and ICAMs are known to correlate with clinical outcome and development of neurological sequelae in TBM patients (8). The significant positive correlation between FA and PMs suggest that FA may be used as a noninvasive surrogate marker of meningeal disease activity of patients with TBM. Similar observations have been made in brain abscess as well as in neonatal meningitis patients in the literature (5, 9). A significant positive correlation between signal intensity measured on Gd enhanced MRI and TNF- α, ICAM confirms the role of these molecules in BBB breakdown. The decrease in FA and meningeal signal intensity at three months of follow up associated with clinical improvement suggests down regulation of these PMs. We conclude that DTI metrics helps in understanding the pathophysiology of the disease and may be useful in monitoring the effect of antimicrobial therapy in TBM patients.

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