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Full Length Research Paper

Differences in DNA repair kinetics of lesions induced by hydrogen peroxide in lymphocytes from premenopausal breast cancer patients and healthy Women resident in Great Buenos Aires

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Nontraditional risk factors in breast cancer have been intensely focused on in recent years. In addition, emphasis has been placed on the gradual variation in preventive paradigms, where identification of susceptible groups of population is of interest. We have evaluated the differences in the repair of oxidative induced DNA damage between pre-menopausal breast tumors patients, and healthy women. Comet assay was chosen as a feasible technique to evaluate DNA repair. Peripheral blood lymphocytes (PBL) were exposed to 100 µM hydrogen peroxide for 5 minutes in ice and then allowed to recover at 37°C for 120 minutes. Results showed that basal DNA damage in patients was not higher than in controls. However, there were significant differences in the amount of DNA repaired at 120 minutes (p <0, 05). The amount of DNA repaired at 120 minutes was 84.5% in the control population versus 63.2% in the premenopausal patient population. Rate of DNA repair was determined in two zones of the curve: from 0 to 30 minutes between the recognition and incision of the lesion when DNA migration reached its maximum and 31 minutes when the comet tail began to shorten until minute 120. The slope of DNA increase in the comet tail during the first ten minutes was significantly lower in patients ($m_{control}$=10.99 *vs* $m_{patients}$=4, 34), Student t test (p< 0.05), while no differences were found during rejoining. We conclude that comet assay is able to discriminate DNA repair efficiency between breast cancer patients and healthy women.

Key words: DNA repair; breast tumor; peripheral blood lymphocytes; comet assay; oxidative damage, genetic susceptibility

INTRODUCTION

Among strategies to evaluate cancer susceptibility in women, repair of oxidative DNA lesions have been focused in recent years. There is evidence of a greater amount of oxidative modified bases in DNA from breast cancer cells and PBL from cancer patients (Matsui et al., 2000; Rajeswari et al., 2000; Shahidi et al., 2007).Because of DNA in the cells is frequently damaged there is a need for efficient repair mechanisms to deal with lesions than can be converted into mutations that are often involved in activation of proto oncogenes or inactivation of tumor suppressor genes. Important sources of mutagens are reactive oxygen species (ROS) from external environment, or from cell metabolism. DNA repair processes have evolved to cope with a great array of lesions. Oxidative damage is corrected mainly by Base Excision Repair (BER) and more bulky lesions through Nucleotide Excision Repair (NER). There are other systems that complete the dispositive for DNA recovery after damage. These include: Transcription Coupled Repair (TCP), Mismatch Repair (MMR), Single Strand Annealing (SSA) and Homologous Recombination Repair (HRR), and Non Homologous End Joining (NHEJ).

(Zheng et al., 2005; Waris and Ahsan, 2006).

Increase in DNA oxidative damage seems to be related to a genetic deficiency in DNA repair, although age and lifestyle are relevant to the prevalence of damage in human cells (Caporaso, 2003; Waris and Ashen. 2006; Maynard et al., 2009).

Identification of a population with a higher susceptibility to oxidative premutagenic lesions would help in addressing prevention strategies for breast cancer prone women. Of all women who develop breast cancer, 5% to 10% may have a germline mutation of BRCA1 and BRCA2. DNA analysis for mutations in BRCA1 and BRCA2 genes is informative about the lifetime risk for developing a breast cancer, a risk that ranges from 40% to 85%. Although this potential risk is high enough to justify the testing, this type of DNA analysis is too expensive to be utilized for Public Health wide screening in preventive programs. Moreover, there are controversial results about breast cancer susceptibility as a multifactor trait resulting from interaction of low penetrance alleles of genes such as BRCA1, BRCA2, CHEK2, TP53 and ATM (Ahmed and Rahman, 2006 ; Antoniou and Easton, 2006; Baynes et al., 2007). An important category for this polygenic model for breast cancer inheritance includes those genes involved in the repair of DNA oxidative lesions including OGG1, XRC1 or APE1. (de Sanjose et al., 2003; Cierniková ET AL., 2005; Paz-Elizur et al., 2008; Vuillaume et al., 2009). Giving the current state of the art in genotyping**;** there is a need for less expensive and biologically relevant functional tests to evaluate different damaging agents that elicits different DNA repair mechanisms. There are different approaches to categorize risk population according to DNA repair capacity: genotyping and functional evaluation (de Sanjose et al., 2003; Cierniková et al., 2005; Kotsopoulos et al., 2007; Vuillaume et al., 2009). Functional tests would be advantageous. Since their results are the consequence of the activity of many genes concerted in determining one complex phenotype. In this respect, Single Cell Gel Electrophoresis or Comet Assay is an outstanding example.

