DNA damage and its association with the plasma Malondialdehyde levels among patients with Cervical Cancer-A case control study.

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Abstract

Background/Objective:

The purpose of the present study was to estimate the DNA damage using comet assay in patients with cervical cancer and to find out its correlation with the oxidative stress marker (Plasma Malondialdehyde, MDA) and to compare the parameters in cases with the age-matched controls.

Materials and Methods:

This study included 49 cervical cancer cases and 49 age-matched controls to measure the DNA damage parameters such as Comet length, head diameter, percentage of DNA in the comet head, tail length, percentage of DNA in comet tail using comet assay technique and the oxidative stress marker (Plasma Malondialdehyde) using TBARS ELISA method.

Results:

The comet parameters such as Comet length, tail length, percentage of DNA in comet tail, representing the DNA damage were significantly high in the cervical cancer cases than in the age-matched controls. The comet parameters such as Head diameter, the percentage of DNA in the comet head, representing the undamaged/mild DNA damage were significantly high in age-matched controls than in cervical cancer cases. A positive correlation between the plasma MDA and the comet tail length was observed.

Conclusion:

The increased levels of Comet parameters and their positive association with plasma MDA indicate the high level of DNA damage in cervical cancer patients than the controls. Such a combination of comet assay and estimation of plasma MDA can be used as a predictive test along with the existing methods such as PAP smear, etc., for early detection and risk assessment of cervical cancer in individuals who belonged to the age group of 30-39 years, with parity of two to four and had a history of the early age at first pregnancy with a positive family history of cervical cancer.

1. Introduction

1.1 Burden

Cervical cancer is the 4th commonest cancer worldwide according to 2018 statistics of WHO [1], and it is the 4th leading cause of cancer-related deaths worldwide (7.5%) as per Global cancer statistics [2, 3]. In India, cervical cancer is the second commonest cancer and the 3rd leading cause of cancer-related death in 2018. In Tamilnadu, it is the 2nd leading cause of cancer-related deaths (22.5%) [2, 4]. It has been estimated that the incidence of cervical cancer in India by the year 2020 will be 20% more than the incidence reported in 2010 [5]. Unlike the other cancers, cervical cancer strikes very early in women's life, and the affected age group ranges from 21 to 67 years [6].

1.2 Causative factors

The risk factors for cervical cancer are poor genital hygiene, HIV, Human papilloma virus (HPV), chlamydia, multiple sexual partners, use of diethylstilboestrol, oral contraceptives, intrauterine devices, smoking, early or late pregnancies, and multiple full-term pregnancies [7–11].

1.3 HPV infection and DNA damage

Among these risk factors, HPV infection is considered the most important risk factor, contributing to the etiology in 99% of cervical cancer cases worldwide [12]. The carcinogenic strains, such as HPV 16, 18, 31, and 33, are associated with cervical cancer [13].

In HPV infections, the viral integrated host genome secretes the oncoproteins such as E6 and E7 that alters the physiological cell cycle. An altered cell cycle increases the production of damaged or mutant DNA (Deoxy-ribose nuclei acid), which results in the accumulation of genomic instability [14–18].

An increased level of genomic instability causes monoclonal proliferation of cervical epithelial cells resulting in cervical cancer [14–18]. The level of DNA damage can be measured from the nucleus of circulating lymphocytes [19]. Hence, the estimation of the DNA damage at a single cellular level is essential.

Single Cell Gel Electrophoresis (SCGE) assay or comet assay detects the single-stranded breaks and alkali labile sites. It is a simple, low cost, and quick technique that can be done using fewer cells (~ 10000), making it an ideal genetic test to have as a screening procedure.

1.4 Lipid peroxidation

In HPV infections, lipid peroxidation produces an increased level of free radicals or reactive oxygen species, which are also cellular metabolism products. The balance between the reactive oxygen species and the cell's antioxidant levels is vital for the normal replication of DNA, protein synthesis, and cellular division [20–22]. Increased production of free radicals tilts the balance between the free radicals and the antioxidant levels; and results in the oxidative stress reaction, which damages the deoxyribose and nucleotide bases. In HPV infections, the expression of E6 oncoprotein increases oxidative stress, which causes lipid peroxidation [23]. The HPV infection is a potent manipulator of DNA damage response (DDR) by which it is responsible for the progression of cervical cancer [24]. The faulty DNA repair and abnormal replication of cells, leading to cellular death or cancer [25, 26].

1.5 Malondialdehyde (MDA)

Malondialdehyde (MDA) is the primary product of lipid peroxidation. It was considered as the global plasma biomarker of lipid peroxidation. The generation of MDA in lipid peroxidation is a complex process. Stress on phospholipids induces hydroperoxide formation, followed by β-cleavage of fatty acid chains, which results in the formation of hydroperoxy-aldehyde.

The released MDA, in turn, cause damage to the cellular organelles and the nuclear membrane and thereby contributes to the disease process [27–29].

MDA estimation is a simple, convenient, cost-effective, and user-friendly method to assess oxidative stress and lipid peroxidation [23, 30, 31]. It was suggested that the estimation of plasma MDA could be used as a screening test for cervical cancer cases to assess the level of oxidative stress [32].

If the amount of DNA damage can be measured by comet assay, then it can be used as a predictive test along with the existing methods such as Papanicolaou's smear (PAP), HPV DNA testing, Visual inspection by acetic acid (VIAA) and visual inspection by Lugol's iodine (VILI) for early detection and risk assessment of cervical cancer in the vulnerable population [33].

Literature regarding the role of genetics is scarcely available for a highly prevalent disease such as cervical cancer. The aim of the present study was to ascertain the DNA damage in cervical cancer cases during their initial diagnosis. Furthermore, To find out whether it can be used as a predictive/screening test. The intention of the present study was not to measure the degree of DNA damage during various stages of cancer as the disease progress. The objective of the present study was to find out the extent of oxidative stress and DNA damage in newly diagnosed cases of cervical cancer before the initiation of treatment by measuring the plasma MDA and comet assay, respectively. Furthermore, measure the association between plasma MDA levels and DNA damage in cases of cervical cancer.

2. Materials And Methods

The present study was carried out in the cytogenetics laboratory of the Department of Anatomy, JIPMER, in collaboration with the Department of Obstetrics & Gynecology and with the Department of Biochemistry, JIPMER, Puducherry from Jan 2018 to Dec 2019. Departmental Postgraduate Research Monitoring Committee (PGRMC) and the Institute Human Ethics Committee (Ref No: JIP/IEC/2017/0370, Dated 05.04.2017) approval was taken for the study.

2.1 Study population:

a) Study group: one – 49 Cervical cancer cases.

Inclusion criteria were as follows: newly diagnosed cervical cancer patients before starting the treatment, clinically and histo-pathologically (Biopsy) proven cancer patients, before starting the treatment. Exclusion criteria were as follows: cervical cancer cases that were already on treatment and cervical cancer cases with comorbid conditions such as Diabetes mellitus, Hypertension, Malignancy, Known Genetic Disorder, Chronic illnesses/infections, renal, liver, thyroid, and lung diseases.

b) Study group: two – 49 controls.

Inclusion criteria were as follows: age-matched people coming to Gynaecology OPD for minor ailments and those who were negative Pap smear for intraepithelial malignancies. Exclusion criteria were as follows: any other cancer, Hypothyroidism, Diabetes Mellitus, Known genetic disorders, Autoimmune disorders.

2.2 Sample size calculation

The sample size of 49 newly diagnosed cervical cancer cases and 49 age-matched controls was calculated using PASS software, version 3.1.2, to compare two means. The minimum expected difference in the tail length between the groups was two, with a standard deviation of 3.5. The sample size was estimated with a 5% level of significance and 80% power. The sample size was calculated using the estimates of all other study parameters from previous studies. Since the difference in the tail length yields a higher sample size, it was considered [33].

2.3 List of Variables observed in the study:

Independent variables such as family history, number of pregnancies, age at first pregnancy, history of smoking, history of alcohol intake, and sexual history were observed. Plasma MDA and comet parameters such as comet length, head diameter, percentage of DNA in the head, tail length, percentage of DNA in the tail were the outcome variables measured. There were no confounding and interacting variables.

2.4 Methodology:

The present study consists of two procedures:

A. DNA damage analysis was carried by comet assay procedure as per Nandhakumar et al., 2011 [34].

B. Plasma MDA levels were estimated using TBARS (ELISA) methods

2.5 Estimation of plasma MDA using the TBARS (ELISA) method.

After the procurement of the TBARS assay kit, it was stored at 2–8°C in the freezer. After collecting the blood from each patient, the blood was centrifuged to separate cells from the plasma. The cells were used for the comet assay procedure, and the plasma was stored at -80°C in the freezer.

