Hippocampal dependent Cognitive dysfunction and Microstructural changes during Early Delayed phase after Whole Body Radiation Exposure

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Target Audience: Researchers, Clinicians and Students.

Introduction: Ionising radiation has been hazardous to human health due to overexposure from natural and artificial sources. Such a radiation exposure causes damage to living tissue and can result in mutations, radiation sickness, cancer and death. It has been suggested that transient impairment of the functioning of the hippocampus is linked to inhibition of hippocampal neurogenesis after acute γ radiation. Studies have reported that irradiation can reversibly alter proliferation, neurogenesis and cell death in the dentate gyrus of adult mice. Also, there are several studies available in literature which reports cranial irradiation induced early and late delayed changes in brain, but there are only few reports which show whole body radiation induced neurological complications. Our previous study has reported metabolic impairment and cognitive dysfunction at 3 months post irradiation[1]. To extend our previous study, the present work is designed to observe radiation induced early delayed microstructural, metabolic and cognitive impairment in hippocampus if any at 5 months post irradiation.

Objective: To evaluate the microstructural, metabolic and Behavior changes during early delayed phase in mice brain after 5 Gy whole body radiation exposure.

Material and methods: A total of 20 C57 male mice (8 to 10 weeks old) were taken and acclimatized for 48 hours in polypropylene cages under standard temperature, humidity conditions prior to group allocation and treatment. Out of 20 animals 10 animals were given 5Gy whole body radiation through Tele 60Co irradiation facility (Bhabhatron II) with source operating at 2.496 Gy/min. The remaining 10 animals served as sham irradiated controls. The Behavioral, DTI and MRS experiments were carried out on 10 animals each at 5 months post irradiation. For behavioral experiments the behavior activity in mice was evaluated as discussed in our previous study[1]. All MR imaging was performed in a Bruker Biospin 7.0 Tesla 30 cm horizontal bore magnet. DTI images were acquired using a multi-slice, multiple-shot spin echo EPI sequence with repetition time (TR) / echo time (TE) = 5000 ms/34.46 ms, number of gradient encoding directions = 46, and b= 672 s mm-2. Java based DTI analysis software was used to generate FA (Fractional Anisotropy) and MD (Mean Diffusivity) maps in hippocampus. The MRS voxel was localised in the hippocampus region of mouse brain (1.5 x 3.5 x 3.0 mm3; 15.75µl). After local field homogeneity optimisation (FASTMAP) and water suppression (VAPOR), MR spectra were acquired using PRESS (Point Resolved Spectroscopy) sequence with TR/TE=2500msec/20msec and 512 averages. MRS raw data (FID) was processed using LC model for quantitation of arbitual concentration of metabolites. Independent Students t test was performed to evaluate the differences in among both the groups.

Result: The data showed impaired cognitive functions, microstructural changes and altered metabolite levels during early delayed phase of whole body radiation induced injury. In behavioural experiments, there was a significant impairment in the cognitive index at 5 months post irradiation in irradiated group as compared to controls (Fig 1). There was no abnormality observed in any group on MRI images. Out of all the DTI parameters measured in this study, most notable differences were observed in FA (Fractional Anisotrophy) and AD (Axial Diffusivity) compared to controls (Fig 2). The result showed significant increase in FA and AD values at 5months post irradiation as compared to controls. No change was observed in MD (Mean Diffusivity) and RD (Radial Diffusivity) in irradiated group as compared to controls. Quantitative analysis of 1H MRS using LC Model reported arbitrary concentration of GABA, glutamine, glutamate(Glu), N acetyl aspartate (NAA), glutamine + glutamate (glx), taurine (tau), myo-inositol (mI) and choline + glycerophosphocholine (tCh) metabolites in reference to water. The results explained significant increase only in myo-Inositol(mI) in irradiated group as compared to controls. Also Taurine was found to be decreased in irradiated group at 5 months but it did not reached to significant level (Table 1).

