Defence Life Science Journal, Vol. 2, No. 3, July 2017, pp. 310-316, DOI : 10.14429/dlsj.2.11674

RESEARCH PAPER

Early Differential Neurometabolite Response of Hippocampus on Exposure to Graded dose of Whole Body Radiation: An *in Vivo* ^{1}H MR Spectroscopy Study

Poonam Rana*, Ahmad Raza Khan, Mamta Aryabhushan Gupta, and Subash Khushu

Institute of Nuclear Medicine and Allied Sciences, Delhi - 110 054, India
**E-mail: poonamrana27@gmail.com*

ABSTRACT

Whole body radiation exposure induced injury may occur during medical or industrial accidents as well as during terrorist radiation exposure scenario. A lot of information is available on alterations in brain function and metabolism post localised cranial irradiation; changes in brain associated with whole body radiation exposure are still limited. The present study has been conducted to assess early differential effect of low and high whole body radiation exposure on hippocampus neurometabolites using *in vivo* proton magnetic resonance spectroscopy (^{1}H MRS). Hippocampal ^{1}H MRS was carried out in controls (n = 6) and irradiated mice exposed to 3 Gy, 5 Gy, and 8 Gy of radiation (n = 6 in each group) at different time points i.e., day 0, 1, 3, 5 and 10 post irradiation at 7 T MRI system. Quantitative assessment of the neurometabolites was done using LCModel. The results revealed significant decrease in myoionisitol (mI)/creatine (tCr) and taurine (tau)/tCr in animals exposed to 5 Gy and 8 Gy dose compared to controls. In 3 Gy dose group, none of the metabolites showed significant alterations at any of the time point post irradiation as compared to controls. Overall our findings suggest differential change in hippocampal volume regulatory mechanism associated neuro-metabolites following whole body radiation exposure with maximum reduction in case of high dose group. We speculate that these alterations may be a consequence of oxidative stress, neuro inflammation or systemic inflammatory response following whole body radiation exposure.

Keywords: Ionising radiation; Central nervous system; Whole body radiation exposure; *In vivo* NMR spectroscopy

1. INTRODUCTION

Radiation exposure is no longer limited to nuclear industries following occupational medicine exposure or occasional accidental exposure. The possibility of an intentional ionising radiation exposure resulting from terrorist attacks is increasing world over and scenarios involving the dirty bomb usage gain more and more attention. In the last decade, it has been understood that even low dose of ionising radiation exposure influences central nervous system (CNS) functions and behaviour both as result of direct effects on the nervous system and indirect through CNS reactivity to the proinflammatory cytokines released during radiation damage of the other systems[1-4]. Hippocampus, the most vulnerable brain region to radiation, has been widely studied for radiation induced delayed injury. Further, some recent studies suggest radiation induced acute alterations in hippocampal neurogenesis as a contributory if not causative factor in the pathogenesis of cognitive dysfunction after irradiation[5,6]. Understanding the acute effect of radiation on hippocampus may provide useful insight into potential approaches to mitigate the delayed cognitive function deficit.

To date lots of information have been acquired in understanding the effect of localised cranial irradiation on brain structure and function; limited literature is available to comprehend the changes in brain associated with whole body radiation exposure. Recent studies have observed altered metabolic and microstructural changes in hippocampus on exposure to high dose of whole body radiation[7,8]. In addition to single high dose radiation exposure induced changes, lately, DTI based microstructural changes along with altered neuro behavioural functions have also been reported in low and moderate dose whole body radiation exposure[9]. It is well recognised that CNS is a dose limiting organ and both low and high dose radiation exposure has differential effect on brain physiological functions. There are evidences of dose and time dependent modulation of genes with diverse function and pathways that are involved during neuroinflammatory response, neurosignalling alterations, oxidative stress or mild morphological changes after irradiation[10]. The disturbed micro environment might have an impact on metabolic response as well. Moreover, studying the changes at low or moderate doses of whole body radiation exposure may provide more useful information that might be helpful in clinical management of humans exposed to radiation accidently. The effect of low or moderate dose of whole body radiation exposure on hippocampus metabolism is yet to be explored.

Proton magnetic resonance spectroscopy (MRS) is an indispensable and valuable tool for studying neurochemical

Received : 24 May 2017, Revised : 19 June 2017
Accepted : 26 June 2017, Online published : 02 August 2017

310

profile of brain regions *in-vivo* in animal models and human. There are several proton MRS based studies available in the literature that report radiation induced metabolic impairment in brain but these studies are limited to fractionated or focal radiation exposure[11-17]. In the present study, to further extend the prospects of MRS in whole body radiation exposure scenario and to understand the differential effect of varied radiation doses in brain, *in vivo* proton MRS has been used to look for early metabolic changes after whole body exposure to different doses of radiation in mice.

