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Simultaneous effect of gamma and Wi-Fi radiation on gamma-H2Ax expression in peripheral blood of rat: A radio-protection note

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ABSTRACT

Introduction: Nuclear medicine patients are isolated in a room after the injection of a radiopharmaceutical. They may be active Wi-Fi option of its smartphone mobile or other environmental radiofrequency waves. The hypothesis of this study was the evaluation of increased biological effects of the simultaneous exposure to gamma-ray and the Wi-Fi waves by measuring the level of the increased double strand-breaks DNA in peripheral blood lymphocyte in the rat.

Materials and methods: Fifty male Wistar rats were exposed for 2, 24, and 72 h only by Wi-Fi, ^{99m} Tc, and simultaneously by Wi-Fi and ^{99m} Tc. The power density levels of Wi-Fi emitter at 15 cm was 4.2nW/ cm^2. An activity of 100 μCi of ^{99m} Tc was injected intraperitoneally. Blood samples were taken by cardiac puncture following general anesthesia. Mononuclear cells are extraction by Ficoll-Hypaque density gradient centrifugation. The number of gamma-H2AX foci per nucleus was counted by flow cytometry. The statistical differences between experimental groups at 2, 24, and 72 h were determined with a repeated measure's analysis of variance. The significant difference between groups at the same time was analyzed with the Kruskal-Wallis Test.

Results: The manner of gamma-H2AX expression was not the same for three groups in time. The number of gamma-H2AX foci between the three groups was a significant difference after 72 h.

Conclusion: Simultaneous Wi-Fi and gamma-ray exposures can increase the number of double-strand break DNA in peripheral blood lymphocytes to exposure of gamma-ray to 72 h after technetium injection in the rat.

1. Introduction

The number of nuclear medicine examinations is increasingly growing, and the principles of radiation protection are important in order to reduce the risk of radiation [1]. Eventually, the best and safest practices will be brought into clinical reality. Wi-Fi systems have been increasingly applied over the last two decades [2]. Laptop computers and mobile phones connected to the internet through Wi-Fi have been increasingly used recently. The biological effects of radiofrequency on the molecular, cellular, and tissue levels have been reported. For instance, Wi-Fi radiation decreases human sperm motility, increase's sperm DNA fragmentation [3,4], and effects microRNA expression in brain tissue [5].

Radiation-induced DNA double-strand breaks (DSBs) lead to H2AX phosphorylation called gamma-H2AX foci. The assessment of gamma-H2AX foci can be introduced as a sensitive biomarker of DSB after exposure to ionizing radiation [6]. The gamma-H2Ax assay is used to count radiation-induced DNA DSBs in peripheral blood lymphocytes (PBLs) in the evaluation of both diagnostic and therapeutic medicine modalities such as radiotherapy [7–9], computed tomography [10–13], diagnostic nuclear medicine [14] and radionuclide therapy [15–17].

Related previous studies have researched the final biological effect of

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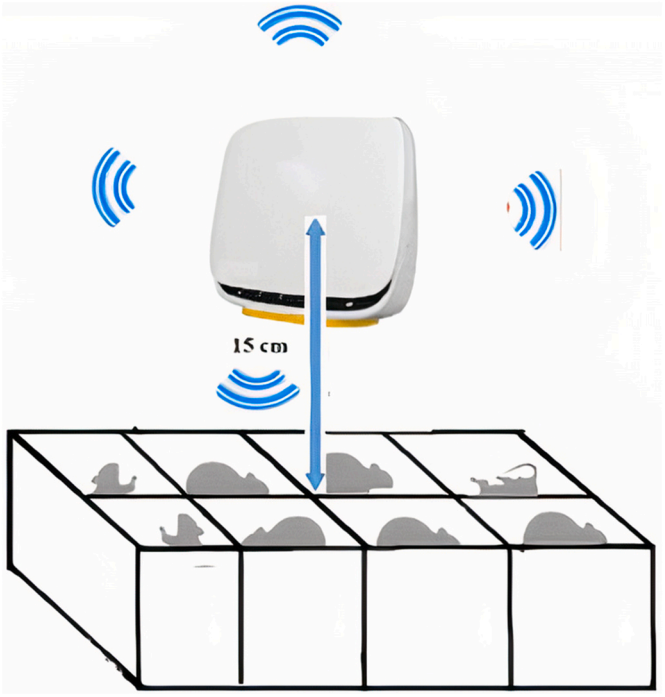


Fig. 1. The rats were in a plastic cage 55 × 32 × 20 cm, but not free to move with restrictions in the cage during exposure. Wi-Fi emitter emitted a continuous wave for 2, 24 h, 72 h, frequency 2.450 GHz frequency band, power density = 4.2 nW/m2 at 15 cm. The cage was surrounded with electromagnetic absorber material backed by metal to isolate outdoor electromagnetic fields from the test setup during exposure.