For the analysis of plasma MDA, the plasma was taken out of the freezer, 6 hours before the MDA-TBARS assay procedure to allow thawing.

Similarly, the TBARS assay kit was also taken out from the freezer for bringing it to room temperature. As per the protocol received with the kit, all the standard solutions and working reagents were prepared from the kit. The number of strips required for the assay was selected and inserted into the frames for use. The unused strips were returned to the freezer to store at 2–8°C for further use.

To a standard well, 50µl of the standard solution was added, and to the sample wells, 40µl of samples along with 10µl of anti-MDA antibody was added. Then, 50µl of streptavidin-HRP was added to all sample wells and standard wells (Not blank control well). The mixture was mixed well, and the plate was covered with a sealer and incubated for 60 minutes at 37°C.

After removing the seal, the plate was washed with 0.35ml of wash-buffer five times with a wash duration of 30 seconds to 1 minute per wash. The plate was blotted with paper towels. To each well, 50µl of substrate solution A and B were added. Then, the plate was incubated for 10 minutes at 37°C in the dark, with a new sealer. After incubation, 50µl of stopping solution was added. Changing of color from blue to yellow was noted immediately. For evaluating the optical density value from each well, the microplate reader was set at 450nm. Within 10 minutes of conversion of color, the plate was transferred to the microplate reader for measuring optical density value. The OD value emitted from each well indicates the level of MDA in plasma of patients and controls.

2.6 Statistical analysis:

The distribution of categorical variables such as family history, history of smoking, history of alcohol intake, and DNA damage was expressed as frequency and percentages. The discrete and continuous variables such as age, number of pregnancies, age at first pregnancy, level of DNA damage, and plasma MDA level were expressed as mean with standard deviation.

The comparison of the level of DNA damage and the plasma MDA levels between the groups were made using an independent Student's t-test. The relationship between the level of DNA damage and plasma MDA levels calculated using correlation analysis. All analysis was done with a 5% level of significance, and $p < 0.05$ was considered significant.

3. Results

3.1 Description of samples (participants) included in the study:

The present study included 49 new cervical cancer cases diagnosed by clinical examination and biopsy before the commencement of treatment and 49 age-matched controls who came to Obstetrics and Gynaecology OPD for minor ailments and turned out to be normal. The samples were assessed for the level of DNA damage and the plasma MDA, and their values were observed and recorded.

3.2 Comparison of comet parameters and plasma MDA in cervical cancer cases and controls:

Comet parameters such as comet length, comet tail length, percentage of DNA in comet tail, and the plasma MDA levels were significantly higher in cervical cancer cases than in controls. Comet parameters such as head diameter and percentage of DNA in the head were significantly higher in controls compared to cervical cancer cases (Table 1). We have analyzed these comet parameters and plasma MDA by categorizing the study participants according to age, parity, number of pregnancies, number of risk factors, and family history of cancer.

Table 1
Comparison of mean of all the comet parameters and plasma MDA in cervical cancer cases and control

S. NO.	PARAMETERS	PATIENTS	CONTROLS	*p*-Value
		MEAN ± S.D	MEAN ± S.D	
1	Comet Length	58.96 ± 4.88	26.22 ± 3.34	0.001
2	Comet Head Diameter	22.33 ± 2.49	24.75 ± 3.22	0.001
3	Percentage of DNA in Comet Head	60.30 ± 9.76	85.37 ± 6.06	0.001
4	Comet Tail Length	36.76 ± 5.16	4.65 ± 1.93	0.001
5	Percentage of DNA in Comet Tail	40.81 ± 7.25	14.63 ± 6.06	0.001
6	Plasma MDA level	11.62 ± 6.53	7.75 ± 5.60	0.001

3.3 Age group-wise comparison of comet parameters and plasma MDA levels:

We observed that the comet length was higher in cervical cancer cases than controls across all the age groups. In cervical cancer cases, comet length was higher in age groups of ≥ 60 years, followed by 30–39 years, 40–49 years, and 50–59 years (Table 2).

Table 2
Age group-wise comparison of the comet parameters and plasma MDA in cervical cancer cases and controls.

Groups		30–39 yrs	40–49 yrs	50-59yrs	≥ 60yrs
Comet length	Cases (n = 49)	59.15 ± 4.39 (n = 3)	58.52 ± 5.99 (n = 9)	56.08 ± 10.90 (n = 19)	59.89 ± 4.81 (n = 18)
	Controls (n = 49)	26.11 ± 2.69 (n = 27)	27.54 ± 5.12 (n = 12)	24.87 ± 1.59 (n = 9)	25.37 ± 0 (n = 1)
Head Diameter	Cases (n = 49)	19.43 ± 4.62 (n = 3)	23.4 ± 2.37 (n = 9)	22.18 ± 2.51 (n = 19)	22.44 ± 1.88 (n = 18)
	Controls (n = 49)	21.38 ± 2.11 (n = 27)	23.45 ± 5.33 (n = 12)	22.81 ± 1.80 (n = 9)	22.68 (n = 1)
Percentage of DNA in comet head	Cases (n = 49)	59.64 ± 3.41 (n = 3)	64.08 ± 13.02 (n = 9)	66.17 ± 10.55 (n = 19)	60.58 ± 7.30 (n = 18)
	Controls (n = 49)	84.37 ± 6.70 (n = 27)	85.78 ± 5.71 (n = 12)	87.60 ± 4.45 (n = 9)	87.19 (n = 1)
Tail length	Cases (n = 49)	39.72 ± 1.44 (n = 3)	35.85 ± 6.30 (n = 9)	36.05 ± 5.14 (n = 19)	37.45 ± 5.00 (n = 18)
	Controls (n = 49)	4.78 ± 2.12 (n = 27)	4.58 ± 1.98 (n = 12)	4.29 ± 1.44 (n = 9)	4.86 ± 0 (n = 1)
Percentage of DNA in comet tail	Cases (n = 49)	40.35 ± 3.41 (n = 3)	40.24 ± 8.31 (n = 9)	40.11 ± 7.30 (n = 19)	42.41 ± 7.30 (n = 18)
	Controls (n = 49)	15.62 ± 6.0 (n = 27)	14.21 ± 5.71 (n = 12)	12.39 ± 4.45 (n = 9)	12.80 ± 0 (n = 1)
Plasma MDA level	Cases (n = 49)	24.22 ± 22.00 (n = 3)	11.18 ± 4.17 (n = 9)	11.71 ± 3.73 (n = 19)	9.64 ± 2.86 (n = 18)
	Controls (n = 49)	7.94 ± 4.26 (n = 27)	8.57 ± 5.27 (n = 12)	6.68 ± 9.20 (n = 9)	2.35 ± 0 (n = 1)

Head diameter was higher in controls compared to cervical cancer cases across all the age groups. In cervical cancer cases, the head diameter was higher in the age group of 40–49 years, followed by the age group of ≥ 60 years, 50–59 years, and 30–39 years (Table 2).

The percentage of DNA in comet head was higher in controls than cervical cancer cases across all the age groups. In cervical cancer cases, the percentage of DNA in comet head was higher in the age group of 50–59 years, followed by 40–49 years, ≥ 60 years, and 30–39 years (Table 2).

Tail length was higher in cervical cancer cases compared to controls across all age groups. In cervical cancer cases, the tail length was higher in the age group of 30–39 years, followed by the age group of ≥ 60 years, 50–59 years, and 40–49 years (Table 2).

The percentage of DNA in the comet tail was higher in cervical cancer cases than controls across all the age groups. In cervical cancer cases, the percentage of DNA in comet tail was higher in the age group of ≥ 60 years, followed by the age group of 30–39 years, 40–49 years, and 50–59 years (Table 2).

The plasma MDA was higher in cervical cancer cases compared to controls across all age groups. In cervical cancer cases, the plasma MDA was higher in the age group of 30–39 years, followed by the age group of 50–59 years, 40–49 years, and ≥ 60 years (Table 2).

3.4 Effect of parity:

Comet length was higher in different parity groups of cervical cancer cases compared to controls. In cervical cancer cases, the comet length was higher in the parity 2–4 followed by the parity ≥ 5 and parity 0–1. However, there were no samples available for the study, in parity group ≥ 5, in controls (Table 3).

Table 3
Parity-wise comparison of the comet parameters and plasma MDA in cervical cancer cases and controls.

