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Comparative Analysis of Comet and Micronucleus Assays in Detecting DNA Repair Across Radiation Dose Ranges

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This study compares the efficacy of the COMET assay and micronucleus (MN) assay in detecting DNA damage and repair across varying radiation dose ranges. The analysis focuses on evaluating the capability of each assay to assess DNA repair at different time points post-radiation exposure. Results indicate that comparing COMET scores at 3 hours and 24 hours post-radiation dose, for radiation doses below 200 cGy, yielded a significant p-value of 8.9E−07. Conversely, comparing MN scores at the same time points and radiation doses showed a p-value of 0.000196. This suggests that the COMET assay is more capable of detecting DNA repair at radiation doses below 200 cGy. In contrast, comparing COMET scores at 3 hours and 24 hours post-radiation dose, for radiation doses above 200 cGy, resulted in a p-value of 0.00340967. Comparing MN scores at the same conditions yielded a p-value of 0.0012. These findings indicate that the MN assay is more adept at detecting DNA repair at radiation doses exceeding 200 cGy. In conclusion**,** the COMET assay proves more suited for low-dose radiation studies, offering high sensitivity in detecting DNA damage and repair at doses below 200 cGy. Conversely, the MN assay demonstrates superior capability in assessing DNA damage and repair at higher radiation doses (>200 cGy). Both assays complement each other, providing a comprehensive toolkit for evaluating DNA damage across a broad spectrum of radiation exposures.

Keywords: Ionizing radiation, DNA Repair, Comet assay, Micronuclei assay, Radiation dose

Introduction

Radiation epidemiology is a crucial field of study that investigates the relationship between radiation exposure and its impact on human populations' health. This interdisciplinary field integrates principles from radiation physics, biology, and epidemiology to elucidate the risks and mechanisms underlying radiation-induced health outcomes. Given the increasing utilization of radiation in industrial, medical, and environmental contexts, comprehending the effects of radiation on human health holds heightened significance in contemporary times.

Ionizing and non-ionizing radiation represent the two main classifications of radiation exposure. Ionizing radiation, encompassing gamma rays, X-rays, beta particles, and alpha particles, possesses adequate energy to dislodge tightly bound electrons from atoms, leading

to ion formation. It is widely acknowledged that ionizing radiation is capable of directly damaging DNA. Conversely, non-ionizing radiation, while lacking the energy for atomic ionization, can still exert significant long-term biological effects. Examples of non-ionizing radiation encompass ultraviolet (UV), visible, infrared, microwave, and radiofrequency radiation. Radiation exposure can emanate from a diverse array of anthropogenic and natural sources. Natural sources comprise cosmic rays, radon gas, and terrestrial radiation originating from naturally occurring radioactive minerals within the earth's crust. Artificial sources encompass nuclear power facilities, industrial applications, medical procedures (such as radiation therapy, CT scans, and X-rays), and remnants from nuclear weapons testing.

The health impacts of radiation exposure are contingent upon various factors, such as dosage, type, duration, and the specific body part exposed. Acute radiation syndrome (ARS) manifests through symptoms like nausea, vomiting, hair loss, skin burns, and in severe instances, fatality, resulting from high doses of radiation received over a short timeframe. Conversely, chronic exposure to low doses of radiation is associated with an increased likelihood of enduring health complications such as cardiovascular ailments and cancer.

Various types of DNA damage, including base modifications, crosslinks, single-strand breaks (SSBs), and double-strand breaks (DSBs), are induced by exposure to radiation, particularly ionizing radiation. Failure to accurately repair these DNA lesions can lead to genetic mutations, genomic instability, and ultimately carcinogenesis. The maintenance of genomic integrity and the mitigation of cancer risk rely on the organism's ability to efficiently rectify DNA damage. Therefore, a fundamental focus in the field of radiation biology involves elucidating the intricate mechanisms governing DNA repair.

Two frequently utilized laboratory techniques for the investigation of DNA damage and repair include the COMET assay, also referred to as single-cell gel electrophoresis, and the micronucleus (MN) assay. Both methodologies offer unique advantages in the identification and quantification of DNA damage, along with the evaluation of repair processes.