Table 1
Descriptive characteristics of FITC.

		time						pvalue
		2h		24		72		
		mean	std	mean	std	mean	std	
group	Wifi	42.58	1.74	41.83	1.21	43.74	4.22	0.048
	Tc	44.16	1.44	43.55	1.16	42.34	0.72	
	Wifi-tc	44.06	1.11	43.45	1.17	46.06	0.74	
p-value		0.042						

ionizing radiation, (e.g., gamma-ray, x-ray) and non-ionizing radiation, (e.g., Wi-Fi) on the cell, tissue, and human body separately. However, nuclear medicine patients after injection of radiopharmaceutical may be exposed to both types of radiation simultaneously [18,19].

The probable effects of Wi-Fi from smart mobile phones on nuclear medicine patients who use them are still not fully known; no organization has proposed restrictions on internet access or cell phone's use in nuclear medicine centers. In this ex vivo study, we evaluated the DNA double-strand breaks by measuring gamma-H2Ax foci per cell values in the PBL of rats simultaneously exposed to gamma-rays of technetium-99 m (^{99m} Tc) and Wi-Fi radiation.

2. Materials and methods

2.1. Animal samples

In this study, eighty five-week-old male Wistar rats (n = 5 per group, aged 8–9 weeks and weight 290 ± 20 g) were maintained and stored under standard conditions. They were in a plastic cage, with a 12-h light/dark cycle, with free access to water and food *ad libitum*, an ambient temperature of 22–25 °C and relative humidity of 45%. Rats were acquired from the Laboratory Animal Center in the Pharmacy School, Kermanshah University of Medical Sciences, Iran. All procedures were approved by the Ethical Committee of the Kermanshah University of Medical Sciences (ethics code No.: 1396.463).

Rats were randomly divided into 12 groups:

Group I: the control group without ionization and non-ionization irradiation

Groups II-IV: the groups with Wi-Fi irradiation at 2, 24 and 72 h respectively.

Groups V-VIII: the groups with gamma irradiation from ^{99m}Tc at 2, 24 and 72 h respectively

Groups IX-XII: the groups with simultaneous gamma from ^{99m}Tc and Wi-Fi irradiation at 2, 24 and 72 h respectively.

The rats were anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg) and were sacrificed after 2, 24 and 72 h after intervention with Wi-Fi or gamma-ray irradiation. Blood samples were collected by cardiac puncture from sacrificed rat and immediately transferred in sample tubes containing heparin with strictly aseptic technique and stored on ice.

2.2. Gamma emission

The ^{99m}Tc without any pharmaceutical carriers was injected intraperitoneally with 100 μCi activities to induce DNA double-strand break and phosphorylation of H2Ax in peripheral blood of rat.

2.3. Wi-Fi exposure facility

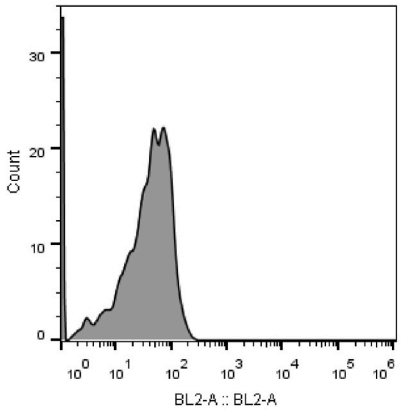
In this experiment, the signal generator system consists of a Wi-Fi emitter (IRAN-CELL company, Iran) which emitted a continuous wave and 2, 24 h, 72 h was determined according to previous studies, frequency 2.450 GHz frequency band, power density = 4.2 nW/m2 at 15 cm. Details of Wi-Fi radiation and rat positioning were described as in Fig. 1. Power of the desktop Wi-Fi was device checked by RF-meter and presented 30 dbm (Aaronia Spectran HF-4060, Germany).