PARITY		0–1	2–4	≥ 5
Comet length	Cases (n = 49)	57.78 ± 0 (n = 1)	58.15 ± 8.22 (n = 42)	57.94 ± 6.71 (n = 6)
	Controls (n = 49)	27.49 ± 5.61 (n = 4)	26.10 ± 3.14 (n = 45)	0 (n = 0)
Head Diameter	Cases (n = 49)	26.52 ± 0 (n = 1)	22.26 ± 2.43 (n = 42)	22.34 ± 6.71 (n = 6)
	Controls (n = 49)	24.27 ± 5.53 (n = 4)	21.52 ± 2.93 (n = 45)	0 (n = 0)
Percentage of DNA in comet head	Cases (n = 49)	60.29 ± 0 (n = 1)	60.16 ± 10.47 (n = 42)	61.58 ± 3.25 (n = 6)
	Controls (n = 49)	87.16 ± 1.62 (n = 4)	85.21 ± 6.29 (n = 45)	0 (n = 0)
Tail length	Cases (n = 49)	31.26 ± 0 (n = 1)	37.02 ± 5.22 (n = 42)	35.80 ± 4.87 (n = 6)
	Controls (n = 49)	3.68 ± 1.07 (n = 4)	4.73 ± 1.97 (n = 45)	0 (n = 0)
Percentage of DNA in the comet tail	Cases (n = 49)	41.71 ± 0 (n = 1)	41.92 ± 7.70 (n = 42)	38.42 ± 3.26 (n = 6)
	Controls (n = 49)	12.83 ± 1.67 (n = 4)	14.78 ± 6.29 (n = 45)	0 (n = 0)
Plasma MDA level	Cases (n = 49)	17.84 ± 0 (n = 1)	21.90 ± 6.88 (n = 42)	18.62 ± 1.42 (n = 6)
	Controls (n = 49)	12.77 ± 4.22 (n = 4)	7.30 ± 5.52 (n = 45)	0 (n = 0)

Head diameter was higher in different parity groups of cervical cancer cases compared to controls. In cervical cancer cases, the head diameter was higher in parity 0–1, followed by the parity ≥ 5 and parity 2–4. However, there were no samples available for the study, in parity group ≥ 5, in controls (Table 3).

The percentage of DNA in comet head was higher in different parity groups of controls compared to cervical cancer cases. In cervical cancer cases, the percentage of DNA in comet head was higher in the parity group ≥ 5 followed by parity group 0–1 and 2–4. However, there were no samples available for the study in parity group ≥ 5 in controls (Table 3).

The comet tail length was higher in different parity group of cervical cancer cases compared to controls. In cervical cancer cases, the comet tail length was higher in the parity group 2–4 followed by the parity group ≥ 5 and 0–1. However, there were no samples available for the study in parity group ≥ 5 in controls (Table 3).

The percentage of DNA in the comet tail was higher in different parity group of cervical cancer cases than controls. In cervical cancer cases, the percentage of DNA in the comet tail was higher in the parity group 2–4 followed by the parity group 0–1 and ≥ 5. However, there were no samples available for the study in parity group ≥ 5 in controls (Table 3).

The plasma MDA was higher in different parity group of cervical cancer cases compared to controls. In cervical cancer cases, the plasma MDA was higher in the parity group 2–4 followed by the parity group ≥ 5 and 0–1. However, there were no samples available for the study in parity group ≥ 5 in controls (Table 3).

3.5 Effects of pregnancy at an early age:

Comet length was higher in both categories of cervical cancer cases (age at 1st pregnancy ≤ 19 years and > 19 years) than controls. Among cervical cancer cases, the comet length was higher in cervical cancer cases with age at 1st pregnancy ≤ 19 years compared to > 19 years (Table 4).

Table 4
Comparison of comet length in cervical cancer cases and controls according to the age at 1st pregnancy

Pregnancy		Age at 1st pregnancy ≤ 19 years	Age at 1st pregnancy > 19 years
Comet length	Cases (n = 49) (Nil = 1)	59.94 ± 9.74	59.49 ± 4.40
		(n = 28)	(n = 20)
	Controls (n = 49) (Nil = 2)	25.46 ± 2.96	26.71 ± 3.52
		(n = 15)	(n = 32)
Head Diameter	Cases (n = 49) (Nil = 1)	22.72 ± 1.77	22.55 ± 3.18
		(n = 28)	(n = 20)
	Controls (n = 49) (Nil = 2)	21.44 ± 3.01	21.90 ± 3.44
		(n = 15)	(n = 32)
Percentage of DNA in comet head	Cases (n = 49) (Nil = 1)	60.38 ± 11.88	60.78 ± 6.29
		(n = 28)	(n = 20)
	Controls (n = 49) (Nil = 2)	87.53 ± 3.89	84.19 ± 6.76
		(n = 15)	(n = 32)
Tail length	Cases (n = 49) (Nil = 1)	37.58 ± 5.73	37.26 ± 4.31
		(n = 28)	(n = 20)
	Controls (n = 49) (Nil = 2)	4.27 ± 1.16	4.93 ± 2.19
		(n = 15)	(n = 32)
Percentage of DNA in the comet tail	Cases (n = 49) (Nil = 1)	41.55 ± 8.00	39.71 ± 6.29
		(n = 28)	(n = 20)
	Controls (n = 49) (Nil = 2)	12.46 ± 3.89	15.80 ± 6.76
		(n = 15)	(n = 32)
Plasma MDA level	Cases (n = 49) (Nil = 1)	10.99 ± 3.51	12.19 ± 9.34
		(n = 28)	(n = 20)
	Controls (n = 49) (Nil = 2)	6.11 ± 7.81	8.35 ± 4.27
		(n = 15)	(n = 32)

The head diameter was higher in both categories of cervical cancer cases (age at 1st pregnancy ≤ 19 years and > 19 years) than in controls. Among cervical cancer cases, the head diameter was higher in cervical cancer cases with age at 1st pregnancy ≤ 19 years compared to > 19 years (Table 4)

The percentage of DNA in comet head was higher in both categories of controls (age at 1st pregnancy ≤ 19 years and > 19 years) compared to cervical cancer cases. Among cervical cancer cases, the percentage of DNA in comet head was higher in cervical cancer cases with age at 1st pregnancy > 19 years compared to ≤ 19 years (Table 4).

The comet tail length was higher in both categories of cervical cancer cases (age at 1st pregnancy ≤ 19 years and > 19 years) than controls. Among cervical cancer cases, the comet tail length was higher in cervical cancer cases with age at 1st pregnancy ≤ 19 years compared to > 19 years (Table 4).

The percentage of DNA in the comet tail was higher in both categories of cervical cancer cases (age at 1st pregnancy ≤ 19 years and > 19 years) than controls. Among cervical cancer cases, the percentage of DNA in the comet tail was higher in cervical cancer cases with age at 1st pregnancy ≤ 19 years compared to > 19 years (Table 4).

The plasma MDA was higher in both categories of cervical cancer cases (age at 1st pregnancy ≤ 19 years and > 19 years) than controls. Among cervical cancer cases, the plasma MDA was higher in cervical cancer cases with age at 1st pregnancy > 19 years compared to ≤ 19 years (Table 4).

3.6 Effects of risk factors:

The comet length was higher in both the categories of cervical cancer cases (≤ 1 and > 1 risk factor) than controls. Among cervical cancer cases, the comet length was higher in cervical cancer cases that had > 1 risk factors (Table 5).

Table 5
Comparison of comet length in cervical cancer cases and controls according to risk factors

Groups		≤ 1 Risk Factor	> 1 Risk Factor
Comet length	Cases (n = 49)	57.69 ± 5.55 (n = 14)	58.28 ± 8.73 (n = 35)
	Controls (n = 49)	26.37 ± 3.49 (n = 43)	25.07 ± 1.73 (n = 6)
Head Diameter	Cases (n = 49)	24.15 ± 2.85 (n = 14)	25.60 ± 1.93 (n = 35)
	Controls (n = 49)	21.78 ± 3.41 (n = 43)	21.47 ± 1.23 (n = 6)
Percentage of DNA in the comet head	Cases (n = 49)	61.96 ± 6.14 (n = 14)	59.63 ± 10.88 (n = 35)
	Controls (n = 49)	85.06 ± 6.23 (n = 43)	87.60 ± 4.37 (n = 6)
Tail length	Cases (n = 49)	34.01 ± 5.84 (n = 14)	37.85 ± 4.48 (n = 35)
	Controls (n = 49)	4.75 ± 1.99 (n = 43)	3.89 ± 1.27 (n = 6)
Percentage of DNA in the comet tail	Cases (n = 49)	38.04 ± 6.14 (n = 14)	41.91 ± 7.44 (n = 35)
	Controls (n = 49)	14.93 ± 6.23 (n = 43)	12.39 ± 4.37 (n = 6)
Plasma MDA level	Cases (n = 49)	11.92 ± 4.39 (n = 14)	11.78 ± 7.26 (n = 35)
	Controls (n = 49)	7.63 ± 4.61 (n = 43)	8.57 ± 11.03 (n = 6)
RF 1 = Early pregnancy, RF 2 = Dysmenorrhea, RF 3 = Family H/O any cancer, RF 4 = Alcoholic, RF 5 = Smoking.			

