Original Article

Radio-adaptive Response in Myocardial Perfusion Imaging Induced by Technetium-99m

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Abstract

Purpose of the Study: Low dose radiation will induce adaptation and following exposure to an adaptive dose, the cells are more resistance to following challenging doses. This phenomenon is known as radio-adaptive response. The aim of this study was to investigate the percentage of apoptotic cells in the peripheral blood samples of the patients which undergo myocardial perfusion imaging (MPI) with technetium-99m (Tc-99m) before thallium scan to assess the induction of radio-adaptive response. **Materials and Methods:** In this study, 97 samples from 74 patients, referred to nuclear medicine center of Mazandaran Heart Hospital for MPI, which had no history of diagnostic, therapeutic, occupational, and radioactive exposures during past 2 years, were provided. The participants were classified into four groups including control, patients which were scanned solely with technetium, the patients which examined by thallium and the last group were the patients that examined by technetium followed by thallium. Then 2 ml Peripheral blood samples were obtained, and after 24 h incubating, the samples were studied by neutral comet assay. Statistical analysis was carried out using Student's *t*-test along with one-way analysis of variance. **Results:** The mean percentage of apoptotic cells in the exposed groups were higher than the control. Furthermore, among exposed groups, the apoptotic cells in thallium group were more than others and this index was significantly lower in the group which was undergone technetium administration before thallium scan. **Conclusions:** These findings suggest that exposure to Tc-99m could induce a radio-adaptive response against the exposure of thallium-201.

Key words: *Myocardial perfusion imaging, radio-adaptive response, technetium, thallium*

Introduction

Nuclear medicine includes both therapeutic and diagnostic modalities, and it provides physiological and functional information to assess therapeutic response and to determine the drug dosage in the body.[1] One of the applications of nuclear medicine is in the cardiovascular system. Myocardial perfusion imaging (MPI) is a noninvasive diagnostic procedure for coronary artery disease assessment. In this procedure, after injection of radiopharmaceuticals, the patient is examined at rest and stress state separately. In addition, nuclear medicine could serve for myocardial viability studies in patients with myocardial infarction history.[2,3]

Thallium-201 (Tl-201), an analog of potassium ion (K^+), and technetium-99m (Tc-99m) sestamibi (trade name cardiolite) are employing in MPI now. Tl-201 have a long physical half-life (73 h) and low energy photons (69–83 keV) which delivers a relatively high effective dose (2.2×10^{-1} mSv/MBq). In comparison, Tc-99m have a short physical half-life (6.2 h) and high energy photons (140 keV) which deliver lower effective doses (9×10^{-3} mSv/MBq in adults) to the patients. These properties make the Tl-201 a less than an ideal agent for imaging because of inducing more DNA damage than Tc-99m.[4,5] Higher effective dose and long physical half-life of Tl-201 limit its usual infusion dosage up to 2–5 mCi.[6,7]

Low dose radiation strengthens the cells against the subsequent higher challenging doses and therefore reducing genotoxic lesions. This phenomenon is so-called the radio-adaptive response which for the first time confirmed by Olivieri *et al*.[8,9] Since then, it was documented by many other investigators.[10] This phenomenon later was approved by studying various endpoints including cellular damage, DNA damage,

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chromosomal aberration, micronucleus formation, neoplasm formation, or apoptosis induction.[11-13] Some studies have linked this phenomenon to repair mechanisms, immune system, and molecular pathways.[14-16]

Comet assay is a microelectrophoresis technique that directly and quantitatively detects DNA damage. This method is sensitive, fast, reliable, and relatively inexpensive. It does not need cell culture, and the results could be obtained within hours after sampling. Microscopic analysis of cells in neutral comet assay does not have the complexity of chromosomal analysis in metaphase and sister chromatid exchange. It can be done with high accuracy and rapidity without special expertise in cytogenetic fields while damages induced by low dose radiation at about few cGy could be detected.[17,18]

In this study, the mean percentage of apoptotic cells in peripheral blood samples of patients that did MPI by Tc-99m followed by injecting Tl-201 and the patients who examined by Tc-99m and Tl-201 separately analyzed and compared using neutral comet assay. The aim of this study was to assess radio-adaptive response among patient which exposed to low dose (Tc-99m) radiation before receiving a higher dose (Tl-201).

Materials And Methods

Subjects

In this study, 97 samples from 74 patients which have been referred to nuclear medicine imaging center of Mazandaran Heart Hospital (Sari, Iran) between April and December 2015 were provided. The patient with no history of chemotherapy, radiotherapy, angiography, or any kind of radiation exposure during past 2 years and no history of clastogenic drug consumption were included in the study. The informed consent was obtained from participants.