2.4. Peripheral blood mononuclear cells isolation

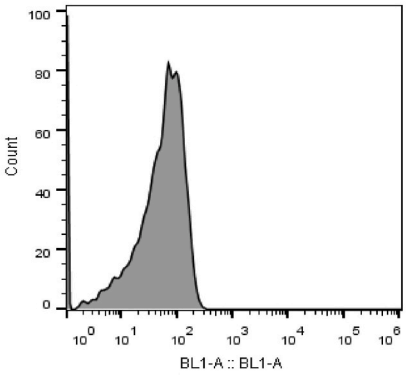
Using gradient centrifugation method, the peripheral blood mononuclear cells (PBMCs) were isolated as follows at Room Temperature (RT). Two ml of blood samples transferred into a heparinized tube and 2 cc of PBS was added to it in Falcon tubes (15-mL). Then, the prepared blood–PBS mixes in the previous stage, slowly was poured on the 4 cc of Ficoll-Paque in a Falcon tube (50-mL). After the centrifugation of mixed solution at a relative centrifugal force of 700×*g* for 25 min, the thin layer containing PBMCs carefully collected with a sampler. The cells washing was performed twice with 10 mL of RPMI-1640 medium (Sigma, USA) supplemented with 10% fetal bovine serum (FBS; Bio idea, Iran) and Peni- Strep (Gibco) by centrifuging at 600×*g* for 5 min.

2.5. Flowcytometry analysis

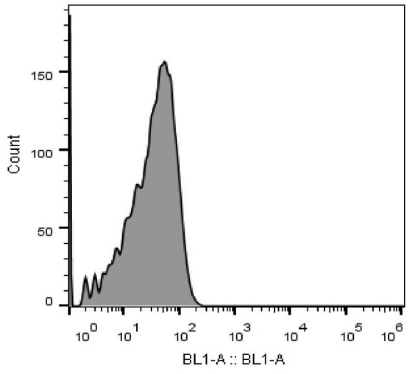
Using Neubauer hemocytometer, 10^2 lymphocytes per sample counted, collected and transferred to a 15-mL Falcon tube. The counted lymphocyte cells were washed with PBS twice and centrifuged in each washing stage. Instantly, the fixation process was performed 1 mL of paraformaldehyde (4%) solution for 10 min in room temperature. The permeabilization of the antibody in the cell was used with 1 mL of prechilled ethanol 70% (−20 °C) for 20 min. The washing stage was done again. Without any agitation, the cells were incubated with 10 μL of anti-gH2AX antibody (Cell Signalling Tech., USA) at 400 × dilution in PBS/1% BSA for 1 h. The incubation was applied with 10 μL of goat anti-mouse Alexa Fluor-488 antibody at 1000 × dilution in PBS/1%BSA for 30 min after washing once. The cells were washed with PBS but once in this stage. In all mentioned procedures from counting to add both antibodies, the Centrifugation was at 500×*g* for 5 min at 4 °C and the washing was done by 1 mL of ice-cold PBS. The PBMCs were incubated