The head diameter was higher in both the categories of cervical cancer cases (≤ 1 and > 1 risk factor) than in controls. Among cervical cancer cases, the head diameter was higher in cervical cancer cases with > 1 risk factors (Table 5).

The percentage of DNA in the comet head was higher in both the categories of controls (≤ 1 and > 1 risk factor) compared to cervical cancer cases. Among cervical cancer cases, the percentage of DNA in comet head was higher in cervical cancer cases with ≤ 1 risk factor (Table 5).

The comet tail length was higher in both categories of cervical cancer cases (≤ 1 and > 1 risk factor) than in controls. Among cervical cancer cases, the comet tail length was higher in cervical cancer cases that had > 1 risk factor (Table 5).

The percentage of DNA in the comet tail was higher in both categories of cervical cancer cases (≤ 1 and > 1 risk factor) than in controls. Among cervical cancer cases, the percentage of DNA in the comet tail was higher in cervical cancer cases that had > 1 risk factor (Table 5).

The plasma MDA was higher in both categories of cervical cancer cases (≤ 1 and > 1 risk factor) than in controls. Among cervical cancer cases, the plasma MDA was higher in cervical cancer cases with ≤ 1 risk factor (Table 5).

3.7 Effect of family history of cancer:

The positive family history of cervical cancer was present in 43% of cervical cancer cases and 6% of controls. The comet length was higher in both categories of cervical cancer cases (positive and negative family history of cancer) than in controls. Among cervical cancer cases, the comet length was higher in cervical cancer cases with a positive family history of cancer (Table 6).

Table 6
Comparison of comet length in cervical cancer cases and controls according to family h/o cancer.

Groups		Positive family H/O cancer	Negative family H/O cancer
Comet length	Cases (n = 49)	58.48 ± 4.76 (n = 21)	57.84 ± 9.70 (n = 28)
	Controls (n = 49)	24.36 ± 0.53 (n = 3)	26.34 ± 3.41 (n = 46)
Head Diameter	Cases (n = 49)	22.44 ± 2.70 (n = 21)	22.40 ± 2.37 (n = 28)
	Controls (n = 49)	21.81 ± 0.75 (n = 3)	21.74 ± 3.32 (n = 46)
Percentage of DNA in the comet head	Cases (n = 49)	61.94 ± 12.26 (n = 21)	59.06 ± 7.36 (n = 28)
	Controls (n = 49)	86.91 ± 6.38 (n = 3)	85.27 ± 6.09 (n = 46)
Tail length	Cases (n = 49)	37.24 ± 5.56 (n = 21)	37.14 ± 4.89 (n = 28)
	Controls (n = 49)	2.92 ± 0.35 (n = 3)	4.76 ± 1.94 (n = 46)
Percentage of DNA in the comet tail	Cases (n = 49)	40.64 ± 7.28 (n = 21)	40.13 ± 7.36 (n = 28)
	Controls (n = 49)	13.08 ± 6.38 (n = 3)	14.72 ± 6.09 (n = 46)
Plasma MDA level	Cases (n = 49)	12.09 ± 9.20 (n = 21)	11.27 ± 3.57 (n = 28)
	Controls (n = 49)	4.55 ± 2.21 (n = 3)	7.96 ± 5.70 (n = 46)

The head diameter was higher in both categories of cervical cancer cases (positive and negative family history of cancer) than in controls. Among cervical cancer cases, the head diameter was higher in cervical cancer cases with a positive family history of cancer (Table 6).

The percentage of DNA in the comet head was higher in both categories of controls (positive and negative family history of cancer) than in cervical cancer cases. Among cervical cancer cases, the percentage of DNA in comet head was higher in cervical cancer cases with a positive family history of cancer (Table 6).

The comet tail length was higher in both categories of cervical cancer cases (positive and negative family history of cancer) than in controls. Among cervical cancer cases, the comet tail length was higher in cervical cancer cases with a positive family history of cancer (Table 6).

The percentage of DNA in the comet tail was higher in both categories of cervical cancer cases (positive and negative family history of cancer) than in controls. Among cervical cancer cases, the percentage of DNA in the comet tail was higher in cervical cancer cases who had a positive family history of cancer (Table 6).

The plasma MDA was higher in both categories of cervical cancer cases (positive and negative family history of cancer) than controls. Among cervical cancer cases, the plasma MDA was higher in cervical cancer cases with a positive family history of cancer (Table 6).

3.8 Correlation analysis between comet parameters and plasma MDA in cervical cancer cases and controls:

The positive correlation between the plasma MDA and two comet parameters such as comet tail length and percentage of DNA in comet head was observed in cervical cancer cases. Correlation analysis between the plasma MDA with the comet parameters in both cases and controls is shown in Table 7. The standard curve obtained by using the Bioassay human MDA kit is shown in Fig. 1.

Table 7
Correlation between Plasma MDA with all comet parameters among cervical cancer cases and controls.

	Comet Parameters	Correlation coefficient (r)	2 tailed significance (*p* -Value)
Plasma MDA level in (Cervical cancer Patients)	Total Comet Length (μm)	-0.132	0.367
	Comet Head Diameter (μm)	-0.314	0.028
	% of DNA in Comet Head	0.057	0.700
	Comet Tail Length (μm)	0.049	0.740
	% of DNA in Comet Tail	-0.099	0.498
Plasma MDA level in (normal healthy controls)	Total Comet Length (μm)	0.138	0.343
	Comet Head Diameter (μm)	0.168	0.248
	% of DNA in Comet Head	-0.046	0.752
	Comet Tail Length (μm)	-0.034	0.817
	% of DNA in Comet Tail	0.046	0.752

3.9 The parts of the comet and the degree of DNA damage:

The parts of the comet captured using an Olympus BX53 bright field microscope are shown in Fig. 2, and the degree of DNA damage from grade-0 to grade-4 is shown in Figs. 3,4,5,6,7. The comets observed in cervical cancer cases and controls are shown in Figs. 8 and 9.

4. Discussion

Cervical cancer is the fourth commonest cancer according to WHO [1] and the fourth leading cause of cancer-related deaths (7.5%) worldwide, as per Global cancer statistics [2, 3]. Early diagnosis and treatment against cervical cancer are crucial for reducing the mortality and morbidity of cervical cancer. Various risk factors cause increased production of free radicals and alter the homeostasis between the pro-oxidants and antioxidants. This imbalance leads to excessive accumulation of free radicals that damage the cellular constituents such as lipids, carbohydrates, proteins, and nucleic acids (DNA), resulting in genetic instability [35–39]. Such genetic instability over a while precipitates as cervical cancer [23, 26, 40, 41]. Double-stranded DNA breaks can be diagnosed and quantitatively measured by alkaline comet assay. In alkaline comet assay, parameters such as comet length, head diameter, percentage of DNA in the comet head, comet tail length, and percentage of DNA in the comet tail are used to ascertain and quantify the DNA damage. Excessive free radicals cause lipid peroxidation leading to the release of the end products such as MDA [42, 43].

4.1 Importance of comet parameters:

During comet assay, because of DNA damage, the minor fragments of DNA tend to migrate away from the nucleoid mass (comet head) during electrophoresis resulting in an increase in the comet tail length and the percentage of DNA in the comet tail. The diameter of the comet head and the percentage of DNA in the comet head indicates the *dispersion* of un-migrated nuclear mass within the comet head (undamaged DNA). The length of the comet tail indicates the *dispersion* of migrated (damaged) DNA fragments in the comet tail, and the percentage of DNA in the comet tail indicates the *amount* of migrated DNA fragments (damaged) within the comet tail. Hence, the total comet length indicates the dispersion of migrated (damaged) DNA fragments in the comet tail and un-migrated (undamaged) DNA fragments in the comet head. Among all the parameters of the comet assay, comet tail length (TL) and percentage of DNA in comet tail (%T) were considered as important markers to measure the intensity of DNA damage because these two parameters indicate the precise quantity of DNA damage [44, 45].

