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Original Research Article

Assessment of Radiation-Induced Cytogenetic Alterations Following Hysterosalpingography in Women Undergoing Infertility Evaluation

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Abstract

Background: Hysterosalpingography (HSG) is a widely used fluoroscopic procedure for evaluating uterine cavity abnormalities and tubal patency in women undergoing infertility assessment. Although radiation exposure during HSG is relatively low, concerns remain regarding its potential cytogenetic effects. The present study aimed to evaluate cytogenetic damage induced during HSG using the cytokinesis-blocked micronucleus assay.

Methods: This hospital-based cross-sectional study included 32 women undergoing HSG for infertility evaluation. Peripheral venous blood samples were collected before and after the procedure. Cytogenetic damage was assessed using the cytokinesis-blocked micronucleus assay in cultured peripheral blood lymphocytes. Micronucleate binucleated cells, total micronuclei, nuclear buds, and nucleoplasmic bridges were quantified. Radiation exposure parameters including fluoroscopy time, dose-area product (DAP), cumulative air kerma, and effective radiation dose were recorded. Statistical analysis included paired t-test, Pearson correlation analysis, and multivariable linear regression.

Results: The mean age of participants was 29.8 ± 4.2 years. Following HSG, micronucleate binucleated cells increased significantly from 8.72 ± 2.11 to 12.47 ± 2.83 per 1000 cells ($p<0.001$). Significant increases were also observed in total micronuclei (9.34 ± 2.36 vs. 13.59 ± 3.07; $p<0.001$), nuclear buds (1.41 ± 0.67 vs. 2.19 ± 0.88; $p<0.001$), and nucleoplasmic bridges (0.81 ± 0.44 vs. 1.36 ± 0.61; $p<0.001$). Micronucleus induction demonstrated significant positive correlations with fluoroscopy time ($r=0.612$), DAP ($r=0.684$), cumulative air kerma ($r=0.703$), and effective radiation dose ($r=0.658$) (all $p<0.001$). Multivariable regression identified DAP ($\beta=1.182$, $p=0.002$) and fluoroscopy time ($\beta=0.064$, $p=0.005$) as independent predictors of increased micronucleus frequency.

Conclusion: Hysterosalpingography induces measurable cytogenetic damage despite relatively low radiation exposure. The extent of genomic instability is significantly associated with procedural radiation parameters, highlighting a dose-dependent biological effect. Optimization of fluoroscopic techniques and strict adherence to radiation safety principles may help reduce cytogenetic damage while maintaining the diagnostic utility of HSG.

Keywords: Hysterosalpingography; Cytogenetic Damage; Micronucleus Assay; Ionizing Radiation; Genomic Instability.

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Introduction

Infertility affects nearly 10–15% of couples worldwide and remains a major reproductive health concern [1]. Tubal pathology and uterine cavity abnormalities account for a significant proportion of female infertility cases, making accurate assessment of the female reproductive tract an essential component of infertility evaluation [1]. Hysterosalpingography (HSG) is one of the most commonly employed radiological investigations for assessing the morphology of the uterine cavity and the patency of the fallopian tubes [2]. The procedure involves the administration of iodinated contrast material into the uterine cavity followed by fluoroscopic and radiographic imaging, allowing visualization of uterine and tubal abnormalities that may contribute to infertility [2]. HSG continues to be widely utilized because of its diagnostic accuracy, accessibility, and cost-effectiveness in infertility workup [3]. Despite its clinical utility, HSG exposes patients to ionizing radiation, with the reproductive organs, particularly the uterus and ovaries, receiving the highest absorbed doses

during the examination [4]. Previous dosimetric studies have reported effective radiation doses ranging from approximately 1.2 to 3.1 mSv, while ovarian doses may vary from 2.7 to 9.0 mGy depending on fluoroscopy time, imaging technique, and equipment characteristics [4,5]. Although these doses are considered relatively low and fall within accepted diagnostic limits, concerns remain regarding the potential biological effects of radiation exposure on reproductive tissues and peripheral blood cells [5].