The patients were classified into four groups. Group A including 23 patients were sampled before MPI considered as control, Group B consist of 30 patients that underwent MPI with Tc-99m, Group C including 23 patients which had Tl-201 injection for MPI, and Group D consist of 21 patients which were examined by MPI in two stages, first by injecting Tc-99m then followed by injecting Tl-201 as their treatment process.

Neutral comet assay

Two ml peripheral blood sample were taken and collected in ethylenediaminetetraacetic acid (EDTA)-disodium containing microtubes within 5 min after radiopharmaceutical drugs injection. The samples were incubated for 24 h at 37°C and 5% CO_2.

After this 24 h incubation and centrifugation of peripheral blood samples, the supernatants were discarded and 140 μL of low melting agarose (Fermentas, Poland) which prepared at 75% concentrations in distilled water, were mixed with 10 μL of remainders. Then 50 μL of this mixture poured over each window of comet slides which had been coated with a supporting layer of 1% normal melting point agarose (Fermentas, Poland). The samples were covered with coverslips and kept in 4°C for about 5 min in dark to allow solidification of agarose. Then slides were placed in horizontal dish containing fresh lysis solution at a final pH of 10 (contains: NaCl [Merck, Germany], Tris-Base [Merck, Germany], 10% dimethyl sulfoxide [Merck, Germany], 1% N-lauryl sarcosine [Sigma, USA], 1% Triton X-100 [Sigma, USA]), and kept at 4°C in dark for 30 min.

After that the slides were washed with electrophoresis buffer (at a final pH of 8.2–8.4 containing: Tris-Base and boric acid, N_2-EDTA) and then put in a membrane horizontal electrophoresis chamber filled with fresh electrophoresis buffer at 20 volts, 9 mA for 10 min which made DNA migration and comet appearance.

After washing the slides with distilled water for 5 min and inserting in 96% alcohol, they dried at room temperature. The slide stained with 50 μL (20 μg/ml) ethidiume bromide solutions (Merck, Germany) then covered with coverslips and the slides were analyzed with a fluorescent microscope (Nikon E800) WL 516–546 nm and barrier filter with 590 nm wave length and magnification power ×200.

After counting a total number of 500 cells per slide visually, the mean percentage of apoptotic cells in four groups determined using this equation:

Percentage of apoptotic cells = (Number of apoptotic cells/ Total cell count) ×100.

Statistical evaluation

Statistical analysis was carried out by SPSS software (SPSS Inc, Chicago, IL). The comparison of apoptotic cell percentages was done using one-way analysis of variance test and to compare the mean of each group with other groups, the Tukey's test were used. $P < 0.05$ was considered a significant level. The study was approved by Ethics Committee of the Institute.

Results

Group A including 23 patients (8 male and 15 female) with the mean age of 57 ± 12. Group B consist of 30 patients (10 male and 20 female) with mean age 58 ± 12 and Group C including 23 patients (10 male and 13 female) with mean age 58 ± 10 and Group D consist of 21 patients (7 male and 14 female) with mean age 59 ± 12.

The outlines of examined cells and the mean percentage of apoptotic cells were shown in Figure 1 and 2 respectively. This index for all of the exposed groups including Group B (Tc-99m), Group C (Tl-201), and Group D (Tc-99m followed by Tl-201) was higher than the controls. The mean percentage of apoptotic cells in Group C was more than Group B, while for Group D, it was less than Group

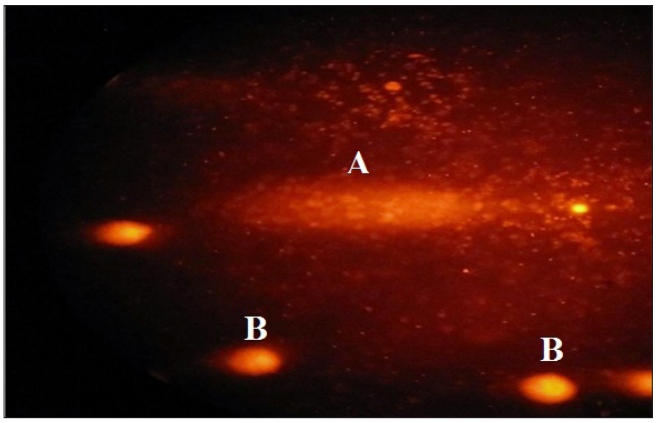

Figure 1: Apoptotic cell (a) normal cells (b) outlines (× 200)