4.2 Observation from the present study:

We have analyzed these comet parameters and plasma MDA by categorizing the study participants according to age, parity, number of pregnancies, number of risk factors, and family history of cancer. We observed that cervical cancer was more prevalent above the age group of 50 years and more prevalent in multiparous individuals, especially with parity between 2–4. The positive family history of cervical cancer was present in 43% of cervical cancer cases and 6% of controls; this underscores the importance of the family history of cancer and genetic predisposition in cervical cancer. The history of early pregnancy (before 19 years of age) was present in 57% of cervical cancer cases and 31% of controls; this reiterates the association of early age of 1st pregnancy in causing cervical cancer. More than one risk factors such as early pregnancies, dysmenorrhea, family history of cancer, alcoholism, and smoking were present in 75% of cervical cancer cases and 12% of controls; this signifies the positive association of risk factors in the causation of cervical cancer.

4.3 DNA damage in different age groups:

We categorized the study participants into four different groups, such as 30–39 years, 40–49 years, 50–59 years, and ≥ 60 years of age. Five comet parameters and plasma MDA levels were compared between the cervical cancer cases and controls among all the age groups. The mean of four comet parameters such as comet length, tail length, percentage of DNA in the comet tail, head diameter, and plasma MDA levels was found to be increased in cervical cancer cases compared to controls. The difference between them was statistically significant. However, the comet head diameter in the age group of

≥ 60 years was more in cervical cancer cases than in control and, the difference between them was statistically insignificant. The percentage of DNA in the comet head was increased in controls compared to the cervical cancer cases in all age groups, and the difference between the cervical cancer cases and controls was statistically significant. In the age group of ≥ 60 years, the percentage of DNA in the comet head was more in controls compared to cervical cancer cases, and the difference between the cervical cancer cases and controls was statistically insignificant. Hence, most comet parameters were higher in cervical cancer cases than controls in almost all age groups except the head diameter and the percentage of DNA in the comet head.

The comet parameters and the plasma MDA levels were compared between different age groups in cervical cancer cases. The comet parameters such as the comet tail length, percentage of DNA in tail, plasma MDA levels were increased in age group of 30–39 years, head diameter was increased in age group of 40–49 years, percentage of DNA in comet head was increased in age group of 50–59 years, and comet length was increased in age group of ≥ 60 years. Among all comet parameters and plasma MDA levels, three comet parameters and plasma MDA levels were found to be increased in the age group of 30–39 years. Hence, the risk of developing cervical cancer may be relatively more in the age groups of 30–39 years compared to all other age groups.

Sadeghi et al. observed that the women with no abnormalities, mild to moderate dysplasia, severe dysplasia, and CIS, invasive cervical carcinoma belonged to the age of 22.1, 22.8, 25.7, 31.9 years, respectively [46].

We observed that most of the (DNA damage) parameters were significantly increased in the age group of 30–39 years in cervical cancer cases compared to the other age groups. And this indicates that the risk of developing cervical cancer is more in the people falling under the age group of 30–39 years. However, Gandhi et al. observed more DNA damage in the age group of 41–45 years. As age increases, an increasing level of DNA damage was observed by Prabhakar et al., and Sobti et al., [47–49]. However, in our study, no such observation was made.

4.4 Parity-wise DNA damage in cervical cancer:

The study participants were categorized into three parity groups like parity one, parity two to four, and parity ≥ five. Furthermore, we observed that the parity distribution among the study participants (both cervical cancer cases and controls) was uneven i.e., most of the participants in this study fell under the category of parity two to four. Hence, we compared the difference between cervical cancer cases and controls only in parity groups of two to four.

Five comet parameters and plasma MDA levels were compared between the cervical cancer cases and controls among the parity groups. The median of four comet parameters such as comet length, head diameter, tail length, percentage of DNA in the comet tail, and plasma MDA levels was found to be increased in cervical cancer cases compared to controls belonged to the parity group of 2–4, and the difference between the cervical cancer cases and controls was statistically significant. The percentage of DNA in the comet head was increased in controls compared to cervical cancer cases belonged to the parity group of 2–4, and the difference between the cervical cancer cases and controls was statistically significant. The majority of comet parameters and the plasma MDA levels were higher in cervical cancer cases than controls in the parity group of two to four.

A similar distribution of study participants (cervical cancer cases and controls) into the parity group of two to four was reported by Elizabeth et al.. Similar to our findings, Elizabeth et al. also reported that the comet parameters were higher in cervical cancer cases than controls in the parity group of two to four [50]. Hence, the risk of developing cervical cancer may be relatively more in the parity group of two to four compared to other parity groups. Gandhi et al. reported that DNA damage was increased in individuals with multiple pregnancies [47]. Prabhakar et al. and Sobti et al. classified the parity groups into P1-3, P4-6, P7 and found that there was a significant difference in the comet assay parameters between the parity groups. Prabhakar et al. and Sobti et al. reported that excessive DNA damage was observed in individuals with a higher number of pregnancies than individuals with a lower number of pregnancies, indicating that the DNA damage increased as the number of pregnancies increased [48, 49].

4.5 DNA damage in early pregnancy:

According to age at 1st pregnancy, we categorized the study participants into two categories, 1) 1st pregnancy at ≤ 19 years of age; 2) 1st pregnancy at > 19 years of age. Five comet parameters and plasma MDA levels were compared between the cervical cancer cases and controls among two categories. The median of the three comet parameters such as the comet length, comet tail length, percentage of DNA in comet tail was found to be increased in cervical cancer cases compared to controls that belonged to category 1 (≤ 19 years of age at 1st pregnancy) and the difference between them was statistically significant. However, the comet head diameter was increased in cervical cancer cases compared to controls, and the difference was statistically insignificant. The percentage of DNA in the comet head was found to be increased in controls compared to cervical cancer cases of category 1 (≤ 19 years of age at 1st pregnancy), and the difference was statistically significant.

When we compared the comet parameters and plasma MDA levels between two categories of cervical cancer cases; we observed that most of the comet parameters that denote the DNA damage were found to be increased in category 1 of cases compared to category 2 of cases, and the difference between two categories was statistically significant. A higher number of cervical cancer cases (n = 28) were under category 1 compared to controls (n = 15). Furthermore, this indicates that the risk of developing cervical cancer is more in an individual with early age pregnancy.

Similarly, Kurl et al. Sobti et al., and Edebiri et al. reported that the people who married at an early age were more prone to develop cervical cancer [49, 51, 52]. Gandhi et al. observed that the DNA damage was increased in cervical cancer cases who became pregnant at an early age (14–16 years). He divided the early pregnancies into two groups (14–16, 17–19) and found a significant increase in DNA damage in patients with early age pregnancy (14–16 years) [47].

4.6 Effects of risk factors:

The study participants were categorized into two groups according to the number of risk factors they had, i.e., group 1 with less than or equal to one risk factor and group 2 with more than one risk factor. And we observed that the risk factor categorization of cervical cancer cases and controls was unevenly distributed. The median of three comet parameters such as comet length, tail length, and percentage of DNA in comet tail was increased in cervical cancer cases compared to controls who had the risk factors more than one. And the difference between the cervical cancer cases and controls was statistically significant.

However, the comet head diameter and the plasma MDA levels were more in cervical cancer cases than controls who had the risk factors more than one, and the difference between the cervical cancer cases and controls was statistically insignificant. The percentage of DNA in comet head was increased in controls compared to cervical cancer cases who had the risk factors more than one. And the difference between the cervical cancer cases and controls was statistically significant. Hence, most comet parameters were higher in cervical cancer cases than controls in the individuals who had the risk factors more than one, except the head diameter and percentage of DNA in the comet head.

When we compared comet parameters and the plasma MDA levels between cervical cancer cases with less than or equal to one risk factor and cervical cancer cases with > 1 risk factors, we observed that most of the parameters that denote the DNA damage were found to be increased in cervical cancer cases who had > 1 risk factors when compared to cervical cancer cases who had ≤ 1 risk factor, and the difference between the two categories was statistically significant. A higher number of cervical cancer cases (n = 35) were found to be under the category of more than one risk factor than the controls (n = 6); this indicates that the risk of developing cervical cancer is more in individuals who had the risk factors more than one.

Gandhi et al. reported that DNA damage was increased in cervical cancer patients who had the risk factors such as multiparity, early pregnancies, and low socioeconomic status, i.e., the cervical cancer cases that had more than one risk factor [47].

