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Evaluation of DNA Damage in Lymphocytes in Percutaneous Thoracic Mass Biopsies Performed with Computed Tomography

Bilgisayarlı Tomografi İle Yapılan Perkütan Torasik Kitle Biyopsilerinde Lenfositlerdeki DNA Hasarının Değerlendirilmesi

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ABSTRACT

Purpose: The increase in the medical and industrial uses of radiation in the last century has caused people to be exposed to higher doses of radiation. Although the harms of high-dose radiation on human health are known, the effects of low-dose radiation on health have not yet been fully elucidated. In this study, we aimed to investigate DNA damage in lymphocytes in patients with lung, pleura/thoracic wall masses planned for percutaneous thoracic mass biopsy with computed tomography (CT).

Methods: Sixteen patients referred to the Radiology Clinic of a public institution hospital with lung, pleura/thoracic wall masses and scheduled to undergo a CT-guided percutaneous biopsy were included in the study. All the biopsies were performed with a 128-slice CT device (Definition AS, Siemens Medical Solutions, Forchheim, Germany). Lymphocytes were analyzed using the comet assay in the venous blood samples taken from the patients before and after the biopsy procedure. DNA damage was quantitatively evaluated with the imaging analysis method.

Results: In the CT analysis data of the study group, the mean scan distance was found to be 19.92±3.60 sec, the mean total milliampere-seconds was 807.43±304.51, and the mean dose-length product was 765.44±278.36 mGy.cm. The mean comet score was 200.50±40.54 for the cells that migrated before the procedure and 237.37±27.85 for those migrating after the procedure. The post-procedure comet scores significantly increased compared to the pre-procedure comet scores (p=0.038).

Conclusion: Post-procedure DNA damage was detected in patients who underwent CT-guided percutaneous biopsy.

Keywords: Computed tomography, Comet assay, DNA damage, Ionizing radiation

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ÖZ

Amaç: Son yüzyılda radyasyonun tıbbi ve endüstriyel kullanımlarındaki artış, insanların daha yüksek dozlarda radyasyona maruz kalmasına neden olmuştur. Yüksek doz radyasyonun insan sağlığına zararları bilinmesine rağmen düşük doz radyasyonun sağlık üzerindeki etkileri henüz tam olarak aydınlatılamamıştır. Bu çalışmada, bilgisayarlı tomografi (BT) ile perkütan torasik kitle biyopsisi planlanan akciğer, plevra/torasik duvar kitleleri olan hastalarda lenfositlerdeki DNA hasarını araştırmayı amaçladık.

Materyal Metod: Bir kamu kurumu hastanesinin Radyoloji Kliniğine akciğer, plevra/torasik duvar kitleleri ile başvuran ve BT eşliğinde perkütan biyopsi planlanan 16 hasta çalışmaya dahil edildi. Tüm biyopsiler 128 kesitli CT cihazı (Definition AS, Siemens Medical Solutions, Forchheim, Germany) ile yapıldı. Hastalardan biyopsi öncesi ve sonrası alınan venöz kan örneklerinde comet assay ile lenfosit analizi yapıldı. DNA hasarı, görüntüleme analiz yöntemi ile kantitatif olarak değerlendirildi.

Bulgular: Çalışma grubunun BT analiz verileri değerlendirildiğinde; ortalama tarama mesafesi 19,92±3,60 sn, ortalama toplam miliamper-saniye 807,43±304,51 ve ortalama doz-uzunluk çarpımı 765,44±278,36 mGy.cm olarak bulundu. İşlem öncesi göç yapan hücrelerde ortalama comet skoru 200,50±40,54 ve işlem sonrası göç yapan hücrelerde 237,37±27,85 olarak tespit edildi. İşlem sonrası comet skorları, işlem öncesi comet skorlarına göre anlamlı olarak arttı (p=0,038).

Sonuç: BT eşliğinde perkütan biyopsi yapılan hastalarda işlem sonrası DNA hasarı saptandı.

