ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

An increased degree of oxidative DNA damage in Iraqi males with breast tumors

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Manuscript Info

Manuscript History:

Received: 15 July 2015
Final Accepted: 22 August 2015
Published Online: September 2015

Key words: male breast cancer, DNA oxidative damage, 8-OHdG.

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Abstract

Reactive oxygen species (ROS) play a pathogenic role in carcinogenesis by inducing oxidative DNA damage, ROS-induced DNA oxidation leads to a multitude of modifications to DNA bases, with 8-hydroxy-2-deoxyguanosine (8-OHdG) representing the most frequent one.
The current study aims to assess the oxidative DNA damage in Iraqi males with breast tumors using 8-hydroxyl-2-deoxygunosine (8-OHdG) levels and numbers of DNA lesions.
Serum, urine samples and post operative tumor specimens were taken from 20 males with newly diagnosed breast tumors (age 24-65 years) and serum and urine samples of 20 healthy controls (age range 24-65 years). The level of 8- OHdG was measured by ELIZA and numbers of DNA lesions was estimated by special formula.
Our data showed a statistically significant increased ($p < 0.0001$) in the mean levels of serum and urine 8-OHdG and DNA lesion in benign and malignant breast tumors as compared to the control groups with augmented elevations in these markers in malignant breast tumor tissues as compared to the benign ones.
Conclusion: - Oxidative DNA damage is significantly associated with breast cancer risk, both serum and urine 8-OHdG levels are useful markers for DNA modification in the cells. 8-OHdG levels contribute to the detection of the degree of DNA damage in persons with breast tumors.

INTRODUCTION

Breast cancer in men is considered to be a rare disease, accounting for less than 1% of all breast cancer cases and 0.1% of cancer mortality in men [1]. However, it is one of the most common neoplastic diseases in Iraq according to the Iraqi Cancer Registry Center, with 2987 new cases expected each year in both gender accounting for 19.59% of all new diagnosed cancer case [2] . To our knowledge, there are no epidemiological studies about male breast cancer (MBC) in Iraq. Studies are limited to single case reports or small case series, describing only clinical or pathological findings on each patient. The pathophysiological change of MBC is not yet well known.

Previous studies proved that tumor initiation and progression are predominantly driven by acquired genetic alteration, yet DNA damage plays a central role in this process [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, estrogens and diet, result in reactive oxygen species (ROS) [5].

ROS play a pathogenic role in carcinogenesis by inducing oxidative DNA damage, modulating gene expression, altering different signaling pathways and leading to a deregulation of cell proliferation and apoptosis. ROS-induced DNA oxidation leads to a multitude of modifications to DNA bases; with 8-hydroxy-2-deoxyguanosine (8-OHdG) representing the most frequent one [6-9].

8-OHdG, is a guanine adduct used as an index of DNA oxidative damage, induces a point mutation in the daughter DNA strands, accumulates in cell DNA and causes mis-pairing, thus demonstrating its mutagenic and potentially carcinogenic role [10]. Several studies have demonstrated that the 8-OHdG in bodily fluids can act as a biomarker of oxidative stress [11-15] and 8-OHdG is commonly used as a marker to evaluate oxidative damage in disorders including chronic inflammatory diseases [11]. It is reported that elevated levels of 8-oxo-deoxyguanosine adducts in DNA play a fundamental role in breast cancer [16-18].
To our knowledge, this is the first Iraqi study to assess the degree of oxidative DNA damage and DNA lesions in benign and malignant breast tumors in Iraqi male utilizing 8-OHdG in the blood, urine and tumor cells. Moreover, we assessed the effects of age, and body mass index on the degree of DNA damage.

Materials and methods:

Study population:

The current study has been approved by the Al-Nahrain medical ethics committee. Forty males were participated in the study; twenty males were newly diagnosed with breast tumors (untreated yet) with an age range of 24-65year. They were admitted to the Department of Surgery at Al-Khadymia Teaching Hospital during the period from December, 2004 to December, 2006. Twenty healthy males were also included in this study as the normal controls (age range 24-65 year) who have no family history of any type of cancer were involved in the study.
All participants were asked to complete a questionnaire on their medical histories and lifestyles, including genetic disease, medication, alcohol consumption, smoking, etc.