4.7 Effect of family history of cancer:

When we compared the comet parameters and plasma MDA levels between the cervical cancer cases and controls with respect to the family history of cervical cancer, we observed that the median of three comet parameters such as the comet length, comet tail length, percentage of DNA in comet tail was found to be increased in cervical cancer cases compared to controls that had a positive family history of cervical cancer and the difference between the cervical cancer cases and controls was statistically significant. However, the plasma MDA was more in cervical cancer cases than controls who had a positive family history of cervical cancer, where the difference between the cervical cancer cases and controls was statistically significant. The percentage of DNA in the comet head was increased in controls compared to the cervical cancer cases that had a positive family history of cervical cancer, and the difference between the cervical cancer cases and controls was statistically significant. The comet head diameter was more in controls than cervical cancer cases, and the difference between the cervical cancer cases and controls was statistically insignificant. Hence, the majority of comet parameters were higher in cervical cancer cases than control in individuals who had a positive family history of cervical cancer, except for the comet head diameter and percentage of DNA in the comet head.

Zoodsma et al. found that the risk of developing cervical cancer was more in the individuals with a positive family history of cervical cancer, especially the first-degree female relatives of cancer cases compared to controls [53].

When we compared the comet parameters and plasma MDA levels between the cervical cancer cases with and without a family history of cervical cancer, we observed that most of the comet parameters that denote the DNA damage were found to be increased in cervical cancer cases that had a positive family history when compared to cervical cancer cases that had a negative family history, and the difference between two categories was statistically significant. A higher number of cervical cancer cases (21 cases, 42%) were found to be under the category with a positive family history of cervical cancer, compared to the controls (3 controls, 6%). And this indicates that the risk of developing cervical cancer is more in individuals with a positive family history of cervical cancer. A similar finding was noted and reported in the different populations, such as the German population by Fischer et al., the American population by Brinton et al., and the Swedish population by Furgyik et al. [54–56].

On comparison of comet parameters and plasma MDA, the total comet length, comet head diameter, comet tail length, percentage of DNA in the comet tail, and plasma MDA were significantly higher in cervical cancer cases than controls. Except for the percentage of DNA in the comet head, which was statistically higher in controls than the cervical cancer cases. Similarly, Udumudi et al., Carlos Alvarez-Moya et al., and Gandhi et al. stated that the cervical cancer cases had more DNA damage (comet tail length) than the age-matched controls. Udumudi et al. and Carlos Alvarez-Moya et al. analyzed the DNA damage among the various stages of cervical cancer, and they found out that the DNA damage increased (comet tail length) as the stages of cervical cancer increased [33, 47, 57]. Smitha et al. analyzed the role of oxidative stress marker (MDA) in cervical cancer cases. They measured the plasma MDA in cervical cancer cases and compared with the age-matched controls and found a significant increase in the plasma MDA in cervical cancer cases than the controls [58]. Smitha et al. observed that the increase in plasma MDA level was accompanied by the decrease in antioxidants levels (SOD, Vit C & Zinc) in cervical cancer cases [58].

4.8 Correlation analysis between plasma MDA & comet parameters in both cervical cancer cases and controls:

Correlation analysis was done between the plasma MDA levels and various comet parameters in both cervical cancer cases and controls. The correlation coefficient (r-value) and 2-tailed significance (*p*-value) were derived from the data and presented in Table 7 and Fig. 1.

In cervical cancer cases, the plasma MDA levels were positively correlated with the comet tail length and percentage of DNA in the comet head and, negatively (reverse) correlated with the total comet length, percentage of DNA in the comet tail, and head diameter.

In controls, we observed that the plasma MDA levels were positively correlated with the total comet length, percentage of DNA in the comet tail and head diameter, and, negatively, correlated with the comet tail length and percentage of DNA in comet head.

From the above data, we can infer that those parameters that were positively correlated with plasma MDA in cervical cancer cases were negatively correlated with plasma MDA in controls (comet tail length and percentage of DNA in comet head). The parameters that were negatively correlated with plasma MDA in cervical cancer cases were in turn positively correlated with plasma MDA in controls (total comet length, percentage of DNA in the comet tail, and head diameter). In cervical cancer cases, the two important comet parameters that denote the increased DNA damage, viz. the comet tail length and percentage of DNA in comet tail, ought to be increased along with the plasma MDA levels. However, we observed that only the comet tail length was increased when plasma

MDA levels were increased. Even though a positive correlation between the comet tail length and plasma MDA was observed in the present study, the results were statistically insignificant. In controls, the correlation between the plasma MDA levels and comet parameters was statistically insignificant. However, the previous studies showed a positive correlation between the comet tail length and the plasma MDA levels [59, 60]. Gao et al. studied the effect of DNA damage by introducing environmental carcinogens (Benzopyrene) in 45 female mice to induce oxidative stress. He reported that if the dose of a carcinogen was increased, then there was an increase in the plasma MDA levels and DNA damage (comet tail length) [60].

Similarly, Beevi et al. studied 45 newly diagnosed cases of squamous cell carcinoma of the cervix and reported that the oxidative stress markers such as plasma MDA, nitric oxide, nitrite, nitrate levels were increased in cervical cancer cases, and antioxidant markers such as erythrocyte superoxide dismutase, catalase, glutathione peroxidase, glutathione transferase levels were decreased in cervical cancer cases. Beevi et al. finally concluded that the lipid peroxidation markers and nitric oxide products were increased in cervical cancer cases, which denotes the imbalance in the oxidant-antioxidant system [59]. An increase in oxidative stress status (plasma MDA levels) with the progression of cervical cancer was noted by Smitha et al. [58]. In the present study, even though we have a similar sample size to that of the previous studies, we have found that only the comet tail length was positively associated with the plasma MDA levels, albeit statistically insignificant. On the other hand, the percentage of DNA in the comet tail had no positive correlation with the plasma MDA levels in cervical cancer cases or the controls.

The evidence from the present study is inconclusive with respect to the positive correlation between the comet tail length and the plasma MDA levels. Such inconclusive evidence might be due to the smaller sample size in the present study. Any ambiguity may be dispelled if the association between the plasma MDA and comet parameters is studied in a larger sample size. Establishing the positive or the negative correlation between the plasma MDA levels and the two most important comet assay parameters (comet tail length and the percentage of DNA in the tail) is important. The relation between the plasma MDA and the comet parameters also underscores the role of the HPV infection.

4.9 DNA damage and accumulation of genetic instability:

Carcinogenesis by HPV: The chronic inflammation caused by viral oncogenes E5, E6, E7 in HPV infections induces oxidative stress. This oxidative stress will, in turn, induces chronic inflammation and cellular damage by promoting further inflammation and the release of various molecules that causes cell damage and ultimately promote carcinogenesis [26].

Tumor suppressor genes such as E6 oncoprotein suppresses the P53 gene, and E7 oncoprotein suppresses the RB gene. The nitric oxide produced during oxidative injury suppresses the P53 and RB gene. Both P53 and RB genes are responsible for the control of the cell cycle. Impairment of these will alter the cell cycle and hence result in carcinogenesis [26]. The inflammation caused by HPV oncoproteins releases the cytokines and growth factors that further accelerates the carcinogenesis. HPV infections release E5, E6, E7 oncoproteins, which stimulate the activator protein − 1 transcription factor (AP-1). This activator protein-1 increases the expression of cyclooxygenase-2 and prostaglandins that lead to cell proliferation, angiogenesis, and decreased apoptosis in cancer cells [26].

4.10 Lipid peroxidation and generation of MDA.

The oxidative stress caused by the HPV infection results in the imbalance of Redox homeostasis and ultimately increase the chronic inflammation in cells. The lipids in the cell membrane are particularly more susceptible to oxidative stress. Reactive oxygen species cause the weakening of the double bond between the two carbon atoms of PUFA. This weakened bond between carbon and hydrogen atoms allows the release of a hydrogen atom from the free radicals. Because of this release of a hydrogen atom from free radicals, the lipid-free radicals are formed, which then undergo oxidation and produce lipid peroxyl radicals, which in turn produce lipid hydro-peroxide. Lipid hydro-peroxide is an unstable compound, which gets fragmented to produce MDA and four hydroxynonenal [27].

