Evaluation of markers of oxidative DNA damage in females with breast tumors

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Abstract

Background: DNA damage reflects a balance between oxidative stress and DNA repair ability which associates with breast cancer risk.

Objective: Assessment of the oxidative DNA damage in women with breast tumors using comet tail length (CTL) and comet tail moment (CTM) to measure the extent of single strand DNA breaks in addition to 8-hydroxy deoxy guanosine (8-OHdG) levels and numbers of DNA lesions.

Methods: Blood leukocytes and post operative tumor specimens were taken from 40 females with newly diagnosed breast tumors (age 24-75 years) and leukocytes of 40 healthy controls (age range 24-50 years). The cells were subjected to single cell agarose gel electrophoresis and the severity of DNA damage was quantitated by computer image analysis. The level of the 8-OHdG was measured by ELISA and numbers of DNA lesions was estimated by special formula.

Results: There were highly significant differences($P<0.001$) in the mean levels of leukocyte CTL, CTM, serum 8-OHdG and DNA lesion in benign and malignant breast tumor as compared to the control groups with augmented elevations in these analytes in malignant breast tumor tissues as compared to the benign ones. A Significant increase ($p<0.001$) in the mean tissue 8-OHdG values was reported in the invasive malignant carcinoma as compared with noninvasive subgroup. The leukocyte means of CTL, CTM, 8-OHdG of malignant breast tumor patients with an age ≤ 48 years and BMI >24 were significantly higher than their counterparts ($p<0.05$, $P<0.001$, respectively). There were strong positive correlation between both leukocytes ($r=0.71$, $r=0.83$; $P<0.001$) and tissue ($r=0.69$, $r=0.83$; $P<0.001$) CTL, CTM with the concentration of serum 8-OHdG in total breast tumors.

Conclusion: The 8-OHdG and comet assays are useful, sensitive markers for monitoring the severity of DNA modification and damage in breast tumor and could be used to identify persons with increased cancer susceptibility.

Keywords: Comet test, 8-hydroxy deoxy guanosine, oxidative DNA damage, Breast tumors.

Introduction

Breast cancer is the most common malignant tumor and the leading cause of death in woman world-wide with 1.5 million new cases being estimated in the year 2001 [1].

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According to Iraqi Cancer Registry Center, breast cancer is remained the commonest malignant tumor in female population [2].

Although tumor initiation and progression are predominantly driven by acquired genetic alterations, yet DNA damage plays a central role [3]. These damages are caused by many factors including environmental factors (exposure to chemicals), nutrition, and natural cellular processes [4]. Ionizing radiation, an established etiologic agent for breast cancer, and other suspected risk factors, such as chemical

carcinogens, alcohol, estrogen and diet, result in reactive oxygen species (ROS), oxidized bases, bulky DNA adducts and DNA strand breaks. Under these circumstances, women may develop cytogenetic alterations, such as deletions, amplifications and/or mutations in critical oncogenes and tumor suppressor genes, leading to cellular transformation and neoplasm. Higher levels of DNA damage and deficient DNA repair may predispose individuals to breast cancer [5].

Oxidation of DNA in human cells occurs as a consequence of its attack by the free radicals arising endogenously as well as exogenously .Internal sources of the free radicals include ROS released during cellular respiration, or by leukocytes as a part of the defense against foreign organisms .Tobacco smoke and intermediates of xenobiotic metabolism (products of mixed function oxidase reactions) are other sources [6, 7].

Various biomarkers have been used to determine cellular DNA damage. The single cell gel electrophoresis or comet assay is one such state-of- the art technique for quantitative DNA damage and repair *in vivo* and *in vitro* in any eukaryotic cell and some prokaryotic cells. This technique is rapid, non-invasive, sensitive, visual and inexpensive as compared to the conventional techniques and is a powerful tool to study factors modifying mutagenicity and carcinogenicity. It has rapidly gained importance in the fields of genetic toxicology and human bio monitoring [8].

Particular types of ROS are responsible for different kinds of DNA damage. The OH˙ generated from H2O2 in the immediate vicinity of DNA, is able to damage the deoxyribose backbone of DNA and all of the four

DNA bases in various ways, including the generation of 8-hydroxyguanosine), the hydrolytic product of which is the 8-hydroxydeoxyguanosine (8-OHdG) [9]. This 8- OHdG has been used as a "fingerprint" of OH˙ attack and it is the most commonly measured and the most widely studied DNA oxidation product [10, 11]. The OH˙ is also responsible for DNA single and double-strand breaks and damage to tumor suppressor genes and other macromolecules [12].

In this study we assayed for the first time the degree of DNA damage and DNA lesions in benign and malignant breast tumors in Iraqi female utilizing the single cell gel electrophoresis (comet assay) and the level of 8-OHdG in the blood and tumor cells. Moreover, we assessed the effect of age, and body mass index on the degree of DNA damage.

Subjects and Methods

The study was part of PhD research that was conducted on 80 females; of these, 40 were newly diagnosed untreated breast tumors with an age range of 24-75years. They were admitted at the Department of Surgery in Al-Khadymia Teaching Hospital during the period of December, 2004 to May, 2006. Moreover, 40 healthy women (age range 24-50 years) with no family history of any type of cancer were involved as controls.

