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ORIGINAL ARTICLE

EFFECTS OF DIFFERENT RADIATION DOSES ON *ESCHERICHIA COLI*

Semiha Pelin Kulaksız[1], Celal Çağlar[1], Huda Avvad[1], Shukran Alhmidi[1], Mohamad Ali Alhussein[1], Sheven Huseen[1], Alaattin Özen[2]

[1]Eskişehir Osmangazi University School of Medicine, Eskişehir, TÜRKİYE
[2]Eskişehir Osmangazi University School of Medicine, Department of Radiation Oncology, Eskişehir, TÜRKİYE

ABSTRACT

Aims: The purpose of this study was to investigate the impact of various radiation doses on cellular elements using electron microscopy and to determine if non-irradiated cells in the same environment were also affected. Radiotherapy treatment is widely used for different types of cancers as it can prevent cell division and death through direct and indirect mechanisms.

Methods: To conduct the study, standard strain *Escherichia coli* bacteria were reproduced in six Petri dishes, with one serving as the control. The other dishes were divided into two halves, and increasing radiation doses (2, 3, 6, 10, and 20 Gray) were administered to one side of each dish. Using a transmission electron microscope, the samples were examined, and changes in the cell elements were recorded.

Results: The findings indicated that as the radiation doses increased, the negative effects on the bacteria were more pronounced. In the samples exposed to higher doses of 10 and 20 Gray, the bacteria's integrity was significantly impaired, resulting in a mud-like structure that made the evaluation of cell elements challenging. Furthermore, the non-irradiated area showed similar morphological deteriorations but at a lesser rate.

Conclusion: This study demonstrated the effects of various radiation doses on *Escherichia coli* bacteria and the impact on non-irradiated cells in the same environment, as seen through transmission electron microscopy.

Keywords: Cellular morphology, *Escherichia coli*, quorum sensing, radiation

INTRODUCTION

Radiotherapy is a cancer treatment that can halt cell division and cause cell death through direct and indirect mechanisms. The direct mechanism works by damaging DNA, while the indirect mechanism relies on the action of hydroxyl and superoxide radicals. Furthermore, damage to both tumors and healthy tissues can occur through communication between cells outside the targeted radiation zone. This phenomenon is referred to as the abscopal effect and the bystander effect (1-3). In certain cases, radiotherapy can produce a systemic anti-cancer response, which is known as the abscopal effect. Studies have suggested that radiotherapy-induced DNA damage may activate the immune system, leading to this effect. The abscopal effect is the ability of localized radiation to trigger an anti-tumor response in areas of the body that were not directly targeted by the treatment (4). The bystander effect of radiotherapy, on the other hand, refers to the biological effects of radiation on cells whose nuclei were not directly irradiated. This effect can cause DNA damage, chromosomal instability, mutation, and apoptosis. Bystander signals can also modify the balance between proliferation, apoptosis, quiescence, or differentiation (2). In the present day, immune-oncology is employed to treat cancer patients, and the abscopal effect on cancer cells has been observed in this setting. Furthermore, the bystander effect has been implicated in the development of radiation pneumonia, especially in areas of the lung that have not received radiotherapy (1-5). Cell-to-cell communication is vital for the survival of non-irradiated cells, which can result in non-targeted effects where untreated or uninfected cells exhibit effects induced by signal

Address for Correspondence: Semiha Pelin Kulaksız, Eskişehir Osmangazi University School of Medicine, Eskişehir, TÜRKİYE
e-mail: pelinklksz@icloud.com
ORCID iDs of the authors: SPK: 0000-0001-7816-5057; CÇ: 0000-0002-4875-6400; HA: 0000-0002-8319-2516; SA: 0000-0001-9974-3434; MAA: 0000-0003-3740-4642; SH: 0000-0003-1487-6807; AÖ: 0000-0001-9219-6001.
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transduction. Tunneling nanotubes are significant in triggering apoptosis in non-targeted cells as a consequence of irradiation (6). Studying the structural and indirect effects of radiotherapy on cells using electron microscopy can be beneficial for medical education and encourage new research. The purpose of this observational study is to investigate the effects of different radiation doses on cell structures through transmission electron microscopy (TEM) and assess any effects on non-irradiated cells in the same environment. We chose to focus on the E. coli strain due to the relatively higher cost associated with preparing cell culture.

