Pilot Study for the Establishment of Biomarkers for Radiation Damage after Computed Tomography in Children

Brunhild M. Halm MD, PhD, RDMS; Adrian A. Franke PhD; Jennifer F. Lai MS; Xingnan Li PhD; Laurie J. Custer BS; Ian Pagano PhD; Robert V. Cooney PhD; Helen C. Turner PhD; and David J. Brenner PhD

Abstract
Computed tomography (CT) is an imaging modality that exposes patients to ionizing radiation (IR). We review and report findings from our pilot study evaluating whether blood markers are altered in 17 children undergoing medically indicated CT scans. Blood was drawn before (‘pre-CT’) and 1 hour after (‘post-CT’) CT scans. Plasma carotenoids, tocopherols, Q10, ascorbic acid (AA) and uric acid (UA) were analyzed by RP-HPLC with diode-array and electrochemical detection. Dehydroascorbic acid (DHAA) was calculated by subtraction from total AA. Total antioxidant capacity (TAC) was measured using the ORAC assay. Cytokines were quantified using a multiplex immunoassay. γ-H2AX foci were visualized using immunofluorescence. Mean pre- and post-CT changes were compared using t-tests; P-levels < .05 indicated significance. All major plasma lipid soluble antioxidant levels were lower post- vs pre-CT (P < .05) possibly from the scavenging of free radicals formed by CT-induced IR. Average AA levels increased (134%) while DHAA levels were decreased (29%) post-CT, probably due to intracellular recycling of AA from DHAA. TAC levels in lipophilic and hydrophilic extracts were unchanged, suggesting that other antioxidants may have assisted in free radical quenching, which would corroborate their lower concentrations post-CT. Cytokine levels were unchanged and dose-dependent increases in γ-H2AX foci, a measure of double strand DNA breaks, were observed (P = .046, n=3 children). Our results suggest that CT-derived IR can influence the antioxidant system and may elicit detrimental responses on the cellular level of young children. When possible and if appropriate non-IR based techniques such as ultrasound or magnetic resonance imaging should be used.

Keywords
ionizing radiation, computed tomography, children, micronutrients, γ-H2AX, free radicals

Introduction
Computed tomography (CT) is an essential imaging modality that allows rapid, painless, and accurate imaging of most organ systems due to its high resolution and fast imaging capabilities. CT use in the United States has risen substantially over the past few decades especially in the emergency department and is the largest medical source of ionizing radiation (IR) in the United States. Between 1995 and 2008, the number of CT scans performed in the pediatric emergency department (ED) increased five-fold while the number of ED visits during the same time frame did not change. The CT rise in children is primarily for diagnostic accuracy in conditions such as trauma, seizures, complicated pneumonias, and abdominal pain and is attributed largely to improved resolution and faster acquisition times, thereby eliminating the need for sedation. A dramatic increase in CT imaging of pediatric patients in the ED suffering from abdominal pain from 1998 to 2008 was reported recently while a substantial increase in CT use from 1995 to 2003 in the evaluation of children with head trauma was noted. A study evaluating children with suspected ventricular peritoneal shunt malfunction found that they received a median 2.6 head CT scans per year. Attributable lifetime cancer risk has been estimated at one fatal cancer per 1000 pediatric head CT scans and it is estimated that 2% of all future cancers may be caused by diagnostic medical radiation with a higher risk for young children owing to their higher radiosensitivity and longer life expectancy than adults.

Two recently published large epidemiological studies assessing IR and cancer risk in children and young adults exposed to medically indicated CT scans reported that a cumulative dose of 50 to 60 mGy received from CT scans could triple the risk of developing leukemia and brain cancers and that cancer incidence was 24% greater in those exposed to CT scans than those not exposed. In addition, some investigators have found that intellectual development may be adversely and permanently affected in children receiving IR to the head. Furthermore, general (non-pediatric focused) hospitals are less likely to use pediatric-specific radiation reduction protocols and instead use pediatric-specific radiation reduction protocols and instead use techniques that are likely to result in children being exposed to adult-size radiation doses, which are significantly higher than those used for children. This is of great concern in light of a recent report on imaging frequency that estimated 89.4% of pediatric CT scans performed in the ED were done at primary adult facilities.