Blood sampling:

Three milliliters (ml) of venous blood were withdrawn pre-operatively from patients and controls into plain test tube and allowed to clot at room temperature and the serum was separated by centrifugation at 300rpm and at -20^{0}C into small aliquots for the measurement of the serum 8-OHdG levels.

Tissue sampling:

For each patient, a piece of tissue was taken from the site of the breast tumor and embedded in formaldehyde, processed in the histopathology laboratory and the stained slides were examined by senior pathologist. According to the histopathological reports, 11 patients were having benign breast tumors and other 9 were suffering from malignant breast tumors. A second piece of tissue was stored at -20^{0}C for tissue DNA extraction.

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 [19]. The concentration of DNA was determined according to the following formula: [O.D.260nm X Dilution factor X 50µg/ml= µg/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 50µg/mL [20].

Urine sampling:-

Urine sample from patients with breast cancer were collected before surgery. Similarly, urine samples collection were obtained from controls. All the samples were centrifuged at 10000g for 10 min to remove any precipitate. The supernatants were stored at-40 ^{0}C until analyses.

ELIZA of 8-hydroxyl -2- deoxy guanosine (OHdG) concentration:

8-hydroxydeoxyguanosin level was measured using. Enzyme linked immunosorbent assay (ELIZA), (BIOTECH-Elx800/England), according to the procedure of the kit cayman USA (Item No. 58920).

Estimation of DNA lesion: the value of 8-OHdG was converted to the number of lesions/ 10^6 DNA bases by assuming that guanine constitutes 21.5% of mammalian DNA using the following formula [21].
One lesion /10^5 guanines= 1/0.465 or 2.15 lesions/ 10^6 DNA bases.

Statistical analysis:

The data of the research were stored in Microsoft Excel Spread sheet and analyzed on the computer using the Statistical version 8 and Microsoft Excel programs (Microsoft Office 2007). The results were expressed as mean ±standard deviation of the mean (SD). Student t-test was applied to compare the results of patient and control groups. Differences were considered statistically significant if the p value is lower than 0.05.

Results:

The Characteristics of patients (benign and malignant breast tumor) and controls groups in relation to the present age, their smoking status body mass index (BMI) and site of the tumor are summarized in table (1).

The mean age of control was 42 years (range: 24-65), while the mean age for men with benign breast tumor 50years (range: 24-63), and for men with breast cancer 55years (range: 49-65) respectively. All control men were non smokers while 36.4% of patients with benign and 55.6% of the patients with malignant breast tumors were smoking 1-10 cigarettes daily. On the other hand, BMI did not significantly difference between cases and control.

The tumor was localized in the right side in 54.5% of patients with benign tumors and 33.3% of patients with malignant breast tumors, while in the left side in 45.5% of patients with benign and 66.% of patients with malignant breast tumors in that order.

In general, the susceptibility to cancer is characterized by high DNA damage, which is the result of low repair capacity.

Table (2) reveals the mean values of serum and urine 8-OHdG samples of all participants included in the study. The mean± SD 8-OHdG value in serum of control group was 96.5±25.6ng/ml while the mean values of 8-OHdG in those with benign and malignant tumors were 129±14.8ng/ml, and 197.8±4.1ng/ml, respectively.

Statistical analysis using student t-test showed a highly significant elevation in the mean value of 8-OHdG in both malignant and benign groups as compared to control group (*p*<0.0001), also t-test revealed that the 8-OHdG was higher in the serum of patients with malignant breast cancer as compared to benign breast tumor (*p*<0.0001).