Single cell gel electrophoresis has emerged as one of the more utilized methods for DNA repair evaluation; this procedure allows the evaluation of DNA repair kinetics for lesions induced by different damaging agents, like oxidants, on a minute frame basis. This technique can be further strengthened by using repair enzyme for lesion recognition, different inductors, temperatures or time frames (Collins, 2004; Cossio Ayala et al., 2004; Shahidi et al., 2007).

In order to evaluate if Comet Assay would allow us to distinguish between breast cancer patients and healthy women, we looked at its capacity to repair those lesions induced by hydrogen peroxide and studied a group of 30 breast cancer women before receiving any antineoplastic treatment and 30 healthy women residing both in Buenos Aires capital city and Great Buenos Aires area.

MATERIAL AND METHODS

Subjects

Patients group

Premenopausal women were derived from the Gynecological Service at Hospital Zonal General de Agudos *Dr. Isidoro Iriarte in Quilmes*, Great Buenos Aires. All subjects involved were positive for histological diagnosis of breast cancer. A physician from the Gynecological service provided us the clinical record from those women that fit inclusion criteria. Informed consent was given to every participant in this study, prior to data collection. All women were interviewed and sampled before the beginning of treatment. Data were collected about family history for breast or ovarian cancer, smoking habit, and parity. Those women with history of previous consumption of oral contraceptives, hormonal replacement therapy or with a body mass index (BMI) over 30, suffering from chronic diseases like diabetes or from any acute condition clinically detected were excluded. Another exclusion criterion was exposure to diagnostic X ray or anesthesia in the previous two weeks, as well as exposure to known genotoxics in their workplace.

Control group

The control group included healthy females residing in Great Buenos Aires. The women in this group did not have a history of chronic or acute disease such as those previously mentioned. They did not use oral contraceptives or hormonal replacement therapy, and at the time of sampling or two previous weeks, were not taking any medication. Members of this control group had not been exposed to X-ray irradiation, or anesthesia, and also had no exposure to known genotoxics in their workplace. Women were interviewed to gather data about their personal and family health history, smoking habits and occupation. (Møller et al., 2000)

Isolation of PBL (PBL)

Venous blood was obtained from patients and healthy women through venipuncture. PBL were isolated by centrifugation (15 min, 260 × g) in a density gradient of histopaque-1077 (Sigma). The final concentration of lymphocytes was adjusted to 1×10^5 cells/ml with MEM

medium added to cell suspensions.

Hydrogen peroxide treatment

Sample corresponding to time 0 was taken immediately before treatment. Then cells were exposed in an ice bath to 100 µM H_2O_2 for 5 min in the darkness, then centrifuged for 5 min at 1000 rpm, resuspended in MEM medium not supplemented with serum and allowed to recover at 37°C. Samples for comets assay evaluation were taken avoiding temperature fluctuations by using a dry bath. Sampling times were: (time in min) 0, 5', 10', 30', 60', 90' and 120'.