All the subjects were non-smokers non-alcoholic. Personal data on each subject were collected in terms of family history, menstrual history, menopause status, parity, age at first pregnancy, and laterality of the affected breast (Table 1).

Five milliliters (mL) of venous blood were withdrawn pre-operatively from patients and controls into heparinized tube to isolate leukocyte for comet assay. Another three mls of blood

were collected in plain test tube and allowed to clot at room temperature and the serum was separated by centrifugation at 3000rpm (750 Xg) and stored at -20˚C into small aliquots for the measurement of the serum 8-OHdG levels.

From each patient, a piece of tissue was taken from the site of the breast tumor and embedded in formalin, processed in the histopathology laboratory and the stained slides were examined by senior pathologist. According to the histopathological reports, 15 patients were having benign breast tumors and the other 25 were suffering from malignant breast tumors. A second piece of tissue was kept in cold phosphate buffered saline for the comet assay while the third piece was stored at -20 ˚C for the tissue DNA extraction.

Isolation of cells from the blood and breast tissue [13]: Neutrophils were isolated from heparinized blood samples by the method of Nath *et al* [14].

A small piece of the breast tumor tissue was placed in 1-2 mls of ice cold mincing solution containing 20mM EDTA and 10% dimethyl sulphoxide, and then filtered. The filtrate was centrifuged at 4000rpm for 30 minutes at 4ºC to precipitate the remaining intact cells, and the cells viability was determined by 0.1% trypan blue exclusion cell˜s number was adjusted to the concentration of 10^6cells/mL [14].

Extraction of DNA from fresh frozen breast tumor tissue: An organic (phenol) extraction method was used and the optical density of the extracted DNA immediately measured at 260nm using tris EDTA buffer as a blank solution [13]. The concentration of DNA was determined according to the following formula:

$$[O.D\,260nm \times Dilution\,factor \times 50\mu g/ml = \mu g/mL]$$

A pure DNA solution gave an A_{260} / A_{280} of more than 1.8 and one absorbance unit indicate a DNA concentration of 50 µg/mL [15].

Agarose Gel electrophoresis of the extracted DNA: The intact DNA was identified using agarose gel electrophoresis in a tri hydroxy methyl amino methane borate buffer (µ= 0.089 M, pH= 8) at 70V (6mA) for 4hr. The gel was stained with an ethidium bromide (10mg/ml) for 20 minutes, visualized under the UV transilluminator and photographed (Figure 1)

Comet assay (single cell gel electrophoresis): The comet assay was performed as described by Singh *et al*., 1988 [16] with some modifications. Briefly, regular microscope slides were coated by dipping in solution of 1% high-melting-point agarose. To the coated slides, 75µl of low melting point agarose (37ºC) were mixed with the 5-10µl of cell suspension (~10000cells) and left on ice packs until the agarose layer hardens (3 to 5 minutes). The slide was dipped into cold, freshly made lysing solution (1% Triton-X-100 and 10% dimethyl sulphoxide) for 24 h at 4°C. The slides were placed in a horizontal gel electrophoresis unit containing cold electrophoresis buffer solution (300 mM NaOH, 1 mM Na_2EDTA, pH = 13, 4°C) for 20-60 minutes to allow the DNA to unwind and to resolve alkali-labile sites. The electrophoresis is run at 0.7 V/cm (25 V, 300 mA) for 25 min at 4°C. Following electrophoresis, the slides were neutralized three times (5 min each) with 400 mM Tris–HCl (pH 7.5), stained with 100µl 1X ethidium bromide and scored immediately or dried by placing them in cold absolute alchohol for two minutes then rehydrate in chilled deionized water for 30 minutes, and stain with ethidium

bromide. The slides were analyzed using the LAI Comet Assay Analysis System (Loats Associates, Westminster, MD). The digitized images of a total 50 random nuclei from two duplicate slides were scored. The median comet tail length and moment of 50 cells /slide was determined from duplicate slides [13, 17].

Cells with damaged DNA appear as fluorescent comets with tails of DNA fragments (figure 2a), whereas normal, undamaged DNA does not migrate far from cell origin (figure 2b). Comet tail length represent the distance of DNA migration from the body of the nuclear core and it is used to evaluate the extent of DNA damage. The comet tail moment is defined as the product of the percentage of cellular DNA in the comet tail and the length of DNA tail migration [17, 8].

ELISA of 8- hydroxyl deoxy guanosine (OHdG) concentration: The ELISA trays were coated with a 100µl of 10µg/ml mouse antihuman 8-OHdG monoclonal antibody (*Crescent chemical co., Inc. US*) and kept for an overnight at 4ºC. After thorough washing, 50µl of either filtered serum (0.45µ Millipore filter), or 1µg/ml DNA digested with 0.5ml of 60% formic acid in an evacuated and sealed test tubes (at 140 ºC for 30 min) was added . A standard solution was applied to corresponding wells .To each sample and standard containing wells, add 50µl of reconstituted primary antibody and incubate at 4ºC for overnight. After thorough washing, 100µl of 1/450 of the reconstituted secondary antibody (Horse radish peroxidase-conjugated rabbit anti mouse antibody; *Sigma, USA*) was added to each well. Mix and incubate at room temperature for one hour then add 100µl per well of enzyme substrate solution. Mix uniformly and the trays were

incubated at room temperature in dark for 15 minutes. Add 100µl of the reaction terminating solution (1M phosphoric acid)**,** read the absorbance at 450nm. The sample 8-OHdG concentration was calculated from the standard curve by plotting the absorbance versus the logarithm of the standard 8-OHdG concentration [18].

