ORIGINAL ARTICLES

Serum Lipids, Proteins and Electrolyte Profiles in Rats Following Total Body Irradiation

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ABSTRACT

Objective: *Serum lipid and electrolyte imbalances are common in critically ill patients undergoing radiation therapy. Although multiple disease states and medication may be responsible for the development of these disorders, the aim of this research is to sequentially document the effect of total body radiation on body function utilizing the sequential changes in the serum lipids, electrolytes and protein in rats.*

Methods: *Serum protein and lipids contents were assessed using kits while electrolytes were assessed with flame photometry in rats exposed to total body irradiations of 1.27 Gy/min in cumulative doses to the fourth irradiation at five-day intervals.*

Results: *Total cholesterol and triacylglycerols serum levels were significantly reduced by irradiation (*$p < 0.05$*). No significant differences between experimental and control groups for HDL-C serum levels were detected. Serum electrolyte concentration remained within the normal range after each total body irradiation. Sodium, bicarbonate and chloride were significantly (*$p < 0.05$*) higher than control while potassium and creatinine were significantly reduced after the first irradiation only. Sodium/potassium ratio was significantly (*$p < 0.05$*) elevated. Serum protein was significantly (*$p < 0.05$*) elevated with increasing radiation.*

Conclusion: *There are subtle but significant changes in serum lipids, electrolytes and protein after total body irradiation of normal rats. These variations could be due to non-specific stress reactions; as such, they are important markers in radiation induced injury diagnosis.*

Keywords: Lipid profile, protein, radiation injury, serum electrolytes

Perfiles de Lípidos, Proteínas y Electrolitos Plasmáticos en Ratas tras Irradiación Corporal Total

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RESUMEN

Objetivo: *Los desequilibrios de lípido y electrolito plasmáticos son comunes en los pacientes críticos sometidos a terapia radioactiva. Aunque los múltiples estados de la enfermedad y la medicación pueden ser responsables del surgimiento de estos trastornos, el objetivo de esta investigación es documentar de manera secuencial el efecto de la radiación corporal total sobre la función corporal,*

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utilizando los cambios secuenciales en los lípidos, electrolitos y proteínas plasmáticos en las ratas.
Métodos: *Los contenidos de lípidos y proteínas plasmáticos fueron evaluados utilizando kits, en tanto que los electrolitos fueron evaluados mediante fotometría de llama en ratas expuestas a irradiaciones corporales totales de rayos X de 1.27 Gy/min, en dosis cumulativas hasta la cuarta irradiación en intervalos de cinco días.*
Resultados: *El colesterol total y los niveles plasmáticos de triacilgliceroles fueron reducidos significativamente por la irradiación (*$p < 0.05$*). No se detectaron diferencias significativas entre; os grupos experimentales y de control en relación con los niveles plasmáticos de colesterol HDL. La concentración de electrolito plasmático se mantuvo dentro de los límites normales luego de cada irradiación corporal total de rayos X. La relación sodio/potasio fue significativamente elevada (*$p < 0.05$*). La proteína plasmática se elevaba significativamente (*$p < 0.05$*) al aumentar la radiación.*
Conclusión: *Tras la irradiación corporal total de las ratas normales, se producen cambios sutiles pero significativos en los lípidos, electrolitos y proteínas del plasma. Estas variaciones podrían ser debidas a reacciones de estrés no específicas, y como tal, son marcadores importantes en el diagnóstico de las lesiones inducidas por la radiación.*

Palabras claves: Perfil lípido, proteína, lesión por radiación, electrolitos plasmáticos

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INTRODUCTION

X-rays are potent toxicants. Whole-body exposure to X-ray radiation can alter the general physiology of an animal (1). Radiation therapy (RT) has evolved to become one of the cornerstones of treatment for various types of cancers (2). Ionizing radiation inflicts its adverse cellular effects through the generation of oxidative stress factors that unleash large-scale destruction or damage of various biomolecules (3, 4). These free radicals react with body tissues and generate lipid peroxidation, DNA lesions and enzyme inactivation, all of which are mediators of radiation damage.

Radiation exposure alters cellular transport processes, which may induce nausea, vomiting, diarrhoea, pancytopenia and anorexia and contribute to the fluid and electrolyte imbalance during chemotherapy (5, 6). Other effects could include, decrease in salivary secretion which is accompanied by a rise in salivary sodium concentration (7), decreases in total body water, extracellular fluid space, plasma volume, plasma sodium concentration, with haemoconcentration (8), and electrolyte disturbances (9–11). Lehner *et al* (12) reported no changes in the levels of electrolytes, creatinine, urea, uric acid and acid or alkaline equivalents in irradiated animals while Holten and Christiansen (13) observed decreased serum protein and albumin levels which could be due to fragmentation and degradation (14).