Ionizing radiation is a well-established genotoxic agent capable of inducing DNA damage through direct interaction with cellular DNA and indirect generation of reactive oxygen species [6]. Such damage may result in chromosomal breaks, deletions, translocations, and the formation of micronuclei, which are small extranuclear bodies containing chromosomal fragments or whole chromosomes that fail to incorporate into daughter nuclei during cell division [7]. Cytogenetic biomarkers, particularly micronucleus frequency in peripheral blood lymphocytes, are recognized as sensitive indicators of chromosomal instability and radiation-induced genetic damage [7]. The assessment of these biomarkers provides valuable information regarding the biological effects of radiation beyond conventional physical dose measurements [7].

The Cytokinesis-Blocked Micronucleus (CBMN) assay has emerged as a validated and widely accepted technique for evaluating radiation-induced cytogenetic damage in human populations [8]. Compared with physical dosimetry alone, biological dosimetry using the CBMN assay offers direct evidence of cellular response to radiation exposure and has been extensively employed in occupational, environmental, and medical radiation studies [9]. Micronucleus formation reflects cumulative chromosomal damage and is considered a reliable surrogate marker of genomic instability and potential long-term health risks associated with ionizing radiation exposure [9]. So, in this context, the present study was aimed to evaluate cytogenetic damage induced during hysterosalpingography by assessing micronucleus formation in peripheral blood lymphocytes.

Materials and Methods

Study Design and Setting: This hospital-based cross-sectional study was conducted in the Department of Radiodiagnosis in collaboration with the Department of Obstetrics and Gynaecology at a Multi-Speciality Hospital over a period of 24 months from January 2023 to December 2024.

The study was designed to evaluate cytogenetic damage induced by ionizing radiation exposure during hysterosalpingography (HSG) procedures in women undergoing infertility evaluation. Ethical approval was obtained from the Institutional Ethics Committee prior to commencement of the study, and written informed consent was obtained from all participants before enrollment.

Study Population and Sample Size: The study included women referred for HSG as part of the routine diagnostic workup for primary or secondary infertility. Participants aged between 20 and 40 years who were scheduled to undergo HSG during the proliferative phase of the menstrual cycle (between the 7th and 10th day) were considered eligible for inclusion. Women with a history of previous exposure to diagnostic or therapeutic radiation within the preceding six months, occupational radiation exposure, smoking, alcohol consumption, chronic systemic illnesses, autoimmune disorders, malignancy, recent viral infections, or current use of medications known to influence chromosomal stability were excluded. Patients with pregnancy, active pelvic inflammatory disease, abnormal uterine bleeding, or known hypersensitivity to iodinated contrast media were also excluded from the study. So, a total of 32 consecutive eligible women undergoing HSG during the study period were recruited using a convenient sampling technique.

Hysterosalpingography Procedure: All HSG examinations were performed using a digital fluoroscopy unit by experienced radiologists following a standardized protocol. The procedure was scheduled during the early proliferative phase of the menstrual cycle to avoid the possibility of pregnancy. After placing the patient in the lithotomy position and maintaining aseptic precautions, a sterile cervical cannula was introduced through the external cervical os. A water-soluble non-ionic iodinated contrast medium was slowly injected into the uterine cavity under fluoroscopic guidance. Sequential fluoroscopic images were obtained to assess uterine cavity morphology, tubal patency, and peritoneal spill of contrast material. Radiation exposure parameters including fluoroscopy time, dose-area product (DAP), and cumulative radiation dose were recorded for each participant directly from the fluoroscopy console immediately after completion of the procedure.