Comet assay

Alkaline single cell gel electrophoresis was performed according to Singh et al (1988) technique with modifications. In brief, 10 µL of PBL suspension were mixed with 75 µL of 75% low melting point agarose (BDH) at 37°C and layered on normal agarose pre-coated frosted slides, covered and placed in the refrigerator for 10 min to allow jellification. Cover-slips were then removed and the slides immersed in a freshly-prepared lysis solution (1 mL of Triton-X 100 (BDH Chemicals Ltd., Poole, UK), 10 mL of DMSO (Sigma-Aldrich, St. Louis, Missouri) and 89 mL of lysis stock solution: 2.5 M NaCl (BDH Chemicals Ltd., Poole, UK), 100 mM EDTA (BDH Chemicals Ltd., Poole, UK) and 0,010 M Tris-HCl (Sigma-Aldrich, St. Louis, Missouri), pH 10.5 for one hour at 4°C . After lysis, slides were immersed in alkaline solution: 300 mM NaOH (BDH Chemicals Ltd., Poole, UK) and 1 mM EDTA for 20 min. Then samples were electrophoresed for 20 min at 23 V and 290 mA (0.9 V.cm-1). Temperature was maintained at 20°C. All procedures were carried out under dim light. After electrophoresis, slides were removed from the electro-phoresis tank. Washed by dropping distilled water over tilted slides so as to remove salts and detergents, then 4 ml of neutralization solution (0.4 M Tris-HCl, pH 7.5) was dropped over slides during 5 minutes. Slides were then allowed to dry at room temperature in a dust free chamber.

Comets evaluation

Scoring was performed by the same technician following a simple blind procedure. Dry slides were stained with an aqueous solution of ethidium bromide (0.02 mg/mL) before examination under a fluorescence microscope (Carl Zeiss), excitation filter: i = 515-560 nm; barrier: I = 590 nm), magnification 1000x. 50 cells per slide were analyzed (one slide for each time point). Classification of comets include five arbitrary damage levels according to the amount of DNA in the "comet" tail: level 0: no damage; 1: low damage, 5-20 %; level 2: medium damage, 20-40%; level 3: high damage, 40-90 %. Cells in level 3 and 4 were considered damaged. "Comets" with more than 50% of material in the tail and no nuclei detectable was classified as "clouds" and not scored. (Collins, 2004)

Damage index calculation

It is calculated by multiplying the value of a visual scoring damage category (from 0 to 4 by the number of comets classified in each category:

ID= n0 (0) +n1 (1) +n2 (2) +n3 (3) +n4 (4)

Where n = number of cells in the damage level
(Anderson y cols 1997)

Percentage of final DNA repair (FDR)

% FDR= [(ID_{120} – ID_0)x 100/ID_o]- 100

Where: ID_{120} = Damage index at 120 minutes
ID_0 = Damage index at the beginning (basal level)
(Collins 2004)

Statistical analysis

Data resulting from comet assay were expressed as mean ± SD of Damage index from each sampling point in the repair kinetics curves respectively from patients and controls.

The data was analyzed using- paired Student's t test for Dependent Samples. Differences in DNA repair kinetics and repair percentage was evaluated through Box Whisker Plot test

RESULTS

Characteristics of patient and control group are shown in Tables 1 and 2. Table Nº 1 summarizes clinical and histological information from patients as well as family history, smoking status and data on damage index and repair efficiency. The predominant type of tumors in the patients with breast cancer was invasive ductal carcinoma (IDC). Two out of thirty were lobular invasive carcinoma (LIC), and two were medullar invasive. Seven cases out of 30 were in stage II. Smoking was present in eleven cases, all of them smoked between 10 and 20 cigarettes per day. Family history was assessed in five