Long-term radiation-associated changes in cholesterol concentration have been observed in the Japanese atomic bomb survivors (15), and in various occupationally-exposed groups (16, 17), presumably as a result of some change in liver metabolism. It has also been associated with coronary diseases and stroke (18–20). On the other hand, beneficial effects of extremely low frequency electromagnetic fields (ELF-EMF) have also been reported. In diet-induced hypercholesterolaemic rabbits, pulses of EMF lowered total cholesterol and triacylglycerol levels (21). Similar results have been found in rats (22) and mice (23) fed on control diets. In this study, we aim to sequentially document the effect of acute cumulative total body irradiation on body function utilizing the sequential changes in the serum lipids, electrolytes and protein in rats.

SUBJECTS AND METHODS

Male Wistar rats weighing between 180–250 g aged 5–7 weeks were obtained and kept at the Animal House of the Faculty of Medical Sciences for this study. During the entire treatment period, animals were kept at room temperature of 28 ± 2°C with 12-hour light/dark cycles. The rats were fed with standard rat chow and water *ad libitum*.

The animals were randomly assigned to five cages of five animals each; with group 1 serving as control and receiving no radiation, Groups 2, 3, 4 and 5 were irradiated once, twice, three times, and four times, respectively. The time interval between total body radiations was five days apart. An X-ray generator (200 kV and 20 mA, Villa Systemi Medicali, Italy) with a filter (0.5 mm Cu and 0.5 mm Al) was used for the experiments. The dose of radiation determined using a dose meter was 1.27 Gy/min. The experiments were conducted according to international protocols for the use of animals in experimental studies and the study received ethical approval from the Faculty of Medical Sciences Ethics Committee, Madonna University, Nigeria.

The rats were sacrificed under pentobarbital (50 mg/kg body weight) anaesthesia immediately after the final irradiation for the group. Their blood was taken from the abdominal aorta, immediately centrifuged at 5000 × *g* for 3 minutes, and the plasma was prepared. Thus, in the test group, a set of five rats received radiation once, twice, thrice and four times, which may be regarded as test groups one, two, three and four, respectively.

Total cholesterol, triglycerides and HDL-cholesterol were analysed enzymatically using kits obtained from Randox Laboratories Limited, Crumlin, United Kingdom (UK). Plasma LDL-cholesterol was determined from the values of total cholesterol and HDL-cholesterol using the Friedewald's formula, LDL-C = TC–(HDL-C)–[triglyceride/5] (24).

Using an automated method, 1 to 200 dilutions with deionised water was made for each sample and standard before being flamed. The concentration of sodium, potassium bicarbonate, chloride, urea and creatinine was obtained *via* an emission flame photometer. Serum levels for total protein were measured by using an enzymatic kit (Randox Laboratories, UK)

Normally distributed data were analysed using parametric tests, *ie* Student's paired *t*-test and analysis of variance (ANOVA). A value of $p < 0.05$ was considered significant. All statistical analyses were performed using the computer programme GraphPad Prism software version 5 (GraphPad Software, San Diego, California, USA). The data are presented as the mean ± standard error of mean (SEM) for the individual groups.

RESULTS

Results for the serum electrolyte values are presented in the Table. The sodium values were significantly ($p < 0.05$) increased with the first and second radiation; values for the third and fourth radiations, though increased, were not statistically significant. The same was observed for the chloride ion (Cl-) levels, bicarbonate ion (HCO^{3-}) levels which were significantly ($p < 0.05$) elevated after the first radiation but not significant by the second, third and fourth radiations. Potassium ion (K^+) and creatinine (CRT) levels were only significantly ($p < 0.05$) reduced after the first irradiation. Serum urea values showed no significant change with radiation exposures, while the protein content was significantly ($p < 0.05$) elevated only after the fourth radiation exposure (Fig. 1).

There were significant differences between experimental and control groups for triacylglycerols (113.44 ± 12.37 *vs* 53.20 ± 7.62) and total cholesterol serum levels (132.80 ± 11.21 *vs* 95.83 ± 7.19) after the first exposure (day 5); values for the triacylglcerols were significantly higher with the second [day 10] (46.13 ± 8.35) and third [day 15] (82.54 ± 4.97) radiation exposures but not with the fourth (day 20) radiation exposures [$p < 0.05$] (Fig. 2). However, the cholesterol values were not significant with the second and third exposures but with the fourth exposure (101.82 ± 6.02) [$p < 0.05$] (Fig. 3). There were no significant differences between experimental and control groups for HDL-C concentrations, and serum levels (Fig. 4). For the LDL cholesterol levels, values observed were lower for days 5 and 20; these values were computed from the Friedewald formula (24) [Fig. 5].