The COMET assay is known for its high sensitivity in detecting minimal levels of DNA damage at a single-cell resolution, proving valuable in evaluating DNA damage caused by low doses of radiation. This method entails encapsulating cells in agarose gel, lysing the cells to release DNA, and exposing the gel to electrophoresis. The damaged DNA moves out of the nucleus, creating a COMET -like tail, from which the assay derives its name. The distance of DNA migration serves as a quantitative indicator of DNA damage [1-2].

The micronucleus assay is highly efficient in detecting chromosomal damage, especially at elevated radiation levels where chromosomal aberrations are more prevalent. This method detects micronuclei, minute extranuclear structures that arise when chromosome fragments or entire chromosomes are unable to integrate into daughter nuclei during cellular division. The presence of micronuclei frequency is indicative of genomic instability and chromosomal harm [3−4].

The comprehension of the impacts of radiation exposure on health heavily relies on radiation epidemiology. By thoroughly understanding DNA damage and repair mechanisms and utilizing advanced laboratory methods such as COMET and micronucleus assays, researchers can enhance the precision of assessing the possible risks associated with radiation exposure and developing effective protective measures. In a context of increasing radiation exposure, these endeavors play a crucial role in safeguarding public health.

Study Design and Sample Collection

This study examines DNA damage and repair mechanisms in human blood cells exposed to ionizing radiation through the micronucleus (MN) assay and the COMET assay. Peripheral blood samples were obtained from **24 cancer patients** at Alexandria University Main Hospital with their informed consent. Samples were collected using BD Vacutainer before radiotherapy, 3 hours post-radiotherapy, and 24 hours post-radiotherapy which produced from linear accelerator to deliver therapeutic X-rays to a patient's tumor.

We processed the collected samples on the same day as most of the assays have to be done on fresh blood at Alexandria University Cancer Research Cluster, Micronuclei Count.

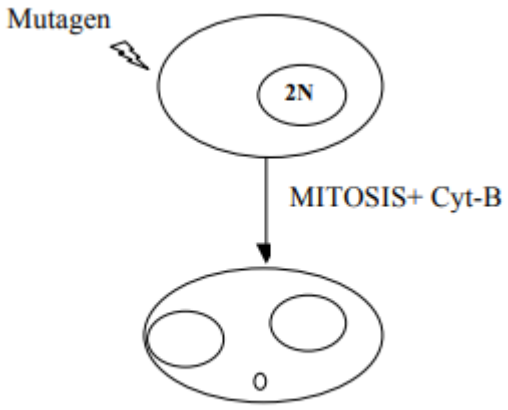
Regarding experimental replication and statistical reliability, we ensured the following:

- Blood samples were collected from 24 patients, with each patient's sample processed in triplicate.
- Each sample involved the preparation of 6 slides, with 100 cells scored per slide to enhance the robustness of the data.
- Data were analyzed using statistical software (Excel 2013, Microsoft, Redmond, WA, USA), with appropriate error analysis included to validate the conclusions.

The radiotherapy schedule was fractionated, with blood samples collected after the first fraction at 3 hours and 24 hours. The “24 h post-radiotherapy” refers to 24 hours after the first fraction.

Experimental Procedures

Micronucleus Count: Micronuclei counts are the standard available current method for measuring DNA damage, dependent on cell culture, which needs 72 hours to produce results as shown in **Fig1**. [5,6].



Scheme of MN formation in binucleated cells

Fig.1.

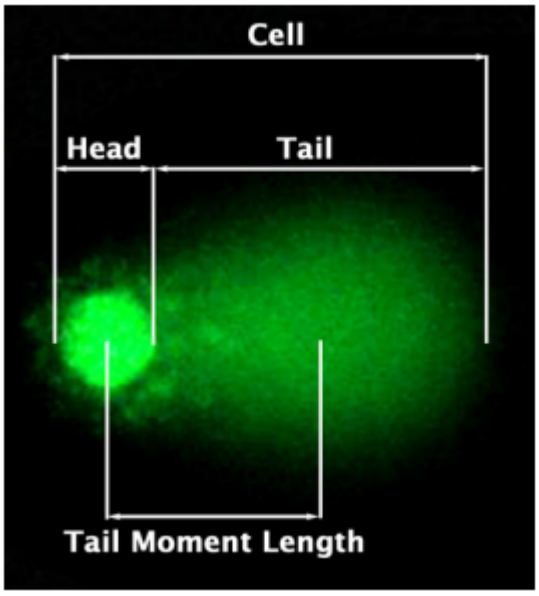


Fig.2.

