Evaluation of HPRT Gene mutation and Comet Assay in Some Breast Cancer Patients Undergoing Radiotherapy

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Abstract

Introduction: Radiotherapy is a toxic cellular treatment that destroys rapidly dividing cells such as cancer cells. The strategy for HPRT mutation examination in human's cells was commonly used to establish in vivo history along with mutation rates in children and adults inhabitants subjected to experienced and unfamiliar external mutagens. As just a practical approach to human biosurveillance studies, the comet assay has been introduced over the past years as biomarker to identify ionizing radiation Consequences with Patients undergoing radiotherapy in breast cancer.

Materials and Method: This study was carried out on thirty Iraqi women patients with BC patients undergoing radiotherapy about 20-30 Gy locally gamma cells at Al-Amel National Hospital for cancer Management in Baghdad during time 2-13 years, non-smokers and non- alcoholic, aged (35-55 year), with stage (grade) I-III, as well as thirty apparently healthy individuals females collected randomly from population living Baghdad, Old age (35-55) Which are non-alcoholic non-smokers as group of control. Around the ongoing research comet-test and hprt mutation test could be applied to study damages in DNA for genetic two endpoints For patients with cancer throughout radiation therapy.

Results: The present study showed significant increase ($p<0.05$) in the *HPRT* gene mutation assay for the in breast cancer patients undergoing radiotherapy as compared with the control group. Also there were found that the values of comet tail moment and tail length was increased significantly ($p<0.05$) in the human lymphocyte in these for breast cancer patients undergoing radiotherapy as compared with the control group.

Conclusions: Present results revealed that there is a probability of utilizing human lymphocytes changes as useful biodosimetric markers for the detection of human exposure to ionizing radiation, the data gathered also demonstrated the utility of the HPRT gene mutation and (Alkaline) comet test as just a precise alternative biological marker for routine preventative care of cancer patients receiving radiation therapy.

Keyword: *Radiotherapy, Breast Cancer, HPRT gene, comet assay.*

Introduction

Approximately (1 million) reported cases diagnosed globally each year; breast cancer has become the most commonly reported cancer, accounting for about 20 percent of all new female cancers. Radiation is a functional therapy for cancer treatment but in 5%-10% of patients it results in extreme late radiation toxicity. In oncology Ionizing radiation has been effectively and widely used.

Recent developments in cancer radiation therapy have enhanced the diagnosis of cancer victims, but have led to health problems[1]. Radiotherapy kills cells in the exposed position by destroys their DNA material.[2]. Radiation therapy is still the most common technique

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of treating cancers victims..About Eighty percent of cancer sufferers receive radiation therapy at a certain time, either for therapeutic or curative reasons[3]. Since radiation exposure neither distinguishes for either normal or even malignant cells. Symptoms can grow in patients throughout therapy course for a several weeks following treatment or months or years later[4]. The exposure to radiotherapy causes more damage to cells of the body, particularly DNA, and the level of damage to cells relies on the dose of radiation received. Radiation therapy is among the most successful options for r treatments of cancer. The prevalence of breast cancer becomes significant, but after several years of radiation treatment, most patients will again be cured or free of cancer indications[5].

Hypoxanthine guanine phosphoribosyl transferase (HPRT) is a purine rescue enzyme which induces the transformation of hypoxanthine and guanine onto the corresponding nucleotides 5- monophosphate and 5- monophosphate guanosine[6]. Numerous in vivo studies of mutations HPRT gene in human cell cultured have presented evidence for, exposure level, age and genetic effect on mutation rate[7]. Enhanced frequency of mutations with advancing age in healthy human individuals is typically demonstrated[8, 9].