The mean±SD 8-OHdG urine sample for the controls was 98.9±16.7ng/ml, while the levels in patients with benign and malignant breast tumor were 146.1±18.3 and 173.7±17.03, respectively. Statistical analysis using student t-test showed a significant increase in urinary 8-OHdG (*p*<0.0001) in the benign and malignant groups as compared to the control group (*p*<0.0001).

Also in table 2, the mean±SD serum and urine values of number of DNA lesion (expressed as an arbitrary units) of the control group were 0.73±0.19 and 0.75±0.13 respectively, while mean values of benign (0.97±0.1; 1.1±0.14) and malignant breast tumors (1.5±0.03; 1.1±0.14) were significantly elevated above the mean control values (*p*<0.0001). between breast tumors groups, t-test revealed highly significant elevation (*p*<0.0001) in the mean value of serum number of DNA lesion in males with malignant tumor as compared to those with benign tumors, while the was no significant different between mean value of urine number of DNA lesion in males with malignant tumor as compared to those with benign tumors.

Table (3) demonstrates the mean values of tissue 8-OHdG and DNA lesions, in male patients with benign and malignant breast tumors. The mean±SD of tissue 8-OHdGand the number of lesions in malignant breast tumors group was 267.8±22.3ng/ml, 2.03±0.5 respectively, whereas in the benign beast tumor, the mean tissue 8-OHdG level was 129.4±2.1ng/ml and the mean of DNA lesions was 0.98±0.05. Student t-test revealed highly significant increase (*p*<0.0001) in the tissue 80OHdG mean values of malignant breast tumor as compared to the benign breast tumor.

Table (4) clarifies the effects of body mass index and age on the mean values of serum 8-OHdG and number of DNA lesions in controls and male patients with benign and malignant breast tumors. The mean serum of 8-OHdG of controls, benign and those who suffer from malignant breast tumor with age ≤50year (97.47±29.8, 115.8±11.09 and 194.33±4.5ng/ml respectively) were significantly increased above the mean 8-OHdG values of those with age >50 year (*p*<0.0001). 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*<0.0001)

Table.1: Characteristics of the studied population

VARIABLE	CATEGOYIES	CONTROL	BREAST TUMORS	
			BENIGN	BENIGN
Age(years)	Mean± SD total	42.1±12.7	50.4±11.2	54.7±5.9
Smoking status	Mean± SD			
Smokers	**(1-10/day)**	-	**5.17±0.71** 4 (36.4%)	**7.25±0.62** 5 (55.6%)
Nonsmokers		20	**6.47±0.41** 7 (63.6%) *p=0.009*	**8.50±0.39** 4 (44.4%) *P=0.007*
BMI (Kg/m^2)	Mean± SD	24.9±1.4	26.0±2.4	25.9±1.9
Location of tumor	Right breast Left breast		6 (54.5%) 5 (45.5%)	3 (33.3%) 6 (66. %)

Table2: The mean (±standard deviation) values of serum 8-hydroxy deoxy guanosine (8-OHdG), urine (8-OHdG) and DNA lesions of male controls, and patients with benign and malignant breast tumors.

	Mean concentration of			
	Serum 8-OHdG (ng/ml)	DNA lesions (lesions/10^6)	Urine 8-OHdG (ng/ml)	DNA lesions (lesions/10^6)
Control N=20	96.5±25.6	0.73±0.19	98.9±16.7	0.75±0.13
Benign N=11	129±14.8	0.97±0.1[a]***	146.1±18.3	1.1±0.14[a]****
Malignant N=9	197.8±4.1	1.5±0.03[a]****,[b]****	173.7±17.03	1.1±0.14[a]****,[n.s]

[a]t-test: comparison of the total, benign, malignant groups with control: *** p<0.0001, ****p<0.0001.
[b]t-test: comparison of the benign with malignant groups: ****<0.0001, not significant.

Table3: The mean (±standard deviation) values of tissue 8-hydroxy deoxy guanosine (8-OHdG) , and DNA lesions in male patients with benign and malignant breast tumors.