Table 1: Patients group

Case	Age	Histological diagnosis	Family History	Smoking	Number of Cig/day	Damage index at 0	Damage index at 120	Percentage of repair at 120 minutes
M-1	42	IDC	No	yes	<10	45	59	68,9
M-2	43	IDC	No	yes	<10	56	67	80,36
M-3	37	LIC*	Yes	yes	<10	52	80	46,16
M-4	50	IM*	No	no		59	87	52,55
M-5	51	IDC	No	no		48	59	77,1
M-6	47	IDC	No	no		64	89	60,94
M-7	49	IDC	No	yes	<20	42	57	64,29
M-8	43	IM*	No	no		46	54	82,61
M-9	49	IDC	Yes	no		58	69	81,04
M-10	40	IDC	No	yes	<10	46	54	82,61
M-11	42	IDC	No	no		49	56	85,72
M-12	52	IDC	No	no		51	68	66,67
M-13	53	IDC	No	yes	<10	60	81	65
M-14	51	IDC	No	no		47	58	76,6
M-15	47	IDC	No	yes	<10	58	78	65,52
M-16	50	IDC*	No	no		67	82	77,62
M-17	51	IDC	No	no		45	62	62,23
M-18	48	IDC	Yes	yes	<5	53	70	67,93
M-19	50	IDC	No	no		58	87	50
M-20	40	IDC	No	si	<20	56	76	64,29
M-21	47	IDC*	Yes	no		59	66	88,14
M-22	41	LIC	No	yes	<10	48	60	75
M-23	42	IDC*	No	no		69	87	73,92
M-24	46	IDC	No	no		55	65	81,82
M-25	45	IDC	No	no		64	84	68,75
M-26	45	IDC	No	no		52	76	53,85
M-27	50	IDC	Yes	no		47	59	74,47
M-28	42	IDC	No	no		64	83	70,32
M-29	43	IDC*	No	yes	<10	82	124	48,79
M-30	40	IDC	No	no		40	70	25
Mean	**45.8**					**54,67**	**72,23**	**67,94**
SE	**0.8**					**1,68**	**2.73**	**2,54**

Abbreviations: IDC: invasive ductal carcinoma, IM: Invasive Medullary. LIC: Lobular Invasive Carcinoma [Gathani et al, 2005, AJCC Cancer Staging Manual, 2010] Asterisk indicate patients in Stage II

Table 2: Control group

Control	Age	Family History	Smoke	Number of Cig/day	Damage index at 0 minutes	Damage index at 120 minutes	Percentage of repair at 120 minutes
C-1	28	no	yes	<10	39,2	36,2	107
C-2	21	no	no		38,3	40,2	95,04
C-3	34	no	no		41,2	40,1	101
C-4	47	no	yes	>10	57,9	67,6	83,25
C-5	35	no	yes	<10	54,6	54,8	99,64
C-6	45	no	yes	<10	48,4	48,5	99,8
C-7	29	no	yes	<10	41,8	39,1	102
C-8	53	no	yes	>10	60,4	90.1	41.3
C-9	37	yes	no		44,7	45	99,33
C-10	43	no	no		44,6	40,1	104,5
C-11	47	no	no		47,6	48,2	98,74
C-12	51	no	no	>10	58,4	89,5	46.71
C-13	49	no	no	>10	48,7	43,3	88,98
C-14	43	no	no	<10	51,6	41.69	80,81
C-15	45	no	no	>10	62,8	78,2	75,48
C-16	47	no	no		55,8	69,4	75,63
C-17	48	no	yes	>10	57,5	72,1	74,61
C-18	43	no	yes	<10	54,7	61,5	87,57
C-19	47	no	no		58,7	68,7	82,97
C-20	36	no	no		44,1	45,2	97,51
C-21	49	no	no		53,6	54,6	98,14
C-22	46	no	no		44,3	48,4	90,75
C-23	50	yes	no		47,6	50,1	94,75
C-24	52	yes	yes	<10	58,8	65,3	88,95
C-25	49	no	no		49,9	54,3	91,19
C-26	47	no	no		47,8	46,5	102,7
C-27	48	no	yes	<10	48,6	48,2	100
C-28	46	no	no		55,4	68,9	75,74
C-29	47	no	no		52,9	42.65	80,63
C-30	45	no	no		49,5	43.85	88,59
Mean	**43,6**				**50,65**	**55.30**	**93,17**
SE	**1.4**				**1.21**	**2,67**	**1.76**

cases, mother two cases, and sister in three; histological diagnose were confirmed as ductal invasive only in two relatives.

No differences in basal damage or repair kinetics were observed according to histological classification, family history or smoke habit.