CT involves significant exposure to IR, which can elicit detrimental cellular responses such as DNA lesions, base
damage, and protein cross-links, all of which can significantly increase the risk of developing cancer.\textsuperscript{1,14} DNA double-strand breaks (DSBs), the principle DNA cytotoxic lesion, can induce the phosphorylation of the core histone variant H2AX (to γ-H2AX)\textsuperscript{15-17} and the ensuing formation of γ-H2AX clusters (foci) that occurs at sites of DNA DSBs\textsuperscript{18,25} with one focus indicating one DNA DSB.\textsuperscript{15,21}

Carotenoids, tocopherols, and retinol are lipid-phase micronutrients (LPM) that function as important antioxidants to reduce oxidative stress and/or prevent oxidative damage.\textsuperscript{22-27} Coenzyme Q10 (Q10) is a LPM that functions as an electron/proton carrier during cellular respiration.\textsuperscript{28,29} Ubiquinol-10 (UL10) is the chemically reduced form of Q10 and has been shown to function as a free radical scavenger that protects against cellular oxidative injury and stress\textsuperscript{30} and minimizes damage to low-density lipoproteins in vitro by dehydrogenation to ubiquinone-10 (UN10).\textsuperscript{30} Thus, the UL10/UN10 and UN10/TQ10 ratios have been postulated as useful measures of oxidative damage.\textsuperscript{31,34} Vitamin C (L-ascorbic acid) is a hydrophilic antioxidant that protects against free radical damage\textsuperscript{35} by scavenging various reactive oxygen and nitrogen species.\textsuperscript{36}

Cytokines are signaling molecules released by cells in response to noxious stimuli and act as intercellular mediators by binding to specific receptors.\textsuperscript{37,38} Cytokines can be induced after radiation exposure and may have important regulatory roles during recovery after exposure (reviewed in\textsuperscript{39,40}).

Here we review our previous results and report our new findings from a pilot study that aimed to evaluate whether the following compounds are altered in young children undergoing CT scans: plasma antioxidants (tocopherols, carotenoids, coenzyme Q10);\textsuperscript{41} plasma redox status (UL10/UN10, ascorbic acid (AA) and dehydroascorbic acid (DHAA)/total AA); total antioxidant capacity (TAC), DNA DSBs (γ-H2AX foci);\textsuperscript{42} and levels of 10 pro- and/or anti-inflammatory cytokines.

**Methods**

**Patient Recruitment.** Seventeen pediatric patients (0.25-6 years old) undergoing medically indicated CT scans were enrolled in the emergency or radiology department at Kapi‘olani Medical Center for Women and Children (Honolulu, Hawai‘i) after receiving signed consent from their legal guardian. Blood draw times, CT scan times, and CT doses were documented. The Western Institutional Review Board, University of Hawai‘i Committee on Human Services, and Columbia University Institutional Review Board approved this pilot study.