Comet test is really sensitive and needs a geneticist who is able to interpret the outcomes. This test is commonly performed in the search for DNA material damage [10]. From the last few years; comet assay has been adopted as a valuable method for human biomonitoring s analyses[11,12]. Thus biomonitoring research using cytogenetic tools are restricted to distributing lymphocytes and include propagating cell population groups,Comet testing may be introduced to propagating and non-propagating cells as well as Tissue cells which are the first hotspots for touch with mutagenic factor.[13,14] The objective of the study is to use HPRT gene mutation and comet as a biological Markers to identify the negative impacts of ionizing radiation susceptibility in victims with breast cancer having receiving radiation therapy

Materials and Methodologies

Collection of subjects and blood samples: During the period January 2018 till January 2019, this study was carried out on thirty Iraqi women patients with breast cancer after radiotherapy treatment, non-smokers and non- alcoholic, aged (30 - 59 year),with stage (grade) II, breast cancer patients treated at Al-Amel National Hospital for cancer Management in Baghdad during time 2-13 years, As well as thirty apparently healthy individuals females collected randomly from population living Baghdad, aged (30 - 59 year) which are non-smokers, non- alcoholic as control group, all of them (100 %) were females. Samples of Blood were obtained from healthy control and cancer sufferers previous to radiation therapy and upon progressive doses of radiation of 20-30 Gy. Locally gamma cells. Five ml of human peripheral blood from all select subjects were collected and placed into sterile plain tube that contained lithium heparin. The blood was placed in a cool - box under aseptic conditions and transfer to the laboratory.

Assay procedure: Mutation of HPRT gene test has been conducted as mentioned by Coa et *al*[11].The DNA (comet) test was operated as mentioned by Moller *et al*[15].

Microscopic examination: Binucleated as well as multinucleated cell types from each 1000 lymphocytes have been record under microscopy illumination in the two sets of cultures. Microscopy (magnification 1000X), the Mutant Hprt Gene Frequency "Mf-hprt" has been measured with the use of formulation below: [11].

"Mf-HPRT (‰) = (binucleated and multinucleated cells for every 1000 lymphocytes while grown with 6TG)(binucleated & multinucleated cells for 1000 lymphocytes if grown without 6-TG)] 1000."

A maximum of 100 seemingly at random captured comets (100 of each slide) have been investigated using only a luminescent microscope linked to an image processing system via a camera. The following comet criteria have been examined to measure actually damage to DNA: the length of the tail (TL) and the moment of the tail.

Tail length (DNA migration size)is closely linked to the Dimensions of a fragment of DNA and is described In to the micrometers. This has been measured cell midpoint. The tail moment was

Measured As product of just the length of the tail and the comet-tail DNA portion.

Data Analysis and Statistics: The information for these research have been assembled in to the computerized database as well as the frequency. Distribution and statistical explanation (mean, SE) were

dissolved utilizing SPSS statistical programstatistical tests of the variability (ANOVA) test as well as the least significant difference (LDS) test has been used with a likelihood of less than 0.05 (p<0.05) variability.

Results and Discussions

Mf-HPRT mutated gene frequent test has been conducted on blood lymphocytes cells which hav been gained from 30 Iraqi breast cancer patients women, aged (30–59) year and duration of radiotherapy is more than 2-13 years, as well as 30 healthy individuals women as control group which age ranged (30-59 years). Table 1. showed (hprt) for 30 patients with cancer throughout radiationtherapy for each patient Mf - hprt expanded with the dose of radiation therapy.mutation assay of MfHPRT gene was conducted according to explanation by [11]. Mutant frequency of *HPRT* gene has been determined as (binucleated), (trinucleated) as well as (quadrinucleated) CB lymphocyte cells for each 1000 lymphocytes. In tissue culture with and without 6-thioguanine were identified by Giemsa staining (Fig. 1).