Baseline damage index in patients did not differ from that of control group; nor with that reported by the authors in previous studies (Cossio Ayala et al., 2004).

Repair kinetics was calculated from (t_0), previous to exposure to hydrogen peroxide, to 120 min (t_{120}), and generated a curve that reached a peak around minute 30. At t_{120} damage index in patient's cells was 72, 23 ± 2.73, that was significantly higher than damage index in control cells ($p < 0.05$) at the end of recovery period: 67, 55.30 ± 2. (Figures 1 and 2).

Faster increase in damage index occurred between minutes 10 and 30. During this time period there were significant differences between patients and controls in the rate of production of DNA incisions (Student t from comparison of curves slopes: 0.897, $p< 0.05$, Figure 3.) Whereas in the second phase when DNA in comet tails begin to shortens, there were no significant differences between patients and controls with exception of five "slow" samples that showed the highest migration peak between minute 30 and minute 45.

Amount of DNA repaired in patients was significantly lower than in controls. A whisker plot analysis showed it

Box plot of DNA repair kinetic in patients expressed as mean sd Damage index in each sampling point

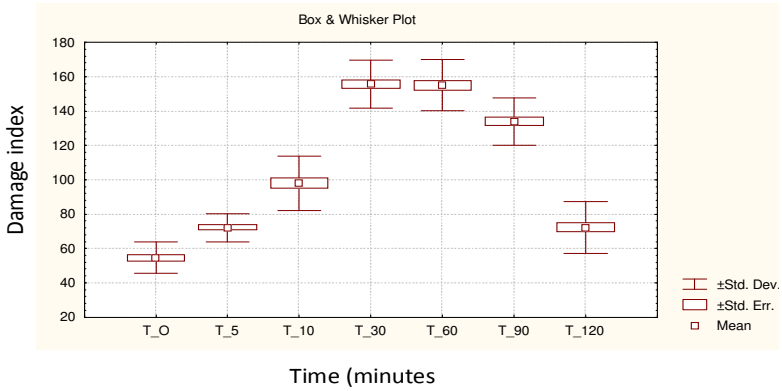

Figure 1: The maximum of Damage index is reached around minute 30 but there is a plateau until minute 60, from that point diminishing in DNA migration is significantly lesser than in controls. Dispersion in comet length is also greater than in controls

Box plot of DNA repair kinetic in controls expressed as mean ± sd Damage index in each sampling point

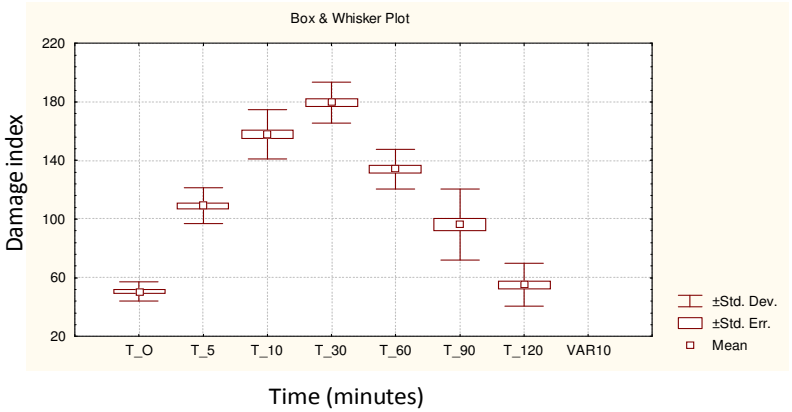

Figure 2: Maximum peak of Damage index is reached around minute 30, at minute 120, DNA migration have been reduced until a level near the baseline although variability in rejoining is greater than that at minute 0.