Anahtar Kelimeler: Bilgisayarlı tomografi, Comet assay, DNA hasarı, İyonlaştırıcı radyasyon

1. Introduction

Ionizing radiation is a type of radiation that carries enough energy to ionize atoms or molecules as it passes through matter. Life on Earth is always exposed to ionizing radiation from natural sources, such as radon gas. However, the increase in the medical, military and industrial uses of radiation in the last century has resulted in people being exposed to higher doses of radiation. Although the harm of high-dose radiation to human health is known, the effects of low-dose radiation on health have not yet been entirely elucidated [1,2].

In recent years, developments in the computed tomography (CT) technology in the field of health and the use of multi-slice CT have allowed for the thin-section examination to be performed in a single breath-holding process [3,4]. The lower the theoretically known kilovolt (kV) values, the lower the radiation dose received [5]. Accordingly, it is necessary to minimize the radiation dose received by the patient as much as possible by applying the smallest kV value that will not affect the radiological diagnosis.

DNA is constantly exposed to physical agents, such as ultraviolet light, ionizing radiation, and thermal degradation; biological or reactive oxygen species, such as viruses and toxins; and chemical agents, such as hydrolysis and alkylators [6]. DNA damage mostly occurs in the primary structure of the double helix, and this alteration is called the base sequence. This altered sequence can disrupt the ordered helical structure of molecules by introducing chemical bonds and bulky adducts that do not fit into the standard double helix. These resulting structures can cause the single- or double-stranded DNA strand

to be broken and chromosomes to be rearranged [7]. This DNA damage in various pathological and physiological conditions can be detected using the comet assay method [8].

Comet assay is considered to be a fast, sensitive and fairly simple method for detecting DNA damage at the level of individual cells. In the detection of DNA single-strand breaks, alkaline labile regions, and cross-links, the comet assay combines the simplicity of biochemical techniques with a unique single cell approach to cytogenetic analysis [9]. This method was introduced by Ostlin and Johanson [10] and further developed by Singh et al. [11]. In brief, the comet assay depends on the principle of negatively charged DNA fragments moving toward a positive charge in an agarose gel in response to an electric field. DNA helixes that open after contact with alkali or endonucleases increase DNA migration. DNA is visualized by fluorescence microscopy after staining with a DNA-binding dye [12]. Migrating DNA helixes appear like 'comets'. The size and shape of the comet and the distribution of DNA within the comet are associated with the extent of DNA damage [13].

Until recently, the comet assay was mainly used as a testing method in academic and scientific studies. However, currently, the comet assay is perceived as a potentially emerging tool for genotoxicity testing and regulatory applications [9]. Today, clinicians request a CT-guided percutaneous thoracic mass biopsy in patients presenting to the emergency department or followed up in an inpatient clinic for other reasons, who have complaints of chest pain and shortness of breath and who are considered to be eligible for this biopsy procedure after the detection of a thoracic mass on CT. Due to the high demand, patients are exposed to further radiation in the biopsy procedure in addition to diagnostic purposes. Therefore, in our study, we aimed to investigate the effects of radiation dose exposed during the biopsy procedure on lymphocyte DNA in patients who underwent a percutaneous thoracic mass biopsy under the guidance of non-contrast-enhanced CT.

2. Material and Method

Study Group

After receiving approval from the Clinical Research Ethics Committee of Suleyman Demirel University Faculty of Medicine (date: 07.10.2020, decision number: 304), the study included a total of 16 patients who underwent a percutaneous thoracic mass biopsy under CT guidance at Suleyman Demirel University between February 2020 and August 2021. Before the procedure, the age and disease status of the patients were questioned. Patients with low saturation, unstoppable cough, or bleeding diathesis and those that could not be properly positioned for the procedure were excluded from the study. Before the examination, all the patients were informed to increase the quality and safety of the procedure.

Venous blood samples were taken for the comet assay before and after the procedure in all patients included in the study. For the comet assay, 500 µl of heparinized blood + 100 µl of dimethyl sulfoxide (DMSO) + 400 µl of Roswell Park Memorial Institute (RPMI) 1640 Medium were prepared in cryotubes and stored at -80 °C until analysis [14].

