

**Rh factor is associated with individual radiosensitivity: A cytogenetic study**Meysam Khosravifarsani<sup>1</sup>, Ali Shabestani Monfared<sup>2</sup>, Sajad Borzoueisileh<sup>3</sup>

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**Abstract**

**Introduction:** Radiosensitivity is an inherent trait, associated with a raised reaction to ionizing radiation on the human body. In radiotherapy and radiation protection fields, individualization of the patient's treatment is one of the main topics. With the goal of determining biomarkers capable of anticipating normal tissue side reactions, we studied the association between the Rh factor and radiosensitivity.

**Methods:** This experimental study was carried out from January to June 2014 among 50 normal responders with A blood group (25Rh+ and 25Rh-) between the ages of 22 and 23 in Babol, Iran. Human peripheral blood samples were taken from subjects and, using CBMN assay, the biological effects of gamma irradiation, including the frequency of micronuclei (MN) and nuclear division index (NDI), were measured. A data analysis was performed using SPSS version 18 to determine the independent and paired samples t-tests.

**Results:** A significant increment occurred in the frequency of MN in group Rh+ ( $196 \pm 18.23$ ) compared with Rh- ( $169 \pm 17.11$ ) following irradiation ( $p < 0.001$ ).

**Conclusions:** The Rh factor might be a predicting marker in an individual's radiosensitivity to ionizing radiations. However, we believe that additional investigations are needed to prove this hypothesis.

**Keywords:** Radiosensitivity, CBMN assay, Nuclear division index (NDI)

**1. Introduction**

Exposure to ionizing radiation generates several reactive oxygen species (ROS), like hydroxyl radical, hydrogen peroxide, aqueous electron, and superoxide in the aqueous medium. Such ROS have harmful effects on macromolecules such as lipids, proteins, and DNA and damages cellular function, leading to multiple disorders and dysfunctions in the human body (1, 2). As several studies in the literature have indicated, radiation response is a multi-factorial scenario that depends on several parameters, such as the nuclear material content, cell reproduction, tissue and organ revival, and biological repair. From a biological viewpoint, it is important to consider how radiation response may moderate the responses of tissues and organs to gamma irradiation. From this aspect, genetic properties are highly crucial in most diseases and disorders, such as ataxia-telangiectasia (AT) (3), Xeroderma Pigmentosum (4), and breast cancer (5), and an important characteristic in the radiosensitivity of cells, tissues and organs (5). Approximately 10% of normal individuals in similar physical and environmental conditions exhibit incremented response to ionizing radiations (6). Increased radiosensitivity of breast cancer patients has been reported in several different studies (7-9). Hence, identifying a prognostic marker for radiotherapy is one of the main goals of the radiation biology and radiation protection field. If the personal complication risks of ionizing radiation are recognized, the risk could be reduced in the small ratio of highly sensitive people through dose reduction in patients and the defining of more limited rules in radiation protection regulations (10). Some origins of

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radiosensitivity, including physiological, genetic, and epigenetic sources, have been proposed in cancer patients and normal individuals (11, 12). Several studies have marked that ABO blood groups are associated with the risk of ischemic heart disease and developing severe manifestation of atherosclerosis (13). Results from the Framingham study and other reports showed the occurrence of ischemic heart disease might be higher in subjects with blood group A (13). In addition, patients with blood groups A or O are more often afflicted with pernicious anemia (14). The incidences of gastric carcinoma (15), peptic ulcer (16), and ovarian cancer (5) are much more extensive in persons with blood types A, O, and B, respectively. The AB blood group is associated with an incremented risk of infectious disease. Nonetheless, it was explained that the ABO blood group is an inherent characteristic (17). Thereafter, the alleles for the blood groups were recognized in the same place on chromosome 9 at 9q34.1–q34.2 (18). These findings demonstrated that one's blood group is a genetic trait. Despite such investigations, a preliminary clinical study showed that the radiation response of type O patients suffering from carcinoma of the cervix is better than those of other blood groups (19). Meanwhile, an elevated frequency of micronuclei (MN) in the A blood group was reported in peripheral blood lymphocytes of normal individuals (20). Regarding these findings and given the variation in radiosensitivity in the normal population-this study aimed to evaluate chromosomal radiosensitivity of peripheral blood lymphocytes in normal individuals with Rh<sup>-</sup> and Rh<sup>+</sup> factors.

## **2. Material and Methods**

### **2.1. Research design and setting**

In this experimental study, informed consent was acquired from 50 healthy human volunteers (25 Rh<sup>+</sup> and 25 Rh<sup>-</sup>) in the A blood group who ranged in age from 22 to 23 years old.

### **2.2. Blood sampling**

All responders completed a questionnaire to provide information on their lifestyle and exposure to chemical and physical agents. Having a history of known irradiation, smoking, drug treatment, and alcohol consumption were exclusion criteria. Blood samples were taken from responders under sterile conditions using heparinized syringes. Finally, samples were divided into two separate groups (control and exposed blood samples). All of them were transferred in cold environmental conditions.