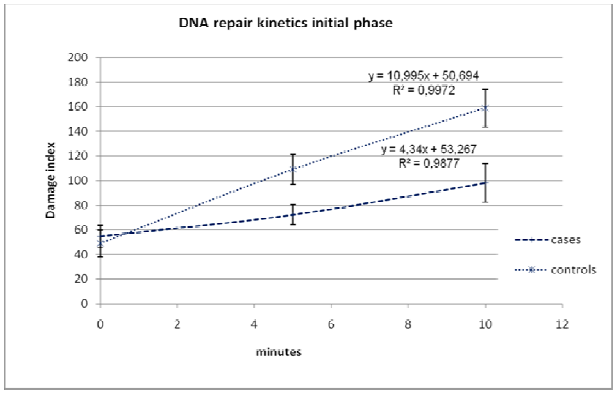

Figure 3. Differences in slope are significant in the first 10 minutes, where recognizing and incision takes place

DNA repair in controls and patients expressed as the percentage of DNA repair according to Damage index at 120 related to that at baseline t_0

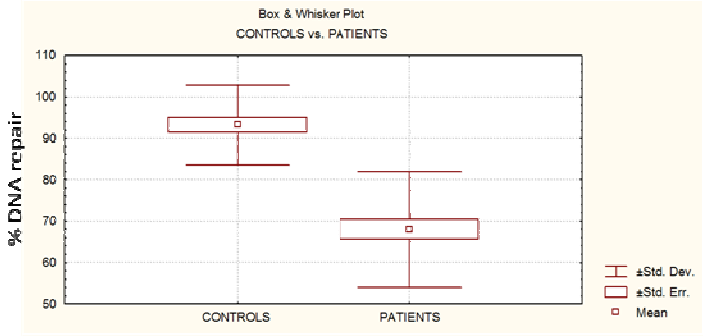

Fig 4 There is a significant difference in the percentage of DNA repaired at 120 minutes($p < 0.05$). . Variability in patients doubles that of cases

graphically and also a greater variation in DNA repair percentage among patients (Figure 4).

DISCUSSION

Approaches to study differences in DNA repair capacity in cancer patients include those based in genotyping of affected subjects through restriction fragment length polymorphism (RFLP), single nucleotide polymorphism (SNP) and ultimately microarrays, that points to a relationship between allelic variants in genes from cellular DNA repair network and cancer susceptibility. Characterization of DNA repair capacity often includes a functional tests because the genotype-based test are not only more expensive and technically complex to be applied in biomonitoring and clinical practice, but also because sometimes their results are not informative neither specific enough. (Sterphone et al., 2010; Baynes et al., 2007) Among functional tests Flow cytometry, Alkaline unwinding, Micronucleus test, Pulse Field Gel Electrophoresis and Alkaline Single Cell Gel Electrophoresis or Comet Assay are commonly used. (Sarkaria et al., 1998; Ahuja and Saran 2001; Giannotti et al., 2002; Hartmann et al., 2003; Ponzinibbio et al., 2008; Hartmann and Speit, 2009)

Assessment of DNA repair capacity in peripheral blood lymphocyte is often evaluated as a risk biomarker, but also would be relevant for disease evolution and response to chemo and radiotherapy. (Palyvoda et al., 2003; Kopjar et al., 2006; Sterphone et al., 2010)

The aim of this study was to use Comet Assay in order to test DNA repair in PBL in breast cancer female patients and healthy women and to classify them according their ability to cope with oxidative lesions. The main finding in this study is the difference in repair capacity between patients and controls and remarkably in the kinetics of single strand repair during the first ten minutes in the phase of recognition of lesions and incision by glycosylases.

Comet assay reveals variations in damage distribution among cells, but central tendency measures are successfully achieved. When visual scored is utilized, one of the more frequently used parameters is Damage index; that is the weighted result from distribution of damage in discrete categories. That parameter has been significantly related to the amount of DNA in comet tail (Collins, 2004), that why it is extensively utilized, it express where tail DNA amount distribution is shifted to. Damage index can be used to express average DNA in comet tail in each measured point with reproducibility (Garcia et al., 2004).