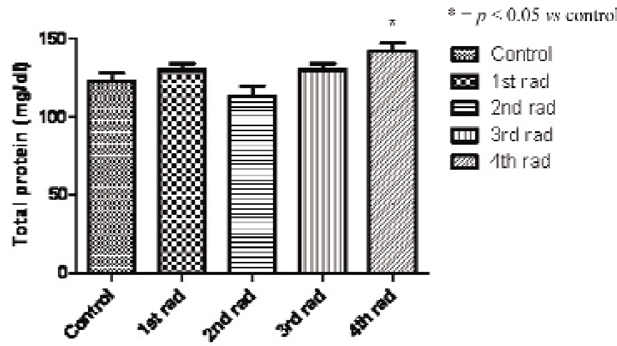

Fig. 1: Serum total protein levels in control and experimental groups.

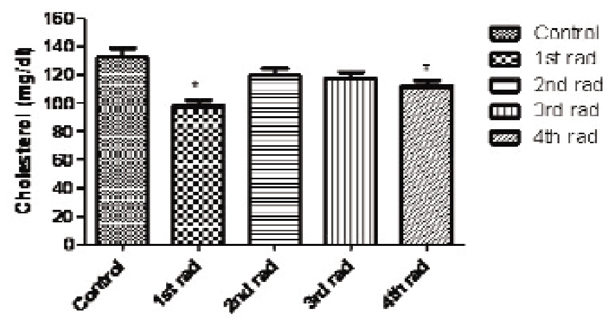

Fig. 2: Serum total cholesterol levels in control and experimental groups.

DISCUSSION

Table : Serum electrolytes values in rats following total body irradiation; results presented as mean ± SEM

(mmol/l)	control	1st irradiation	2nd irradiation	3rd irradiation	4th irradiation
Na^+	127.83 ± 7.12	139 ± 1.65*	136 ± 2.37*	135.14 ± 3.18	129.14 ± 4.53
K^+	11.5 ± 1.94	7.11 ± 0.54*	8.26 ± 0.49*	7.9 ± 0.64*	9.47 ± 1.28
HCO^{3-}	17.33 ± 2.12	19.86 ± 0.91*	18.43 ± 0.61	18.86 ± 1.37	16.29 ± 0.89
Cl^-	103.83 ± 7.10	113.57 ± 2.17*	111 ± 2.94	104.86 ± 2.96	101.57 ± 2.33
Urea	7.27 ± 0.79	6.69 ± 0.15	7.79 ± 0.49	7.34 ± 0.29	7.37 ± 0.49
Creatinine	40.83 ± 8.38	32.71 ± 1.67*	40 ± 4.50	31.71± 3.53	41.14 ± 2.30
$Na^+:K^+$	11.16 ± 3.67	19.55 ± 3.06*	16.46 ± 4.84	17.11 ± 4.97	13.64 ± 3.54

* $p < 0.05$ compared with control values

Na^+ = sodium ion; K^+ = potassium ion; HCO^{3-} = bicarbonate ion; Cl^- = chloride ion

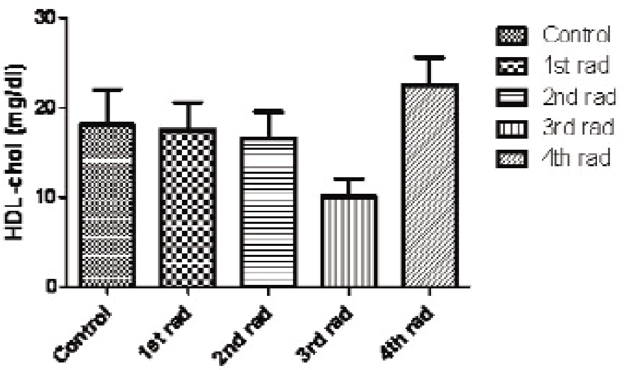

Fig. 3: Serum HDL-cholesterol levels in control and experimental rats.

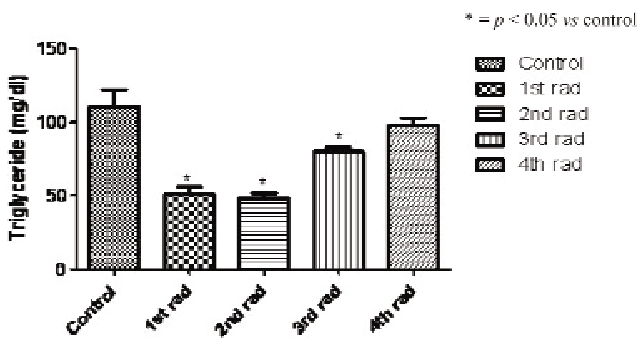

Fig. 4: Serum triglyceride levels in control and experimental rats.