The E5, E6, E7 oncoproteins in HPV infection is responsible for two outcomes. One, they cause interruption of the cell cycle, fragmentation of DNA, and ultimately carcinogenesis. Two, they cause an increase in oxidative stress and lipid peroxidation which produce MDA at increased levels [26]. Therefore, any increase in DNA damage due to carcinogens should also result in the associated increase in the plasma MDA levels. In the present study, the plasma MDA levels are significantly increased in cervical cancer cases than in controls. Likewise, there was a significant increase in DNA damage in the cervical cancer cases compared to controls. However, if we consider their interrelation among the cervical cancer cases alone, there is a weak positive correlation. We consider this to be a paradox and need further investigation with increased sample size.

However, irrespective of the positive or negative correlation between the DNA damage and plasma MDA, we have observed a significant increase in the comet parameters of cervical cancer cases, which may be sufficient to conclude that there is extensive DNA damage in cervical cancer cases compared to the controls. This fact enables us to use comet assay (to estimate the DNA damage) as one of the tools for screening cervical cancer cases. Likewise, there is a significant increase in the plasma MDA levels in cervical cancer cases than in the controls. This fact also enables us to use the estimation of plasma MDA levels (lipid peroxidation) for screening cervical cancer cases.

Currently, screening methods such as the PAP smear, VIAA, VILI, and HPV DNA testing are used as a screening tool for early diagnosis of cervical cancer (precancerous stage) [33, 41]. The objective of the present study was to find out whether there is significant DNA damage in newly diagnosed cervical cancer cases before the treatment is initiated, compared to the controls. We found that there was significant DNA damage in cervical cancer cases compared to controls. Hence, the assessment of DNA damage with the help of comet assay in peripheral blood cells and the estimation of plasma MDA using the TBARS method can be used as a screening test to augment the existing screening methodology for cervical cancer.

5. Conclusion

In our study, we observed that there was a statistically significant difference in the comet assay parameters in cervical cancer cases compared to controls, which denotes that there is a significant increase in DNA damage in cervical cancer cases compared to controls. Likewise, there was a significant increase in plasma MDA levels in cervical cancer cases than controls, which also denotes the increased lipid peroxidation and oxidative stress. The information from the present study is inconclusive to consider a positive or a negative correlation between the comet assay parameters and plasma MDA in cervical cancer cases. In essence, the estimation of DNA damage by comet assay and plasma MDA by TBARS assay may act as a positive predictive tool for screening cervical cancer in cancer in individuals who belonged to the age group of 30–39 years, with parity of two to four and had a history of the early age at first pregnancy with a positive family history of cervical cancer.

6. Declarations

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ii. **Conflicts of interest/Competing interests**: No Conflicts of interest to declare.

iii. **Ethics approval**: Approval was obtained from JIPMER Institute Ethics Committee.

iv. **Consent to participate**: A written informed consent was obtained from all the study participants.

v. **Consent for publication**: Consent was obtained from all the study participants.

vi. **Availability of data and material**: Available

vii. **Code availability** – Not applicable.

viii. **Author Contributions**

Conceptualization: RSSSN, LC. Data Acquisition: SG. Data analysis or interpretation: SG, LC, PA. Drafting the manuscript: RSSSN, SG. Critical revision of the manuscript: SSSN, LC, PA. Approval of the final version of the manuscript: All authors.

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Figures

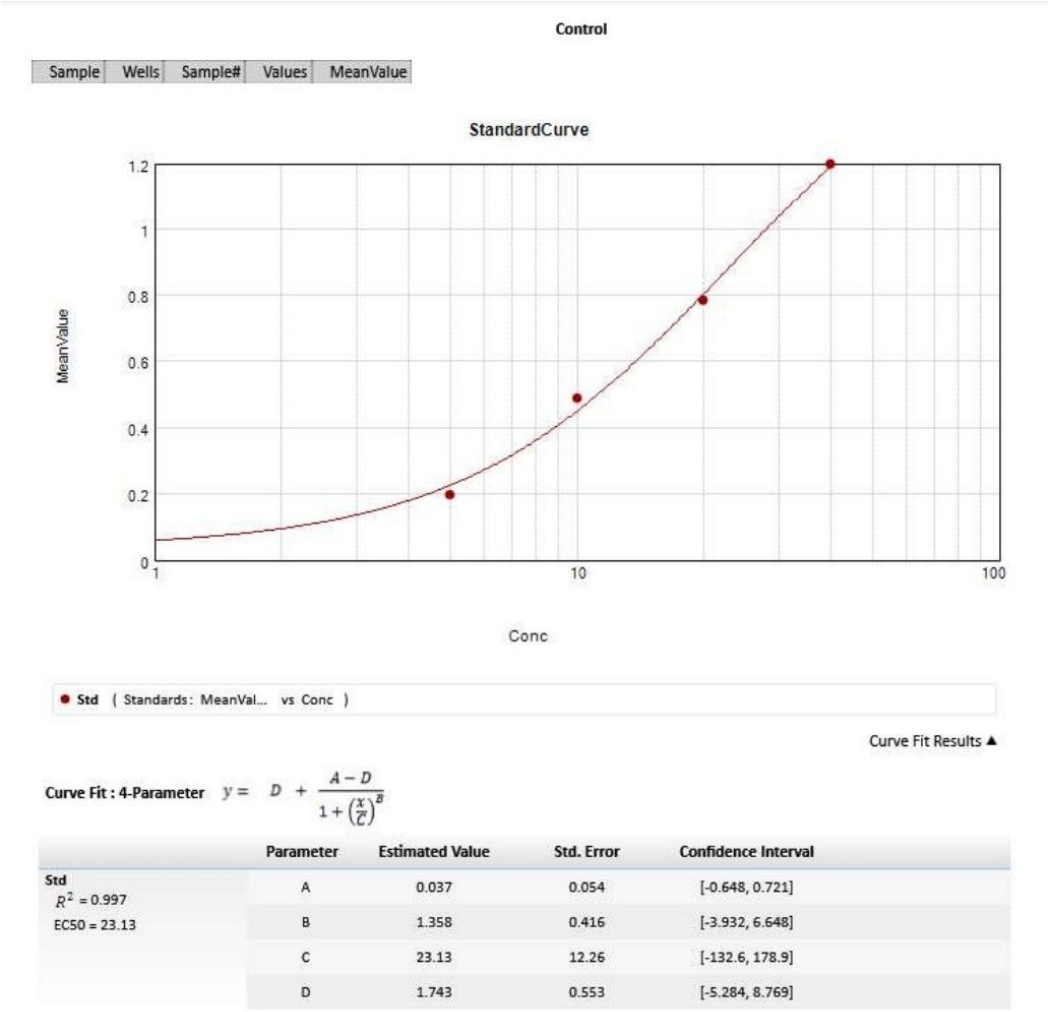

Figure 1

Standard curve showing the correlation between plasma MDA and comet parameters.

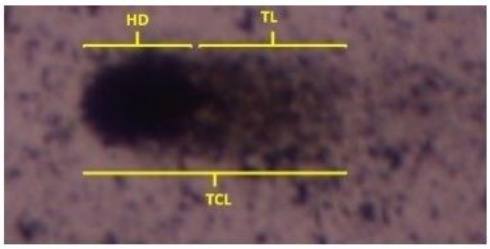

Figure 2

Photograph showing the parts of the comet

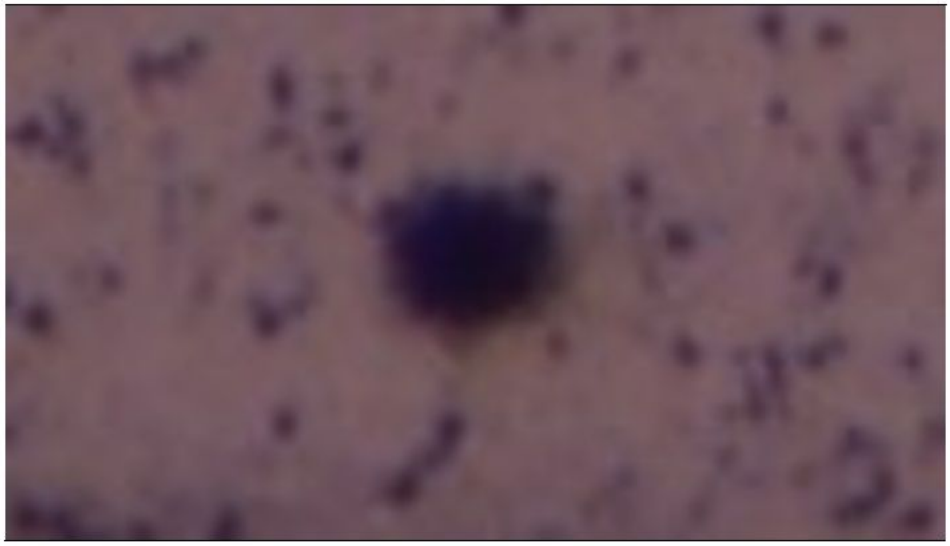

Figure 3

Photograph showing the comet with no DNA damage (grade-0)