Premutagenic oxidative lesions have been documented in breast cancer. Breast cancer etiology includes environmental, genetic and hormonal factors influencing the initiation as the first event in the neoplastic development. Ability of the cell to cope with DNA damage is crucial to cell's fate; under this approach it is possible to say that DNA repair of oxidative lesions is an essential component of breast cancer susceptibility, and that repair capacity as expressed in the Comet assay could be considered as a cancer risk biomarker.

Baseline DNA damage in PBL in breast cancer patients in the actual series was not significantly different from that of control group, these results agree with that of Alapetitte et al (1999) and Rajaee-Behbahani et al (2001). Djuzenova et al (2006) that found similar pretreatment baseline damage in PBL from patients and controls but most of the authors reported a baseline level significantly higher in patients than in healthy subjects (Rajeswari et al., 2000; Martin 2001; Kopjar et al., 2006; Gamulin et al. 2010, Sterpone et al, 2010)

There are controversial results regarding the factors which can affect genomic stability in PBL from cancer

patients; among those that have been invoked, tumor stage, women's ages, BMI and genetics, are under scrutiny (Smith et al., 2003; Antoniou and Easton, 2006, Harsimran et al., 2009, Gamulin et al. 2010, Caseira Cabral et al., 2010, Milosević-Djordjević et al., 2010; Santos et al., 2010).

Basal DNA damage is one of the consequences of genomic instability; one of the causes considered for this phenomenon is tumor stage at the time of patient recruitment. It is accepted that proneness to chromosome or microsatellite instability increases as tumor progress (Udumudi, 1998; Kopjar et al., 2006; Gonzalez-Angulo et al., 2007). But regarding PBL, it is not clear if non tumor cells from a cancer patient express more damage as tumor progress (Rossner et al., 2005; Doak, 2008; Gochhait et al., 2009; Harsimran et al., 2009). In this series, most of the patients were in the first cancer clinical stage. From seven patients recruited in stage II, three (43%) showed a significantly greater baseline increase (Student t $p< 0.002$), while no one out of 23 in stage I showed a baseline higher than the control mean.

There are other factors that have been directly related to pretreatment DNA damage as patient's age and Body Mass Index. BMI in any particular subject from both groups was below 25, but age of some members in control population might have influence the results.

DNA repair kinetics was significantly slower in patients than in controls in agreement with the results of studies from authors including Rajeswari et al., (2000), Martin (2001) Smith at al (2003), Kopjar et al (2006) Gamulin et al (2010) Sterpone et al (2010) and Santos et al (2010).

Oxidative DNA lesions are repaired mainly by base excision repair system that has gained importance as a factor in cancer initiation. Oxidative damage is ubiquitous and frequent, some of the resultant base modifications like 8-oxoguanine are highly mutagenic, and its repair implicates base excision repair deficiencies as a cancer susceptibility factor, but also includes nucleotide excision repair and others repair pathways (Jaloszynski et al., 1997; Colleu-Durel et al., 2004; Latimer et al., 2005, Nyaga et val., 2006).

Evaluation of efficiency to remove oxidative lesions provoked by sub lethal doses of hydrogen peroxide is biologically significantly, because it is an operational measure of excision repair systems that act concomitantly.

Our results shows a slower kinetics repair of hydrogen peroxide provoked lesions in PBL from cancer patients as compared with healthy women that were matched for several reported influential variables. During the first ten minutes post challenge the patients' lengthening of DNA migration was slower than in control´s, so there are significant differences between both slopes in the first phase of the kinetic curve, (between minutes 0 and 30, including a more striking differential slope from minute o to 10) but not in the second phase from minute 31 up to minute 120, where tail shortening took place.

Significant difference in slope between minute 0 and 10, as we found became evident because we considered more sampling points than the usual in literature reports, this increment result in a more accurate characterization of the DNA repair kinetics. A "fine DNA repair kinetics" with two minutes interval between sampling for comet evaluation was done, but this approach was not informative for consistent patterns in tail DNA content, from minute 15 to minute 60. Only in the first ten minutes there was a tendency to more speed in the increase of DNA migration in cells from healthy people than that of cases. Sampling at 5 and 10 minutes was in accordance with this previous result.