Discussion: Cognitive health of an organism is considered to be maintained by the capacity of hippocampal precursors to proliferate and differentiate. Ionizing radiation have been shown to inhibit neurogenesis by generation of short lived ROS species which leads to persistent oxidative stress that extends upto several months or even years thus leading to onset of cognitive impairements. The decline in cognitive index at 5 months post irradiation could be due to radiation induced persistent oxidative stress. Myoinositol (mI), a sugar like molecule and an osmolyte, is considered as a glial marker and an increase in its content is believed to represent glial proliferation. Its concentration is altered in many brain disorders including MCI and Alzheimer's disease. Few other studies on humans have reported association of higher mI with reduced cognitive abilities [2,3]. Further, increased FA in hippocampus could be associated with coherent arrangement of reactive astrocytes. Although FA is mostly associated with white matter because of high anisotrophy compared to gray matter (GM). There is a recent study on patients of post concussive symptoms following mTBI which report increased FA in GM but no evidence of radiological changes in brain [4]. Another recent study on animals offers an interesting hypothesis linking increased FA in GM two months following mTBI with gliosis [5]. Our results also correspond with the similar changes. The increase in FA is related to gliosis which is further characterized by an increase in AD, but no change in RD. The role of gliosis is further confirmed by increased mI in our study. All structural and metabolic changes observed in our study might be associated with reduced cognitive functions as well.

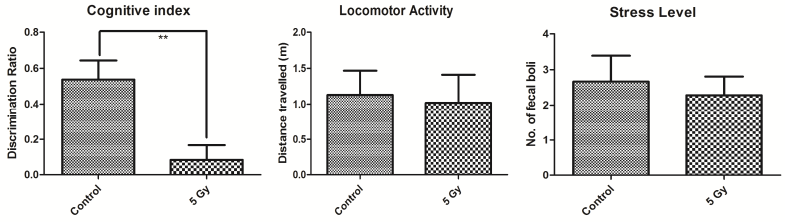

FIGURE 1: Bars showing a) Cognitive index b) Locomotor Activity c) Stress Level

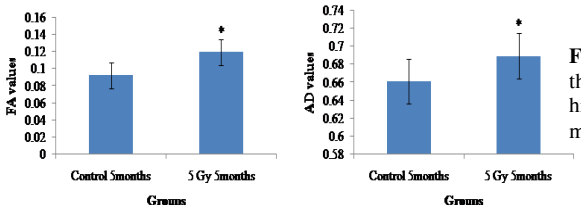

FIGURE 2: Bars showing the FA and AD values in hippocampus region at 5 months in both the groups.

TABLE 1: Concentration of metabolites in control and irradiated group

Groups	Pcr	Glu	mI	Tau	Gpc+Pch	NAA+	Glx
Control 5 Months	195.2±31.04	205.19±17.4	122.06±6.6	291.12±20.19	52.58±4.0	183.6±12.8	309.01±14.6
5 Gy 5 Months	197.53±31.5	205.06±25.9	136.7±16.2*	278.9±24.9	50.8±4.2	170.1±14.9	310.2±31.5

References: 1.Gupta M, Rana P, Haridas S, et.al. Int. Soc. Of Mag. Resonance in Med. 2014. 3174. **2**. Siger M, Schuff N, Zhu X, et.al. Alzheimer Dis Assoc Disord. 2009; 23(1):57-62. **3.** Beacher F, Simmons A, Daly E, et.al. Arch Gen Psychiatry. 2005 ;62(12):1360-5. **4.** Bouix S, Pasternak O, Rathi Y, et.al. PLoS One. 2013;8(6):e66205 **5**. Budde MD, Janes L, Gold E, et.al. Brain 2011:134: 2248–2260.

Conclusion: In the present study Behavior, microstructural and MRS studies together were able to elucidate early delayed changes due to 5Gy whole body radiation in mice brain. These findings might be valuable in evaluating the radiation induced pathologies leading to gliosis and hence cognitive dysfunction. However, further correlative studies for mRNA gene level expression for gliosis are required to understand the mechanism behind the whole body radiation induced cognitive deficit.