### **2.3. Gamma irradiation**

The aliquots of blood samples were exposed to gamma radiation of a low LET <sup>60</sup>Co source (Theratron780 manufactured by Canada) at dose rate of 70 CGy/min, with source-to-sample distance (SSD) = 80 cm, a field size of 10 × 10 cm, and at room temperature. Blood samples were irradiated with a total dose of 2 Gy, and a vial of unexposed blood samples was kept as the control group. The sample irradiation was performed by an authorized person.

### **2.4. Cytokinesis-block micronucleus (CBMN) assay**

The CBMN assay was carried out using the standard technique proposed by earlier investigations (21), with minor modifications (22). Hence, 0.5ml of peripheral blood samples were added to 4.5ml of a cell culture medium (RPMI-1640) containing sodium bicarbonate supplemented with 10% fetal calf serum (FCS), antibiotics, and 1% L-glutamine. One hundred microliters of Phytohaemagglutinin (PHA, SIGMA) was added to stimulate lymphocytes. Forty-four hours after PHA stimulation, Cytochalasin-B (Cyt-B, Sigma) at a final concentration of 6µg/ml was added, and binucleated lymphocytes were harvested after 72 h. The cells were then collected by centrifugation at 2,000 RPM for 10 min (BOECHO U-320 R), and supernatant was decanted. Two to three microliters of fresh hypotonic solution 0.075 M KCl was added to the solution remaining at the bottom of tubes and centrifuged at 1,200 RPM for 7 min. Again, the supernatant was poured off, and 5ml of fixative including methanol:glacial acetic acid (6/1) were quickly mixed with solution at the bottom of the tubes. After 20 min, the tubes were centrifuged at 1,200 RPM for 7 min. the induction of MN was evaluated using a double-blind score of 1000 binucleated cells (BNC) with a light microscope set to 40x magnification. Only BN cells were included in the microscopic analysis. All slides were coded before analysis.

### **2.5. Statistical analysis**

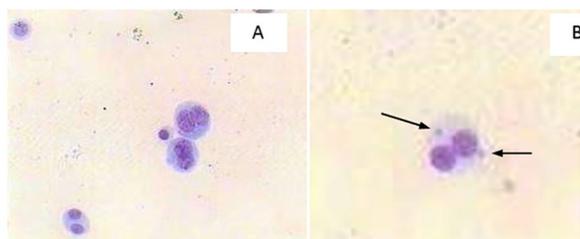
The statistical analysis was carried out using Microsoft Excel 2007. Independent-Sample t-test was used to determine the statistical significance of the difference between Rh<sup>-</sup> and Rh<sup>+</sup> samples. In addition, a paired-samples t-test was used to analyze the difference between the control and exposed samples.

### 2.6. Research ethics

The study was approved by the Ethical Committee of the Faculty of Medical Sciences at Babol University of Medical Sciences (Babol, Iran).

### 3. Results

The results of this study showed an increased mean frequency of MN in binucleated cells after irradiation (Figure 1). This result confirms the induction of MN frequency following gamma irradiation. As Table 1 shows, the mean values of MN in binucleated cells were  $196 \pm 18.23$  and  $169 \pm 17.11$  for Rh<sup>+</sup> and Rh<sup>-</sup> samples, respectively (Table 1). The results indicated that the average MN is significantly higher in Rh<sup>+</sup> than in Rh<sup>-</sup> in the irradiated group ( $p < 0.001$ ) (Table 1). Table 2 shows that the frequency of MN cells significantly increased in both Rh<sup>-</sup> and Rh<sup>+</sup> irradiated (2Gy) samples whereas the percentage of binucleated, trinucleated, and tetranucleated cells decreased after irradiation. The NDI value for the Rh<sup>+</sup> group was 1.81 and 1.39 before and after irradiation, respectively. For the Rh<sup>-</sup> type, the parameter declined from 1.85 to 1.40 following gamma irradiation. Regarding the NDI in Table 2, no significant difference emerged between Rh<sup>-</sup> and Rh<sup>+</sup> samples after irradiation.



**Figure 1.** Increased frequency of micronuclei (MN) in both Rh<sup>-</sup> and Rh<sup>+</sup> samples after gamma irradiation (2Gy). A: Mononuclear and binuclear lymphocyte cells before irradiation, B: Binuclear lymphocyte cell bearing MN. Arrows show the appearance of MN during gamma irradiation.

**Table 1.** Frequency of micronuclei (MN) in binucleated cells (BNC)

Dose	MN (micronuclei) Yield in BNC (binucleated cells)	
	Rh <sup>+</sup>	Rh <sup>-</sup>
0Gy	$3.75 \pm 0.53$	$5.35 \pm 0.90$
2Gy	$196 \pm 18.23$	$169 \pm 17.11$

**Table 2.** Percentage of mononucleated (MNC), binucleated (BNC), trinucleated (TNC), and tetranucleated (TeNC) cells in non-exposed (0Gy) and exposed (2Gy) samples.  $NDI = \frac{M1+2(M2) +3(M3) +4(M4)}{N}$ , where M1–M4 presents the number of cells with one to four nuclei and *N* is the total number of viable cells.