Estimation of DNA lesion: The value of 8-OHdG was converted to the number of lesions/ 10^6DNA bases by assuming that guanine constitutes 21.5% of mammalian DNA using the following formula [18]:-

One lesion/10^5guanines=1/0.465 or 2.15 lesions /10^6DNA bases

Statistical analysis

The data were analyzed using Statistica version 6 and Microsoft Excel. The results were expressed as mean± standard deviation (SD). Student t-test was used to compare the results of patients and control groups. Simple correlation coefficient (r) was performed to test the relation between different markers of DNA damage. Differences were considered statistically significant if the *p* value is lower than 0.05.

Results

Table 2 reveals the mean values of leukocyte comet tail length (CTL), comet tail moment (CTM), number of DNA lesions, and serum 8-hydroxy deoxy guanosine (8-OHdG) of controls, and female patients with benign and malignant breast tumors. The mean ±SD CTL value in leukocytes of the control group was 2.4±0.05µm, while the mean value of CTL in those with benign and malignant tumors was4.7±0.13µm, 15.5±0.27µm, respectively.

Statistical analyses using student t-test showed a highly significant elevation in the mean value of CTL in both malignant and benign groups as

compared to control group(P<0.001). Between group t-test revealed that the mean CTL was higher in the sample of patient with malignant breast cancer as compared to benign breast tumor. The mean ±SD level of CTL for malignant invasive (15.4±0.30μm) is not significantly different from the non invasive tumor values (15.9±0.56μm).

The means ± SD CTM of control leukocyte was 7.8±0.10. In patient with benign and malignant breast tumor the mean ±SD CTM were 12.4±0.14 and 36.1±0.33, respectively. Statistical analyses using student t-test showed significant increase in CTM (P <0.001) in benign and malignant as compared to those of control group. Between groups t-test revealed statistically significant elevation (P <0.0001) of mean CTM in malignant breast tumor as compared to benign breast tumor.

Also in table 2, the mean± SD serum values of 8-OHdG and, number of DNA lesions (expressed as an arbitrary units) of the control group were 16.7±0.54ng/ml, and 0.1±0.004, respectively .While mean values of the benign (27.1±1.91; 0.2±0.01) and malignant breast tumors (152.6±8.06; 1.2±0.06) were significantly elevated above the mean control values (P <0.001). Between breast tumor groups, t-test revealed highly significant elevation (P <0.001) in the mean values of 8-OHdG and, number of DNA lesions in females with malignant tumor as compared to those with benign tumors.

Table 3 demonstrates the mean values of tissue CTL, CTM, DNA lesions, and serum 8-OHdG in female patients with benign and malignant breast tumors .The mean± SD CTL and CTM of malignant breast tissue cells were 4.6 ± 3.66 μm, and 13.9±4.3, respectively .These values were highly

significantly increased (P <0.001) above the mean values of benign breast tissue cells values (117.2±6.50; 48.1±14.95, in an order). Between malignant sub groups t-test revealed no significant differences between invasive carcinoma and noninvasive malignant breast carcinoma mean values.

The mean ±SD of tissue 8-OHdG and the numbers of DNA lesions in malignant breast tumor group was 294.8±59.68ng/ml, 2.3±0.42, respectively. Whereas in the benign breast tumor, the mean tissue 8-OHdG level was 29.3ng/ml ±6.95 and the mean of DNA lesions was 0.3±0.06. Student t-test revealed highly significant increase (P <0.001) in the tissue 8-OHdG mean values of the malignant breast tumor as compared to the benign breast tumor subtypes. Yet, there was a significant increase (P <0.001) in the mean tissue 8-OHdG levels in the invasive malignant carcinoma as compared with noninvasive carcinoma subgroups.

Table 4 Clarify the effects of body mass index and age on the mean values of leukocyte CTL, CTM, , number of DNA lesions, and serum 8-OHdG in controls and female patients with benign and malignant breast tumors. Statistical inference revealed no significant differences in the means of the CTL values in control(2.4±0.06 μm) benign (4.6±0.15 μm) and malignant breast tumors(15.0±0.46) patients with an age of ≤48year as compared to their respective mean values of those with a mean age>48(2.5±0.18 μm, 5.1±0.27 μm, 15.8±0.33 μm, respectively).

The mean leukocytes CTL of the controls and females with benign breast tumors with BMI≤24 were 2.4±0.07μm, 4.7±0.14 μm, in an order. These values were comparable and statistically not different from the mean value of those

with BMI>24 (2.4±0.09μm, 5.2±0.39 μm, one by one). In women with malignant breast tumor with BMI≤24, the mean CTL level was 16.4±0.43μm which is significantly higher (*P* <0.001) than the mean values observed from malignant breast tumor patients with BMI>24 (14.8±0.33μm).