We have not found a substantial amount of literature reports that focused on the first 10 minutes. In one of the few, Smith et al., 2003 reports a result that is in disagreement with ours. They found a greater tail moment for breast cancer patients' cells than that of healthy controls at ten minutes of incubation for DNA repair. That implicates a greater DNA incision rate in PBL from patients rather than more remaining damage as they found; that greater amount of damage in cell patients could be related to the short period of incubation that was allowed for repair in there. We assessed that slowness in repair depends on the first part of the curve, where DNA is being incised, this is in agreement with reports from Alapettite et al (1999), Blasiack et al (2004), El-Zein, (2010). Alapetitte also identified two phases in the repair curves as we have described, although not covering the same time intervals.

Blasiack et al (2004) studied the repair kinetic of lymphocyte DNA from breast cancer patients before, during and after chemotherapy. Cells were challenged in vitro with hydrogen peroxide or doxorubicin. Comet assay plus formamidepirimidine glycosilase (FPG) revealed a slower removal of formamidopirimidine or alkylated lesions in patient cells.

Sterphone et al (2010) also found less repair ability of radiation induced lesions in PBL from breast cancer patients, when compared to healthy controls in that series. Impairment of their DNA repair capacity was associated with the development of radiation sensitivity but not with polymorphisms in excision repair genes XRCC1, OGG1 and XRCC3.

When using in vitro repair test, cells are exposed to a huge amount of a damaging agent like hydrogen peroxide, repair mechanism have to cope with a great array of lesions that appear suddenly. It is accepted that removal of small oxidative adducts like 8-oxoguanine primarily involves BER that acts rapidly; most of repair occurs through short patch BER, initiated either by monofunctional or bifunctional glycosylase, while those more complex lesions requires the involvement of long patch repair BER, and also NER when appears and helical distortion of the DNA double helix (Rastogi et al.,

2010) Involvement of more complex mechanisms are expected to need more time to cut, excise, polymerize, and seal the larger gap. Complex repair kinetics could be related to that overlapping of mechanism coping preferentially with particular lesions (Kastan and Bartek, 2004).

In our study we found as smaller slope in the first phase of repair curves, when DNA migration is increasing as the result of the action of enzymes like OOG1, or apurinic endonuclease 1 (APE1), that recognize abasic sites. That points to endonucleolytic activity (expressed through more nicks and greater DNA migration) as determinant for the initial rate of repair as suggested by Hjertvik et al (1998).

Considering that genotyping studies have revealed association between some allelic variant and repair kinetic in breast cancer patients (Latimer et al., 2010), the slower speed in recognizing and cutting DNA backbone could be the expression, in a functional test like comet assay, of the deficiency in DNA repair of oxidative lesions in PBL of breast cancer patients.

Some authors have evaluated repair, through comet assay, extending the observational period to between 4 and 24 hours, and even a longer timeframe (Cavallo et al., 2003; Milić and Kopjar, 2004; Sanchez-Suarez et al., 2008;. Previous studies have found subpopulations of cells showing greater DNA migration several hours after initial genotoxic challenge. These findings suggest a kind of persistent lesion. From another line of evidence there have been reported DNA lesions that are difficult to remove (Rastogi et al., 2010).

It is not well understood if such lesions could be the cause of lethality in “heavily damaged cells”, leading to less baseline damage. Comet Assay allows DNA damage evaluation only in viable “less damaged” cells where DNA migrates into the tail. The absence of differences in background DNA damage between cancer patients and healthy subjects, found by us and another authors, could be the result of such a negative selection for cells bearing persistent or unrepairable lesions.

These features have some implications regarding the analysis of DNA repair kinetics in whole PBL cells, in patients with breast cancer or normal population. One of the perspectives would be to better classify risk, as screening test. Another may be the evaluation of DNA damage and repair capacities in cancer patients. It could be considered as a novel procedure for stratification and pre evaluation of sensitivity to oncospecific treatment and toxicity. Those studies require larger samples and an epidemiological screening design.

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