MATERIAL AND METHODS

Our study was supported by Eskişehir Osmangazi University Scientific Research Projects Commission with project number 2020-2983. Standard strain *Escherichia coli* bacteria were reproduced at the end of 12-24 hours in the ETUV device in a total of 6 Petri dishes in Eskişehir Osmangazi University Faculty of Medicine, Department of Microbiology. Each Petri dish was divided in half symmetrically with the help of a marker. Then, 2, 3, 6, 10, and 20 Gray (Gy) doses were administered once individually to only one side of each Petri dish surface in Eskişehir Osmangazi University Faculty of Medicine, Department of Radiation Oncology. The irradiation process was done with 6 MV photons from a single field from a 180-degree angle using the Varian Trilogy device, with a 100 cm silver sulfadiazine on the Petri dish surface. The studies required for the examination with electron microscopy were started 24 hours after the irradiation. After all radiation doses, the examination was carried out at the same time, and the examination at different times for different doses was left for future studies due to the limited budget of the study. At the concentrations determined for examination by electron microscopy, the samples were taken from each Petri dish into Falcon tubes. The samples were centrifuged with sodium phosphate buffer at 1000 g for 10 minutes twice, washed, and then taken into 2.5% glutaraldehyde prepared in 0.1 mL sodium phosphate buffer. After 1 night of waiting at +4 °C, the cells were washed with buffer and then placed in 1% osmium tetroxide and were subjected to secondary fixation. Prepared 3% molten agar was added to the cells washed with buffer again and a homogeneous cell-agar mixture was obtained. Small drops of this mixture were instilled onto the slide with the help of a pipette and allowed to freeze. Then, they were divided into small pieces with a razor blade and gently put into brown glass bottles. After block staining with 1% uranyl acetate for 15 minutes, firstly dehydration with gradually increasing alcohol series and then transparency with propylene oxide was performed. Cells taken into Araldite-based embedding material were left to polymerize at 600 °C for 48 hours and were made into blocks. Full thin sections (60 nm) taken with an ultramicrotome (Leica Ultracut R) of the samples were taken on the grit, stained with uranyl acetate-lead citrate, examined in TEM and the findings were recorded.

Statistical Analysis

No statistical comparison method was used in the study since a parametric data evaluation was not performed.

RESULTS

Cellular morphology changes were observed in the cells on the side of the Petri dish, where a radiation dose of 2 Gy was applied. The cell ends took a bottle-shaped appearance, and there were several ghost cells. An atypical morphology was evident, and the cells were emptied. Large-sized hollow structures were formed in the cytoplasm. The cell membranes and the cell walls were damaged, and the apical parts of the cells were melted (Figure 1). On the other hand, it was determined that the damage findings such as the melting of the cytoplasm or change in shape in the cells in the non-irradiated area of the Petri dish were still present, although not at the same extent as the cells in the irradiated area (Figure 2). Ghost cell formation was observed, and their number increased in the part where a radiation dose of 3 Gy was applied (Figure 3). In the non-irradiated area of the Petri dish (Figure 4), vacuole formations were detected with some deterioration in the morphology of some cells, but the ghost cells in this group were negligible compared to the cells of the irradiated group. Significant vacuole formation, electron-dense appearance, lysis, and advanced damage were observed in cells administered at a dose of 6 Gy (Figure 5). In the non-irradiated area of the Petri dish, atypical morphological changes and vacuole formations in the cells, and ghost cells were observed (Figure 6). It was determined that advanced damage findings, atypical cell appearances, vacuole formations, and cell wall and cytoplasm damage occurred in cells that received a 10 Gy radiation dose (Figure 7). Morphological deterioration, electron-dense appearance, and vacuole formation were observed in the cells in the non-irradiated area of the Petri dish (Figure 8). Advanced damage and slight bleb formation were observed in the cells administered at a 20 Gy dose, and morphological deterioration, electron-dense appearance, and lysed cell formations were detected in the cells in the non-irradiated area of the Petri dish (Figures 9 and 10).

DISCUSSION

Radiotherapy treatment leads to cell division and cell death with direct action mechanisms in 20% of cases and indirect action mechanisms in 80%. The indirect effect occurs through superoxide and hydroxyl radicals, while the direct effect is formed by direct DNA damage. As a result of the direct effect, rupture, and breaks occur in the DNA. Therefore, the cell G2 and/or M phases do not proliferate, and cell death occurs. The indirect effect causes ionization and oxidation, which then form hydroxyl radicals as a result. The toxic molecules formed because of free radical reactions cause cell death (6). The cellular responses to irradiation are manifested in several structural and functional changes to cells and cellular organelles. The radiation-induced changes in the cellular and organelle membranes play a significant role in the development of acute