The mean leukocyte CTM of malignant breast tumor patients with an age ≤ 48 years (36.7±0.37) was significantly higher than the mean CTM of the same disease group with an age >48year(33.7 ± 0.68). Furthermore, only females with benign breast tumors with BMI>24 exhibited a significant elevation in mean CTM (13.4±0.4) above the values of those with BMI> 24.

The mean serum 8-OHdG, of controls and those who suffer from malignant breast tumor with an age≤48 year (16.4±0.59ng/ml, and 171.6±12.7 ng/ml, respectively) were significantly increased above the mean 8-OHdG values of those with age > 48 years (*P* <0.05). Furthermore within the control, benign and malignant breast tumor groups the serum 8- OHdG concentration were significantly elevated in those with BMI> 24 as compared to patients with lower BMI (*P* <0.01, *P* <0.05, respectively).

Figure 3 and 4 reveal strong positive correlation between the leukocytes CTL and CTM and concentration of serum 8-OHdG in total breast tumors(r=0.71, r=0.83; *P* <0.001).Similar significant relationship was also observed between the tissue CTL and CTM and concentration of serum 8-OHdG in total breast tumors(r=0.69, r=0.83; *P* <0.001).

Table 1: Characteristics of the studied population[*]

VARIABLE	CATEGORIES	CONTROL N=40	BREAST TUMORS	
			BENIGN N=15	MALIGNANT N=25
Age(years)	Total	36.85±6.76	37.33±16.36	51±5.67
Age at menarche (years)	Total	12.4±0.87	11.9±0.89	11.92±1.28
	≤ 12.5	22 (55%)	12 (80%)	17 (68%)
	13-14	18 (45%)	3 (20%)	8 (32%)
Parity	≥ 1	23 (57.5%)	11 (73.3%)	23 (92%)
	Nulliparous	3 (7.5%)	1 (6.7%)	1 (4%)
	Unmarried	14 (35%)	3 (20%)	1 (4%)
BMI (Kg/m²)	Total	25.2± 3.17	22.3± 1.84	25.54± 4.33
Location of tumor	Right breast	-	8 (53.4%)	14 (56%)
	Left breast	-	5 (33.3%)	8 (32%)
	Bilateral	-	2 (13.3%)	3 (12%)

[*]Results are expressed as mean ±standard deviation.

Table 2: The mean (± standard deviation) values of leukocyte comet tail length, comet tail moment, number of DNA lesions, and serum 8-hydroxy deoxy guanosine (8-OHdG) of female controls, and patients with benign and malignant breast tumors.

		Mean concentration of			
		Comet tail length(μm)	Comet tail moment	8-OHdG (ng/ml)	DNA lesions (lesions/10^6bases)
Control N= 40		2.4±0.05	7.8±0.10	16.7±0.54	0.1±0.004
Benign N= 15		4.7±0.136[a***]	12.4±0.14 [a***]	27.1±1.91 [a***]	0.2±0.01 [a***]
Malignant	**Total N= 25**	15.5±0.27[(a,b)***]	36.1±0.33 [(a,b)**]	152.6±8.06 [(a,b)**]	1.2±0.06 [(a,b)***]
	Invasive N= 19	15.4 ±0.3[a(a,b)***]	36.3 ±0.38 [(a,b)***]	145.7±9.16[a***]	1.1 ±0.06[(a,b)***]
	Non Invasive N= 6	15.9±0.56[a***]	35.4±0.65 [(a,b)***]	174.3±14.85[(a,b)***]	1.3±0.01[(a,b)***]

[a]t-test :comparison of the total, benign, malignant breast tumor groups with control: [***]$p<0.001$
[b]t-test :comparison of benign group with malignant breast tumor groups: [***]$p<0.001$
[c]t-test :comparison of invasive malignant breast tumor with non invasive subgroup: Not significant

Table 3: The mean (± standard deviation) values of tissue comet tail length, comet tail moment, number of DNA lesions, and breast tissue 8-hydroxy deoxy guanosine (8-OHdG) in female patients with benign and malignant breast tumors.

		Mean concentration of:			
		Comet tail length(μm)	Comet tail moment	8OHdG (ng/ml)	DNA lesion (lesions/10^6bases)
Benign tumors N= 15		4.6±3.66	13.9±4.31	29.3±6.95	0.3±0.06
Malignant tumors	**Total N= 25**	17.2±6.50 [a***]	48.1±14.95 [a***]	294.8±59.68 [a***]	2.3±0.42 [a***]
	Invasive N= 19	17.3±0.2 [a***]	47.6±0.49 [a***]	290.7±14.91 [a***]	2.3±0.1 [a***]
	Non Invasive N= 6	16.7±0.39 [a***]	49.9±0.83 [a***,b*]	307.6±15.91[a***, b*]	2.4±0.1 [a***]

[a]t-test : comparison of benign group with malignant breast tumor groups: [***]$p<0.001$.
[b]t-test: Between invasive and noninvasive malignant breast tumor groups t-test: [*]$p<0.05$.

Table 4: The effect of body mass index and age on the mean (± standard deviation) values of leukocyte comet tail length, comet tail moment, number of DNA lesions, and serum 8-hydroxy deoxy guanosine (8-OHdG) in controls and female patients with benign and malignant breast tumors.