COMET Assay. This technique involves the following steps:

1. Preparation of slides with a layer of Normal Melting Agarose (NMA).
2. Deposition on the slides of the blood embedded in Low Melting Agarose (LMA).
3. Cellular lysis to remove cellular membranes.
4. Rinsing in electrophoretic buffer.
5. Unwinding in electrophoretic buffer to unroll DNA strands.
6. Electrophoresis in basic conditions (pH 13).
7. Neutralization of alkaline products.
8. Dehydration with alcoholic passages.
9. Staining of DNA and visualization of COMET t images as shown in **Fig.2**.
10. Quantification of DNA damage.

Table 1. Paired Two-Tailed t-Test Comparing Comet Scores at 3 Hours and 24 Hours Post-Radiation Dose

	Variable 1	*Variable 2*
Mean	216.98	146.36
Variance	2104.01	1308.86
Observations	24	24
Pearson Correlation	0.590	
Hypothesized Mean Difference	0	
Df	23	
t Stat	9.077	
P(T<=t) one-tail	2.299E-09	
t Critical one-tail	1.7139	
P(T<=t) two-tail	4.6E-09	
t Critical two-tail	2.07	

Table 2. Paired Two-Tailed t-Test Comparing MN Scores at 3 Hours and 24 Hours Post-Radiation Dose

	Variable 1	*Variable 2*
Mean	12.15	8.17
Variance	22.68	18.28
Observations	24	24
Pearson Correlation	0.79	
Hypothesized Mean Difference	0	
Df	23	
t Stat	6.61	
P(T<=t) one-tail	4.75E-07	
t Critical one-tail	1.71	
P(T<=t) two-tail	9.49E-07	
t Critical two-tail	2.07	

Table 3. Paired Two-Tailed t-Test Comparing COMET Scores at 3 Hours and 24 Hours Post-Radiation Dose, for radiation dose <200 cGy

	Variable 1	*Variable 2*
Mean	214.96	143.36
Variance	2112.45	1456.631
Observations	16	16
Pearson Correlation	0.65	
Hypothesized Mean Difference	0	
Df	15	
t Stat	7.98	
P(T<=t) one-tail	4.45E-07	
t Critical one-tail	1.75	
P(T<=t) two-tail	8.9E-07	
t Critical two-tail	2.13	

Table 4. Paired Two-Tailed t-Test Comparing MN Scores at 3 Hours and 24 Hours Post-Radiation Dose, for radiation dose <200 cGy

	Variable 1	*Variable 2*
Mean	12.66	8.53
Variance	23.36	23.41
Observations	16	16
Pearson Correlation	0.76	
Hypothesized Mean Difference	0	
Df	15	
t Stat	4.89	
P(T<=t) one-tail	9.8E-05	
t Critical one-tail	1.75	
P(T<=t) two-tail	0.00026	
t Critical two-tail	2.135	

Table 5. Paired Two-Tailed t-Test Comparing COMET Scores at 3 Hours and 24 Hours Post-Radiation Dose, for radiation dose >200 cGy

	Variable 1	*Variable 2*
Mean	221.02	152.35
Variance	2358.6	1117.545
Observations	8	8
Pearson Correlation	0.45	
Hypothesized Mean Difference	0	
Df	7	
t Stat	4.34	
P(T<=t) one-tail	0.0017	
t Critical one-tail	1.9	
P(T<=t) two-tail	0.0034	
t Critical two-tail	2.36	

Table 6. Paired Two-Tailed t-Test Comparing MN Scores at 3 Hours and 24 Hours Post-Radiation Dose, for radiation dose >200 cGy

	Variable 1	*Variable 2*
Mean	11.13	7.44
Variance	22.76	8.96
Observations	8	8
Pearson Correlation	0.976	
Hypothesized Mean Difference	0	
Df	7	
t Stat	5.216	
P(T<=t) one-tail	0.0006	
t Critical one-tail	1.89	
P(T<=t) two-tail	0.0012	
t Critical two-tail	2.36	

Statistical Analysis

The statistical analysis was performed by (Excel 2013 Microsoft Redmond, WA, USA). A two-tailed Student's t-test performed between the experimental groups to determine which groups were significantly different at $P< 0.05$ level.