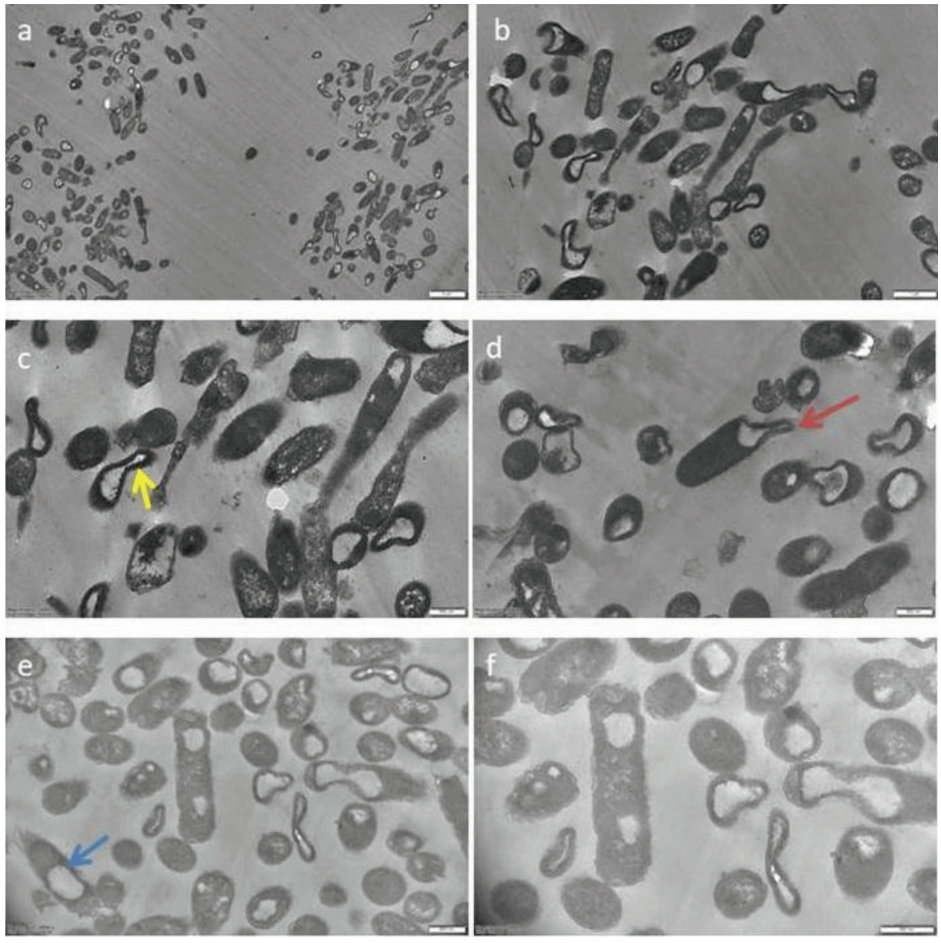

Figure 1: TEM findings of samples taken from the irradiated area of the Petri dish treated with 2 Gy. Yellow arrow: bottle-shaped appearance, blue arrow: large-sized hollow structures in the cytoplasm, red arrow: melted apical parts of the cells, **a:** magnification x5000, **b:** magnification x12000, **c:** magnification x20000, **d:** magnification x20000, **e:** magnification x12000, **f:** magnification x20000.

TEM: Transmission electron microscopy, Gy: Gray

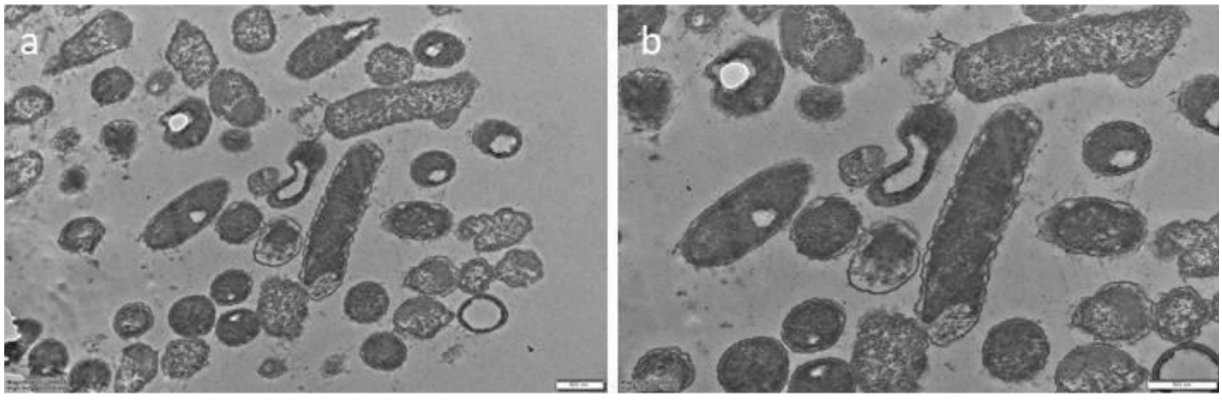

Figure 2: TEM findings of samples taken from the non-irradiated area of the Petri dish treated with 2 Gy, **a:** magnification x20000, **b:** magnification x30000.