**CT Parameters and Radiation History** of each child expressed as dose in relative numbers were described previously (Table 1).\textsuperscript{31,42}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|c|c|}
\hline
Patient ID & Gender & Height (cm) & Weight (kg) & Age (m) & CT dose (mGy-cm) & Effective dose (mSv) & CT location & CT type & Contrast use & Multi-vitamin Intake & Radiation history (dose in relative numbers)* \\
\hline
1 & M & 68.2 & 11.9 & 24 & 372.85 & 2.50 & Head & Axial & no & yes & 0.02 \\
2 & M & 64.0 & 9.8 & 14 & 372.85 & 2.50 & Head & Axial & no & no & 0.50 \\
3 & M & 99.0 & 14.5 & 24 & 376.58 & 11.30 & Abdomen & Helical & yes & no & 1.02 \\
4 & M & 95.0 & 13.9 & 36 & 147.37 & 4.42 & Abdomen-Pelvis & Helical & yes & no & 0.01 \\
5 & M & 118.0 & 28.0 & 60 & 104.13 & 2.08 & Abdomen-Pelvis & Helical & yes & no & 0.05 \\
6 & F & 96.0 & 14.0 & 36 & 340.89 & 2.28 & Head & Axial & no & yes & 0.02 \\
7 & M & 65.0 & 10.0 & 17 & 310.71 & 2.08 & Head & Axial & no & no & 2.53 \\
8 & M & 123.0 & 40.6 & 72 & 355.10 & 1.42 & Orbit & Axial & yes & no & 2.05 \\
9 & F & 118.0 & 20.0 & 60 & 195.30 & 0.78 & Orbit & Axial & yes & yes & 0.00 \\
10 & F & 102.0 & 14.7 & 48 & 426.12 & 2.86 & Head & Axial & no & yes & 4.37 \\
11 & M & 105.0 & 15.5 & 48 & 426.12 & 2.86 & Head & Axial & no & yes & 0.02 \\
12 & M & 102.0 & 18.6 & 72 & 525.55 & 2.10 & Head & Axial & no & no & 14.12 \\
13 & M & 112.0 & 19.0 & 48 & 236.14 & 6.14 & Mastoid bone & Helical & yes & no & 1.00 \\
14 & F & 114.0 & 18.7 & 48 & 106.48 & 1.28 & Chest & Helical & yes & no & 0.05 \\
15 & M & 65.0 & 7.1 & 3 & 92.46 & 1.57 & Neck & Helical & yes & yes & 0.03 \\
16 & M & 92.0 & 12.1 & 21 & 426.12 & 2.86 & Head & Axial & no & yes & 1.01 \\
17 & M & 66.0 & 11.2 & 15 & 340.89 & 2.28 & Head & Axial & no & yes & 0.00 \\
\hline
\end{tabular}
\caption{Demographics of Study Participants}
\end{table}

*Each head, chest, abdominal CT is 1.0 unit and pelvis 5.0 units, chest X-ray 0.01 units (postero anterior) and 0.02 units (lateral), abdominal or plevic X-ray 0.35 units. From Ref: Mettler FA Radiology 2008: 248(1), 254-263; Valentin, J Ann ICRP 2007:37(1),1-79; American Nuclear Society Radiation Dose Chart available at: http://www.ans.org/pi/resources/dosechart/. Published 2012, accessed July 2, 2012. Table re-used with permission from Halm, 2014.\textsuperscript{41}
Sample Collection and Processing. Peripheral whole blood was drawn by venipuncture into sodium heparin vacutainer® tubes (2.5 – 4.0 mL) from each child immediately before (‘pre-CT’) and one hour after (‘post-CT’) their scheduled CT exams as previously reported.\textsuperscript{41,42}

Chemicals and reagents used were reported previously.\textsuperscript{41,42} Randomly methylated beta-cyclodextrin (Trappsol) was purchased from Cyclodextrin Technologies Development Inc. (High Springs, FL)

Extraction and analysis of UL10, UN10, carotenoids, tocopherols, and retinol was performed using our well-established HPLC assay with minor modifications \textsuperscript{30,43} as previously described.\textsuperscript{31}

Quantitative determination of Cytokines. Concentrations of plasma cytokines (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, GM-CSF, IFN-γ and TNF-α) were assayed using an ultrasensitive multiplex immunoassay (In Vitrogen, Camarillo, CA) per manufacturer’s instructions with slight reductions in sample and reagent volumes to maximize sensitivity and minimize interferences. Median fluorescent intensities of each cytokine were obtained using the Luminex\textsuperscript{®} 200\textsuperscript{TM} dual-laser based fluorescent analyzer (Luminex Corp., Austin, TX) and quantified against a standard curve using GraphPad Prism 5 software (La Jolla, CA). Plasma and quality control samples were kept at -80°C, thawed immediately before use, and measured in duplicate on a 96-well magnetic plate.