Blood Sample Collection: Peripheral venous blood samples were collected from each participant at two time points. The first sample was obtained immediately before the HSG procedure to establish baseline cytogenetic status. A second blood sample was collected 24 hours after completion of the procedure to assess radiation-induced cytogenetic alterations. Approximately 5 mL of venous blood was drawn aseptically from the antecubital vein into sterile heparinized vacutainer tubes and

transported to the cytogenetics laboratory for further analysis.

Cytokinesis-Blocked Micronucleus Assay: Cytogenetic damage was evaluated using the Cytokinesis-Blocked Micronucleus (CBMN) assay following the standardized protocol described by Fenech with minor modifications. Briefly, 0.5 mL of whole blood was cultured in 4.5 mL of RPMI-1640 medium supplemented with 15% fetal bovine serum, antibiotics, and phytohemagglutinin to stimulate lymphocyte proliferation. Cultures were incubated at 37°C in a humidified atmosphere containing 5% carbon dioxide for 72 hours. At 44 hours after culture initiation, cytochalasin-B was added at a final concentration of 6 µg/mL to inhibit cytokinesis and facilitate identification of binucleated cells.

At the end of the incubation period, cells were harvested by centrifugation and treated with a hypotonic potassium chloride solution, followed by fixation using methanol-acetic acid fixative. Fixed cell suspensions were dropped onto clean microscopic slides, air dried, and stained with 5% Giemsa stain. Prepared slides were coded and examined independently by two trained observers who were blinded to the sampling time and clinical details of the participants.

Assessment of Micronucleus Frequency: Micronucleus frequency was determined according to the criteria established by Fenech. For each sample, a minimum of 1,000 well-preserved binucleated lymphocytes were evaluated under a light microscope at 1000× magnification. Micronuclei were identified as round or oval chromatin-containing structures with staining intensity and texture similar to the main nucleus but clearly separated from it. The number of micronucleate binucleated cells and the total number of micronuclei were recorded. Cytogenetic damage was expressed as the frequency of micronucleate binucleated cells per 1,000 binucleated lymphocytes.

Outcome Measures: The primary outcome measure was the change in micronucleus frequency before and after HSG exposure. Secondary outcome measures included the relationship between micronucleus frequency and radiation exposure parameters such as fluoroscopy time, dose-area product, and cumulative radiation dose. Demographic and clinical characteristics including age, type and duration of infertility, and relevant reproductive history were also recorded and analyzed.

Statistical Analysis: Data were entered into Microsoft Excel and analyzed using Statistical Package for Social Sciences (SPSS) software version 26.0. Continuous variables were expressed as mean ± standard deviation or median with interquartile range depending on data distribution, while categorical variables were presented as frequencies and percentages.

Normality of data was assessed using the Shapiro-Wilk test. Pre- and post-procedure micronucleus frequencies were compared using the paired Student's t-test for normally distributed variables or the Wilcoxon signed-rank test for non-normally distributed variables. Correlations between cytogenetic damage and radiation exposure parameters were evaluated using Pearson's correlation coefficients as appropriate. A p-value of less than 0.05 was considered statistically significant.

Results

The study included 32 women undergoing HSG for infertility evaluation. The mean age of participants was 29.8 ± 4.2 years, with the majority belonging to the 26–30 years age group (40.6%), followed by 31–35 years (28.1%) and ≤25 years (21.9%). The mean duration of infertility was 4.1 ± 2.3 years.

Primary infertility was more common than secondary infertility, accounting for 68.8% and 31.2% of cases, respectively. The mean body mass index (BMI) was 24.6 ± 3.8 kg/m², with over half of the participants (53.1%) having normal BMI.

Previous pelvic surgery and a history of pelvic inflammatory disease were reported in 15.6% and 9.4% of participants, respectively, indicating that most women had no major predisposing gynecological conditions (Table 1).