Dose	Mononucleated cells (MNC) (%)		Binucleated cells (BNC) (%)		Trinucleated cells (TNC) (%)		Tetranucleated cells (TeNC) (%)		Nuclear Division Index (NDI)	
	Rh <sup>+</sup>	Rh <sup>-</sup>	Rh <sup>+</sup>	Rh <sup>-</sup>	Rh <sup>+</sup>	Rh <sup>-</sup>	Rh <sup>+</sup>	Rh <sup>-</sup>	Rh <sup>+</sup>	Rh <sup>-</sup>
0 Gy	45.1	38.3	34.1	40.4	15.2	19.1	5.6	2.2	1.81	1.85
2 Gy	70.6	69.7	20.1	21.2	8.2	8.4	1.1	0.7	1.39	1.40

### 4. Discussion

Approximately 20% of the radiotherapy patients showed a wide range of normal tissue reactions (23). Recognizing patients with high, moderate, or low radiosensitivity would help develop a good plan for the radiotherapy of patients with the highest cure rate in terms of the patient's individual adaptation (10). The explanation of criteria that modify the total effects of ionizing radiation is one of the main subjects of research efforts in radiobiology. Some studies have explained that a correlation exists between tumor response and the level of 8-oxo-dG in the urine of lung and breast cancer patients (23). Hence, several studies have been initiated to investigate whether radiosensitivity is an individualized subject in patients' radiation response. The main purpose of this study was to investigate the role of Rh in radiation response of healthy people. Hoeller et al. showed that individual radiosensitivity measured by lymphocytes might be a good way to predict fibrosis in breast cancer patients after radiotherapy (24). In the field of radiobiology, the CBMN Cyt assay for PBL is a suitable bio-dosimetry device to measure in-vivo and in-vitro radiosensitivity and cancer reproducibility (25). Lymphocytes are chosen because of their easy availability, simple method of culture, and ease of sample collection. Therefore, in our study, CBMN Cyt assay as one of the standard techniques for genetic toxicology evaluation in human cells was selected to measure chromosomal aberrations in Rh<sup>-</sup>

and Rh<sup>+</sup> healthy people. As expected, the average number of MN increased significantly after the 2Gy gamma irradiation of lymphocyte cells. This result concurs with the earlier findings indicating a higher level of MN after gamma irradiation (21, 26). The current study showed that the mean frequency of MN in the Rh<sup>+</sup> group was roughly 16% higher than in the Rh<sup>-</sup> group. This result might be due to the association of Rh alleles on chromosome 9 and particular alleles of genes responsible for DNA double strand break repair. However, more molecular investigations need to evaluate more precisely the main reason for this event. Elahimanesh et al. indicated that the A blood group has more MN frequency compared to other blood types (20). They showed that group A has a higher frequency of MN than did groups AB, B, and O (20). In another study, Khosravifarsani et al. elucidated that an association exists between handedness and radiosensitivity. They showed that left-handed breast cancer women are more radiosensitive than right-handed women (18). The background MN frequency in the present study was  $3.75 \pm 0.53$  and  $5.35 \pm 0.90$  for the Rh<sup>+</sup> and Rh<sup>-</sup> groups, respectively. This value is in fair agreement with the cited value of IAEA EPR bio-dosimetry manual by International Atomic Energy Agency (27). Regarding the IAEA report, the background MN frequencies range from 0 to 40 per 1000 BN cells (27). For the NDI counted prior to and after irradiation, the value decreased in both Rh<sup>+</sup> and Rh<sup>-</sup> groups after 2Gy gamma irradiation, indicating a reduction in cell proliferation. However, no significant difference occurred between these two blood types after irradiation. As the present study indicated, a decrease yield of binucleated and polynucleated cells was observed after gamma irradiation. In contrast, the frequency of mononucleated cells increased after gamma irradiation. Because carrying out standard Cytokinesis Blocked Micro-nuclei protocol was time consuming, choosing a larger study population was restricted in this project.

### 5. Conclusions

The present study suggested that chromosomal radiosensitivity of lymphocytes in normal individuals with Rh<sup>+</sup> factor is higher than their Rh<sup>-</sup> counterparts. However, this difference is not generalized to other types of cells. This finding revealed that the Rh factor can be considered one of the hereditary traits affecting individuals' radiosensitivity and can be a valuable item in radiation protection regulations. However, further in-vivo and in-vitro studies are needed for validation.

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### Conflict of Interest:

There is no conflict of interest to be declared.

### Authors' contributions:

All authors contributed to this project and article equally. All authors read and approved the final manuscript.

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