Imaging Protocol

After the evaluation of the pre-procedure non-contrast and/or contrast-enhanced thorax CT scans of patients with lung, pleura/thoracic wall masses, the patients were placed in the supine, prone or lateral decubitus position in a way that would minimize complications, such as bleeding and pneumothorax during the CT-guided percutaneous thoracic mass biopsy. All the biopsies were performed with a 128-slice CT device (Definition AS, Siemens Medical Solutions, Forchheim, Germany). A preliminary image was taken with a small field of view on the topogram (guide image) obtained for the preliminary evaluation, and the plane to be biopsied was marked on each patient with a marking pen. Then, the patient's chest was cleaned according to the rules of asepsis, and the working area was covered in a sterile manner. As a short-acting local anesthetic, 10 cc of lidocaine was used in each patient. Subsequently, a 17-G coaxial needle (Geotek, Ankara, Turkey) was advanced toward the mass, and control images were obtained without crossing the pleura. After ensuring the accuracy of the plane, the distance between the needle tip and the lesion was measured on the image, and the pleura was passed in a single attempt. After confirming the presence of a mass in the control image, the chuck of the coaxial needle was removed, and an 18-G semi-automatic biopsy needle (Geotek, Ankara, Turkey) was advanced. A 2 cm-long tissue piece was taken three times by angling the needle tip cranially or caudally and placed in formaldehyde for the pathological examination. The semi-automatic needle was removed,

aspiration was performed with a 20-cc injector, and the coaxial needle was withdrawn. The control image was taken and the procedure was terminated. The stages of the percutaneous thoracic needle biopsy performed in the supine position are shown in Figure 1.

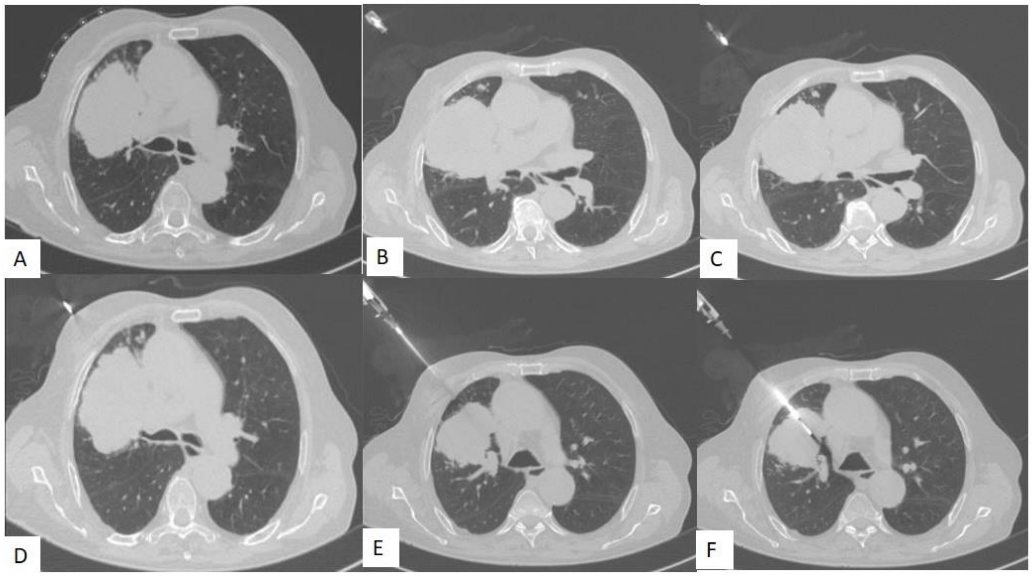

Figure 1: Stages of the computed tomography-guided percutaneous thoracic needle biopsy performed in a 90-year-old male patient in the supine position, showing a peripherally located lobulated contoured mass lesion (order: left to right, top to bottom)

Radiation Dose Calculation During Biopsy

In this study, the effective radiation dose was calculated using a method recommended by the European Working Group as part of the CT quality criteria guide. Accordingly, the effective dose was derived from the product of the dose-length product (DLP, mGy.cm) and a conversion factor (for the examined anatomical region (i.e., chest) (k=0.017 mSv.mGy-1.cm-1), which is indicator of the dose received by the patient throughout the procedure [15]. DLP values used for the calculation of the effective dose were obtained from the protocol automatically provided by the device for each CT section taken. DLP and effective dose values determined in each CT section obtained separately during the biopsy procedure until the needle was advanced and entered into the mass and those obtained throughout the entire procedure were calculated separately for each patient.