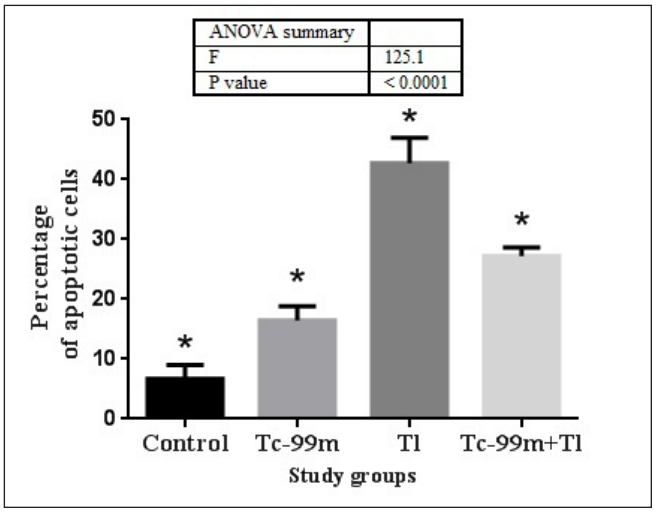

Figure 2: The mean percentage of apoptotic cells of four studied groups (Control = Control group, Technetium-99m = Technetium group, Tl = Thallium group, Technetium-99m + Tl = Technetium and thallium group). All of the differences are statistically significant. Error bars represent the confidence interval 95%

C significantly. There is a statistically significant difference between Group C and Group D too.

The normal and apoptotic cells outlines are shown in Figure 2. The mean percentage of apoptotic cells showed that there is no significant age effect.

Discussion

The mean percentage of apoptotic cells were investigated and among exposed groups, Group B (Tc-99m) had the lowest mean percentage of apoptotic cells due to low effective dose (9×10^{-3} mSv/MBq). Group C (Tl-201) had the highest percentage of apoptotic cells due to high effective dose (about 2.2×10^{-1} mSv/MBq). In addition, this index for Group D (Tc-99m followed by Tl-201) was between Group B and Group C while their received effective doses were higher than Group C.

Our results showed that the damages are higher in exposed groups compared to the controls which are in agreement with Monfared *et al*. study which showed that the mean frequency of total chromosomal aberration in the exposed group was higher than controls. This implies that low doses of radiation could induce damages to the cells.[19]

The results of this study showed that in the Tc-99m group which delivers lower doses, the percentage of damages are lower than the Tl-201 group. Monfared *et al*. conducted a study and find that the DNA damages in peripheral blood samples of radiation worker are higher than control.[20]

The probability of radio-adaptive response in cells pretreated with a low dose radiation before exposure to a high dose is well documented by many investigators. Our results showed that after receiving a conditioning dose (Tc-99m), the damages induced by higher dose (Tl-201) could be adapted. Monfared *et al*. reported a similar finding which means that the chromosomal aberration in the blood samples of patients who underwent thyroid scan before radio-iodine therapy were lower than those who had not received Tc-99m. They concluded that it might be due to radio-adaptive response.[19] Mortazavi *et al*. showed that chromosomal aberrations of high background areas of Ramsar are lower than controls. They have concluded that it may be due to radio-adaptive response.[21,22] Ikushima *et al*. have connected the adaptation event to repair process and reported that the rate of DNA repair in adapted cells was higher than that in nonadaptive cells.[23] Pakniat *et al*. revealed that radio-adaptive response is more prominent in occupationally exposed medical staff than the control group[24] while Monfared *et al*. reported that natural background radiation is more effective in induction of cytogenetic radio-adaptive response than occupational exposure.[25]

Farooqi and Kesavan *et al*. have accepted this phenomenon, but they have reported an important time window for the radio-adaptive response when they studied bone marrow cells of the whole body irradiated mice. Their results showed that when the time interval between the conditioning dose and the challenging dose was 2 h, the frequency of chromosomal aberration was reduced. They revealed that the radio-adaptive response disappeared after a time interval of 13 h, but when the time interval between the conditioning dose and challenging dose was 18.5 or 24 h, only the lower conditioning doses appeared to be effective in inducing the radio-adaptive response.[26]

Conclusions

This study showed in spite of receiving higher exposure dose in Group D who were examined by both Tc-99m and Tl-201 in two stages compare to the Group C who were examined just with Tl-201, the mean percentage of apoptotic cells in Group D were lower than that Group C, which it may be due to induction of radio-adaptive response by low-level exposure of Tc-99m. This study will be continued in nuclear medicine field in future.

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Conflicts of interest

There are no conflicts of interest.

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