Table 1: Baseline Demographic and Clinical Characteristics of Women Undergoing Hysterosalpingography (HSG) (n=32)

Variable	Frequency (%)/Mean ± SD
Age (years)	29.8 ± 4.2
Age group (years)	
≤25	7 (21.9)
26–30	13 (40.6)
31–35	9 (28.1)
>35	3 (9.4)
Duration of infertility (years)	4.1 ± 2.3
Type of infertility	

Primary infertility	22 (68.8)
Secondary infertility	10 (31.2)
BMI (kg/m²)	24.6 ± 3.8
Normal weight (18.5–24.9)	17 (53.1)
Overweight (25–29.9)	11 (34.4)
Obese (≥30)	4 (12.5)
Previous pelvic surgery	5 (15.6)
History of pelvic inflammatory disease	3 (9.4)

BMI: Body Mass Index.

Radiation exposure parameters recorded during HSG demonstrated relatively low-dose diagnostic radiation exposure. The mean fluoroscopy time was 41.8 ± 13.5 seconds, ranging from 18 to 72 seconds. The mean dose-area product (DAP) was 2.38 ± 0.84 Gy·cm², while the mean cumulative air kerma was 11.6 ± 3.9 mGy. The estimated effective radiation dose received during the procedure was 1.84 ± 0.63 mSv. The observed variability in radiation parameters reflected differences in procedural complexity and fluoroscopy duration among participants (Table 2).

Table 2: Radiation Exposure Parameters During Hysterosalpingography Procedure (n=32)

Parameter	Mean ± SD	Range
Fluoroscopy time (seconds)	41.8 ± 13.5	18–72
Dose Area Product (DAP) (Gy·cm²)	2.38 ± 0.84	1.02–4.36
Cumulative Air Kerma (mGy)	11.6 ± 3.9	4.8–21.5
Estimated Effective Dose (mSv)	1.84 ± 0.63	0.82–3.52

DAP = Dose Area Product; mGy = milligray; mSv = millisievert.

A statistically significant increase was observed in all assessed cytogenetic damage parameters following HSG exposure. The mean frequency of micronucleate binucleated cells increased from 8.72 ± 2.11 to 12.47 ± 2.83 per 1000 cells, representing a mean increase of 3.75 ± 1.96 cells ($t=10.83$, $p<0.001$). Similarly, total micronuclei per 1000 binucleated cells increased significantly from 9.34 ± 2.36 to 13.59 ± 3.07 ($t=11.02$, $p<0.001$). Nuclear buds increased from 1.41 ± 0.67 to 2.19 ± 0.88 per 1000 cells ($t=7.49$, $p<0.001$), while nucleoplasmic bridges increased from 0.81 ± 0.44 to 1.36 ± 0.61 per 1000 cells ($t=6.76$, $p<0.001$). Overall, these findings indicate measurable cytogenetic damage following exposure to ionizing radiation during the HSG procedure (Table 3 and Figure 1).

Table 3: Comparison of Cytogenetic Damage Markers Before and After Hysterosalpingography Exposure (n=32)

Parameter	Pre-HSG	Post-HSG	Mean Difference	Test Statistic	p-value
	Mean ± SD				
Micronucleate binucleated cells per 1000 cells	8.72 ± 2.11	12.47 ± 2.83	3.75 ± 1.96	t=10.83	<0.001
Total micronuclei per 1000 binucleated cells	9.34 ± 2.36	13.59 ± 3.07	4.25 ± 2.18	t=11.02	<0.001
Nuclear buds per 1000 cells	1.41 ± 0.67	2.19 ± 0.88	0.78 ± 0.59	t=7.49	<0.001
Nucleoplasmic bridges per 1000 cells	0.81 ± 0.44	1.36 ± 0.61	0.55 ± 0.46	t=6.76	<0.001

Statistical comparison performed using paired Student's t-test.