Comet Assay

Lymphocyte DNA damage was analyzed using the comet assay method in the blood samples taken before and after the biopsy procedure. First, the blood samples kept at -80 °C were allowed to dissolve in a water bath at 37 °C. Then, 20 µl of whole blood sample was mixed with 150 µl of low melting agarose at 37 °C, and 140 µl of this mixture was placed on a slide pre-coated with normal melting agarose. The slides were kept at 4 °C for 5 minutes, and then placed in the lysis solution and kept in the solution for 1 hour. After lysis, the slides were placed in a tank with a cold alkaline electrophoresis buffer (1 mmol/L EDTA and 300 mM NaOH, pH > 13) and left for 30 minutes to open up the DNA helix. Next, electrophoresis (25 minutes at 25V, 300 mA) was performed. Following electrophoresis, the slides were placed in a neutralization buffer with 0.4 M Tris and pH 7.4 and incubated for 5 minutes. After being removed from the neutralization buffer, the slides were kept at room temperature for 1 hour, and 100 cells were randomly analyzed under a fluorescence microscope (Olympus BX-50, Japan) by adding ethidium bromide. During this evaluation, the cells were classified into five categories from 0 to 4, undamaged (no DNA migration) to severely damaged (DNA migrated) [12]. Scoring was undertaken in accordance with the images obtained (Figure 2).

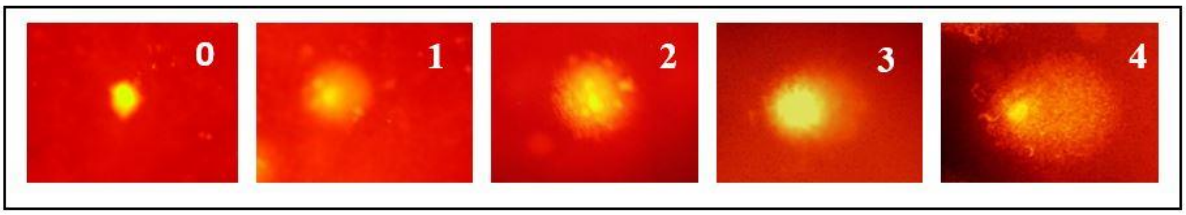

Figure 2: Comet scoring images derived from the study findings. The cells were classified into five categories from 0 to 4, undamaged (no DNA migration) to severely damaged (DNA migrated). 0: cells with no damage, 1: Less damaged cell, 2: moderately damaged cell, 3: very damaged cell, 4: severely damaged cell

Statistical Analysis

Statistical analyses were performed using SPSS v. 21.0 for Windows software package w. The precondition for the normal distribution of data was tested using the Kolmogorov-Smirnov test. One-way analysis of variance was applied to homogeneous data. The Pearson correlation test was used to determine the relationship between the comet score and the scan distance, milliampere-seconds (mAS) and DLP after the procedure. In the evaluation of the results, the upper limit of error margin was accepted as 0.05.

3. Results

Gender (%), mean age, scan distance, mean total mAS and DLP amount are shown in Table 1. In addition, only the intra-group comet score (min-max) before and after biopsy is presented in Table 1. Sixty-five percent of the sample were male and 35% were female, with the mean age being 67.25±8.99 years, mean scan distance 19.92±3.60 sec, mean total mAS 807.43±304.51, and mean DLP 765.44±278.36 mGy.cm.

Lymphocyte DNA damage in blood samples taken from the patients before and after the procedure was analyzed using the comet assay method. Comet scoring was performed according to the image obtained from the study and presented in Figure 2, and the results of statistical analyses are shown in Table 2. Damage was assessed visually, taking into account the frequency of migrating cells and the average tail length. The mean comet score was determined as 200.50±40.54 for the cells that migrated before the procedure and 237.37±27.85 for those that migrated after the procedure. The comet scores obtained before and after the procedure were evaluated with the paired-samples t-test, and the difference was found to be statistically significant (p=0.038). DNA damage was detected in all patients before and after the procedure, but the degree of damage was found to be significantly higher after the procedure. In the statistical analysis of the radiation dose received by the patients and the comet scores, no significant correlation was found between the DLP dose and the comet scores. We observed that the radiation dose received during the procedure increased DNA damage. Figure 3 presents the images of DNA damage before and after the procedure.