			Mean concentration of:			
			Comet tail length(µm)	Comet tail moment	8OHdG (ng/ml)	DNA lesion (lesions/10^{6}bases)
Control N= 40	Age (year)	≤48	2.4±0.06	6.9±0.09	16.4±0.59 [a***]	0.1±0.004
		>48	2.5±0.18	7.9±0.23 [a**]	17.5±1.27	0.1±0.01
	BMI (Kg/m^2)	≤24	2.4±0.07	7.5±0.11	17.1±0.68 [b**]	0.1±0.005
		>24	2.4±0.09	7.8±0.14	15.9±0.87	0.1±0.01
Benign tumors N= 15	Age (year)	≤48	4.6±0.15	12.4±0.1	26.9±2.43	0.2±0.01
		>48	5.1±0.27	12.6±0.2	27.5±3.12	0.2±0.02
	BMI (Kg/m^2)	≤24	4.7±0.14	12.3±0.1	27.5±2.11	0.2±0.01
		>24	5.2±0.39	13.4±0.4 [b**]	24.0±5.00 [b**]	0.2±0.03
Malignant tumors N= 25	Age (year)	≤48	15.0±0.46	33.7±0.68	139.9±12.78	1.1±0.09
		>48	15.8±0.33	36.7±0.37 [a***]	171.6±9.34 [a*]	1.3±0.07 [a**]
	BMI (Kg/m^2)	≤24	16.5 ±0.43 [b***]	36.3±0.52	140.1±10.14	1.1±0.07
		>24	14.8 ±0.33	35.9±0.42	162.4±11.64 [b**]	1.2±0.08