TEM: Transmission electron microscopy, Gy: Gray

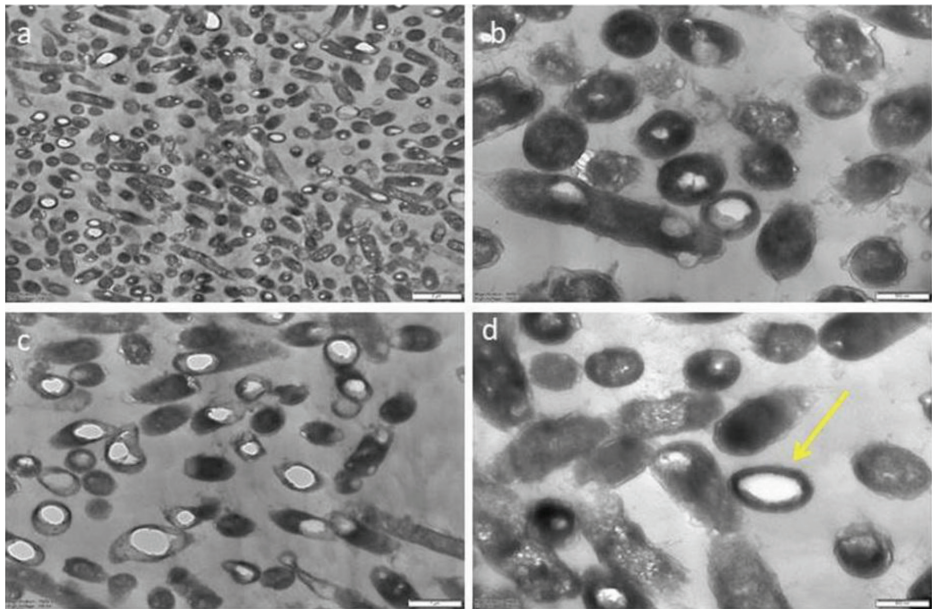

Figure 3: TEM findings of samples taken from the irradiated area of the Petri dish applied 3 Gy. Yellow arrow: ghost cell formation, **a:** magnification x7000, **b:** magnification x30000, **c:** magnification x15000, **d:** magnification x15000.

TEM: Transmission electron microscopy, Gy: Gray

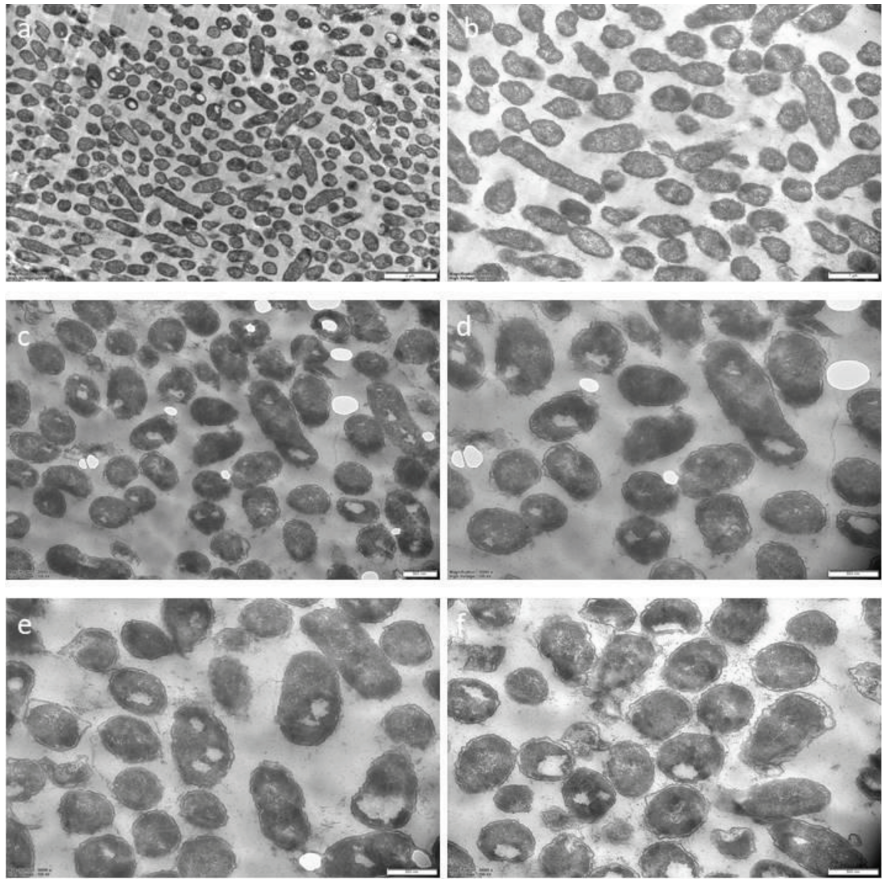

Figure 4: TEM findings of samples taken from the non-irradiated area of the Petri dish treated with 3 Gy, **a:** magnification x8000, **b:** magnification x15000, **c:** magnification x20000, **d:** magnification x30000, **e:** magnification x30000, **f:** magnification x30000.