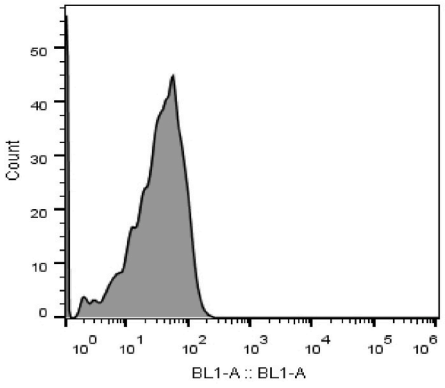
A) Control group



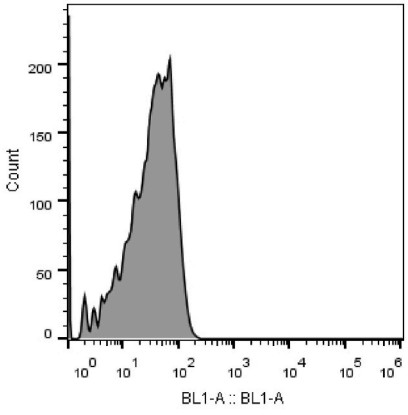
B) Wi-Fi -2h



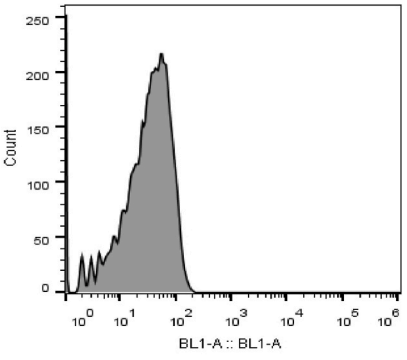
C) Wi-Fi- 24 h



D) Wi-Fi- 72 h

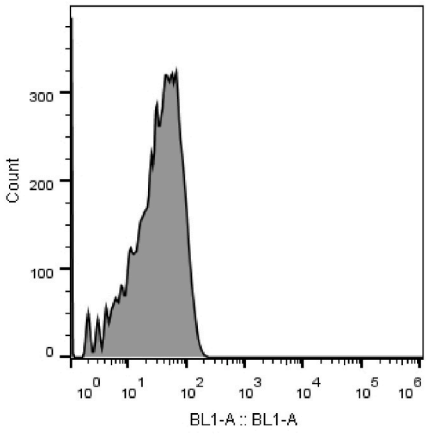


E) Tc – 2h

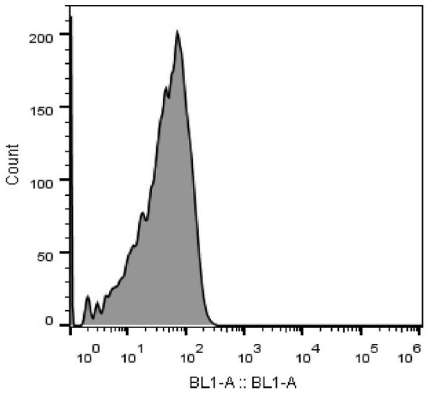


F) Tc – 24h

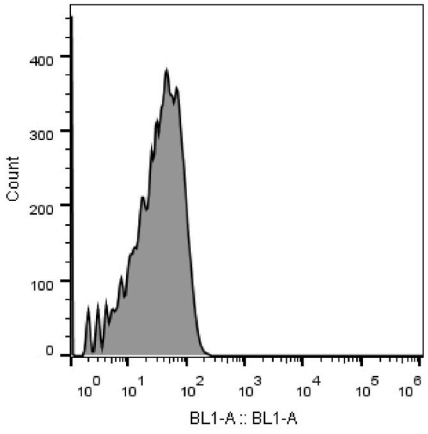
Fig. 2. Some histograms of counted gamma-H2Ax foci through different groups: A) Control without gamma and Wi-Fi radiation, B) Wi-Fi -2h, C) Wi-Fi −24h, D) Wi-Fi −72h, E) Tc – 2h, F) Tc −24h, G) Tc −72h, H) Wi-Fi and Tc −2 h, I) Wi-Fi and Tc −24 h, J) Wi-Fi and Tc −72 h.



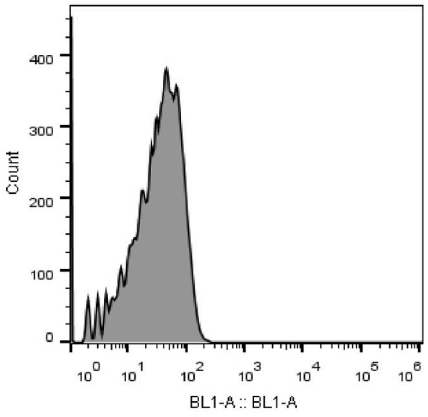
G) Tc-72h



H) Wi-Fi and Tc – 2 h



I) Wi-Fi and Tc –24 h



J) Wi-Fi and Tc –72 h

Fig. 2. (*continued*).

for a few minutes at RT before analyzing. Then, the samples were analyzed using Flow Cytometer (Attune ™ NxT Acoustic Focusing Cytometry, Life Technologies, Applied Biosystems, US). A 488-nm blue light excitation laser was used to analyze, and FlowJo 10 software was used to analyze the flowcytometry data.

2.6. Statistical analysis

Statistical differences in obtained results were considered significant at ($p < 0.05$) using a computer program (SPSS 15.0, SPSS Inc. Chicago, IL, USA). The normal distribution of data was checked with the Shapiro Wilk's test [20] and the statistical differences between experimental groups at 2, 24 and 72 h were determined with a repeated measure's analysis of variance. Pairwise comparisons between groups were performed with Tukey's Honestly Significant Difference (HSD) test. The significant difference between groups at the same time was analyzed using the Kruskal-Wallis Test.

