



## Diagnostic Range Ionizing Radiation and Reactive Oxygen Species Production: an Initial Experience

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**Abstract:** Radiation is a common occurrence in our daily lives that comes from both natural and man-made sources. Ionizing Radiation (IR) causes damage either directly or indirectly through the generation of reactive oxygen species (ROS). Oxidative damage to DNA, lipids, proteins, and many metabolites occurs through a complex series of processes that are enhanced by endogenous signalling which is activated by free radicals. Though literature is abundant on ROS and antioxidants at high doses, no study to the best of our knowledge has assessed the ROS levels after Multi Detector Computed Tomography (MDCT) examination (i.e. in diagnostic range radiation). The aim of the present study was to assess the production of ROS after diagnostic level radiation by MDCT examination and at 24 hour follow up. The study involved fifty patients posted for clinically indicated MDCT which were recruited. The average radiation dose was 2-9 mGy. Three blood samples were drawn, one prior to CT (control sample), within half an hour of CT (post CT) and 24 hrs after CT. 3 ml venous blood was withdrawn in aseptic conditions and immediately serum was isolated for ROS assessment. The blood examination results were compared in immediate and post 24 hour after MDCT and both were compared with control values and correlated with radiation parameters. Our results have shown a significant increase in ROS level in immediate post CT samples compared to prior CT scan samples (control) ( $p$  value  $<0.0001$ ). The ROS levels reduced at 24 hours compared to immediate post CT, however they were still higher than control values. Our findings reflect that there is a rapid increase in free radicals production in the mitochondria after diagnostic level radiation. Detection of higher ROS levels at 24 hours suggests incomplete repair with the presence of some residual oxidative species at 24 hours.

**Keywords:** Computed Tomography (CT Scan), ROS, Free Radicals, ELISA, Low Dose Radiation

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## 1. INTRODUCTION

In recent years, faster and higher resolution studies with dynamic contrast, like CT angiography, cardiac CT, and virtual CT colonoscopy have become available in parallel to the development of high technology devices like the multidetector CT (MDCT) <sup>1</sup>. This has led to a crescendo in the demands for radiological studies for clinicians in an effort to redesign their diagnostic approaches and treatment plans, which has also given rise to an increase in the exposure of patients to ionizing radiation <sup>2, 5, 6, 7</sup>. In England, CT formed 4% of the radiological applications in 1990, compared to the USA for which CT made up 10% of the radiological applications in 2000. However, these procedures were found to be responsible for the large majority of ionizing radiation exposure to patients (40% in England; 65% in the USA) <sup>3</sup>. The increasing amount of ionizing radiation that is received from controllable artificial radiation resources gives rise to possible risks of developing cancer over the course of a lifetime and hence constitutes a threat to public health <sup>8</sup>. Exposure to ionizing radiation (IR) induces various types of DNA damage, of which DNA double-strand breaks are the most severe, leading to genomic instability, tumorigenesis, and cell death. Hence, cells have developed DNA damage responses and repair mechanisms. IR also causes the accumulation of endogenous reactive oxidative species (ROS) in the irradiated cells. The reactive oxygen species (ROS) i.e. hydroxyl radical ( $\cdot\text{OH}$ ), ionized water ( $\text{H}_2\text{O}^+$ ), hydrogen radical ( $\cdot\text{H}$ ) and hydrated electrons ( $\text{e}_{\text{aq}}^-$ ) are rapidly generated by ionizing radiation of cellular water <sup>9-12</sup>. Approximately, ten thousand oxidation reactions harm DNA per human cell per day, which eventually produce single-strand breaks (SSB), and double-strand breaks (DSB). These lesions if not repaired, can interact with essential DNA metabolism, including transcription, translation, recombination, and replication, which ultimately give rise to undesirable outcomes and there are chances of three possible responses: 1) The cell may become senescent, 2) The cell may become apoptotic and, 3) The cell may become malignant<sup>5</sup>. Accumulating evidence indicates that exposure to a high dose of acute IR causes a sustained increase in the production of endogenous ROS over a few hours. Though these bio-effects of radiation particularly ROS production have been researched at high doses of radiation, but how low-level radiation in the diagnostic range stimulates these cellular changes remains largely unexplored and is still considered controversial <sup>13-14</sup>. The aim of the present study is to assess the production of ROS immediately after diagnostic level radiation by MDCT examination at 24 hours follow up.