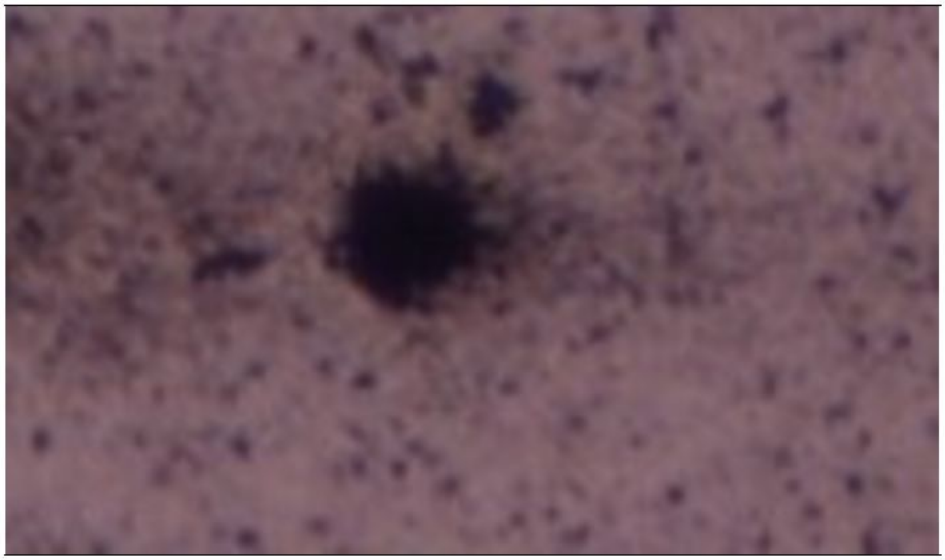

Figure 4

Photograph showing the comet with mild DNA damage (grade-1)

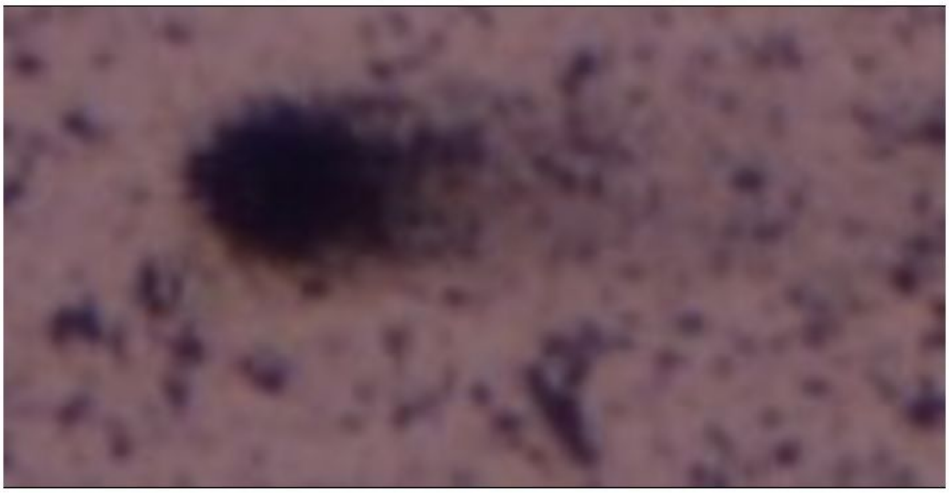

Figure 5

Photograph showing the comet with moderate DNA damage (grade-2):

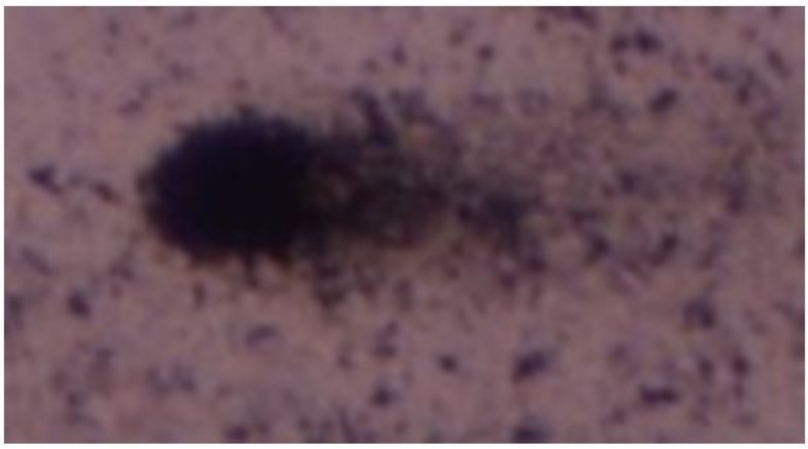

Figure 6

Photograph showing the comet with severe DNA damage (grade-3):

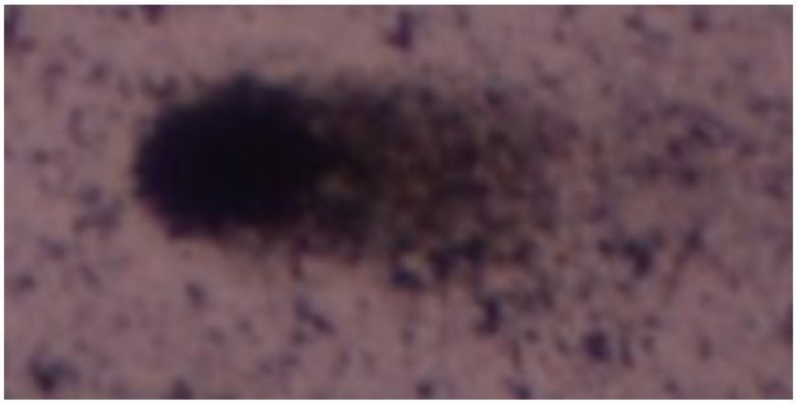

Figure 7

Photograph showing the comet with very severe DNA damage (grade-4):

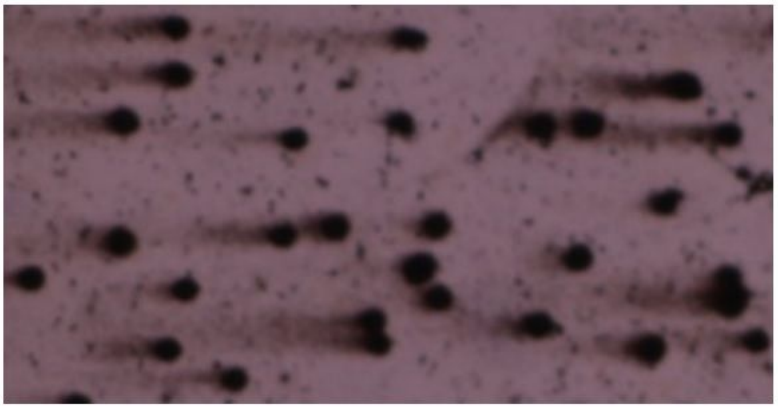

Figure 8

Photograph showing the comets of cervical cancer cases:

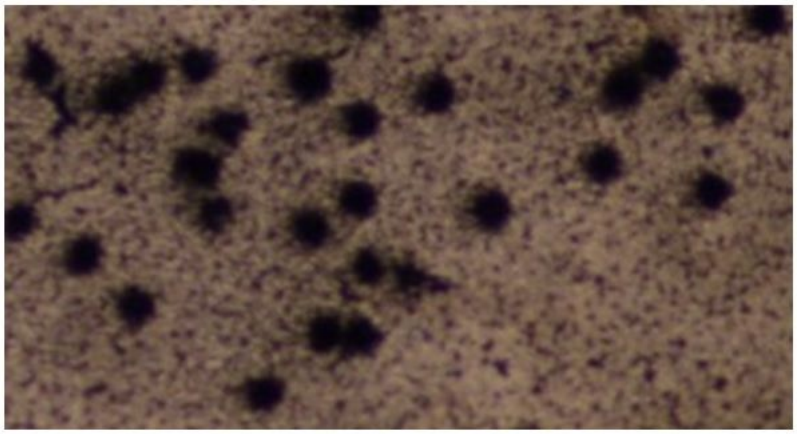

Figure 9

Photograph showing the comets of controls: