

# NMR spectroscopy based study of physiological perturbations during recurrence of symptoms in radiation sickness

P. Rana<sup>1</sup>, A. R. Khan<sup>1</sup>, M. M. Devi<sup>1</sup>, S. Pal<sup>2</sup>, and S. Khushu<sup>1</sup>

<sup>1</sup>NMR Research Centre, INMAS, Delhi, Delhi, India, <sup>2</sup>Division and Cyclotron & Radiopharmaceutical Sciences, INMAS, Delhi, Delhi, India

**Introduction:** The radiation sickness occurs after whole body or significant partial body irradiation of greater than 1 Gy radiation dose. It is a sequence of phased symptoms. Early phase with no specific clinical responses starts with in few hours to few days of radiation exposure followed by a transient recovery phase. However, clinical symptoms associated with major organ system injured reappear after 2-3 weeks of radiation exposure. This manifest illness phase is characterised by intense immunosuppression and is most difficult to manage. Survivability of radiation exposed person depends on this phase and a regular monitoring in illness phase is essential for optimal clinical management. In earlier studies, radiation induced changes in liver and kidney have been observed after 3 weeks of radiation exposure using NMR based metabolomics<sup>1,2</sup>. However, for development of minimal invasive or non invasive techniques for identification of radiation related changes of metabolic markers during illness phase, NMR spectroscopy based study on urine has been proposed.

**Aim and Objective:** The present study was conducted to assess the changes in the metabolite pattern in urine in mice during illness phase after a whole body exposure to  $\gamma$  rays.

**Material & Methods:** Male A1 strain mice of 10-12 weeks of age ( $n = 9$ ) were exposed to LD<sub>50</sub> dose of 5 Gy and control ( $n = 5$ ) were sham irradiated from <sup>60</sup>Co source in a GC-220 AECL-CANADA operating at a dose rate of 0.1916 Gy/min. Urine samples were collected after 5 days and 21 days of irradiation and stored at -80°C till NMR Spectroscopy was carried out. 200  $\mu$ l of centrifuged urine sample was added to 400  $\mu$ l of deuterated phosphate buffer (pH= 7.4) containing 1mM TSP and transferred to 5mm NMR tube. <sup>1</sup>H NMR spectra were acquired at 400.13 MHz, Bruker-AVANCE 400 spectrometer at 298°K. NMR experiment of single pulse sequence with water pre saturation was performed on all urine samples. Typically 64 scans were acquired with a relaxation delay of 2 s, flip angle of 90° and spectral width 15 ppm. All data sets were zero-filled to 32K data points, exponential line broadening of 0.3Hz was applied before Fourier Transform. Peak assignment was determined according to previously reported literature<sup>3</sup>. NMR spectra were segmented into region of 0.04 ppm width. The area for each segmented region was calculated and normalised to the total spectral area of each <sup>1</sup>H NMR spectrum. In order to discern the presence of inherent similarities of spectral profiles, an unsupervised pattern recognition method, PCA was conducted on urine samples.

**Results:** A number of perturbations in endogenous metabolites were observed only in the 1H NMR spectra of urine samples collected 3 weeks post irradiation. PCA was performed on 1H NMR spectra of urine samples from control and irradiated animals and the score plot showed a clear separation between control and irradiated along PC2 (Figure 1), whereas, scores obtained from control and irradiated animals were overlapped on day 5 time point, indicating the effect of irradiation at this time point was very low. On 21<sup>st</sup> day, the prominent changes in endogenous urinary metabolites, confirmed by inspection of NMR spectra and the loading values, comprised an increase in the levels of Ile, val, 2 oxo glutarate, citrate, TMA, creatinine, malonate, acetoacetate, allantoin, fumarate, , phenyl alanine, phenyl acetyl glycine (PAG) tryptophan, hippurate. There was a decrease in formate, pyruvate and acetate levels in irradiated animals when compared to controls. However, after five days of irradiation, not much significant changes in metabolites were observed except for decreased levels of acetate, pyruvate and allantoin in irradiated animals

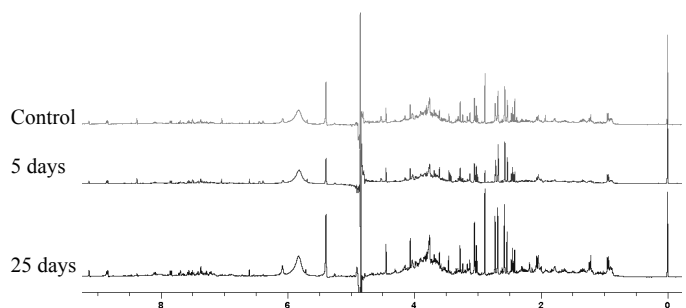


Figure 1: Representative NMR urine spectra from different doses of radiation at 6 hrs post irradiation.

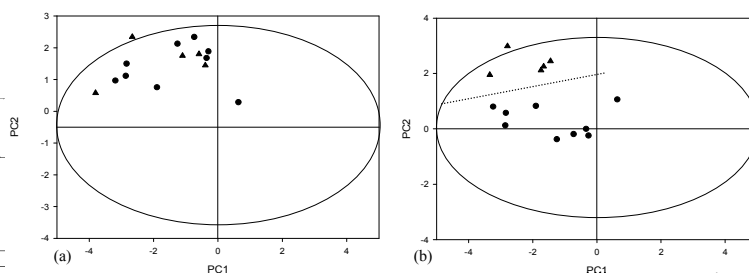


Figure 2: PCA score plot based on <sup>1</sup>H NMR spectra of urine sampled on 5<sup>th</sup> (a) and 21<sup>st</sup> day (b) from control mice (●,  $n = 9$ ) and irradiated group with 5 Gy (▲,  $n = 5$ ) Gy dose

**Discussion:** After five days of radiation, no significant changes in metabolite concentrations were observed in irradiated mice group except for acetate, pyruvate and allantoin. Marked effect on the metabolism by radiation exposure was seen after 21 days of the radiation. The results exhibited an altered energy, amino acid and gut microflora metabolism. Increased Krebs cycle intermediates and decreased pyruvate in urine samples showed disturbed glucose energy metabolism. Increased acetoacetate concentration (ketoneuria) supported disturbance in energy metabolism and suggested a switch in energy metabolism from glucose to fatty acid beta-oxidation. Similarly, increase in amino acid concentration in urine could be due to obstacles to the reabsorption of such amino acids as all of the amino acid has to be absorbed by active transport in kidney. Therefore, it could be deduced that there could be some renal dysfunction. These changes could be the consequence of radiation induced damage to physiological systems during recurrence of clinical symptoms after recovery period. Similar findings had earlier been observed in liver and kidney tissues on 25<sup>th</sup> day post radiation in mice<sup>1,2</sup>.

**Conclusion:** Over all the results based on urine NMR spectroscopy showed that reappearance of clinical symptoms during illness phase of radiation sickness were reflected in urine as physiological perturbations at metabolites levels. The information attained from the study along with biochemical assays could be very useful in assessing the organ dysfunction during radiation sickness.

**References:** 1.Poonam Rana, Ritika et al. (2009) Proc. Intl. Soc. Mag. Reson. Med.Honolulu, Hawaii  
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3.Lindon J.C., et al.(1999) ,Annu. Rep. NMR Spectrosc. 38, 1–88.