## 2. MATERIALS AND METHODS

### 2.1 Inclusion Criteria

After obtaining institutional ethical committee approval Reference number IEC-HR/2019/38/IR and written informed consent of each subject, fifty adult patients who were posted for clinically indicated MDCT abdomen/ chest plus abdomen/ whole body were enrolled for the study.

### 2.2 Exclusion Criteria

The patients who had undergone radiotherapy treatment or recent radiation-based study were excluded from the study.

## 2.3 Sample Collection

The samples were collected in the plain vial (BD vacutainer tubes) under aseptic conditions. Approximately 2 ml of venous blood samples were collected three times from each patient

1. Prior to CT examination (Control sample- Group I).
2. Within 30 minutes of CT scan (Post CT-Group II).
3. After 24 hours of CT scan (24 hrs- Group III).

All samples were handled in the same conditions. After blood withdrawal, the samples were transported to the laboratory at room temperature. The samples were centrifuged for 20 minutes at 3000 rpm to separate serum from blood. The serum was separated and taken in Eppendorf from the blood. Samples were then analysed immediately after the arrival using a commercially available ELISA kit (Bioassay Technology) according to the manufacturer's instructions

## 2.4 MDCT Examination

The MDCT examinations of patients were carried out on Siemens (Elargen Germany) Model Definition AS 64 slice CT scanner at GTB Hospital, Delhi. The conditions of exposure were normal for routine diagnostic procedures. The following imaging parameters were used: 120 kV, 35 mA, rotation time of 0.5 second, 0.6 and 1.2 pitch for abdomen and chest respectively. The radiation dose that has been delivered to the patient was calculated from the DLP and CTDI value using Monte Carlo simulation software.

## 2.5 ROS Technique<sup>15</sup>

The reagents and samples were brought to room temperature. Standards were prepared as instructed. 50  $\mu\text{l}$  of standard solution and 40  $\mu\text{l}$  samples were added in triplicates to wells of pre-coated with human ROS antibody. The anti-ROS antibody was added to the Plate followed by 50  $\mu\text{l}$  of streptavidin-HRP to sample and standard wells and incubated for 60 minutes at 37°C. Plates were washed 5 times with a wash buffer. 50  $\mu\text{l}$  of substrate solution A and B were added to each well and then the plate was incubated for 10 minutes at 37°C in the dark. The stop solution (50  $\mu\text{l}$ ) was added and the blue colour changed to yellow sample concentrations were calculated based on the standard curve.

## 3. STATISTICAL ANALYSIS

The presentation of the categorical variables was done in the form of numbers and percentages (%). On the other hand, the quantitative data were presented as the means  $\pm$  SD and as median with 25th and 75th percentiles (interquartile range). The comparison of the variables which were quantitative in nature was analysed using an independent t test (for two groups) and Paired t-test was used for comparison across follow up.

## 4. RESULTS

The CT scan radiation exposures measured by Monte Carlo simulation software varied from 2 to 9 mSv in our study group. The mean, median, range and comparisons of the measured ROS levels in the three study groups are listed in Table I. Mean group values  $\pm$  standard error of group I

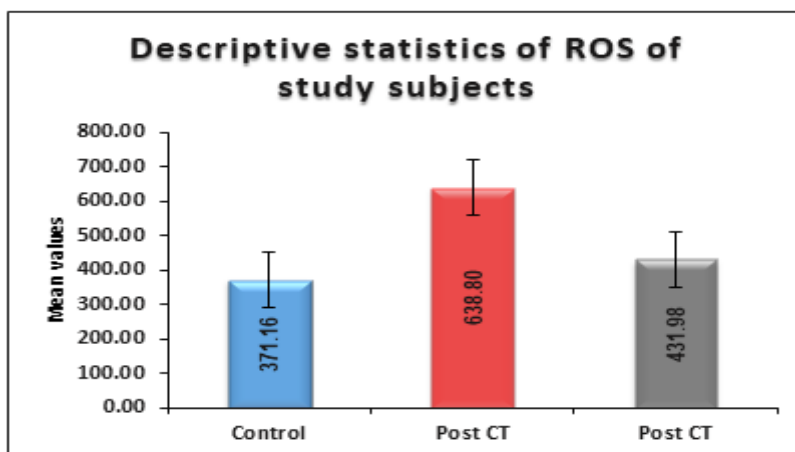
were  $371.16 \pm 245.52$  and  $638.8 \pm 261.03$  in group 2. Mean group values  $\pm$  standard error of group 3 was  $431.98 \pm 268.92$ . There was a highly significant rise in ROS levels in

post CT scan samples (p-value < 0.001). The levels remained high even at 24 hours after the CT scan, though they were less than immediate post CT values. (Table 1)(Figure 1).