The Pearson correlation test was used to analyze the correlation between the post-procedure comet score and the scan distance, total mAS and DLP values. According to the results, there was no statistically significant correlation.

Table 1: Statistical analysis of the variables of the study group

Sociodemographic characteristic	n (%)	Mean ± SD	Median (min-max)
Gender Male Female	 13 (65%) 3 (35%)		
Age		67.25 ± 8.99	
Scan time (sn)		19.92 ± 3.60	
mAS		807.43 ± 304.51	
DLP (mGy.cm)		765.44 ± 278.36	
Pre-procedure comet score (intra-group)			201 (115-264)
Post-procedure comet score (intra-group)			228 (191-286)

Table 2: Pre- and post-procedure comet scores

Evaluation time	Comet score (arbitrary unit)	
	Mean ± SD	P value
Pre-procedure	200.50±40.54	
Post-procedure	237.37±27.85*	0.038

SD: standard deviation. Values given in mean ± SD. The paired-samples t-test was used in the comparison of the two groups.

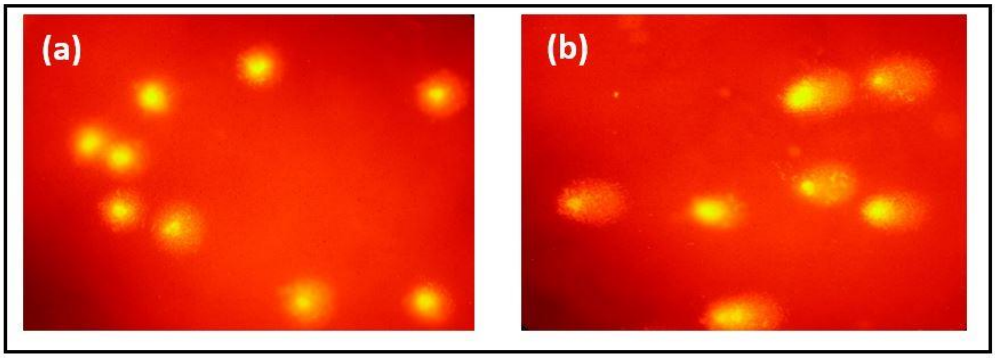

Figure 3: (a) Comet image before the procedure (lymphocyte DNA damage), (b) Comet image after the procedure (lymphocyte DNA damage)

4. Discussion and Conclusion

It is well known that ionizing radiation can directly cause oxidative stress by accumulating energy in cells or increase the formation of free radicals, and oxidative stress can indirectly induce DNA damage [16]. Experimental studies on cellular and molecular radiation biology have shown DNA to be a possible candidate for radiation [17,18]. Physiological and chemical interactions between ionizing radiation and DNA cause damage to nucleotide bases, DNA-DNA and DNA-protein cross-links, and alkali unstable regions, as well as DNA lesions, such as single- or double-strand breaks [19]. Misrepaired double-strand breaks are considered to be the main cause of both chromosomal lesions and gene mutations [20,21].

It is widely accepted that lesions in DNA caused by ionizing radiation can be detected using the comet assay [22].

In a study investigating occupational exposure to ionizing radiation, it was reported that increased levels of reversible DNA damage occurred in the leukocytes of nuclear medicine personnel, and the level of DNA damage was dependent on the type and duration of work. The authors suggested that although most DNA damage detected by the comet assay was repaired, radiation safety needed to be further improved [23]. In a similar study in which human lymphocyte DNA was evaluated using the comet assay among healthcare workers, it was shown that ionizing radiation caused DNA damage [24]. In another study, the effects of 0.1 and 0.4 Gy gamma radiation on human lymphocytes were investigated using the standard and modified comet assay analyses. The authors showed that the parameters of the standard comet assay were significantly higher in the samples exposed to the 4 Gy radiation dose than in those exposed to 0.1 Gy and the control sample [25].