Analysis of total and native AA and DHA. Total and native AA and DHA were analyzed using our previous published method.\textsuperscript{34} Specifically, plasma was diluted 1:1 with 10% metaphosphoric acid (MPA) immediately after centrifugation of blood then vortexed followed by centrifugation at 2500 rpm for 20 min. For the analysis of total AA concentration, DHAA was reduced to AA by adding 50 µL of the supernatant with 150 µL dithiothreitol solution (0.25 g/dL in 0.1M trisodium phosphate) followed by allowing the solution to stand for 30 min at 4°C before re-acidification with 25 µL 40% MPA; the solution was subsequently mixed with 20 µL internal standard (homogentisic acid), 10 mg/L. For native AA determinations, 50 µL of the supernatant was mixed with 150 µL 5% MPA, 25 µL 5% MPA and 20 µL HGA (10 mg/L). Two microliters were added to wells of a 96-well microplate as a standard curve using GraphPad Prism 5 software (La Jolla, CA). Plasma and quality control samples were kept at -80°C, thawed immediately before use, and measured in duplicate on a 96-well magnetic plate.

ORAC reagent preparation. Trolox standard solutions were diluted in 75 mM phosphate buffer (pH 7) for the hydrophilic extracts and in 7% RMCD solution for the lipophilic extracts and prepared at concentrations ranging from 0.19 to 12.5 µM. A stock solution (stock #1) of fluorescein (FL), used as a fluorescence probe, was made by dissolving 0.0225 g in phosphate buffer. The final dilution of the lipophilic extract was 1:400.

Deproteinized ORAC Assay. To 50 µL of the 20x PBS-diluted plasma (from above) was added 50 µL 0.5M perchloric acid then centrifuged at 2000 x g for 10 min. 20 µL of the supernatant was diluted with phosphate buffer. The final dilution of the hydrophilic extract was 1:200.

Quantitative determination of Cytokines. Concentrations of plasma cytokines (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, GM-CSF, IFN-γ and TNF-α) were assayed using an ultrasensitive multiplex immunoassay (In Vitrogen, Camarillo, CA) per manufacturer’s instructions with slight reductions in sample and reagent volumes to maximize sensitivity and minimize interferences. Median fluorescent intensities of each cytokine were obtained using the Luminex\textsuperscript{®} 200\textsuperscript{TM} dual-laser based fluorescent analyzer (Luminex Corp., Austin, TX) and quantified against a standard curve using GraphPad Prism 5 software (La Jolla, CA). Plasma and quality control samples were kept at -80°C, thawed immediately before use, and measured in duplicate on a 96-well magnetic plate.

ORAC Assay. Determination of antioxidant activity was measured using the ORAC assay according to previously established protocols \textsuperscript{45} with slight modifications. Briefly, 100 µL PBS-diluted plasma (20x) was mixed with 50 µL 100% EtOH and 150 µL hexane. The mixture was vortexed, left to sit for 2 min, then centrifuged at 2000 rpm for 5 min. The organic (hexane) layer was removed and the extraction was repeated. The hexane layers were combined, dried under N\textsubscript{2} flow, then reconstituted in 200 µL 7% randomly methylated beta-cyclodextrin (RMCD) solution in acetone:water; 50:50 v/v; 20 µL of this mix was further diluted with phosphate buffer. The final dilution of the lipophilic extract was 1:400.

Hydrophilic ORAC Assay. The remaining aqueous layer (from above) was mixed with 100 µL 0.5M perchloric acid then centrifuged at 2000 x g for 10 min. 20 µL of the supernatant was diluted with phosphate buffer. The final dilution of the hydrophilic extract was 1:200.