[a] Within Age subgroup of benign and malignant breast tumors t-test: $^*p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.
[b] Within BMI subgroup of the controls, benign and malignant breast tumors t-test: $^{**}p<0.01$, $^{***}p<0.001$.
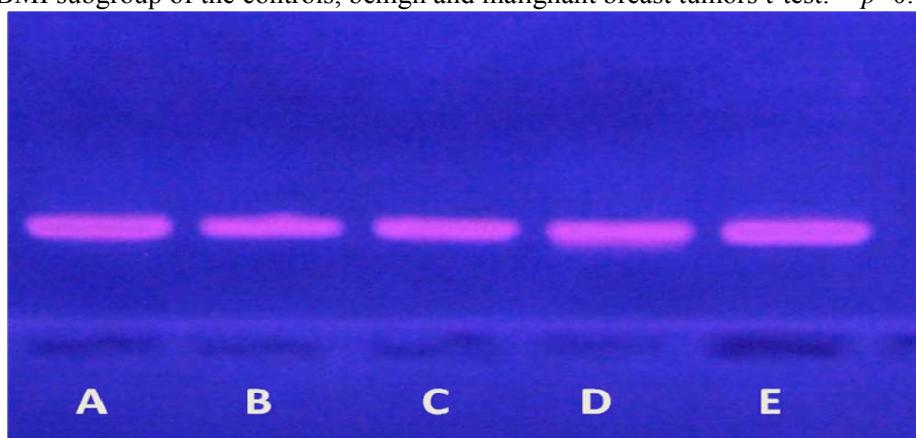
Figure 1: An agarose gel electrophoreticograph of an extracted intact cellular DNA stained with ethidium bromide: *lanes* **A and B are for benign breast tumors whereas** *lanes* **C, D, and E are for malignant breast tumors.**
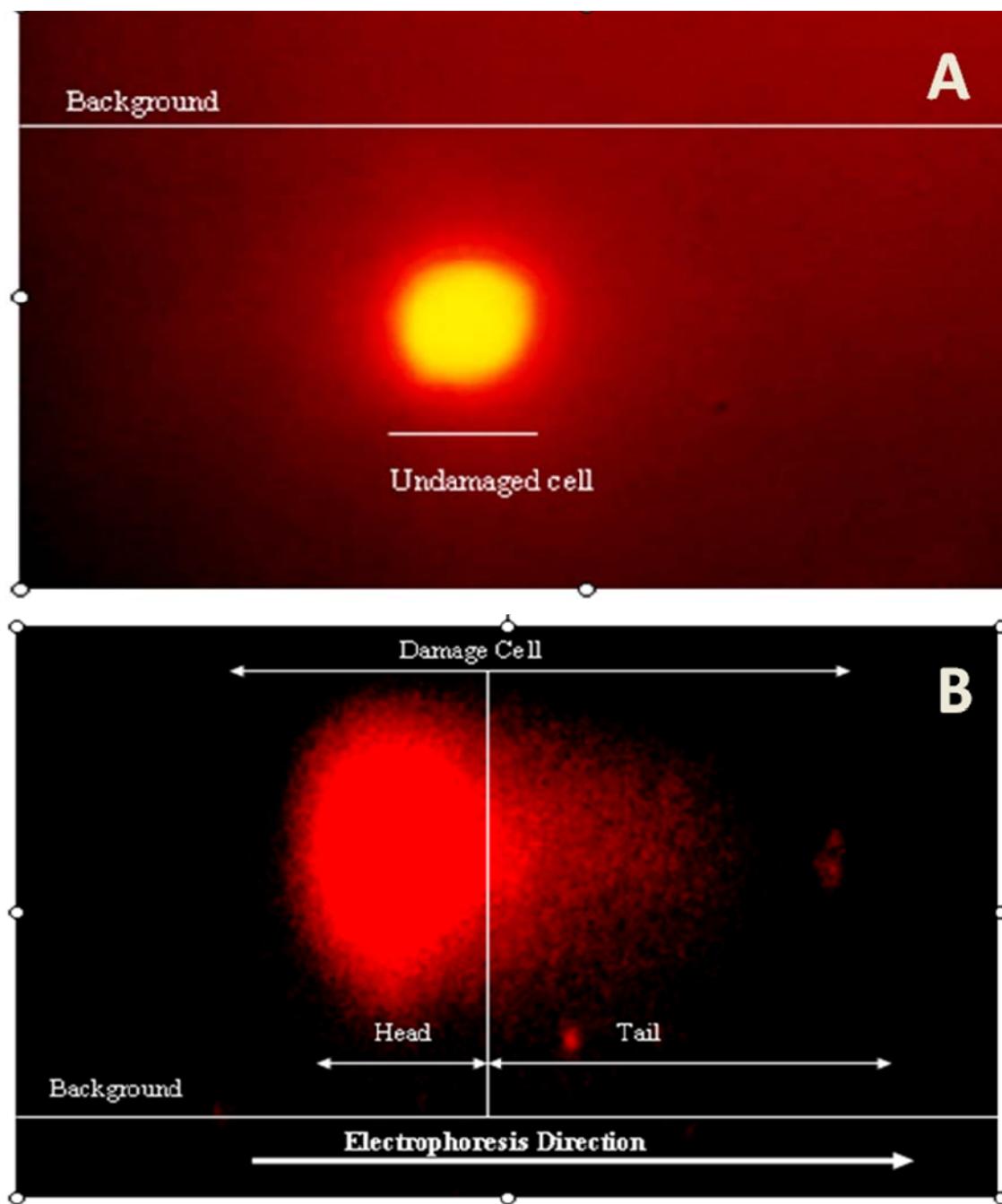
Figure 2: A photograph of a single cell gel electrophoresis. *A: intact cellular DNA which appears as nucleoids. B: malignant breast tumor cell with extensive DNA damage appear as a typical comet with head and tail.*
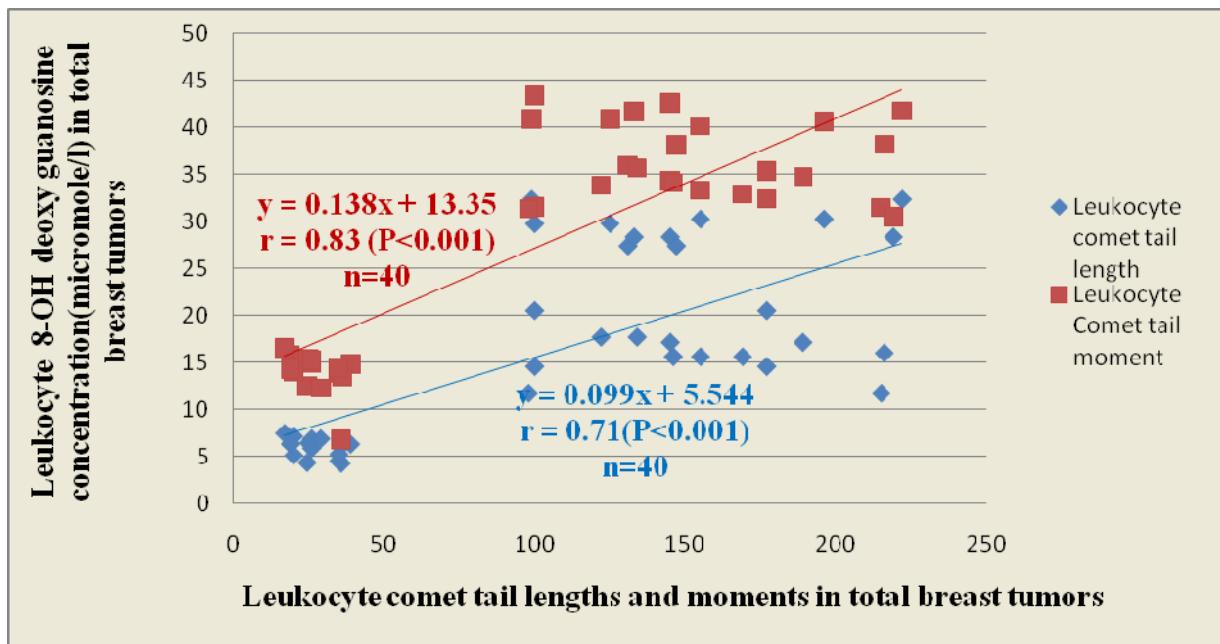
Figure 3: Correlation between Leukocyte comet tail length, comet tail moment, and concentration of serum 8-OHdG in total breast tumors.
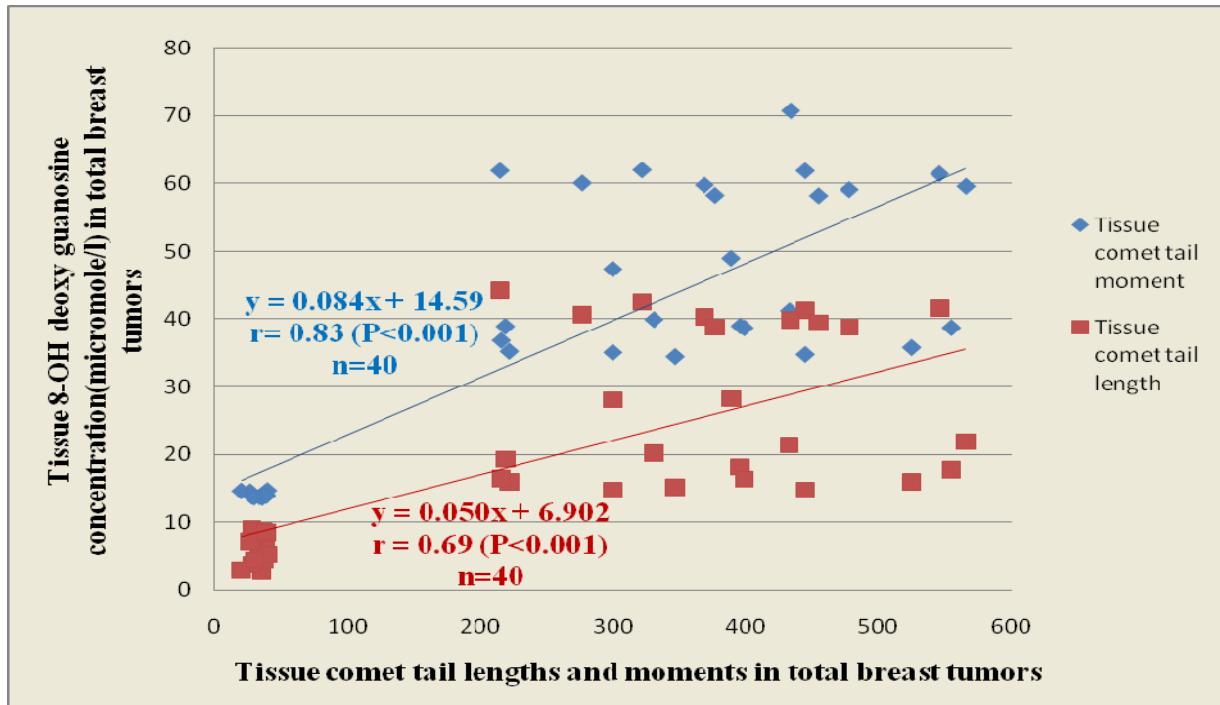

