# Radiation-Induced Early Changes in the Brain and Behavior: Serial Diffusion Tensor Imaging and Behavioral Evaluation after Graded Dose of Radiation

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**Introduction:** In the present scenario, increasing burden of nuclear arsenal and scramble of nuclear power leads to a big threat of radiation exposure to the population at large. In all potential radiation disasters, we are likely to encounter whole body or partial body radiation exposure. The CNS, which has long been considered to be a radioresistant system, is recently proved to be highly susceptible to even low doses of radiation[1]. Various investigators have described the white matter necrosis, demyelination, and vascular changes in the CNS after irradiation[2]. Moreover, few animal studies have shown cognitive impairment, personality changes and gait disturbance after a mild dose of whole body irradiation.[3] Diffusion tensor imaging (DTI) allows tissue structure to be probed and imaged in a microscopic scale, providing clue to the fine architecture of the neural tissue and to changes associated with various physiological and pathological changes. We hypothesize that DTI indices [fractional anisotropy (FA) and mean diffusivity (MD)] can accurately detect and monitor the pathologic changes of radiation-induced damage in brain parenchyma and that these changes are dose and time dependent. To confirm aforementioned hypothesis, serial DTI was performed during early phase of moderate (3 Gy), sub-lethal (5 Gy) and lethal (8 Gy) dose of gamma whole body irradiation.

**Materials and Methods:** The institutional research ethics committee approved the study. Male strain A mice of 6-10 weeks of age (n = 15) and 25-35 g of weight were used for this study. Mice were exposed to mild (3Gy), moderate (5Gy) and severe (8Gy) radiation dose from Tele 60Co unit gamma irradiation facility (Bhabhatron II). Control mice were sham irradiated. Brain MRI was performed at 0h, 1 day, 5 day and 10 days post irradiation (PI) in all the three groups. All MR imaging was performed in a Bruker Biospec 7.0 Tesla 30 cm horizontal bore magnet (Bruker Biospin Ettlingen, Germany). The MRI protocol included Turbo RARE T2-weighted, RARE T1-weighted and DTI. DTI images were acquired using a multi-slice, multiple-shot spin echo EPI sequence with the following parameters: repetition time (TR) / echo time (TE) = 5000 ms/34.46 ms, number of gradient encoding directions = 46, and b= 672 s mm-2. The other parameters for DTI data acquisition were: acquisition matrix = 128 × 128, field-of-view = 2 cm × 2 cm, slice thickness =1 mm, and number of slices =11 (with no interslice gap). The DTI data were processed as described in detail elsewhere.[4] Regional FA and MD values were obtained by placing region of interests (ROIs) **(Fig.1)** on the hippocampus, thalamus, hypothalamus, caudatoputamen (CuP), frontal association cortex (FC), sensorimotor cortex (SMC), cerebral peduncle (CP) and corpus callosum (CC).

***Behavioral studies:*** Open Field Exploratory Paradigm and novel Object recognition test were performed during the light cycle from 10 am- 2 pm by the same experimenter who had been handling the animals throughout.

***Statistical analysis:*** One-way analysis of variance (ANOVA) with multiple comparisons using Bonferroni, Post Hoc test was performed to evaluate the differences in DTI measures among different time points. P values of $\leq 0.05$ were considered to be significant.

**Results:** At any time point, no circumscribed lesions were observed on either T1 or T2-weighted images in any group.

***Temporal changes in DTI measures in 3 grey irradiated mice group:*** Significantly decreased FA values at day 5 PI compared to baseline study were observed in entire brain except for CP **(Fig.2).** No significant difference in FA was observed in any region except for hippocampus at day 10 PI compared to baseline study. In this group, at day10 PI a significant decrease in MD values compared to baseline study was observed in hypothalamus, FC, SMC and cingulum regions.

***Temporal changes in DTI measures in 5 grey irradiated mice group:*** Except for CP and cingulum, all of the region showed significantly decreased FA at day 5 PI as well as day 10 PI compared to baseline study. In 5 grey irradiated mice group a trend of decrease in MD values was observed at day5 and day10 compared to baseline study in all regions, significantly reduced MD values were observed at day5 compared to baseline study only in thalamus, cingulam, and CuP regions.

***Temporal changes in DTI measures in 8 grey irradiated mice group:*** All regions showed significantly decreased FA values at day 5 and day 10 compared to baseline study. In 8 grey irradiated mice group, significantly decreased MD values were observed in FC, SMC, and hippocampus at day10 compared to baseline study.