Results and Discussion

Comparing COMET Scores at 3 Hours and 24 Hours Post-Radiation Dose, for radiation dose <200 cGy showed p-value = 8.9E−07. While, Comparing MN Scores at 3 Hours and 24 Hours Post-Radiation Dose, for radiation dose <200 cGy showed p-value = 0.000196. This indicates that the COMET assay is more capable of detecting DNA repair at radiation doses below 200 cGy (Table 3 and 4).

For radiation doses >200 cGy, comparing COMET Scores at 3 Hours and 24 Hours Post-Radiation Dose showed a p-value of 0.0034, while MN Scores yielded a p-value of 0.0012. This suggests that the MN assay is more adept at detecting DNA repair at higher doses (Table 5 and 6).

The ability of COMET assay to detect the DNA repair should always be compared with the response of irradiated cells. This makes sense since low linear energy transfer (LET) radiation induces lesions at defined rates, close to 1000 SSBs per mammalian cell per 100 cGy (0.3 SSBs per 10^9 Da per 100 cGy). [7,8].

The COMET assay's high sensitivity to low levels of DNA damage allows it to detect single-strand breaks, double-strand breaks, and alkali-labile sites at a single-cell level, particularly useful for low-dose radiation studies [7−9]. It provides quantitative measurements through parameters such as tail length, tail intensity, and tail moment, which are more precise compared to counting micronuclei [10−12]. The COMET assay is also applicable to a wide range of cell types, including non-dividing cells.

In contrast, the MN assay excels in detecting chromosomal fragments or whole chromosomes not included in the main nuclei during cell division, which are more likely at high doses [13−14]. It effectively assesses genomic instability and can detect cumulative damage over multiple cell cycles.

Fig.3 shows the one of the cases after 24 hour exposure of 180 cGy of MN assay indicate a cell of binuclited however of the same patient and the same condition COMET assay showed us different levels of damages range between type II to type III.

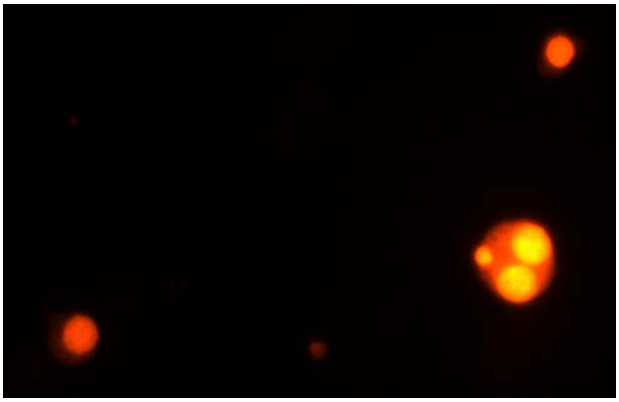


Fig.3

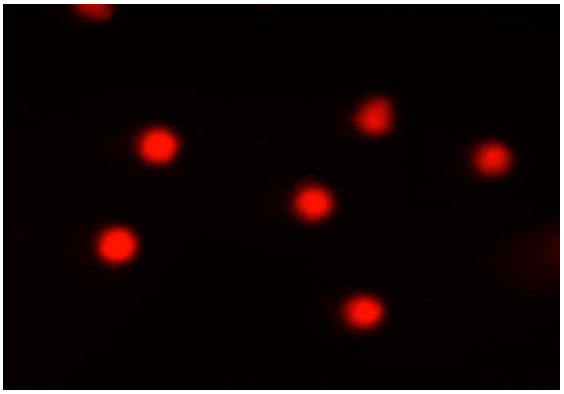


Fig.4

Conclusion

Low-Dose Radiation Detection (<200 cGy): The COMET assay is superior due to its high sensitivity and ability to detect and quantify DNA damage at a finer scale. The MN assay is less sensitive at these levels, as it primarily detects larger chromosomal aberrations.
High-Dose Radiation Detection (>200 cGy): The MN assay provides robust detection of chromosomal damage, evaluates genomic instability, and offers cumulative damage detection over time. The COMET assay, while valuable, may be less effective at distinguishing extensive chromosomal damage and complex rearrangements.

Both assays complement each other, providing a comprehensive toolkit for evaluating DNA damage across a broad range of radiation exposures.

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