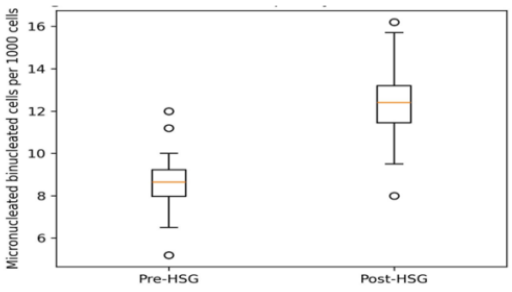

Figure 1: Box-and-Whisker Plot Showing Distribution of Micronucleus Frequency Before and After Hysterosalpingography

Micronucleus frequency expressed as micronucleate binucleated cells per 1000 cells. Horizontal line represents median; box represents interquartile range; whiskers indicate minimum and maximum values.

When stratified according to infertility type, both primary and secondary infertility groups demonstrated significant increases in micronucleus frequency following HSG. Women with primary infertility showed an increase from 8.55 ± 2.04 to 12.31 ± 2.76 cells per 1000 cells (mean increase: 3.76 ± 1.92; p<0.001), while those with secondary infertility demonstrated an increase from 9.10 ± 2.28 to 12.82 ± 3.01 cells per 1000 cells (mean increase: 3.72 ± 2.10; p<0.001).

However, no significant difference was observed in the magnitude of increase between the two groups (p=0.944), suggesting that infertility type did not influence radiation-induced cytogenetic response (Table 4).

Table 4: Comparison of Change in Micronucleus Frequency According to Type of Infertility

Type of Infertility	Pre-HSG	Post-HSG	Mean Increase	p-value
Primary infertility (n=22)	8.55 ± 2.04	12.31 ± 2.76	3.76 ± 1.92	<0.001
Secondary infertility (n=10)	9.10 ± 2.28	12.82 ± 3.01	3.72 ± 2.10	<0.001
Between-group comparison	—	—	—	0.944

Within-group comparisons performed using paired Student's t-test; between-group comparison performed using independent Student's t-test. Correlation analysis revealed significant positive associations between radiation exposure metrics and the increase in micronucleus frequency. The strongest correlation was observed with cumulative air kerma (r=0.703, p<0.001), followed by DAP (r=0.684, p<0.001), effective radiation dose (r=0.658, p<0.001), and fluoroscopy time (r=0.612, p<0.001). In contrast, demographic and clinical variables including age (r=0.142, p=0.438), duration of infertility (r=0.171, p=0.349), and BMI (r=0.108, p=0.556) showed no significant correlation with cytogenetic damage.

These findings suggest that radiation exposure parameters, rather than patient-related factors, primarily contributed to the observed increase in micronucleus formation (Table 5, Figure 2 and 3).

Table 5: Correlation Between Radiation Exposure Parameters and Increase in Micronucleus Frequency

Variable	Correlation coefficient (r)	p-value
Age	0.142	0.438
Duration of infertility	0.171	0.349
BMI	0.108	0.556
Fluoroscopy time	0.612	<0.001
Dose Area Product (DAP)	0.684	<0.001
Cumulative Air Kerma	0.703	<0.001
Effective radiation dose	0.658	<0.001

Pearson correlation coefficient (r) used to assess linear association between variables. Positive values indicate direct correlation.

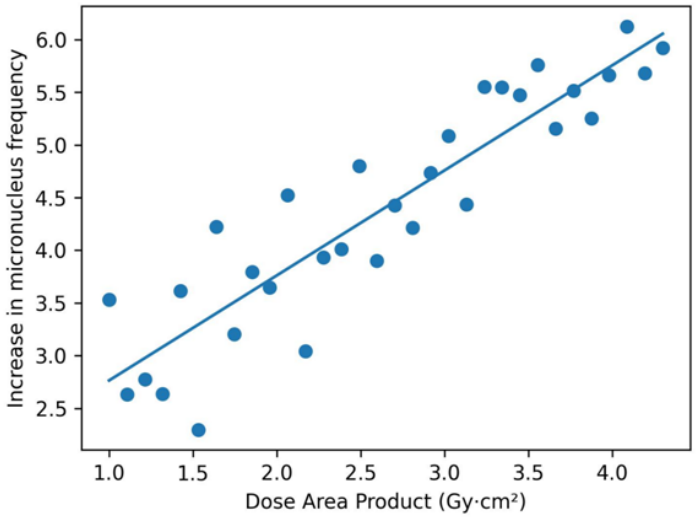

Figure 2: Scatter Plot Demonstrating Correlation Between Dose-Area Product and Increase in Micronucleus Frequency