3. Results

Descriptive statistics for our sample are presented in Table 1. Normality of data was assessed using Shapiro's test. The FITC intensity distribution was normal ($p < 0.05$) for all groups. The Mauchly's Test of Sphericity as a precondition of a repeated measure's analysis of variance (ANOVA) was investigated. Therefore, the Greenhouse–Geisser correction was used in statistical analysis. The results showed that the interaction between time-group effects were not significant ($p > 0.05$). It means, the manner of gamma-H2Ax expression in three groups was not the same with time but the time and group was significant ($p < 0.05$).

Data analysis such as gating of lymphocyte and histograms are represented in Fig. 2. The mean FITC intensity was compared for all groups. The relative percentage of gamma-H2AX cells in the PBL is higher among all groups at all times than the control group and Wi-Fi ($p < 0.001$). This percentage was not significantly different between the three groups at 2 and 24 h. The result of this study showed that the FITC intensity differences were significant in three groups at 72 h ($p = 0.04$). In the other words, the Induced gamma-H2Ax foci values for simultaneous

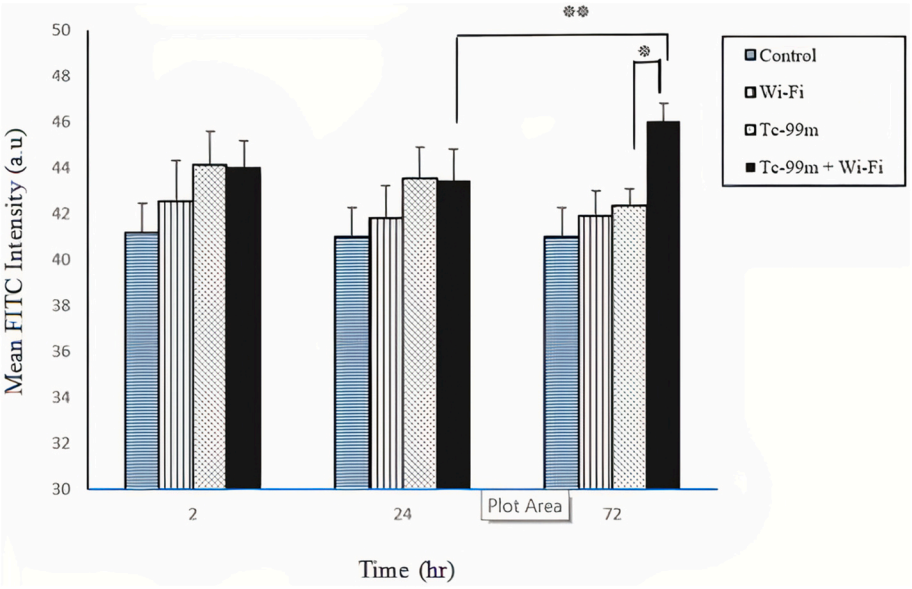


Fig. 3. Comparison of Mean FITC intensity (a.u) in blood lymphocyte cells in exposed rats to Wi-Fi, ^{99m}Tc, and Wi-Fi/^{99m}Tc at 2, 24 and 72 h irradiation using Kruskal-Wallis Test.
*, ** Significant difference was obtained for Wi-Fi /^{99m}Tc rather than ^{99m}Tc and Wi-Fi respectively ($p = 0.04$).

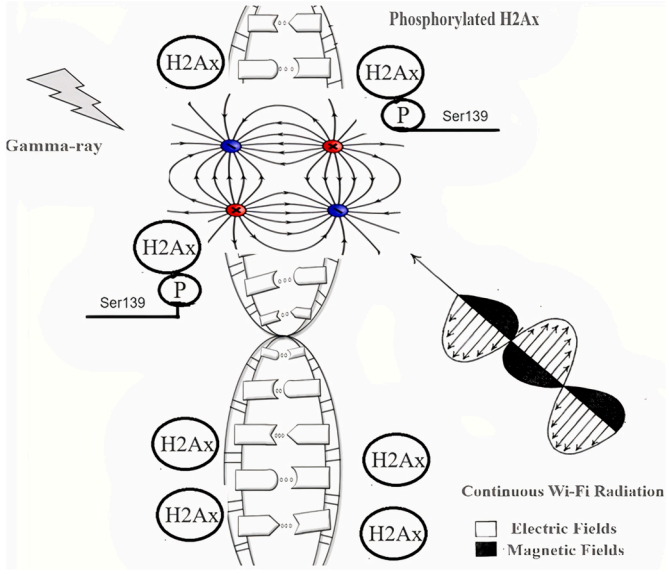


Fig. 4. Ionization radiation-induced DSBs generate quadruple-like static electric fields and the electric field lines as in this figure. The magnetic field of Wi-Fi wave penetrates in the depth of tissues more than the electric field. It may be able the reorientation of charged sticky end of strand-break DNA according to the equation of force for the magnetic field on moving charges.