**Table 1:-Comparison of ROS across follow up of study subjects**

ROS	Mean $\pm$ SD	Median(25th-75th percentile)	Range	Control vs Post CT	Control vs 24 hours after CT	Post CT vs 24 hours after CT
Group 1	$371.16 \pm 245.52$	287.6(185.6-518.6)	88.6-1038.6			
Group 2	$638.8 \pm 261.03$	689.6(398.6-828.6)	158.6-1174.6	<.0001 <sup>†</sup>	0.068 <sup>†</sup>	<.0001 <sup>†</sup>
Group 3	$431.98 \pm 268.92$	370.6(210.6-574.6)	46.6-1038.6			

<sup>†</sup> Paired t-test



**Fig 1:-Comparison of ROS across follow up of study subjects**

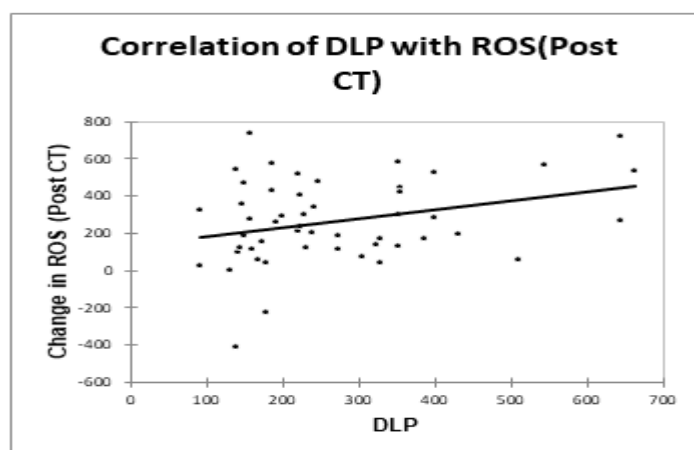
**Table 2:- Correlation of DLP, effective mAs and total Effective dose (mSv) (NRPB) with change in ROS.**

Variables	DLP	Effective mAs	Total effective dose (mSv) (NRPB)
<b>Change in ROS (Post CT)</b>			
Correlation coefficient	0.305	0.199	0.190
P value	0.032	0.166	0.186
<b>Change in ROS (24 hours after CT)</b>			
Correlation coefficient	0.114	0.001	0.050
P value	0.429	0.994	0.731

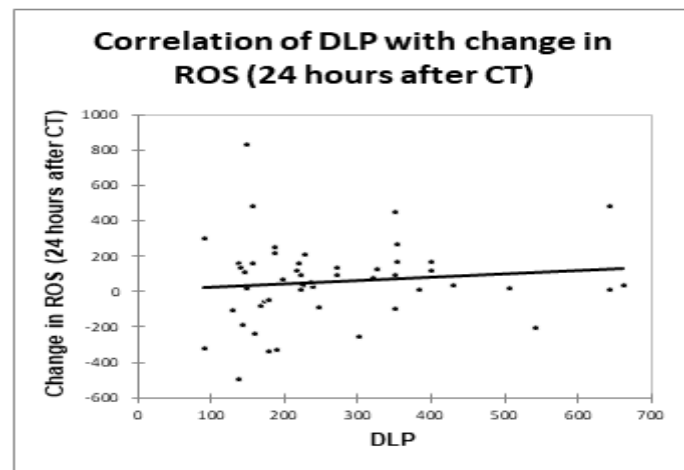
Pearson correlation coefficient

Further, the ROS concentration was correlated with Dose length product and effective dose (mSv) levels as measured on CT scan. A positive significant correlation was seen with a