Deproteinized ORAC Assay. To 50 µL of the 20x PBS-diluted plasma (from above) was added 50 µL 0.5M perchloric acid. The mixture was centrifuged at 2000 x g for 10 min. 20 µL of the supernatant was diluted with phosphate buffer. The final dilution of the deproteinized plasma was 1:200.

Deproteinized ORAC Assay. To 50 µL of the 20x PBS-diluted plasma (from above) was added 50 µL 0.5M perchloric acid. The mixture was centrifuged at 2000 x g for 10 min. 20 µL of the supernatant was diluted with phosphate buffer. The final dilution of the deproteinized plasma was 1:200.

ORAC reagent preparation. Trolox standard solutions were diluted in 75 mM phosphate buffer (pH 7) for the hydrophilic extracts and in 7% RMCD solution for the lipophilic extracts and prepared at concentrations ranging from 0.19 to 12.5 µM. A stock solution (stock #1) of fluorescein (FL), used as a fluorescence probe, was made by dissolving 0.0225 g in phosphate buffer; 50 µL of stock #1 was diluted in 10 mL phosphate buffer to make stock #2. 320 µL of stock #2 was diluted in 20 mL phosphate buffer for a final concentration of 14 µM (working solution). For the assays, 25 µL of sample or standard (trolox) were added to wells of a 96-well microplate and mixed with 150 µL FL working solution (substrate). The plate was incubated for 30 min at 37°C before addition of 25 µL AAPH (31.7 mM) to generate a peroxy radicals and initiate the reaction. The plate was shaken for 10 seconds and the fluorescence of each microwell was recorded at 1-minute intervals for 60 minutes at ambient temperature (37°C) using Gemini XPC fluorescence microplate reader (Molecular Devices, LLC Sunnyvale, CA). The final ORAC values were calculated using the trapezoid method equation:

\[ \text{AUC} = \frac{(f_0+f_1+f_2+\ldots+f_{60})}{f_0} \]
The corresponding net AUC was obtained as follows:
Net AUC = AUC\text{sample} - AUC\text{blank}

The isolation of lymphocytes from whole blood, calculation of organ and blood doses, and subsequent γ-H2AX detection was described previously.\textsuperscript{42}

Statistical Analysis

LPM+Q10, Redox status, ORAC, cytokines, and γ-H2AX. Data analyses were performed using SAS 9.3 statistical software (SAS Institute, Cary, NC) and/or Excel (Microsoft, Seattle, WA). Details were described previously.\textsuperscript{41,42} The significance level was set at $P < .05$.

Results

Characteristics of the participants have been described previously\textsuperscript{41} and are presented in Table 1. The children ranged in age from 3 months to 6 years. Twelve children received CT scans in the head region while the remaining children received CT scans of the abdomen (n = 3), neck (n = 1) or chest (n = 1) region. The CT and effective doses ranged from 92.46 to 525.55 mGy-cm, equivalent to 0.78 to 11.30 mSv, respectively.

In our previous report investigating in vivo changes in LPM levels,\textsuperscript{41} we observed significant decreases in post- versus pre-CT plasma levels of numerous LPM, which were in contrast to the increases (albeit non significant) noted in post-CT plasma levels of UN10 and UL10. These changes are shown in Table 2.