TEM: Transmission electron microscopy, Gy: Gray

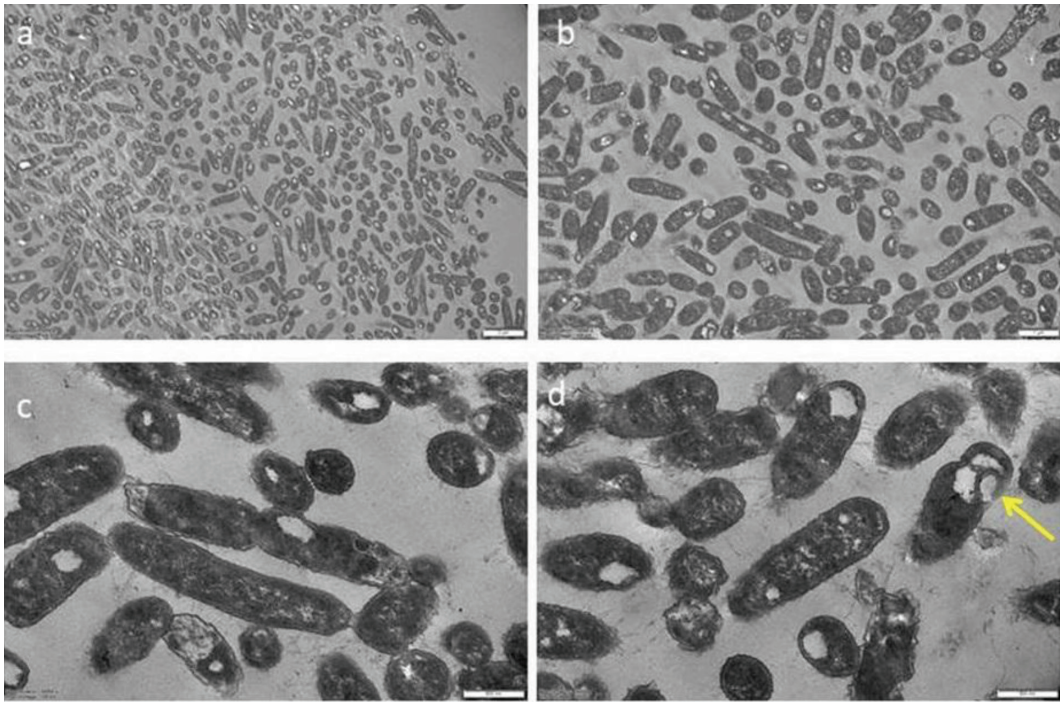

Figure 5: TEM findings of samples taken from the irradiated area of the Petri dish treated with 6 Gy. Yellow arrow: vacuole formation, **a:** magnification x5000, **b:** magnification x10000, **c:** magnification x20000, **d:** magnification x30000.

TEM: Transmission electron microscopy, Gy: Gray

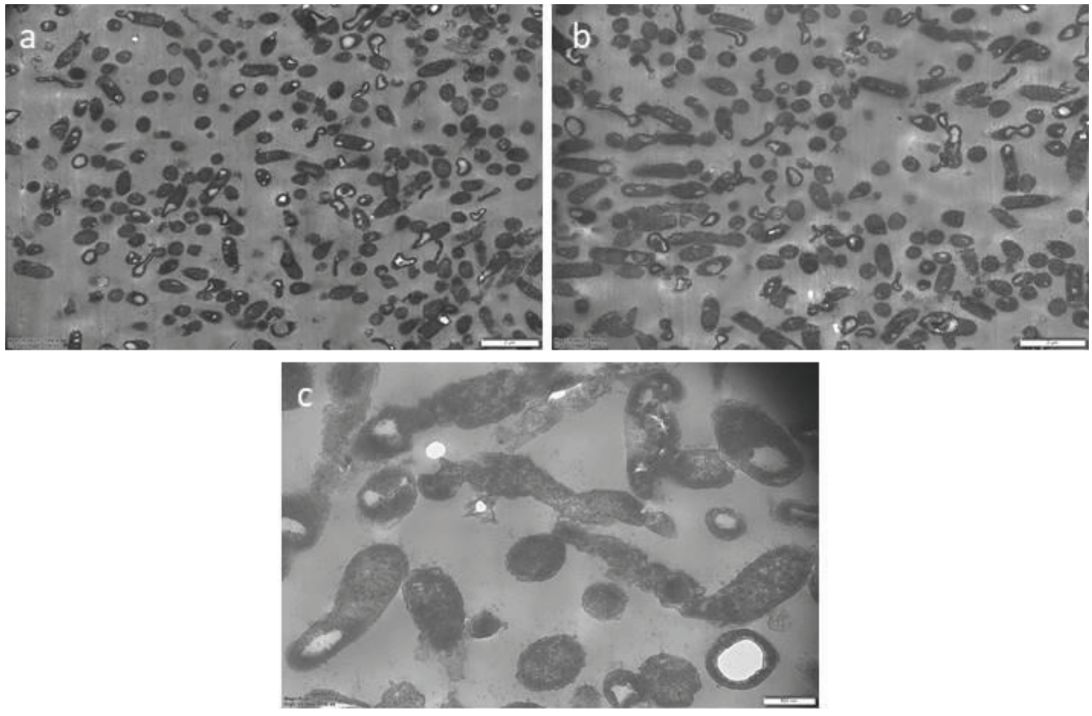

Figure 6: TEM findings of samples taken from the non-irradiated area of the Petri dish treated with 6 Gy, **a:** magnification x7000, **b:** magnification x8000, **c:** magnification x25000.