DAP = Dose Area Product (Gy·cm²); micronucleus frequency expressed as increase in micronucleate binucleated cells per 1000 cells.

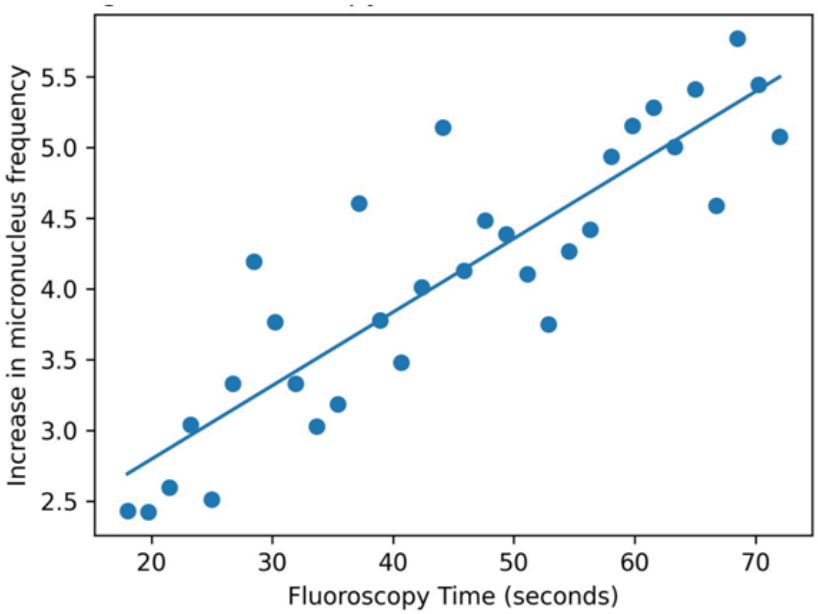

Figure 3: Scatter Plot Demonstrating Correlation Between Fluoroscopy Time and Increase in Micronucleus Frequency

Fluoroscopy time measured in seconds. Correlation assessed using Pearson correlation analysis. Multivariable linear regression analysis identified radiation-related variables as independent predictors of increased micronucleus frequency. Dose-area product emerged as the strongest predictor (β=1.182, p=0.002), followed by fluoroscopy time (β=0.064, p=0.005). Conversely, age (p=0.264), BMI (p=0.484), and duration of infertility (p=0.276) were not significantly associated with cytogenetic damage after adjustment for confounding variables.

The regression model explained 54% of the variance in micronucleus frequency (R^2=0.54; adjusted R^2=0.47) and demonstrated overall statistical significance (F=7.84, p<0.001), indicating that radiation exposure parameters were the principal determinants of cytogenetic alterations following HSG (Table 6).

Table 6: Multivariable Linear Regression Analysis of Factors Associated with Increase in Micronucleus Frequency

Variable	β Coefficient	Standard Error	t-value	p-value
Age	0.041	0.036	1.14	0.264
BMI	0.029	0.041	0.71	0.484
Duration of infertility	0.052	0.047	1.11	0.276
Fluoroscopy time	0.064	0.021	3.05	0.005
Dose Area Product (DAP)	1.182	0.352	3.36	0.002

β = standardized regression coefficient; R^2 = coefficient of determination.