<table>
<thead>
<tr>
<th>Table 2. Pre- Versus Post-CT Changes in Plasma LPM Levels*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Analyte</strong></td>
</tr>
<tr>
<td>UL10 (nM)</td>
</tr>
<tr>
<td>UN10 (nM)</td>
</tr>
<tr>
<td>TQ10 (nM)</td>
</tr>
<tr>
<td>UN10/TQ10 (%)</td>
</tr>
<tr>
<td>tr LUT (ng/mL)</td>
</tr>
<tr>
<td>tr ZEA (ng/mL)</td>
</tr>
<tr>
<td>Tot. tr LUT/ZEA (ng/mL)</td>
</tr>
<tr>
<td>Tot. cis LUT/ZEa (ng/mL)</td>
</tr>
<tr>
<td>tr AH-LUT (ng/mL)</td>
</tr>
<tr>
<td>cis AH-LUT (ng/mL)</td>
</tr>
<tr>
<td>αCRX (ng/mL)</td>
</tr>
<tr>
<td>tr βCRX (ng/mL)</td>
</tr>
<tr>
<td>cis βCRX (ng/mL)</td>
</tr>
<tr>
<td>Tot.LYCOP (ng/mL)</td>
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<tr>
<td>tr LYC (ng/mL)</td>
</tr>
<tr>
<td>5 cis-lyc (ng/mL)</td>
</tr>
<tr>
<td>DHLYC (ng/mL)</td>
</tr>
<tr>
<td>αCAR (ng/mL)</td>
</tr>
<tr>
<td>tr βCAR (ng/mL)</td>
</tr>
<tr>
<td>cis βCAR (ng/mL)</td>
</tr>
<tr>
<td>Tot.βCAR (ng/mL)</td>
</tr>
<tr>
<td>Tot. CAROT (ng/mL)</td>
</tr>
<tr>
<td>βTOC (ng/mL)</td>
</tr>
<tr>
<td>β+γTOC (ng/mL)</td>
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<tr>
<td>αTOC (ng/mL)</td>
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<tr>
<td>Tot.TOC (ng/mL)</td>
</tr>
<tr>
<td>tr RET (ng/mL)</td>
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</tbody>
</table>

*n=17 children. Table re-used with permission from Halm, 2014.\textsuperscript{41}
Our study evaluating the effects of low dose IR from CT scans on lymphocytic γ-H2AX foci (marker of DNA damage) led to observations of dose-dependent increases in γ-H2AX foci post-CT exam ($P = .046$) among the 3 young children examined (patient ID 15, 16 and 17; Table 1). CT-induced mean IR blood doses of 0.22 to 1.22 mGy led to mean pre- to post CT increases of 0.96 to 1.95 foci per cell (Figure 1) with an average doubling (102%) of foci per cell between the lowest and highest IR dose.

None of the 10 cytokines showed significant post- to pre-CT changes (Table 3). Average AA levels increased significantly (6.19±4.75 vs 8.32±5.00; $P = .003$) while DHAA levels were decreased (3.54±3.27 vs 2.47±1.86; $P = .057$) post-CT with borderline significance whereas the redox status (DHAA/total AA) was dramatically lowered (43% to 30%) post-CT ($P = .008$; Table 4). The ORAC assay showed non-significant post-CT changes in mean lipophilic (4.46±2.51 vs 4.33±2.32; $P = .73$), hydrophilic (7.73±2.63 vs 7.45±1.29; $P = .70$) and deproteinated plasma extracts (8.20±3.05 vs 9.44±2.73; $P = .23$, Table 5).

![CT blood dose vs Pre- and Post-CT #foci/cell](image)

*Figure 1. Post-CT (red bars) versus pre-CT (blue bars) changes in lymphocytic γ-H2AX foci from 3 young children as a function of CT-induced IR dose (expressed in blood dose [mGy] and in effective dose [mSv]); the means of the average foci per cell are presented. Error bars represent standard deviations between means of blinded duplicate analyses. Figure re-used with permission from Halm, 2014.*