TEM: Transmission electron microscopy, Gy: Gray

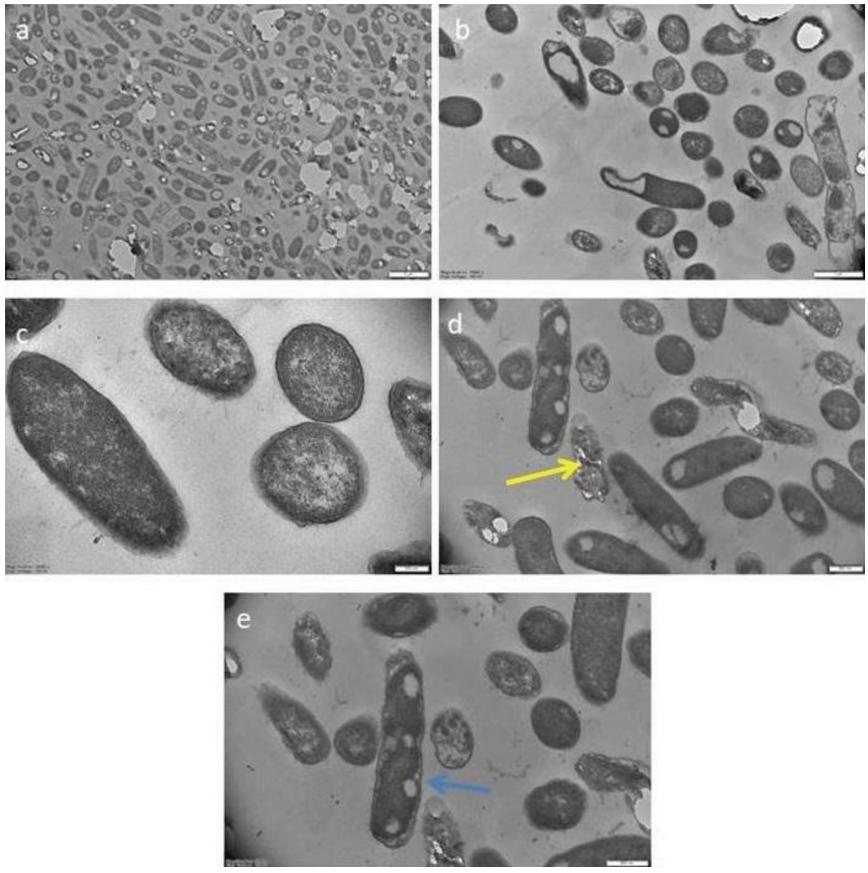

Figure 7: TEM findings of samples taken from the irradiated area of the Petri dish applied 10 Gy. Yellow arrow: atypical cell appearances, blue arrow: vacuole formation, **a:** magnification x6000, **b:** magnification x15000, **c:** magnification x50000, **d:** magnification x20000, **e:** magnification x25000.

TEM: Transmission electron microscopy, Gy: Gray

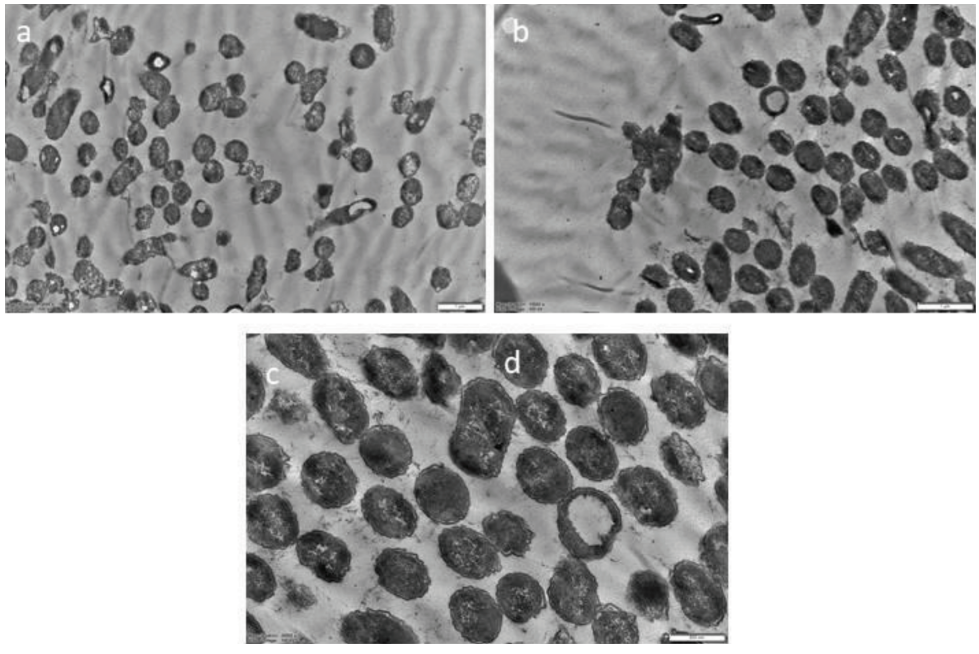

Figure 8: TEM findings of samples taken from the non-irradiated area of the Petri dish treated with 10 Gy, **a:** magnification x12000, **b:** magnification x15000, **c, d:** magnification x30000.