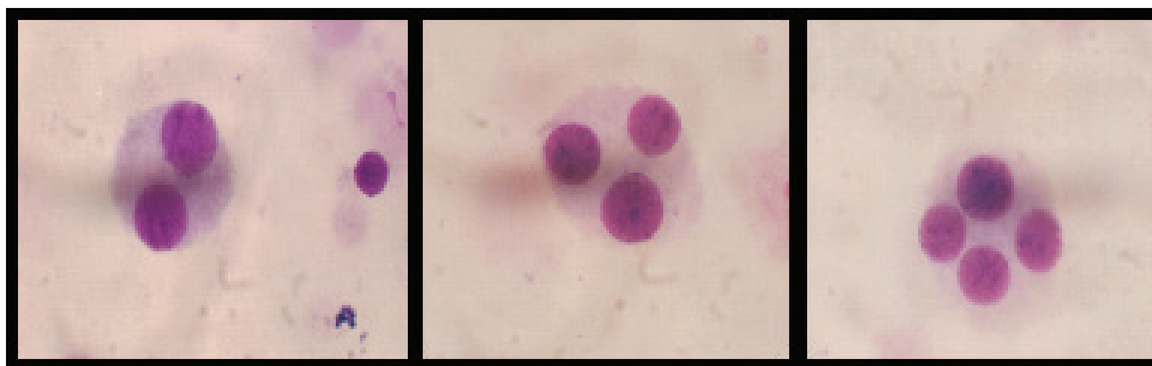

Figure (1): Human lymphocyte cell, blocked by Cytokinesis (A):binucleated (B): trinucleated and (C) qudrinucleated (1000X). genetically determined.

The outcome of Mfs- HPRT gene mutation for breast cancer patients after radiotherapy and control can be seen in Table (1) The Mf-HPRT rate for patients with breast cancerand controls were 0.91–1.21 and 0.82–0.96, accordingly. Difference of MF- HPRT between patients of breast cancer after radiotherapy and controls Has been significantly high (P.< 0.05), Result of HPRT gene analysis in breast cancer high frequency about 1.088 ± 0.0211 ‰ from control 0.890 ± 0.0089 %. Table (1) So the frequency of HPRT test had to identify the geneticallyhazard Among breast cancer survivors after radiotherapy into current Inquiries.

The findings of the HPRT assay in our study demonstrated a significant difference between patients with breast cancer as well as individual in control groups. In current study;breast cancer patients chronically affected to anticancer radiotherapy drag were studied cytogenetically to evaluate the frequencies of HPRT gene mutation, in comparison with control individuals.

Table (1): Mutant frequency- HPRT gene mutation in lymphocytes of breast cancer patients and control groups

Studies groups	No. of samples	Ranges of Mutant Frequency-*HPRT* ‰	Mutant Frequency-*HPRT* (Mean ± SE)‰
Radiation Workers	30	0.96–1.21	1.088 ± 0.0211 a
Control	30	0.82–0.96	0.890 ± 0.0089 b
LSD Value			0.0263
P Value			0.05

- The least significant difference (HPRT 0.05) = 0.0263
- The latter in the column (for comparison of study groups) means that there is no significant difference (p < 0.05).

There are many agents that damage gene and made somatic mutations, *HPRT* gene analysis widely test in many studies to know mutations frequency (MF) *in vivo* by T-lymphocytes culturing in media have 6-thioguanine if cell resistance and have colony that mean lymphocytes have mutant at the hypoxanthine-guanine ribosyltransferase (*HPRT*). *HPRT* used to understand radiotherapy effect in patients or radiations or any genotoxic factor that effect on populations and cancerpatients[11,16,17].HPRT gene is used in this work as a reporter for such mutations. If an appropriate reporter is present; its susceptibility to mutation initiation must be similar to the Mf.HPRT gene essential to tumorigenesis (18).

The findings of this study indicate that perhaps HPRT lymphocyte test is a valuable biomarker For evaluating the possible carcinogenicity of radiation therapy as a cytotoxic cancer agents. Alkaline comet analysis was used as an application biomarker of exposure to assess genotoxic consequences of radiotherapy on peripheral blood cells of 30 Iraqi breast cancers Patients and control groups.

Figure 2 illustrates the usual ordinary and damaged DNACells examined underneath a fluorescent microscope. The results of the alkaline comet assay as tail length, tail moment and % DNA (Mean ± SE) in cancer patients during radiotherapy and control groups,illustrated in Table 2.

The median of comet tail lengths in breast cancer patients, via radiotherapy were 19.21 ± 0.7612 µm, when compared with the control were 16.410 ± 4.031 µm, the observed value was increased significantly in compared with the experimental control group ($p < 0.05$).