***Open field exploratory Behavior:*** Irradiated mice showed a radiation-dose dependent locomotor hyper activity at day 5 PI. Such hyper activity could not sustain at a later interval i.e day 10 post exposures.

***Novel Object Recognition Test:*** A perfect dose dependent decrease in the values of time spent with the novel object was reported at day 5 PI **(Fig.3).** At 10 day PI also there was a significant decrease in the values of time spent with the novel object but values were not in the dose dependent manner.

**Discussion:** To the best of our knowledge, this is the first longitudinal DTI study to establish relationship between DTI indices and neurobehavioral changes in acute phase of whole body radiation-induced brain damage. This translational model of hyperacute and acute brain injury resulting from whole body irradiation showed a reduction in both FA and MD at the time of follow-up studies compared to baseline study in brain parenchyma. With regards to dose effect, our results showed that the higher radiation dose (8 Gy) induce earlier and more severe neurobehavioral changes in the WM than the lower doses (3 and 5 Gy), and these differences could be reflected by the magnitude of change in FA values to some extent. Glial hypothesis is one of the possible mechanisms of radiation-induced brain damage. In support to glial hypothesis, Kurita and colleagues observed rapid apoptotic depletion of oligodendroglial population in white matter within 24 hours after irradiation.[5] There is evidence from animal experiments measuring the extracellular space in brain injury models that diffusion is reduced in astrogliosis. In our study at day 10 PI, there was a dose dependent decline in the FA values as well as cognitive functions as reported in the terms of novel object recognition test. By combining the analysis of DTI indices and behavioral study, our results suggest that DTI may be used in noninvasive monitoring of radiation induced brain damage. This experimental model may be used to assess the neurotoxic adverse effects of irradiation treatment and to test the effectiveness of potential neuro-protective therapies.

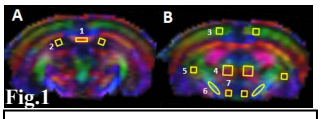

Fig.1

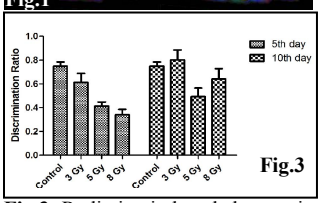

Fig.3

**Fig.3:** Radiation induced changes in the discrimination ratio following 5th day and 10th day PI.

**Fig.1:** Color-coded FA map from a male age matched mouse shows ROI placement on corpus callosum (1), hippocampus (2), sensory-motor cortex (3), thalamus (4), cudato-putamen (5), cerebral peduncle (6), hypothalamus (7).

**Fig.2:** Change in FA values with time. A plot of FA values and time for each group showing disease-related FA change at different time points. * Denotes significant difference in FA values at particular time point compared to baseline study of respective group. # denotes significant difference in FA values at particular time point compared to day1 of respective group. $ Denotes significant difference in FA values at particular time point compared to day5 of respective group.

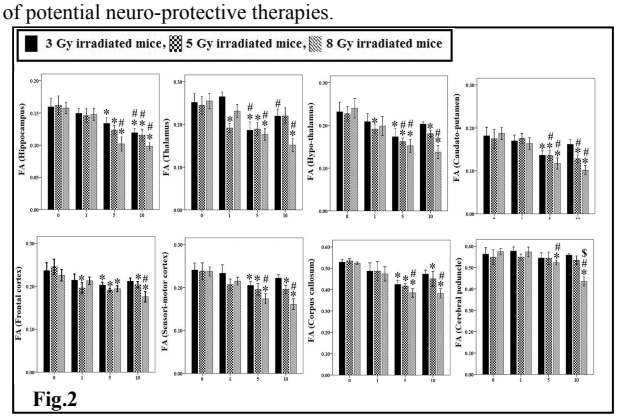

Fig.2

**References :** (**1**) Manda & Reiter. Prog Neurobiol 2010;90(I):60-68; (**2**) Price RE et al.J Med Primatol 2001; 30: 81–87; (**3**) Raber J et al.Radiat Res 2004;162:39–47; (**4**) Saksena S et al. J Gastroenterol Hepatol. 2008;23: e111-e119; (**5**) Kurita H et al. Neurol Res 2001;23:869–74.