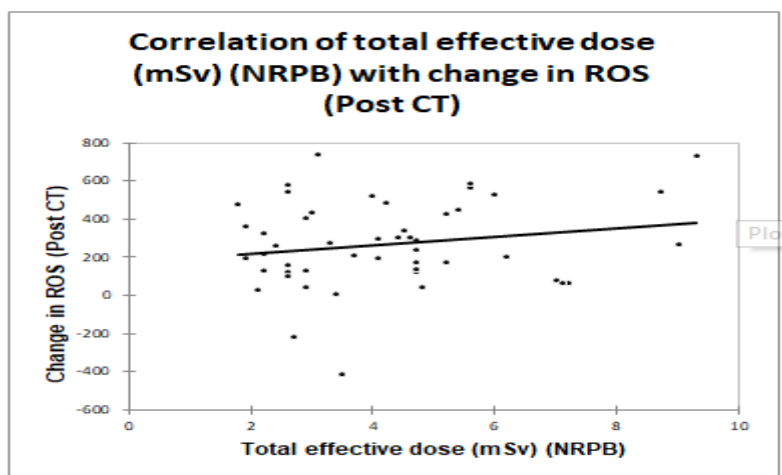
p-value of 0.032 in group II i.e. immediate post CT scan (Table 2, Fig 2, 3). A weak positive correlation was seen with effective dose (mSv) also but it was not statistically significant.



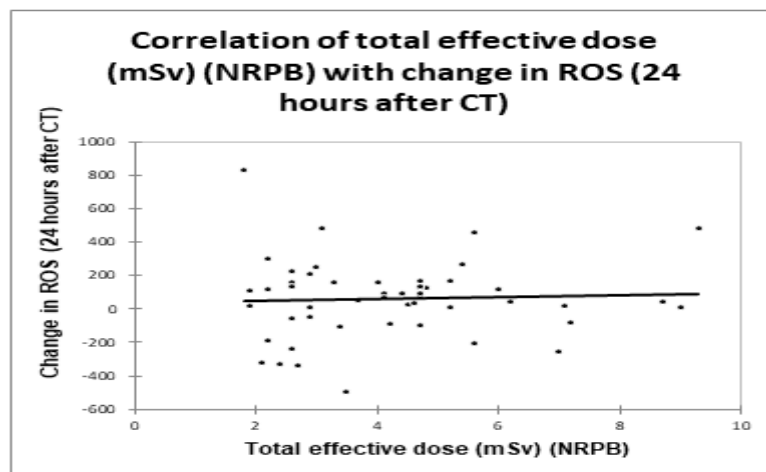
**Fig 2:- Correlation of change in ROS (Post CT) with DLP.**



**Fig 3:- Correlation of change in ROS (24 hours after CT) with DLP.**



**Fig 4:- Correlation of change in ROS (Post CT) with total effective dose (mSv).**



**Fig 5:- Correlation of change in ROS (24 hours after CT) with total effective dose (mSv).**

Correlation of DLP and total effective dose (mSv) has also been found in both between CT examinations within 30 minute and after 24 hours.

## 5. DISCUSSION

Ionizing radiation has been categorized into high dose (>100mSv) and low dose (<100mSv). So far, several studies have reported that exposure of mammalian cells to radiation increased the production of ROS in the irradiated cells. It is

well established that there are reactive oxygen species at high radiation doses<sup>16-19</sup>. There are two mechanisms by which radiation affects the cells, direct ionization and indirect ionization<sup>20</sup>.

### 5.2 Direct Ionization

it occurs when charged particles e.g. Electrons with enough kinetic energy interact with cellular atoms to create free radicals. This phenomenon is called direct because the

interaction occurs directly between a particle and a cellular component without any in-between step<sup>20</sup>.

### 5.3 Indirect Ionization

It occurs when non-charged particles, for example, Photons, interact with cellular water. The energy absorbed by the water molecule results in ion pairs and reactive oxygen metabolites such as hydroxyl radicals. These free radicals interact with cellular atoms and molecules damaging cellular proteins and may form additional free radicals. The process is called indirect because there is an in-between step of H<sub>2</sub>O-based free radical formation<sup>20</sup>. The production of ROS after IR exposure is caused by water radiolysis<sup>8, 9, 10</sup>. The human body is constituted of 70% water, the chances of radiolysis are quite high under the presence of ionizing radiation. In the process, water loses an electron and becomes highly reactive. Then water is sequentially converted to hydroxyl radical ( $\cdot\text{OH}$ ), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), superoxide radical ( $\text{O}_2^{\cdot-}$ ), and ultimately oxygen ( $\text{O}_2$ ). The biological reactivity of superoxide, hydrogen peroxide or hydroxyl radical produced by dissociated  $\text{H}_2\text{O}$  is high, resulting in oxidative damage, and a cascade amplification of biological effects. ROS are formed in organelles such as mitochondria and the endoplasmic reticulum even when they are not exposed to IR<sup>19</sup>. When free radicals come in contact with cellular macromolecules, for example, protein, alter their chemical structures and may cause repairable or non-repairable damage with crucial downstream effects. These lesions if not repaired, can interact with essential DNA metabolism, including transcription, translation, recombination, and replication, which ultimately give rise to undesirable outcomes and there are chances of three possible responses:

1. The cell may become senescent
2. The cell may become apoptotic
3. The cell may become malignant.

To protect the genome integrity, cells possess a sophisticated mechanism of DNA lesions detection and repair, the DNA damage response. Endogenous or exogenous stress can induce several different DNA repair systems, including mismatch repair, base excision repair, nucleotide excision repair, homologous repair, and non-homologous end-joining repair systems. Because of the high reactivity of ROS, the life span of ROS is very short with the surrounding molecules. Accumulating evidence indicates that exposure to a high dose of acute IR causes a sustained increase in the production of endogenous ROS over a few hours. The accumulation of oxidative injuries due to excessive radicals cause cell and tissue injuries, which may cause carcinogenesis. While there are chemical defence mechanisms in the body that eliminates ROS and repair the damaged molecules. The generation of ROS in cells exists in equilibrium with a wide variety of antioxidant defences. These include enzymatic scavengers such as superoxide dismutases (SOD), catalase, and, glutathione peroxidase, as well as non-enzymatic scavengers such as vitamins C and E, glutathione (GSH), lipoic acid, carotenoids and iron chelators. ROS is very unstable in nature, therefore, most of the studies were based on its antioxidants. The antioxidants like SOD, GSH etc. were estimated in reference to ROS. Russo et al<sup>21</sup> reported an increase in GSH level in the exposed group from  $12.37 \pm 1.22$

to  $20.61 \pm 2.16$  as compared to control samples at an effective annual dose ranging from 1.5 to 8.4 mSv. The exposed subjects showed a three-fold increase in hydrogen peroxide as compared with the unexposed subjects ( $2.21 \pm 1.03$  to  $6.51 \pm 1.55$ ). As a result of the increase in hydroxyl radical species, it would be expected to observe a different antioxidant level in the serum of the exposed subjects vs. the controls. In the rat model, Yamaoka<sup>22</sup> studied that at 0.25-0.5 Gy for 4 hours, the SOD activity was significantly increased and persisted in the spleen for at least 12 weeks, liver for 8 weeks, brains and thymuses for 4 weeks and bone marrow for about 1 week. No study was found which directly relates the low dose radiation correlation with ROS at 2-9 mSv radiation doses. In our results, the genetic damage may be induced by the radiation from an MDCT examination as the ROS level significantly increased i.e. from  $371.16 \pm 245.52$  to  $638.8 \pm 261.03$  after immediate radiation and persisted after 24 hours i.e.  $431.98 \pm 268.92$  (Table 1). To better understand the relationship between ROS and CT scan parameters i.e. Dose length product (DLP) and effective doses (mSv) we construct scatter plots. There is a positive correlation that can be seen when comparing ROS with Dose length product and effective dose levels as measured on CT scan (Figure 2, 3, 4 and, 5). A positive significant correlation was seen with a p-value of 0.032 in the immediate post CT scan (Table 2). A weak positive correlation was seen with effective dose (mSv) also but it was not statistically significant (Table 2). More research needs to be done in order to increase radiation protection. Our research will continue in this area to learn more about genome damage and subsequent DNA repair after low dosage exposure. All health workers must do their part to perform diagnostic procedures with the highest level of safety and quality.

## 6. CONCLUSION

The results of our study indicate that there is an increased production of ROS/free radicals at the cellular level after exposure to diagnostic range radiation by MDCT examination which remains high even after 24 hours. This highly sensitive response to diagnostic range radiation has important implications in understanding and assessing the health risks of radiation exposure and it also provides novel insights into the low-dose radiation-induced adaptive response.

## 7. AUTHOR CONTRIBUTION STATEMENT

Dr. (Prof) Anupama Tandon conceptualised the research topic and contributed valuable inputs for the manuscript preparation. Ms. Shikha Sirohi gathered the data and analysed the results and prepared the manuscript for the study. Prof. B.D.Banerjee guides in conducting the tests and workspace in the laboratory. Mr. Ranjeet Kumar supported in data analysis and experimental work.

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## 9. CONFLICT OF INTEREST

Conflict of interest declared none.

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