TEM: Transmission electron microscopy, Gy: Gray

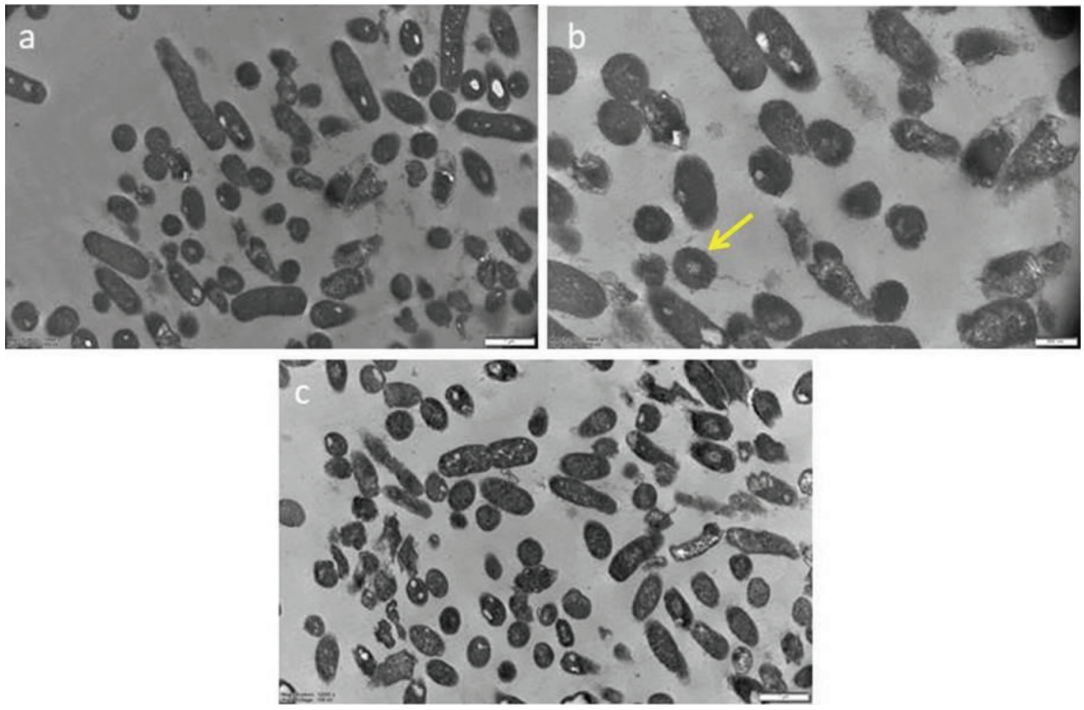

Figure 9: TEM findings of samples taken from the irradiated area of the Petri dish applied 20 Gy. Yellow arrow: bleb formation, **a:** magnification x12000, **b:** magnification x20000, **c:** magnification x12000.

TEM: Transmission electron microscopy, Gy: Gray

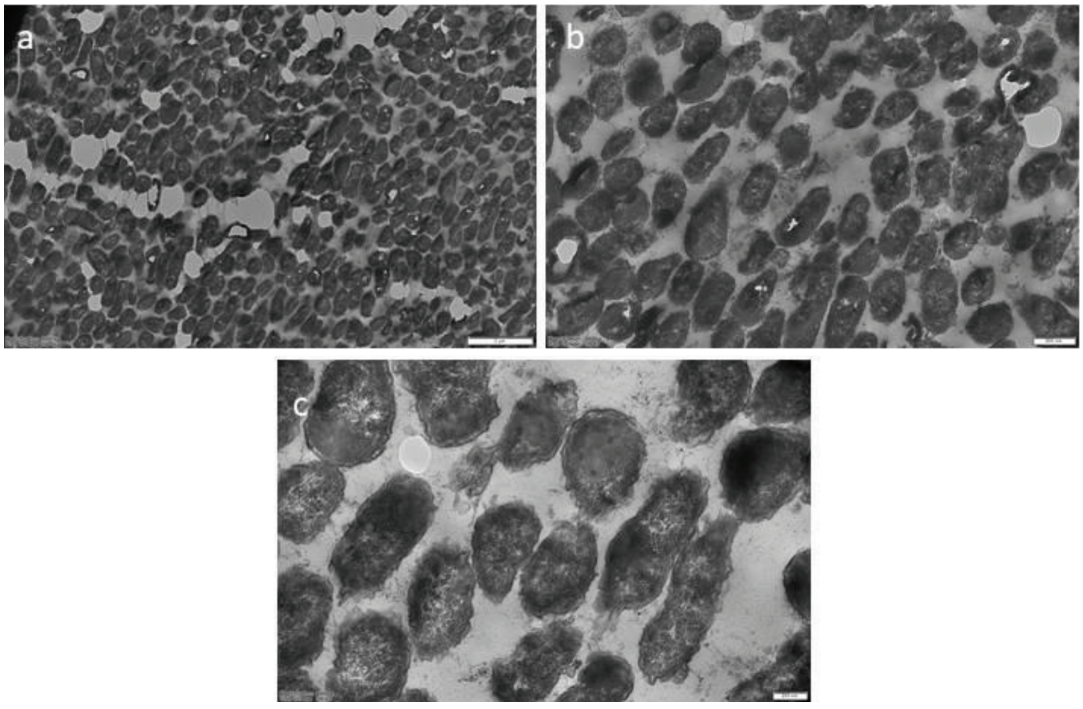

Figure 10: TEM findings of samples taken from the non-irradiated area of the Petri dish treated with 20 Gy, **a:** magnification x8000, **b:** magnification x20000, **c:** magnification x40000.