He et al. (2000) used the comet assay and micronucleus test to determine the genotoxic effects of X-ray radiation on human lymphocytes. The authors applied in vitro radiation doses of 0.00, 0.02, 0.05, 0.10, 0.25, 0.50, 1.00 and 2.00 Gy to the isolated human lymphocytes to compare the relationship and sensitivity of these two detection methods. They reported that the mean comet length increased in a dose-dependent manner, but the results of the micronucleus test were not significant, suggesting that comet analysis was more sensitive than the micronucleus test in X-ray-induced genotoxicity [26]. Wilkins et al. (2002) also used the comet assay to quantify apoptosis based on characteristic DNA fragmentation patterns and stated that this method could be used to determine late phase apoptosis, while Annexin V was effective in showing all stages of apoptosis [27].

In the current study, the experimental data of the radiation dose on peripheral blood lymphocytes exposed to individuals who underwent a CT-guided percutaneous thoracic biopsy were investigated with the comet assay. To this end, blood samples were taken before and after the procedure from 16 patients who underwent a CT-guided biopsy. DNA damage was detected in all patients before and after the procedure, but the degree of damage was found to be significantly higher after the procedure. In the statistical analysis of the radiation dose received by the patients and the comet scores, there was no significant correlation between the DLP dose and the comet scores. Our results suggest that the comet assay can be a useful complement to the standard biodosimetric methods. Detection of immediate DNA damage reflects the simultaneous exposure and actual levels of DNA damage in the peripheral blood lymphocytes of patients undergoing CT-guided biopsy.

As a result, there was a significant difference in the comet scores before and after the CT-guided biopsy procedure. We also observed that the radiation dose received during the procedure increased DNA damage. Future studies that determine more specific parameters of the comet assay and/or investigate the same parameters in larger samples can make further contributions to our results.

Declaration of Ethical Code

In this study, we undertake that all the rules required to be followed within the scope of the "Higher Education Institutions Scientific Research and Publication Ethics Directive" are complied with, and that none of the actions stated under the heading "Actions Against Scientific Research and Publication Ethics" are not carried out.

The study was approved by Clinical Research Ethics Committee of Suleyman Demirel University Faculty of Medicine (date: 07.10.2020, decision number: 304). The study was conducted in accordance with the Declaration of Helsinki, 2013.

References

[1] McLean, A.R., Adlen, E.K., Cardis, E., Elliott, A., Goodhead, D.T., Harms-Ringdahl, M., et al. 2017. A restatement of the natural science evidence base concerning the health effects of low-level ionizing radiation. Proc. R. Soc., 284: 1-7.

[2] Tang, F.R., Loganovsky, K. 2018. Low dose or low dose rate ionizing radiation-induced health effect in the human. J. Environ.Raidoact., 192: 32–47.

[3] Jones, A.E., Kline, J.A**.** 2003. Availability of technology to evaluate for pulmonary embolism in academic emergency departments in the United States. J Thromb Haemost., 1: 2240-2.

[4] Richman, P.B., Courtney, D.M., Friese, J., Matthews, J., Field, A., Petri, R., et al. 2004. Prevalence and significance of nonthromboembolic findings on chest computed tomography angiography performed to rule out pulmonary embolism: a multicenter study of 1,025 emergency department patients. Acad Emerg Med, 1: 642-647.

[5] Webb, W. R., Higgins, C. B. 2011. Thoracic Imaging: Pulmonary and Cardiovascular Radiology. Lippincott Williams & Wilkins, 27: 656-682.

[6] Karabas, H. C., Ozcan, I., Sener, L., Guler, S., Albeniz, I., Erdem, T. 2019. Evaluation of cell and DNA damage induced by panoramic radiography. Nigerian Journal of Clinical Practise, 22 (8): 1041-1048.

[7] Vodicka, P., Vodenkova, S., Opattova, A., Vodickova, L**.** 2019. DNA damage and repair measured by comet assay in cancer patients. Mutat Res Gen Tox En, 843: 95–110.

[8] Kuchařová, M., Hronek, M., Rybáková, K., Zadák, Z., Štětina, R., Josková, V., et al. 2019. Comet Assay and Its Use for Evaluating Oxidative DNA Damage in Some Pathological States. Physiol. Res., 68: 1-15.

[9] Kumaravel, T.S., Jha, A.N. 2006. Reliable Comet assay measurements for detecting DNA damageinduced by ionising radiation and chemicals. Mutation Research, 605: 7-16.

[10] Ostling, O., Johanson, K.J. 1984. Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells. Biochem. Biophys. Res Commun, 123: 291-298.