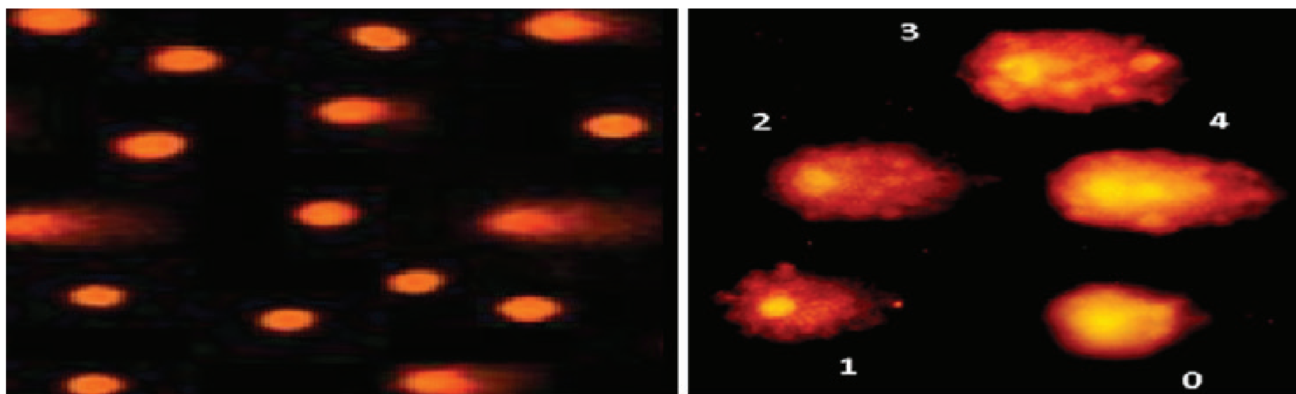

Figure (2): Comet micrographs: normal cells (0) and various levels of DNA migration as damaged DNA cells (1-4). Magnification x 400.

Table (2) Exhibits the findings of the tail moment in comet test for controls and breast cancer patients undergoing radiotherapy. Throughout the comet test, Damage of DNA in Lymphocyte has been shown by tail moment and tail length.. The average tail moment (Mean ± SE) of breast cancer patients undergoing radiotherapy was 12.451 ± 0.551, And that was considerably higher than that (8.310 ± 0.281 of healthy controls ($P < 0.05$). mean of comet Tail DNA % (Mean ± SE) of patients with breast cancer undergoing radiotherapy and healthy controls were 0.1120 ± 0.0061 and 0.00450 ± 0.0005, respectively. Moreover MCR mean for patients with breast cancer was higher significantly than healthy control. ($P < 0.01$). The percentage of DNA tail was high after radiation therapy in the BC patients compared to the healthy control group. ($P < 0.05$). According to the results obtained here, the breast cancer patients are highly significant ($P < 0.05$) compared with healthy control groups.

Victims suffering from breast cancer were exposed to radiotherapy, the therapeutically radiation genotoxic effects has been examined in lymphocytes that circulating across the body and verified as valuable biodosimeters in a number of radiation research [19, 20]. Alkaline comet test this was chosen as a versatile tool for identifying the damage in DNA caused by proven or possibly genotoxic compounds [21].

The present work has shown that radiation therapy is followed by a marked increase in DNA destruction in the lymphocytes cells of patients with breast cancer, this finding was not unexpected as radiation therapy induces a wide range of DNA disruption, varying from double- and singled breaks in DNA strands [11].

Table 2: Statistical Assessments of Comet assay for patients receiving radiation treatment with control groups as measuring values of 100 comets for each subject.

Studies groups	No. of samples	Comet Parameter valuated		
		Taillength (µm) (Mean ± SE)	Tailmoment (Mean ± SE)	Tail DNA % (Mean ± SE)
BC patients	25	19.21^{a}±0.7612	12.451^{a}±0.551	0.1120^{a}±0.0061
Control	25	16.410^{b}±4.031	8.310^{b}±0.281	0.00450^{b}±0.0005
LSD Value		8.230	1.2314	0.0131
P Value		0.05	0.05	0.05

Close column letter (for contrast of study groups) indicates that there was nosignificant difference (p<0,05)-

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq.

Conflict of Interest: Non

Funding: Self-funding

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