TEM: Transmission electron microscopy, Gy: Gray

radiation injury. The observed changes in the cell surface and the alteration of intercellular connections are closely related to the reorganization of the cytoskeletal elements in the irradiated cells. The mitochondria, endoplasmic reticulum, Golgi-complex, and the lysosomal system are considered to be direct intracellular targets of irradiation. The radiation effects result as a direct or indirect consequence(s) of absorbed radiation energy. Some data suggest that all these effects are not strictly specific to radiation and may consider general stress responses (7).

The minimum dose we investigated in our study, 2 Gy, is the conventional radiotherapy dose (8). The dose of irradiation may also play a role in determining the type of cell death. Occurred events are dose-dependent in irradiated cells and can be detected after irradiation. Also, it has been shown that the radiation sensitivities of different cell types are also different from each other. In different studies, significant morphological and functional changes in cell elements were often shown at lower doses (7). In this context, the biggest shortcoming of our study is that the effects of low doses were not investigated. The ghost cell is an enlarged eosinophilic epithelial cell with eosinophilic cytoplasm that indicates coagulative necrosis where there is cell death. The ghost cell without a nucleus appears as a shadow cell (9). In our study, we observed that even with low-dose radiotherapy applications such as 2 Gy, disturbances in cellular morphology were initiated, large-sized structures occurred in the cytoplasm where the cell has been discharged, the cell membrane and the cell wall got damaged, and apical parts of the cells melted. It was observed that the number of ghost cells increased with increasing radiation doses. Cell lysis became apparent, and cell damage increased with the application of 6 Gy. After the application of 10 and 20 Gy, bleb formation increased. On the other hand, examination of the samples taken from other Petri dishes except the control dish showed morphological changes similar to the samples taken from the irradiated regions, albeit less than expected. This result was predicted before the examination, and although radiation was applied using a semi-cutter, the samples were taken as far as possible from the region where radiotherapy was applied to prevent the effect of scattered radiation. The two generally accepted basic mechanisms are the bystander effect and the abscopal effect, which describe the radiation effect seen in cells that are not exposed to radiation. The factors released from cells exposed to radiation and the direct communication of cells via junctions cause these two effects (1-3). The bystander effect is the biological effect that appears similar to irradiated cells in neighboring cells that are not directly exposed to radiation. The bystander effect is reported in many cell groups independently of radiation types. The bystander effect can be seen in the γ-radiation that transfers alpha particles and low linear energy. However, there is no clear information about the type of signal that plays a role in both radiation types (1-3). Abscopal effect tries to explain the effects of radiotherapy that occur not only within, but also outside the treatment area. Today, it is generally accepted, and it probably reveals the effects of radiation on the whole body. Similar to the bystander effect, it starts with local DNA damage in irradiated cells or tissues. This can be considered a triggering effect that marks the teleported area as a "stress" field in the body. The remote radiation effect is the release of short and long-distance reporters to transmit stress signals to remote areas through well-preserved inflammatory and immune response networks. Clinically, the most advanced manifestation of this phenomenon is the shrinkage of tumors in remote areas that cannot be reached by irradiation (1-3). It was understood that bacteria could detect the change in the bacteria population and regulate gene expressions via cell-to-cell communications, which was defined as quorum sensing. It has also been reported that bacteria can communicate not only with their own but also across species. This way, bacteria ensure many functions such as spores, gene transfer, conjugation, bioluminescence, biofilm formation, antibiotic resistance, and virulence factors (10, 11).

Limitations

The most important limitation of our study is that the non-radiotherapy area was not checked by dosimetry and the effect of low-dose radiotherapy effects are not investigated. Currently, only the virtual dose information obtained from the treatment planning system has been defined as the non-dose region and no dosimetric measurement has been done.

CONCLUSION

Although it is speculative, the observation of similar morphological distortions, albeit at a lesser rate, in the samples taken from the region where radiotherapy was not applied can be explained by quorum sensing.

Ethics Committee Approval: *N/A*

Informed Consent: *N/A*

Conflict of Interest: *The authors declared no conflict of interest.*

Author Contributions: *Concept: H.A., S.A., M.A.A., S.H., A.Ö., Design: H.A., S.A., M.A.A., S.H., A.Ö., Data collection or processing: H.A., S.A., M.A.A., S.H., Analysis or Interpretation: H.A., S.A., M.A.A., S.H., A.Ö., Literature Search: S.P.K., C.Ç., H.A., S.A., M.A.A., S.H., A.Ö., Writing: S.P.K., C.Ç., A.Ö.*

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