Discussion

The present study evaluated cytogenetic damage induced by radiation exposure during hysterosalpingography (HSG) using the cytokinesis-blocked micronucleus (CBMN) assay in peripheral blood lymphocytes. The study population predominantly comprised women with primary infertility (68.8%), with a mean age of 29.8 ± 4.2 years and a mean infertility duration of 4.1 ± 2.3 years. These demographic characteristics are comparable to those reported in infertility populations undergoing diagnostic evaluation, where women typically present during the third decade of life and primary infertility remains the most common indication for investigation [1,2,10,11].

The observed mean effective radiation dose of 1.84 ± 0.63 mSv was within the range reported in previous HSG dosimetric studies by Achuka et al., Alzimami et al., Chiegwu et al., and Sulieman et al., which have documented effective doses between 1 and 3 mSv depending on fluoroscopic technique, equipment characteristics, and procedural factors [4,12,13,14]. Although such

doses are generally considered safe from a deterministic radiation injury perspective, accumulating evidence suggests that even low-dose diagnostic radiation may induce measurable biological effects at the cellular and molecular levels [13,14,15,16].

A major finding of the present study was the significant increase in micronucleate binucleated cells from 8.72 ± 2.11 to 12.47 ± 2.83 per 1000 cells following HSG ($p<0.001$). This represents an approximate 43% increase in micronucleus frequency, indicating substantial induction of chromosomal damage after radiation exposure. Micronuclei originate from acentric chromosomal fragments or whole chromosomes that fail to be incorporated into daughter nuclei during mitosis and are widely recognized as sensitive biomarkers of DNA damage and genomic instability [8,9]. Similar findings were reported by Chodankar et al., who demonstrated a significant increase in micronucleus frequency following HSG and concluded that radiation exposure during the procedure produced detectable cytogenetic injury [17]. The consistency between the present findings and previous investigations strengthens the evidence that HSG-associated radiation can induce transient chromosomal alterations despite relatively low absorbed doses [17]. Furthermore, biodosimetric studies by Tamizh Selvan et al., and Pujol-Canadell et al., have consistently shown that micronucleus frequency increases proportionally with radiation exposure, supporting its utility as a sensitive marker of radiation-induced chromosomal damage [8,9].

In addition to micronucleus formation, the present study documented significant increases in nuclear buds and nucleoplasmic bridges following HSG exposure. Nuclear buds increased from 1.41 ± 0.67 to 2.19 ± 0.88 per 1000 cells ($p<0.001$), while nucleoplasmic bridges increased from 0.81 ± 0.44 to 1.36 ± 0.61 per 1000 cells ($p<0.001$). Nuclear buds are considered indicators of gene amplification and elimination of excess DNA, whereas nucleoplasmic bridges reflect misrepaired DNA strand breaks, dicentric chromosome formation, and chromosomal rearrangements [18]. The concurrent increase in these biomarkers suggests that radiation exposure during HSG may induce multiple forms of genomic instability rather than isolated chromosomal fragmentation alone [8,9,19,20]. Similar observations have been reported in radiation bio dosimetry studies by Tamizh Selvan et al., and Pujol-Canadell et al., where increased frequencies of micronuclei, nucleoplasmic bridges, and nuclear buds were interpreted as evidence of persistent DNA damage and imperfect chromosomal repair mechanisms [8,9].

The biological basis of these findings can be explained by the interaction of ionizing radiation with cellular macromolecules. Radiation can directly damage DNA through energy deposition within the nucleus or indirectly through radiolysis of water molecules, resulting in the generation of reactive oxygen species (ROS) [6,7,21,22]. These ROS induce single-strand and double-strand DNA breaks, oxidative base modifications, and chromosomal aberrations [6,7,23,24]. Although cellular DNA repair mechanisms attempt to correct such damage, incomplete or erroneous repair may lead to chromosomal fragments and segregation errors manifested as micronuclei, nuclear buds, and nucleoplasmic bridges [8,9,23]. Because peripheral blood lymphocytes are highly radiosensitive and circulate throughout the body, they serve as an ideal surrogate tissue for detecting systemic cytogenetic effects of radiation exposure [8,9,24].