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Pre-CT</th>
<th>Post-CT</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM-CSF (pg/mL)</td>
<td>2.11-168.1</td>
<td>2.11-204.4</td>
</tr>
<tr>
<td>IFN-γ (pg/mL)</td>
<td>0.3-42.85</td>
<td>0.22-35.03</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>0.44-96.89</td>
<td>0.10-161.48</td>
</tr>
<tr>
<td>IL-10 (pg/mL)</td>
<td>0.16-2.92</td>
<td>0.15-4.27</td>
</tr>
<tr>
<td>IL-2 (pg/mL)</td>
<td>0.94-56.84</td>
<td>0.97±0.96</td>
</tr>
<tr>
<td>IL-4 (pg/mL)</td>
<td>1.67-76.13</td>
<td>1.6-114.84</td>
</tr>
<tr>
<td>IL-5 (pg/mL)</td>
<td>1.91-5.58</td>
<td>1.22-35.03</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>0.89-69.61</td>
<td>1.04-69.93</td>
</tr>
<tr>
<td>IL-8 (pg/mL)</td>
<td>5.36-150.94</td>
<td>3.64-53.15</td>
</tr>
<tr>
<td>IL-10 (pg/mL)</td>
<td>3.28-100.22</td>
<td>2.81-117.11</td>
</tr>
</tbody>
</table>

*N=17 children. **comparison of pre-to post-CT means using student’s t-test.
Table 4. Pre- versus post-CT changes in ascorbic acid, dehydroascorbic acid and total vitamin C*

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Pre-CT</th>
<th>Post-CT</th>
<th>(P^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid ((\mu g/mL))</td>
<td>0.63-14.14</td>
<td>4.30</td>
<td>6.19±4.75</td>
</tr>
<tr>
<td>Dehydroascorbic acid ((\mu g/mL))</td>
<td>0.66-15.18</td>
<td>3.06</td>
<td>3.54±3.27</td>
</tr>
<tr>
<td>Total Vitamin C ((\mu g/mL))</td>
<td>2.83-20.06</td>
<td>8.06</td>
<td>9.74±8.81</td>
</tr>
<tr>
<td>Dehydroascorbic acid/Total Vitamin C(%)</td>
<td>5-92%</td>
<td>43%</td>
<td>43±27%</td>
</tr>
</tbody>
</table>

\*N=17 children. **comparison of pre-to post-CT means using student’s t-test.

Table 5. Oxygen radical absorbance capacity of lipophilic, hydrophilic and deproteinated plasma pre- and post-CT extracts*

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Pre-CT</th>
<th>Post-CT</th>
<th>(P^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>lipophilic plasma extract ((\mu M))***</td>
<td>1.32-9.38</td>
<td>3.74</td>
<td>4.46±2.51</td>
</tr>
<tr>
<td>hydrophilic plasma extract ((\mu M))***</td>
<td>5.13-16.43</td>
<td>6.94</td>
<td>7.73±2.63</td>
</tr>
<tr>
<td>deproteinated plasma extract ((\mu M))****</td>
<td>3.40-15.20</td>
<td>6.99</td>
<td>8.20±3.05</td>
</tr>
</tbody>
</table>

\*N=17 children. **comparison of pre-to post-CT means using student’s t-test. ***1:400 diluted. ****1:200 diluted.

Discussion

IR from CT scans has been well documented to elicit a wide variety of detrimental cellular responses. IR such as x-rays are able to ionize surrounding atoms and molecules and, in the process, generate highly reactive free radicals. In humans, hydroxyl molecules are common targets of ionization due to the abundance of water in the body. The resultant hydroxyl radicals can damage relevant biological systems and can lead to DNA lesions, base damage, and protein cross-links all of which can lead to the induction of fatal cancers. In young children, the damage is most pronounced in children owing to their higher radiosensitivity, higher risk of cumulative exposure, and longer life expectancy than adults.