2. MATERIAL AND METHODS

2.1 Animal Handling and Radiation Exposure

Male strain 'A' mice (n = 6 in each group, 25 g - 30 g) of 10 weeks of age were exposed to a radiation of 3 Gy, 5 Gy, and 8 Gy through Tele ^{60}Co gamma irradiation facility (Bhabhatron II, Panacea Medical, Bangalore, India) with source operating at 2.496 Gy/min in our institute to represent low, moderate and high of radiation exposure respectively. All animals were irradiated with a source to surface distance (SSD) of 80 cm from the centre of the animal and a field size of 35 x 35 mm. Control group consisting of age matched mice (n = 6) was sham irradiated. During the study, all animals were placed in temperature and humidity controlled room (19 °C - 23 °C, 45 per cent - 65 per cent, respectively) subjected to a 12 h light/dark cycle with standard chow and water ad libitum. All animal handling and experimental protocols were conformed to the guidelines stipulated by the Institutional Animal Ethical Committee.

2.2 MRS Acquisition and Processing

To look for acute changes, magnetic resonance (MR) data was acquired from all three irradiated groups and controls at day 0, 1, 3, 5, 10 post irradiation. All magnetic resonance imaging (MRI) and MRS experiments were carried out on a 7 T Bruker Biospec USR 70/30, (AVANCE III) horizontal bore animal MRI system. Anaesthesia was induced by i.p. injection of a mixture of xylazine (10 mg/kg BW) and ketamine (80 mg/kg BW). All animals (both control and irradiated) were placed in prone position on an animal bed and then slid into the centre of the magnet bore. Radio frequency (RF) excitation was accomplished with 72-mm inner diameter (ID) linear birdcage coil and 4 channel phase array mouse brain coil was used for signal reception. Axial and coronal T2W images obtained using rapid acquisition with relaxation enhancement (RARE) sequence (TE, 26 ms; TR, 2.5 s) were used as localisers for placement of spectroscopic voxel. In this study single voxel ^{1}H MRS was performed in hippocampus region in control and irradiated mice groups using a Point Resolved Spectroscopy (PRESS) sequence. The voxel size of 1.5 x 3.5 x 3.0 mm^3 (15.75 µl) was placed within the region of interest with the maximum volume containing only tissue from the intended structure, minimising the contribution from surrounding tissue and also partial volume effects. The local field homogeneity was optimised by adjustment of first and second order shim coils currents using the FASTMAP sequence. The field homogeneity in a 15.75 µl voxel typically resulted in water line widths of 10-15 Hz in live mouse brain. The water signal was suppressed by variable power RF pulses with optimised relaxation delay (VAPOR). Outer volume suppression combined with a PRESS (Point Resolved Spectroscopy) sequence with spectral width of 4006.41 Hz, 2048 data points, 512 averages, 2.5 s TR and 20 ms TE with total acquisition time of 21 minutes was used for acquiring the MR spectra. Eddy current compensation and static magnetic field drift correction were applied during the data acquisitions.

2.3 MRS Spectral Processing and Data Analysis

For quantitative assessment of the brain metabolites, the MRS raw data was analysed using Linear Combination Model (LCModel)[18]. The analysis was performed in the frequency domain with raw data (free induction decay (FID)) as the input. Spectral peaks were assigned in the reference of water peak (4.82 ppm). To determine the detectability of each metabolite from measured spectra, the per cent standard deviation (SD) of each metabolite defined as the Cramer-Rao lower bound (CRLB) criteria, was derived as measurable index for quantitation reliability[19]. The per cent SD was expressed as the percentage of the estimated overall concentration; a per cent SD of ≤ 20 per cent was considered as an acceptable level of quantitation reliability. In this study relative levels in terms of metabolic ratios were used and no absolute concentration was calculated.

2.4 Statistical Analysis

The data for each metabolite were tested for homogeneity of variances and MANOVA with post hoc multiple comparison using Bonferroni was used to compare the means. A difference was considered statistically significant when p value was less than 0.05. The Sigmaplot 11.0 (Systat, USA) package was used for statistical analysis of the spectral data.

3. RESULTS

All MR images were reviewed for gross morphological lesions. No lesions or changes in the image intensities were observed in T_1/T_2 weighted images. Single voxel MR spectra were acquired from hippocampal region of the irradiated and sham irradiated groups and analysed using the LCModel (Fig. 1). The metabolites that were detectable and quantifiable in all three irradiated and sham-irradiated mice brains included creatine plus phosphocreatine (tCr), glycerophosphocholine plus phosphocholine (tCho), N-acetyl aspartate (NAA), glutamine plus glutamate (Glx), myoinositol (mI) and taurine (tau). To reduce the systematic variations among the animals studied and to extract the dominating metabolic changes, metabolite ratios were used in this study. The tCr concentration was used as a normalising factor since its concentration in normal brain is relatively constant, which is reported in earlier studies[13,20]. Earlier MRS studies have also observed no change in the concentration of creatine during acute or delayed response of radiation exposure[21,22].