[11] Singh, N.P., McCoy, M.T., Tice, R.R., Schneider, E.L**.** 1998. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res, 75: 184-191.

[12] Collins, A.R**.** 2004. The comet assay for DNA damage and repair. Molecular Biotechnology, 26: 249–261.

[13] Carolina, A., Sozua, F., Yujra, V.Q., Pisani, L.P., Viana, M.D.B., Castro, G.M.D., et al. 2019. The Use of Single-Cell Comet Assay on Oral Cells: A Critical Review. Anticancer Res, 39: 4011-4017.

[14] Hininger, I., Chollat-Namy, A., Sauvaigo, S., Osman, M., Faure, H., Cadet, J., et al. 2004. Assessment of DNA damage by comet assay on frozen total blood: method and evaluation in smokers and non-smokers. Mutation Res, 558: 75–80.

[15] Hausleiter, J., Meyer, T., Hadamitzky, M., Huber, E., Zanki, M., Martinoff, S., et al. 2006. Radiation dose estimates from cardiac multislice computed tomography in daily practice: impact of different scanning protocols on effective dose estimates. Circulation, 113: 1305-1310.

[16] Kaur, S., Sangeeta, G.K.K., Gautam, N. 2017. Assessment of Radiation Induced DNA Damage in Human Peripheral Blood Lymphocytes Using COMET Assay. Int. J. Life Sci Scienti. Res, 3 (4): 1208-1214.
[17] Hofer, K.G., Lin, X., Schneiderman, M.H. 2000. Paradoxical effects of iodine-125 decays in parent and daughter DNA: A new target model for radiation damage. Radiat. Res., 153: 428–435.

[18] Lara, C.M., Hill, M.A., Jenner, T.J., Papworth, D., O'Neill, P. 2001. Dependence of the yield of DNA double strand breaks in Chinese hamster V79-4 cells on photon energy of characteristic ultrasoft X rays. Radiat. Res., 155: 440–448.

[19] Nikjoo, H., Neill, P.O., Wilson, W.E., Goodhead, D.T. 2001. Computational Approach for Determining the Spectrum of DNA Damage Induced by Ionizing Radiation. Radiation Res., 156:577-583.

[20] Gloss, L.M., Placek, B.J**.** 2002. The effect of salts on the stability of the H2A–H2B histone dimer. Biochem., 41 (50): 14951-59.

[21] Santivasi, W.L., Xia, F. 2014. Ionizing Radiation-Induced DNA Damage, Response, and Repair. Antioxid. Redox Signal., 21 (2): 251–259.

[22] Touil, N., Aka, P.V., Buchet, J.P., Thierens, H., Kirsch-Volders, M. 2002. Assessment of genotoxic effects related to chronic low level exposure to ionizing radiation using biomarkers for DNA damage and repair. Mutagen, 7 (3): 223-32.

[23] Dobrzyn´ska, M.A., Pachockı, K.A., Gajowık, A., Radzikowska, J., Sackiewicz, A**.** 2014. The Effect Occupational Exposure to Ionizing Radiation on the DNA Damage in Peripheral Blood Leukocytes of Nuclear Medicine Personnel. J Occup Health, 56: 379–386.

[24] Garaj – Vrhovac, V., Kopjar, N**.** 2003. The alkaline Comet assay as biomarker in assessment of DNA damage in medical personnel occupationally exposed to ionizing radiation. Mutagenesis, 8 (3): 265-271.

[25] Mikloš, M., Gajski, G., Garaj – Vrhovac, V. 2009. Usage of the standard and modified comet assay in assessment of DNA damage in human lymphocytes after exposure to ionizing radiation. Radiol Oncol, 43 (2): 97-107.

[26] He, J.L., Chen, W.L., Jin, L.F., Jin, H.Y. 2000. Comparative evaluation of the in vitro micronucleus test and the comet assay for the detection of genotoxiceffects of X-ray radiation. Mutat Res, 469 (2): 223-31.

[27] Wilkins, R.C., Kutzner, B.C., Truong, M., Sanchez-Dardon, J., McLean, J.R.N. 2002. Analysis of radiation-induced apoptosis in human lymphocytes: flow cytometry using Annexin V and propidiumiodide versus the neutral comet assay. Cytometry, 48 (1): 14-9.