In this report, we reviewed and reported new findings from our pilot study that investigated whether low-dose IR from medically indicated CT scans would lead to plasma biomarker changes in young children. From our previous study, we observed significant decreases in all major LPM levels post-CT, which we deemed may have been due to the scavenging and degradation of free radicals, a process that would help to prevent cellular and tissue damage formed by the IR. Antioxidants such as tocopherols, carotenoids (e.g., lutein, \(\beta\)-cryptoxanthin, zeaxanthin) and CoQ10 (e.g., UL10) have been shown to remove peroxyl radicals (ROO·) or prevent the formation of hydro-peroxides from radicals such as singlet oxygen (\(O_2^*\)) thus interrupting the propagation of lipid peroxidation and, in the process, becoming radicals. The resulting antioxidant radicals can be considerably stabilized via aromatic delocalization and subsequently reduced back to non-radical forms by AA or other intracellular reductants.

None of the 10 cytokines analyzed showed significant pre- to post-CT changes. Although low dose IR can have anti-inflammatory effects and larger doses can possibly increase serum cytokine concentrations, it is possible that in our study either cytokine release was altered more than one hour after CT or the IR dose was too low to show any detectable effects on cytokine levels.

The parallel increase and decrease in AA and DHAA levels post-CT, respectively, (Table 4) may be due to the intracellular recycling of AA from its oxidized form (DHAA) to maintain adequate AA levels as a self-protection mechanism from irreversible decomposition or as a rebound effect through increased shredding from cellular pools into the circulation after blood levels decreased or as a result of cell death.

The non-significant changes in TAC of lipophilic, hydrophilic, and deproteinated plasma extracts in post-CT samples may indicate that the CT-induced IR did not compromise antioxidant capacity in the blood and suggests that other plasma antioxidant may have assisted in the quenching of free radicals, which would corroborate their decreased concentrations post-CT. Alternatively, the ORAC assay may not be sensitive or specific enough to detect the minimal changes in these antioxidant capacities. The employed ORAC method is an inhibition assay based on the antioxidant capacity of a sample to inhibit the thermally decomposed products of AAPH, an azo-radical initiator, namely alkyl-peroxy (ROO·) radicals. However, quantification of AAPH generated radicals from a previous electron paramagnetic resonance study showed that the thermal decomposition of AAPH generates alkyl-oxy (RO) rather than ROO· radicals thus indicating that the employed ORAC assay may not be scavenging the specific radicals.

As reported previously, the \(\gamma\)-H2AX foci analysis of the 3 children (Table 1) revealed a significant induction of \(\gamma\)-H2AX foci post-CT despite the very low IR doses used - effective doses as low as 1.57 mSv corresponding to a blood dose of 0.22 mGy. The IR doses applied in our study are much lower.
than other studies measuring γ-H2AX foci after CT exams 53,54 which demonstrates the high sensitivity of the employed γ-H2AX assay and indicates the reliability of the assay to evaluate the effects of low dose IR relevant to the general population.

At present, cancer risk estimations for low dose IR are based on the linear-no-threshold (LNT) model. This hypothetical model extrapolates cancer risks from well-verified moderate to high dose IR data from exposed populations (mostly Japanese atomic bomb and Chernobyl survivors) to lower IR dose ranges on the assumption that cellular effects such as DNA damage occur in direct proportion to IR exposure at all levels. In this context, the LNT model implies that no threshold level can be considered risk-free.55,56 Although our findings support the LNT hypothesis and imply a causal role of CT for the observed changes even at very low IR doses, these results are very preliminary and need to be confirmed with larger sample sizes.

Conclusion

The results of our pilot study suggest that low-dose IR has the ability to influence the antioxidant systems and trigger detrimental responses in young children undergoing CT scans. Many of the plasma LPM levels were decreased while dose-dependent increases in γ-H2AX foci (biomarker for DNA DSB) were observed. Children exposed to IR for diagnostic medical reasons are part of a large and growing population. When possible and appropriate CT should be replaced with non-ionizing techniques such as ultrasound or magnetic resonance imaging. Our findings need to be confirmed and expanded in future studies with larger sample sizes.

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Conflict of Interest

None of the authors declare any conflict of interest.

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References