	Mean concentration of	
	tissue 8-OHdG (ng/ml)	DNA lesions (lesions/10^6)
Benign N=11	129.4±2.1	0.98±0.05
Malignant N=9	267.8±22.3****	2.03±0.50****

t-test: comparison of the benign with malignant groups: ****<0.0001.

Table4: The effect of body mass index and age on the mean (± standard deviation) values of serum 8-hydroxy deoxy guanosine (8-OHdG) , urine (8-OHdG) and DNA lesions of male controls, and patients with benign and malignant breast tumors.

			Mean concentration of:	
			8-OHdG (ng/ml)	DNA lesion (lesions/10^6 base)
Control N=20	Age (year)	≤50 (n=15)	97.47±29.8	0.73±0.22
		> 50 (n=5)	93.60±2.7	0.71±0.02
	BMI (Kg/m^2)	≤24 (n=8)	82.6±5.97	0.63±0.04
		> 24 (n=12)	105.75±29.66	0.80±0.22
Benign tumors N=11	Age (year)	≤50 (n=5)	115.8±11.09a,	0.87±0.08
		>50 (n=6)	140.0±4.47a****,	1.06±0.03a****,
	BMI (Kg/m^2)	≤24 (n=4)	112.25±8.95	0.85±0.06a****
		>24 (n=7)	138.57±5.56	1.05±0.04
Malignant tumors N=9	Age (year)	≤50 (n=3)	194.33±4.5a****,b***	1.47±0.03a****,b***
		>50 (n=6)	199.5±2.88a****,b****	1.51±0.02a*** , b****
	BMI (Kg/m^2)	≤24 (n=2)	192.0±2.82	1.45±0.02a***,b***
		>24(n=7)	199.42±2.63	1.51±0.02

[a]t-test: Between age groups≤50 year and > 50 year comparison of control, benign and malignant groups ****p<0.0001.
[b]t-test: Between BMI groups≤24 and > 24 comparison of control, benign and malignant groups ****p<0.0001.

Discussion:

Oxidative stress can cause DNA damage, including oxidative of nucleosides. Oxidative DNA damage may be involved in the development of breast cancer. In this study we investigated the presence of oxidative DNA damage in the breast tumors by using 8-OHdG in the serum and also in urine in a small set of samples.

The significant increase in serum and urine 8- OHdG levels in benign and malignant breast tumors reported here is consistent with Donghui et al[22], and Zora et al. [23] findings who reported high levels of the 8-OHdG in serum and urine of the 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 over helms the capacity for DNA repair [24].

Our result reveled that the concentration of 8-OHdG was increased in malignant breast tumors patients with age >50 year above those of lower age. This finding was in line with kuo et al [25] who reported that the concentration of 8-OHdG is age dependent. However, another study found that the accumulation of oxidative DNA damage was unrelated to age or to smoking and drinking habits [26]. ASami et al [27] revealed that the lymphocytic 8-OHdG levels were mainly dependent upon age and smoking status.

Our study found a statistically significant increase in the serum 8OHdG in the malignant breast tumor tissues of patients with a BMI > 24 Kg/m^2 but the results were lower in patients with benign breast tumor tissues as compared to those of $\leq$ 24 Kg/m^2 BMI. The high BMI associates with an increased endogenous estrogen production may explain the association between obesity and breast cancer [28]. The association between estrogen levels and breast cancer in men is deduced from the fact that obesity has fairly consistently been associated with increased risk and implicated in the etiology of MBC due to higher circulating estrogen levels [29, 30].

Furthermore, the means of numbers of DNA lesion in the sera and urine 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 agree with Donghui et al [22] who found that tissue breast cancer had significant higher level of 8-OHdG/10^6 than control subjects .

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

Conclusion:

Oxidative DNA damage is significantly associated with breast cancer risk, estimation of both serum and urine 8-OHdG levels are useful for oxidative DNA modification in the cells. 8-OHdG could contribute to the detection of the degree of oxidative DNA damage in persons with breast tumors.

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