Wi-Fi and gamma irradiation was significantly increased rather than only gamma irradiation and Wi-Fi wave after 72 h (Fig. 3).

4. Discussion

In this study, the FITC intensity was measured or in the other words induced gamma-H2Ax foci values resulted from the DNA double-strand breaks in the PBL of exposed rats with gamma-rays of ^{99m} Tc, and Wi-Fi radiation, and both them simultaneously. Significant differences the FITC intensity in three groups at 72 h is important because, it can be interesting in nuclear medicine patient who uses the Wi-Fi internet of the smartphone after administration of radionuclide to diagnosis or therapeutic test. These patients may be simultaneously exposed to ionization (e.g. gamma-ray) and non-ionization radiation (e.g. Wi-Fi). To discuss these phenomena, It should be reviewed know the Wi-Fi is electrodynamics wave with variable attitude and the activity of ^{99m} Tc is decreasing through time. Furthermore, the dependent variant is the gamma-H2Ax expression which itself is not constant in time. Looking at the independent and dependent variables, it is clear; the interpretation of the flowcytometry outcome can be a complicated discussion. Non-significant interaction between time-group effects is related to a complication of dependent and independent parameters and different mechanism of Wi-Fi wave and gamma-ray of ^{99m}Tc with molecular and cellular levels.

In the similar study, Arruda-Neto et al. evaluated the viability of a strong electrostatic field for 2 h to the cells that exposed to 3 kGy of the gamma-ray from ^{60}Co in an in vitro experiment. They explained the reorientation of repair proteins such as gamma-H2Ax as an electric dipole in the electric field in capacity [21]. In this study, the electric component of the electrostatic wave is weaker than electrostatic field Arruda's. As a general scientific principle, the penetration of the electric field is less than the magnetic field in the tissue of the rat. In result, the magnetic field is probably more important, particularly in our interoperation. Although, the electric component is stronger than the magnetic field. Hence, irradiation condition and type of the study were different in both them to compare the results but, in this study, their model is borrowed for further analysis, which is as follows: Ionization radiation-induced DSBs generate quadruple-like electric fields. Spontaneous recombination may happen after each radiation induced-strand break in DNA. To the best of our knowledge his phenomenon may be disturbed by irradiation of a magnetic field to the cell. Therefore, the magnetic component of Wi-Fi wave may be able the reorientation of the charged end of strand-break DNA according to the equation of force for the magnetic field on moving charges. This may be caused to decreasing chance of probable recombination and however, increasing gamma-H2Ax expression level (Fig. 4).

This study is the first to focus on the possible radiobiological effect of simultaneous irradiation Wi-Fi and gamma-ray in PBLs of the rat. In future research, it can be used another radiopharmaceutical and biological endpoint such as dicentric or micronuclei chromosomal aberration. Furthermore, it is suggested the evaluation simultaneous effect of low-LET radiation and radiofrequency wave in nuclear medicine therapy or different cancer cell line in radiation therapy. Also, using a large number of rat in groups, the parametric test can help to amplify statistical analysis and more validate results.

5. Conclusion

Simultaneous Wi-Fi and gamma-Ray exposures can increase the number of double-strand break DNA relative to exposure of gamma-Ray to 72 h after technetium injection in PBLs of the rat. It is possible, using the Wi-Fi in smartphone mobile after injection of gamma–emitter radiopharmaceuticals in nuclear medicine patient lead to more induced-radiation biological effect.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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Abbreviations

LET	Linear Energy Transfer
PBS	Phosphate Bovine Serum
BSA	Bovine Serum Albumin
PBL	Peripheral Blood Lymphocyte
99mTc	Technetium-99 Metastable
DSB	Double Strand Break
FBS	Fetal Bovine Serum
RT	Room Temperature

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