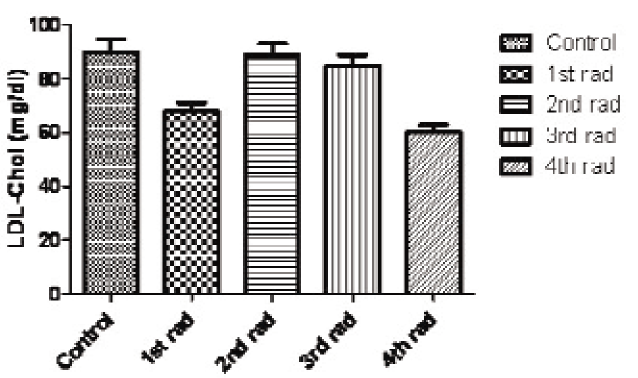

Fig. 5: Serum LDL-cholesterol levels in control and experimental groups.

In the present study, we used the rodent model to test the time-course effects of consecutive exposure to X-ray radiation on serum lipids, electrolytes and proteins. There were significant differences between experimental and control groups for most of the measured indices.

In our studies, we observed an increase in the sodium, bicarbonate and chlorine serum concentrations which was consistent even after the fourth radiation exposure whereas the potassium and creatinine concentrations were reduced; the values for urea fluctuated but were not of any statistical significance. The observed increase of sodium concentration is at variance with Geraci *et al* (8) who have reported significant decrease in plasma sodium concentration which they attributed to a radiation-induced breakdown of the intestinal mucosa. Electrolyte homeostasis is usually a significant function of the kidney, the increase in plasma sodium and decrease in potassium ion levels might be attributed to alterations in renal homeostatic mechanisms. There is substantial evidence regarding the usefulness of electrolyte measurement in the management of disease. The sodium-potassium (Na:K) ratio has frequently been used as a diagnostic tool to identify different disease conditions (25). In the present study, there was a significantly high Na:K ratio in the experimental groups when compared with the control. The elevated sodium/potassium ratio was significant with the first radiation and could be associated with acute stress and inflammation.

Total serum proteins are diagnostically of relative importance in assessing the state of health of the organism, their increase appearing especially in inflammatory processes and tissue dysfunctions (26). Holten and Christiansen (13) had reported that irradiation causes decreased serum protein and albumin levels; our observations were that serum protein levels varied within normal ranges but were significantly elevated with the fourth cumulative radiation exposure.

Lehner *et al* (12) had reported that radiations did not cause any changes with creatinine, urea and uric acid levels. The observations, in the present study, of no significant elevation of serum urea concentrations are in accordance with this; however, we observed a significant reduction in the serum creatinine values after the first radiation, which became normal with the other radiation exposures.

Torres-Duran *et al* (27) had observed no changes in total cholesterol and triacylglycerol serum levels following exposure to radiation. They opined that exposure played no vital role in the serum lipid profiles alteration. Other researchers had reported that cholesterol and triglyceride plasma levels decreased following exposure (21, 28, 29). However, Bellossi *et al* (22, 30) also reported a rebound in triglyceride level 48 and 96 hours after the end of the exposure and concluded that these alterations may be due to a reversible accumulation of either triglycerides or their precursors. Such discrepancies may be indicative of differences in sensitivities among the applied methods of analysis or indicative of significant discrepancies in health consequences after comparable total exposures of different populations under varying conditions (31).

No significant differences were observed between experimental and control groups for serum levels of HDL-C concentrations at any time analysed. Total serum cholesterol levels were significantly reduced with the first and fourth radiation exposure, while triacylglycerols were significantly reduced with the first, second and third radiation exposure. The results of the present study are partially in accordance with these reports (21, 22, 28–30).

The observation of some form of rebound from the measured indices of serum lipids, electrolytes and proteins following increasing doses of radiation may be a form of homeostatic mechanism within the body as it seems to be better equipped to chronic rather than acute stressors such that radiation falls under; an adjustment of these body systems and mechanism can be a method of adaptation to these stressors.

The results indicate that the applied ionizing radiation exposure may induce slight but statistically significant alterations in some serum lipids profile of rats, within the physiological limits. Electrolytic disorders are significant for diagnosis of radiation induced injury and can be important for evaluation of its severity and correct management. The mechanisms for the effects of these ionizing radiations on lipid metabolism are not well understood yet, but they may be associated with non-specific cellular stress reactions.

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