Figure 4: Correlation between Tissue comet tail length, comet tail moment and concentration of serum 8OHdG in total breast tumors.

Discussion

The comet assay protocol used in this study is adequate to detect significant differences in single strand breaks between breast cancer cases and controls. Varieties of tissues have been used in the comet assay. Leukocytes were considered a good marker of actual bodily state as they are more susceptible to the damaging effects of free radicals due to the high percentage of polyunsaturated fatty acids (PUFAs) in their plasma membranes and increased production of free radicals as part of their normal function [7,19]. In this study, DNA damage expressed as comet tail length (CTL) and comet tail moment (CTM) was reported to be higher in leukocytes of benign and malignant breast cases than controls. Similar finding was recorded by Rajeswari et al. [20], who observed a 7-fold difference in CTL between breast cancer cases and controls. Furthermore, the means of comet tail length and CTM t were increased in patient with malignant breast cancer as compared to benign breast tumor. Alice et al. [21] reported that the lower levels of DNA damage in controls were associated with being long-lived, cancer-free, and without a history of cancer among first degree relatives, supporting the notion of their capacity to control endogenous DNA damage.

DNA damage (expressed as CTM) was increased in malignant breast tumors patients with an age >48 year as compared to those of ≤48years and this may be due to a decrease in DNA repair and an accumulation of DNA damage in malignant breast tumors leading to increased susceptibility to DNA damage .This finding was in line with those of Singh et al. [22] who documented that cells from older individuals have less resistance to DNA by *ex vivo* x-ray exposure. Moriwaki et al. [23] and Wei et al. [24] observed a decrease in DNA repair with increase of age. On the contrary, Ramos et al. [25] reported that younger breast cancer patients had a more substantial reduction in capacity to repair UV damage compared with age matched control subjects than did older patients and controls. Moreover, we observed that the age has no effect on the CTL and this finding agrees with cross sectional study on healthy Americans (age range 25- 91 years) that showed no effect of age on the basal level of DNA damage [26].

In women with malignant breast tumor with BMI≤24 Kg/m^2 the mean CTL level was significantly higher ($p<0.001$) than those with higher BMI (>24 Kg/m^2) with no effect of BMI on the CTM. This finding disagreed with those of Smith et al. [27] who recorded that a high BMI may be associated with increased levels of lipophilic aromatic compounds, such as polychlorinated biphenyls, aromatic and heterocyclic amines, and polycyclic aromatic hydrocarbons, stored in breast adipose tissue, leading to a continuous exposure to DNA-damaging agents. Udumudi et al. [28] reported that CTL significantly differed between cancer patients and controls in cervical epithelial cells, as well as in peripheral blood leukocytes. These studies suggested that genetic defects in DNA repair may contribute to higher levels of DNA damage in leukocyte and target tissue in cancer patients.