Significant change was observed only in two metabolite ratios, tau/tCr and mI/tCr in 5 Gy and 8 Gy radiation dose group animals compared to sham irradiated controls. No significant difference was observed in other important metabolites like N-acetyl aspartate (NAA), choline and glutamine/glutamate

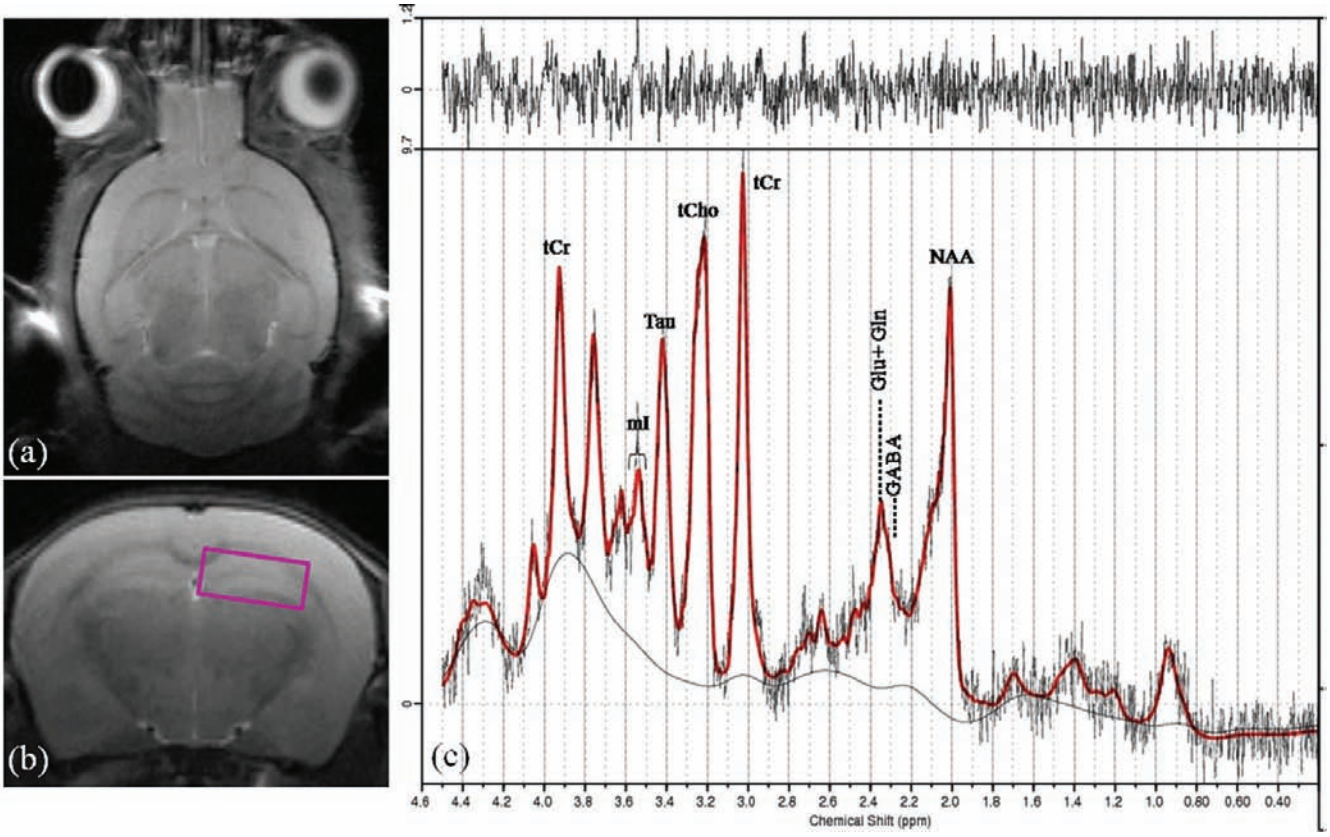

Figure 1. High resolution T2 weighted coronal (a) and axial (b) imaging illustrating the location of voxel used to obtain MR spectra and a representative LCModel processed ^{1}H MR spectrum (c) from hippocampus region of mouse brain.

(Glx). In 8 Gy irradiated mice group, significantly reduced mI/tCr and tau/tCr ratios were observed at day 3 post-irradiation, which continued to decrease till day 10 post irradiation (Fig. 2). Whereas, in case of animals irradiated with 5 Gy of radiation dose, change in mI/tCr was observed only at day 5 but tau/tCr continued to decrease till day 10 post irradiation. Surprisingly, there was no significant change in any of the metabolites at any time point in 3 Gy radiation dose group. The effect of spectral quality on relative intensity levels of mI and tau was analysed using ANCOVA by taking FWHM as a covariate. The results showed insignificant correlation between mI and tau levels and FWHM at all time points.

The combination of tau and mI identified in NMR spectra were also evaluated for their ability to diagnose radiation induced brain injury. Plot of tau/tCr as a function of mI/tCr was able to segregate 8 Gy dose group from controls at day 3, 5 and 10 post irradiation time point (Fig. 3). On the other hand, mice group irradiated with 5 Gy of radiation was segregated