An important observation of the present study was the absence of significant differences in radiation-induced cytogenetic damage between women with primary and secondary infertility. The increase in micronucleus frequency was comparable in both groups (3.76 ± 1.92 vs. 3.72 ± 2.10; $p=0.944$), suggesting that infertility type does not influence cellular susceptibility to radiation-induced chromosomal damage. This finding indicates that the observed cytogenetic alterations are primarily attributable to radiation exposure rather than underlying reproductive pathology. Similar observations have been reported in studies by Matsumoto et al., Perisinakiset al., and Gyekye et al., evaluating the biological effects of diagnostic radiation exposure, where radiation dose rather than baseline clinical characteristics was the principal determinant of cytogenetic injury [5,15,16]. Correlation analysis provided further evidence supporting a dose-dependent relationship between radiation exposure and cytogenetic injury. Significant positive correlations were observed between micronucleus induction and fluoroscopy time ($r=0.612$, $p<0.001$), dose-area product (DAP) ($r=0.684$, $p<0.001$), cumulative air kerma ($r=0.703$, $p<0.001$), and effective dose ($r=0.658$, $p<0.001$). The strongest association was observed with cumulative air kerma, suggesting that absorbed radiation dose is a critical determinant of chromosomal damage.

These findings are consistent with established radiobiological principles and previous bio dosimetry studies by Tamizh Selvan et al., Pujol-Canadell et al., and Wei et al., demonstrating a dose-dependent increase in micronucleus frequency following radiation exposure [6,8,9]. The significant dose-response relationship observed in the present study enhances the biological plausibility of the findings and supports the validity

of micronucleus frequency as a sensitive biomarker of low-dose radiation exposure [8,9,17,25,26].

The multivariable regression analysis further strengthened these observations by identifying radiation-related variables as independent predictors of increased micronucleus frequency. Dose-area product (β=1.182, p=0.002) and fluoroscopy time (β=0.064, p=0.005) remained significant predictors after adjustment for age, BMI, and duration of infertility, whereas demographic variables showed no significant association. The regression model explained 54% of the variability in micronucleus induction (R^2=0.54), indicating that procedural radiation exposure accounts for a substantial proportion of observed cytogenetic damage. These findings emphasize the importance of radiation optimization strategies during HSG, including minimizing fluoroscopy time, using pulsed fluoroscopy, restricting field size, and adhering to the "As Low As Reasonably Achievable" (ALARA) principle [14]. Previous dosimetric investigations Achuka et al., Alzimami et al., Chiegwu et al., and Sulieman et al., have similarly demonstrated that optimization of procedural parameters can significantly reduce patient radiation burden while maintaining diagnostic efficacy [4,12,13,14].

Limitations

The present study was limited by its relatively small sample size and single-center design, which may restrict the generalizability of the findings. Cytogenetic damage was assessed only in peripheral blood lymphocytes and at a single post-exposure time point, precluding evaluation of long-term persistence or repair of DNA damage. Additionally, the study did not include a control group or assessment of other biomarkers of oxidative stress and genomic instability.

Conclusion

The present study demonstrated that hysterosalpingography is associated with a significant increase in cytogenetic damage markers, including micronuclei, nuclear buds, and nucleoplasmic bridges, indicating measurable genomic instability following exposure to low-dose diagnostic radiation.

The magnitude of cytogenetic damage showed significant positive correlations with fluoroscopy time, dose-area product, cumulative air kerma, and effective radiation dose. Radiation exposure parameters emerged as independent predictors of micronucleus induction, whereas patient-related factors showed no significant influence. These findings highlight the biological effects of HSG-associated radiation exposure and emphasize the importance of optimizing procedural techniques and adhering to radiation protection principles to minimize unnecessary exposure in women undergoing infertility evaluation.

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