This study showed that DNA damage (expressed as CTL) was increased in malignant subtypes as compared to controls. This observation agrees with Alice et al. [21] finding of a

consistent association of increased endogenous DNA damage (indicated by higher CTL values) with the rise in cancer risk. Statistical analysis revealed no differences in CTL and CTM between the invasive and non invasive malignant breast carcinoma subtypes which mean that women with non invasive carcinoma subtype are under the risk of conversion to an invasive carcinoma type.

In tissue analyses our study showed that the CTL and CTM in malignant breast tissue were increased more than in benign breast tissue. This increase in DNA oxidative damage in malignant breast tissue is probably due to increase proliferation of this type of tissue which leads to increase accumulation of damaged DNA in breast tissue. The malignant noninvasive carcinoma was found to have higher CTM values than invasive carcinoma. A number of epidemiological studies, primarily on lung and skin cancers have suggested that deficiency in DNA repair capacity, accumulation of DNA damage, acceleration of gene rearrangements (deletions, insertions, and amplifications) are involved in human carcinogenesis [29, 30]. Alice *et al.* [21] reported a rise in the risk of breast and thyroid cancers with the increase in the values of CTL and CTM or only CTM, respectively.

The increased serum 8-OHdG level in benign and malignant breast tumors reported herein is consistent with Donghui *et al.*[31],and Zora *et al.* [32] findings who reported high levels of the 8-OHdG in serum and/ or urine of cancer patients. Moreover, elevated levels of 8-OHdG were observed in serum of patients with malignant tumors compared with those with benign tumors. It is possible that an

accumulation of damage due to 8-OHdG formation overwhelms the capacity for DNA repair [33].

Our result revealed that the concentration of 8-OHdG was increased in malignant breast tumors patients with an age >48 year above those of lower age. This finding was in line with Kuo *et al* [34] observation who reported that the concentration of 8-OHdG is age dependent. On the contrary, one study found that the accumulation of oxidative DNA damage was unrelated to age or to smoking and drinking habits [35]. Asami *et al.* [36] revealed that the lymphocytic 8-OHdG levels were mainly dependent upon age and smoking status. We observed that the oxidative damage expressed as 8-OHdG was significantly increased in malignant breast tumor tissues of patients with a BMI >24 but were lower in patients with benign breast tumor tissues as compared to those of ≤24 Kg/m^2 BMI. The high BMI associates with increased endogenous estrogen production may explain the association between obesity and breast cancer [37]. Mizoue *et al* [38] found intensive association between BMI and 8-OHdG levels among smokers with no apparent relations between BMI and 8-OHdG levels among nonsmokers. On the contrary, Trie *et al.* [39] recorded significant negative correlations of the 8-OH-dG level with BMI.

Because 8-OHdG is known to represent one of the major forms of oxidative DNA damage, many researchers have measured 8-OHdG in tissues or urine as a marker of oxidative stress [34]. We observed an increase in the mean concentration of tissue 8-OHdG in malignant breast cancer as compared to the values of the benign breast tumors. This finding agrees with those of Donghui *et al.*[31] who reported

that tissue from breast cancer had significant higher level of 8OHdG than normal control breast tissue. For malignant subtypes there was a significant elevation in the mean concentration of tissue 8-OHdG in the non invasive above the values of invasive malignant breast carcinomas. This result is consistent with those of Parshad *et al.* [40] who revealed a decreased repair of X-ray induced DNA damage in lymphocytes of eight women with pre invasive malignant breast lesions. This observation indicates that oxidative DNA damage may play a role in placing these women at increased risk of conversion to an invasive subtype. Furthermore, the means of numbers of DNA lesion in the sera of patients with benign and malignant breast tumors were significantly higher than the control values. Yet, the increase was much augmented in malignant breast tumors. This result agrees with Donghui *et al.* [32] who found that tissue breast cancer had significant higher level of 8OHdG than control subjects but he found the mean±SD of 8-oxo-dG/10^6 were $3.9\pm7.2/10^6$ and $1.1\pm1.4/10^6$ for cases and controls, respectively. Poirier and Weston [41] found that the concentration of benzpyrene-DNA adduct from malignant tumors taken from smokers to be 0.65-$5.33/10^6$ DNA bases. Ottender and Lutz [42] work on rat liver, revealed that carcinogen-DNA adduct concentration is associated with a 50% incidence of liver cancer which ranged from 53 to 2083 adducts/10^8 nucleotides for aflatoxin and dimethylnitrosamine carcinogens.

The mean of number of DNA lesions was high in older patients with malignant tumors (> 48 years). Yet, it was not influenced by the increase in patient BMI. In malignant subtypes there

were no significant differences in the number of DNA lesions in both sera and tissues of patients with noninvasive as compared to those of invasive malignant breast tumors.

In Conclusion: DNA damage is significantly associated with breast cancer risk. The comet assay could be used for measuring the levels of DNA damage in patients with breast tumors whereas 8-OHdG is a useful marker for DNA modification in the cells. Both tests could contribute to the detection of the degree of DNA damage in persons with breast tumors.

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