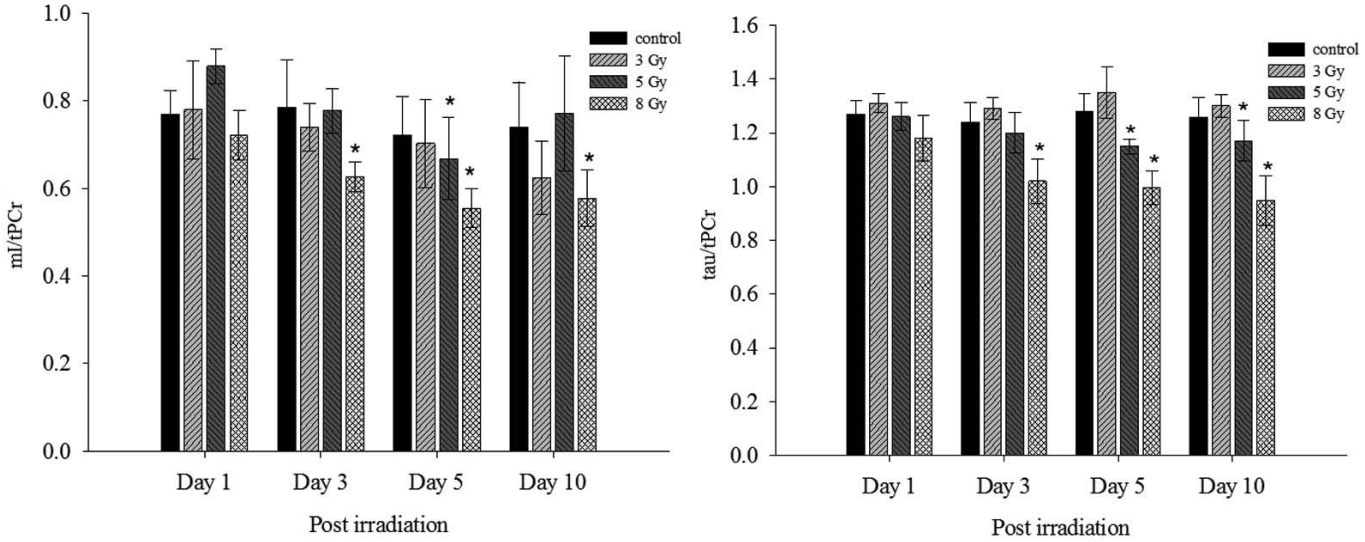

Figure 2. Temporal changes in mI/tCr and tau/tCr ratios in irradiated groups compared to controls.

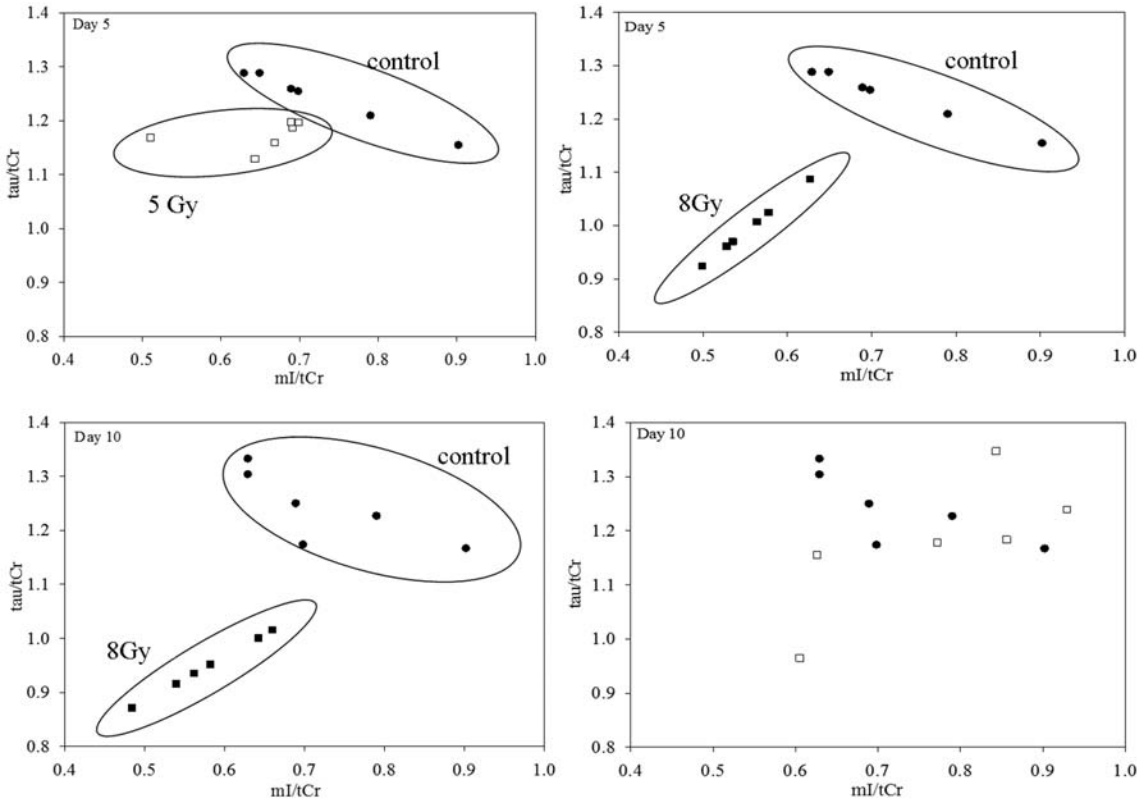

Figure 3. Predictive ability of metabolite marker combination for identification of phenotypes for radiation exposure.

from controls based on a plot of tau/tCr as function of mI/tCr at day 5 post irradiation only (Fig. 3).

4. DISCUSSION

The role of central nervous system is not fully understood in whole body radiation induced acute radiation sickness (ARS). Now it has been investigated that CNS is responsive to ionising radiation and its electrical activity and neurochemical metabolism can also be affected with low doses[3]. Therapeutic localised radiation induced changes in brain have been widely studied; however, acute effects of whole body irradiation on brain function and metabolism are still in infancy. In the present study using ^{1}H MRS, we have investigated the metabolite fluctuations in hippocampus on exposure to varied doses of whole body radiation compared to controls. Our results showed reduced mI and taurine levels in the hippocampus region of the mouse brain in 5 and 8 Gy dose groups. Myoinositol is a sugar which is found only in glial cell and is a constituent of membrane lipid, a key precursor in the phosphoinositate signal transduction and neuronal calcium signalling. It is also considered as a marker of glia because it is actively transported into astrocytes[23]. On the other hand, tau plays an important role for maintaining cellular intergrity in CNS. It provides cytoprotection, osmoregulation, neuromodulation to brain in humans and animals[24]. Indeed, both mI and tau are one of the many organic osmolytes that are present in brain together as a part of the volume regulation process and are believed to be located primarily in glia and absent in the neurons[25,26].

Earlier, altered tau and mI levels in brain have been considered to be associated with osmotic dysregulation and neuroinflammation[7,8]. It has been shown that radiation induced neuroimmune and inflammatory response[3,27,28] may bring out electrophysiological and biochemical alterations[27]. Altered mI is usually interpreted to indicate as glial activation and macrophage infiltration[29]. The change in mI levels post irradiation in our study supports biochemical alterations associated with neuroinflammatory response of glial cells[30].

Several studies have supported another hypothesis that radiation induced injury is driven in part, via increased oxidative stress through generation of free radicals[31-33]. Presence of multipotent precursor or neurogenic stem cells in the subgranular zone of hippocampus make it exquisitely sensitive to suppression by various stressors, including radiation and oxidative stress. Astrocytes, like other glial cell are an important component of cellular composition of hippocampus and metabolic processes and biosynthetic activities of astrocytes may be altered when exposed to oxidative stress. In support of it, few studies have reported oxidative stress induced osmotic dysregulation in astrocytic cell lines[34-36]. According to Kimelberg[37], due to poor ATP production during oxidative stress, ATP driven transport and ion pumps/channels such as Na^{+}-K^{+}-ATPase, Ca^{2+}-ATPase and Na^{+}-Ca^{2+} exchangers might have been impaired resulting in astrocyte cell swelling and lowering the intracellular Na levels. The reduced levels of mI and tau in this study may reflect maintenance of volume regulation for hypo-osmolarity like situation developed due to radiation induced oxidative stress. Results of our study are consistent with another in vitro NMR spectroscopy based study that showed oxidative stress induced reduction in the levels of mI, taurine, hypotaurine in astrocytes[34,36].

Furthermore, studies have suggested the role of microenvironment, in particular, neuroinflammation for inhibition of neurogenesis, which subsequently may contribute to neurocognitive impairment post irradiation[32,38]. Literature also shows association of altered mI levels with the onset of cognitive decline in conditions fostering neuroinflammation, such as HIV and Alzheimer's disease[39,40]. Altered mI levels in 5 Gy and 8 Gy dose group animals observed in our study are in accordance with our recently observed neurobehavioral dysfunction and altered DTI parameters following graded dose of irradiation. We speculate that the change in tau and mI levels in our study might be a result of both oxidative stress led osmotic disturbances and radiation induced neuroinflammation that may have profound long lasting effect on neurocognitive function at the later part of life.

Earlier studies have reported that the radiation induced changes largely depend on the dose received[26,41]. Yin[41], *et al.* observed dose dependent radiation induced changes in expression pattern of genes involved in signal transduction, ion regulation and myelin associated metabolic functions. However, in our study, we observed differential change where maximum reduction in tau and mI levels was observed in high dose i.e. 8Gy of radiation dose and change started appearing in this dose at day 3 post irradiation and persisted till day 10. In case of 5Gy dose group, both mI and tau levels were decreased at day 5 post irradiation compared to controls. Later on, mI level was found to recover but tau continued to decrease till day 10 post irradiation. Reverting back of mI level to normal after 5th day of irradiation in 5 Gy dose group indicated reversible changes. It showed that with time progression, anti oxidant system of the body was able to cope up with radiation induced oxidative stress in case of intermediate dose. We did not observe any significant change in any of the metabolites in low dose (3 Gy) group. However, our earlier DTI study showed microstructural changes in animals irradiated with 3 Gy of radiation dose[9]. It could be interpreted that radiation induced subtle changes might have occurred in brain in case of 3 Gy dose group but could not be detected due to poor sensitivity of in vivo MRS compared to DTI technique.

Differential response of 8 Gy dose group compared to 5 Gy and 3 Gy irradiated animals could further be explained as an additional indirect effect of systemic inflammatory response on CNS after whole body irradiation. Recently, correlation of C reactive protein, a sensitive marker of systemic inflammatory response and altered cerebral mI/Cr levels have been observed in human subjects depicting a risk factor for late life cognitive impairment and dementia[42]. Furthermore, extent of damage during 8 Gy of irradiation would be more prominent compared to rest of the two doses (3 Gy and 5 Gy) as 8 Gy is close to lethal dose. Based on metabolite combinations graphs, mI and tau were identified as marker of high dose radiation dose group only as they was able to differentiate only high dose radiation group from low and medium dose group.

The study concludes that ^{1}H MRS reveals differential response of brain volume regulatory mechanism associated metabolites (mI and tau) against different doses of whole body radiation with maximum changes in high dose group. The metabolic perturbations also suggests that the effect of whole body radiation exposure on CNS should not be underestimated and further agrees that hippocampal metabolic alterations may be a consequence of systemic inflammatory response following whole body radiation exposure.

REFERENCES

1. Nyagu, A.I. & Loganovasky, K.N. Neuropsychiatric effects of ionizing radiation. Kiev: Publishing House Chernobylinterinform, 1998 [in Russian].
2. Wong, C.H. & Van, der Kogel A.J. Mechanism of radiation injury to the central nervous system: implications for neuroprotection. *Mol. Intervent.,* 2004, **4**, 273-284. doi: 10.1124/mi.4.5.7
3. Gourmelon, P.; Marquette, C.; Agay, D.; Mathieu, J. & Clarencon, D. Involvement of the central nervous system in radiation induced multi organ dysfunction and/or failure. *Brit. J. Radiol*., 2005, **27**, 62-68. doi: 10.1259/bjr/15393845
4. Stewart, F.A.; Akleyev, A.V.; Hauer-Jensen, M.; Hendry, J.H.; Kleiman, N.J.; MacVittie, T.J.; Aleman, B.M.; Edgar, A.B.; Mabuchi, K.; Muirhead, C.R.; Shore, R.E. & Wallace, W.H. ICRP statement on tissue reactions/early and late effects of radiation in normal tissues and organs – Threshold doses for tissue reactions in a radiation protection context. IRCP Publication 118. *Ann. IRCP*, 2012, **41**(1/2).
5. Mizumatsu, S.; Monje, M.L.; Morhardt, D.R.; Rola, R.; Palmer, T.D. & Fike, J.R. Extreme Sensitivity of adult neurogenesis to low doses of X irradiation. *Cancer Res.*, 2003, **63**, 4021-4027.
6. Zou, Y.; Corniola, R.; Leu, D.; Khan, A.; Sahbaie, P.; Chakraborti, A.; Clark, D.J.; Fike, J.R. & Huang, T. Extracellular superoxide dismutase is important for hippocampal neurogenesis and preservation of cognitive functions after irradiation. *Proc. Natl. Acad. Sci.* USA, 2012, **109**, 21522-7. doi: 10.1073/pnas.1216913110
7. Rana, P.; Khan, A.R.; Modi, S.; Hemanth, Kumar B.S.; Javed, S.; Tripathi, R.P. & Khushu, S. Altered brain metabolism after whole body irradiation in mice: A preliminary *in vivo* MRS Study. *Int. J. Radiat. Biol*., 2012, **89**, 212-218. doi: 10.3109/09553002.2013.734944
8. Gupta, M.; Rana, P.; Trivedi, R.; Kumar, B.S.; Khan, A.R.; Soni, R.; Rathore, R.K. & Khushu, S. Comparative evaluation of brain neurometabolites and DTI indices following whole body and cranial irradiation: a magnetic resonance imaging and spectroscopy study. *NMR Biomed*., 2013, **26**, 1733-1741. doi: 10.1002/nbm.3010
9. Trivedi, R.; Khan, A.R.; Rana, P.; Haridas, S.; Hemanth, Kumar B.S.; Manda, K.; Rathore, R.K.; Tripathi, R.P. & Khushu, S. Radiation induced early changes in the brain and behaviour: Serial diffusion tensor imaging and behavioural evaluation after graded doses of radiation. *J. Neurosci. Res.*, 2012, **90**, 2009-2019. doi: 10.1002/jnr.23073
10. Mahmoud, Ahmed; Atkinson A.S. & Wong, C.S. Early

gene expression profile in mouse brain after exposure to ionizing radiation. *Radiat. Res.,* 2006, **165**, 142-154. doi: 10.1667/RR3485.1

11. Atwood, T.; Payne, V.S.; Zhao, W.; Brown, W.R.; Wheeler, K.T.; Zhu, J.M. & Robbins, M.E. Quantitative magnetic resonance spectroscopy reveals a potential relationship between radiation-induced changes in rat brain metabolites and cognitive impairment. *Radiat. Res*., 2007, **168**, 574-81. doi: 10.1667/RR0735.1
12. Atwood, T.; Robbins, M.E. & Zhu, J.M. Quantitative in vivo proton MR spectroscopic evaluation of the irradiated rat brain. *J. Magn. Reson. Imaging*, 2007, **26**, 1590-1595. doi: 10.1002/jmri.21095
13. Chan, Y.L.; Yeung, D.K.W.; Leung, S.F. & Cao, G. Proton magnetic resonance spectroscopy of late delayed radiation-induced injury of the brain. *J. Magn. Reson. Imaging,* 1999, **10**, 130-137. doi: 10.1002/(SICI)1522-2586(199908)10:2<130::AID-JMRI4>3.0.CO;2-R
14. Sundgren, P.C.; Nagesh, V.; Elias, A.; Tsien, C.; Junck, L.; Hassan, D.M.G.; Lawrence, T.S.; Chenevert, T.L.; Rogers, L.; McKeever, P. & Cao, Y. Metabolic alterations: a bio marker for radiation induced injury of normal brain: An MR spectroscopy study. *J. Magn. Reson. Imaging,* 2009, **29**, 291-297. doi: 10.1002/jmri.21657
15. Sundren, P.C. MR spectroscopy in radiation injury. *Am. J. Neuroradiol*., 2009, **30**, 1469-1476. doi: 10.3174/ajnr.A1580
16. Bálentová, S.; Hnilicová, P.; Kalenská, D.; Murín, P.; Hajtmanová, E.; Lehotský, J. & Adamkov, M. Effect of whole-brain irradiation on the specific brain regions in a rat model: Metabolic and histopathological changes. *Neurotoxicology*, 2017, **60**, 70-81. doi:10.1016/j.neuro.2017.03.005
17. Pospisil, P.; Kazda, T.; Hynkova, L.; Bulik, M.; Dobiaskova, M.; Burkon, P.; Laack N.N.; Slampa, P. & Jancalek, R. Post-WBRT cognitive impairment and hippocampal neuronal depletion measured by in vivo metabolic MR spectroscopy: Results of prospective investigational study. *Radiother. Oncol*., 2017, **122**(3), 373-379. doi: 10.1016/j.radonc.2016.12.013
18. Provencher, S.W. Automatic quantitation of localized in vivo ^{1}H spectra with LCModel. *NMR Biomed*. 2001, **14**, 260-264. doi: 10.1002/nbm.698
19. Cavassila, S.; Deval, S.; Huegen, C.; Ormondt, D-van & Graveron-Demilly, D. Cramér–Rao bounds: an evaluation tool for quantitation. *NMR Biomed.,* 2001, **14**, 278–283. doi: 10.1002/nbm.701
20. Oberg, J.; Spenger, C.; Wang, FH.; Andersson, A.; Westman, E.; Skoglund, P.; Sunnemark, D.; Norinder, U.; Klason, T.; Wahlund, L.O. & Lindberg, M. Age related changes in brain metabolites by ^{1}H MRS in APP/PS1 mice. *Neurobiol. Ageing*, 2008, **29**, 1423-1433. doi: 10.1016/j.neurobiolaging.2007.03.002
21. Usenius, T.; Usenius, J.P.; Tenhunen, M.; Vainio, P.; Johansson, R. & Kauppinen, R. Radiation induced changes in brain metabolites as studied by ^{1}H nuclear magnetic resonance spectroscopy *in vivo*. *Int. J. Radiat. Oncol. Biol. Phys.,* 1995, **33**, 719-724. doi: 10.1016/0360-3016(95)02011-Y
22. Kaminaga, T. & Shirai, K. Radiation induced brain metabolic changes in the acute and early delayed phase detected with quantitative proton magnetic resonance spectroscopy. *J. Comput. Assist. Tomo.,* 2005, **29**, 291-297. doi: 10.1097/01.rct.0000161422.95625.8a
23. Griffin, J.L.; Bollard, M.; Nicholson, J.K. & Bhakoo, K. Spectral profile of cultured neuronal and glial cells derived from HRMAS ^{1}H NMR spectroscopy. *NMR Biomed.,* 2002, **15**, 375-384. doi: 10.1002/nbm.792
24. Ripps, H. & Shen, W. Taurine: A very essential amino acid. *Mol. Vis.,* 2012, **18**, 2673-2686.
25. Dutton, G.R.; Barry, M.; Simmons, M.L. & Philibert, R.A. Astrocyte taurine. *Ann. NY Acad. Sci.,* 1991, **633**, 489-500. doi: 10.1111/j.1749-6632.1991.tb15638.x
26. Lang, F.; Busch, G.L. & VoElkl, H. The diversity of volume regulatory mechanisms. *Cell Physiol. Biochem.,* 1998, **8**, 1-45. doi: 10.1159/000016269
27. Lestaevel, P.; Grandcolas, L.; Paquet, F.; Voisin, P.; Aigueperse, J. & Gourmelon, P. Neuroinflammatory response in rats chronically exposed to (137) Cesium. *Neurotoxicology*, 2008, **29**, 343-348. doi: 10.1016/j.neuro.2008.01.001
28. Linard, C.; Marquette, C.; Mathieu, J.; Pennequin, A.; Clarencon, D. & Mathe, D. Acute induction of inflammatory cytokine expression after gamma irradiation in the rat: effect of an NF-kappaB inhibitor. *Int. J. Radiat. Oncol. Biol. Phys.,* 2004, **58**, 427-434. doi: 10.1016/j.ijrobp.2003.09.039
29. Bokemeyer, M.; Ding, X.Q.; Goldbecker, A.; Raab, P.; Heeren, M.; Arranitis, D.; Tillmann, H.L.; Lanfermann, H. & Weissenborn, K. Evidence for neuroinflammation and neuroprotection in HCV infection associated encephalopathy. *Gut*, 2011, **60**(3), 370-377. doi: 10.1136/gut.2010.217976
30. Rosen, Y. & Lenkinski, R.E. Recent advances in magnetic resonance neurospectroscopy. *Neurotherapeutics*, 2007, **4**, 330-345. doi: 10.1016/j.nurt.2007.04.009
31. Robbins, M.E.C. & Zhao, W. Chronic oxidative stress and radiation-induced late normal tissue injury: a review. *Int. J. Radiat. Biol.,* 2004, **80**, 251–259. doi: 10.1080/09553000410001692726
32. Monje, M.L. & Palmer, T. Radiation injury and neurogenesis. *Curr. Opin. Neurol.,* 2003, **16**, 129-134. doi: 10.1097/00019052-200304000-00002
33. Limoli, C.L.; Giedzinski, E.; Baure, J.; Rola, R. & Fike, J.R. Altered growth and radiosensitivity in neural

precursor cells subjected to oxidative stress. *Int. J. Radiat. Biol.,* 2006, **82,** 640-647.
doi: 10.1080/09553000600887816

34. Flogel, U.; Niendorf, T.; Serkova, N.; Brand, A.; Henke, J. & Leibfritz, D. Changes in organic solutes, volume, energy state, and metabolism associated with osmotic stress in a glial cell line: a multinuclear NMR study. *Neurochem. Res.,* 1995, **20**, 793-802.
doi: 10.1007/BF00969691
35. Noseworthy, M.D. & Bray, T.M. Effect of oxidative stress on brain damage detected by MRI and *in vivo* ^{31}P NMR. *Free Radical. Biol. Med.,* 1998, **24**, 942-951.
doi: 10.1016/S0891-5849(97)00383-3
36. Brand, A.; Leibfritz, D. & Landsberg, C.R. Oxidative stress-induced metabolic alterations in rat brain astrocytes studied by multinuclear NMR spectroscopy. *J. Neurosci. Res.,* 1999, **58**, 576–585.
doi:10.1002/(SICI)1097-4547(19991115)58:4<576::AID-JNR10>3.0.CO;2-D
37. Kimelberg, H.K. Swelling and volume control in brain astroglial cells. In: Gilles R, Hoffman EK, Bolis L, editors. Advances in comparative and environmental physiology and osmolarity control in animal cells. Berlin, Heidelberg: *Springer-Verlag;* 1991, pp 81-117.
doi: 10.1007/978-3-642-76226-0_4
38. Monje, M.L.; Mizumatsu, S.; Fike, J.R. & Palmer, T.D. Irradiation induces neural precursor cell dysfunction. *Nat. Med.,* 2002, **8**, 955-962.
doi: 10.1038/nm749
39. Huang, W.; Alexander, G.E.; Daly, E.M.; Shetty, H.U.; Krasuski, J.S.; Rapoport, S.I. & Schapiro, M.B. High brain myo inositol levels in the pre dementia phase of Alzheimer's disease in adults with Down's syndrome. *Am. J. Psychiatry.,* 1999, **156**, 1879-1886.
40. Siger, M.; Schuff, N.; Zhu, X.; Miller, B.L. & Weiner, W. Regional myo inositol concentration in mild cognitive impairment using ^{1}H magnetic resonance spectroscopy study. *Alzheimer Dis. Assoc. Disord.*, 2009, **23**, 57-62.
doi: 10.1097/WAD.0b013e3181875434
41. Yin, E.; Nelson, D.O.; Coleman, M.A.; Peterson, L.E. & Wyrobek, A.J. Gene expression changes in mouse brain after exposure to low dose ionizing radiation. *Int. J. Radiat. Biol.,* 2003, **79,** 759-775.
doi: 10.1080/09553000310001610961
42. Eagan, D.E.; Gonzales, M.M.; Tarumi, T.; Tanaka, H.; Stautberg, S. & Haley, A.P. Elevated serum C reactive protein relates to increased cerebral myo inositol levels in middle aged adults. *Cardiovasc. Psychiat. Neurol.,* 2012.
doi: 10.1155/2012/120540

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

GRANT SUPPORT

This work was performed as a part of DRDO sponsored R&D project INM 308. The authors are grateful for the financial support from Defence Research & Development Organisation (DRDO), Ministry of Defence, India.

CONTRIBUTORS

Dr Poonam Rana, PhD, At present working as Scientist 'E' in NMR Research Centre, INMAS, Delhi, and currently involved in identification of imaging and metabolic markers for radiation stress using advanced MR techniques. Her area of research interest include MR imaging, Metabolomics and NMR spectroscopy.
She was involved in study concept, designing, statistical analysis. Interpretation and critical revision of manuscript.

Dr Ahmad Raza Khan, PhD worked as research fellow at NMR research centre in DRDO funded project in NMR Research Centre, INMAS, Delhi.
In the present work was, he has contributed in acquiring and analysis of data and drafting of manuscript.

Ms Mamta Aryabhushan Gupta, MSc, worked as research fellow at NMR research centre in DRDO funded project in NMR Research Centre, INMAS, Delhi.
She contributed in acquisition and statistical analysis of the data

Dr Subash Khushu, PhD, At present, working as Scientist 'G' and Departmental head of NMR Research Centre, INMAS, Delhi. His main areas of research interests are MRI imaging, Functional imaging, MR spectroscopy.
He was involved in study designing, obtaining funds and provided administrative, technical and material support for carrying out this research activity.