Some Immunological and Cytological Aspects of The Breast and Uterus Cancer Patients After Radiotherapy

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Abstract

The role of radiotherapy in immunological and cytological function of patients with carcinoma of breast and uterus were investigated: Using rosette forming T lymphocytes, white blood cells and lymphocytes count and distribution of micronuclei as parameters . After irradiation . the number of peripheral blood lymphocytes and white blood cells was reduced . The percentage of rosette forming T lymphocytes were also decreased while NBT percentage of poly morpho nucleocytes were increased with radiotherapy . Peripheral blood lymphocytes appears in different forms and shows formation of micronuclei . The number of micronuclei was significantly increased with irradiation especially in binuclear lymphocytes compared with that before irradiation .

Introduction

Immune system play's agreat role in defence mechanism against many diseases (1). T and B lymphocytes , which are the major elements of the immune system , exhibits many variations in their function and proliferation through immune diseases such as tumors (2). Radiotherapy used as amost common treatment in many tumor diseases (3). Irradiation caused immunosuppresssive of the host showing by depression of T and B lymphocytes (4) . Forthermore , radiotherapy depresses lymphocytes activity for several months after treatment (5). Radiation also induced lymphopenia in cancer patients , lymphocytes of breast cancer patients are more sensitive than that of healthy individuals (6) .

Cytological alterations was occurred in peripheral blood lymphocytes by the effect of irradiation , lymphocytes of patients exposed to radiation exhibits various abnormalities like chromosomal abberation and micronuclei (7). Numbers and frequency of micronuclei was increased in lymphocytes of cancer patients after treated with radiotherapy (8).

The present study was designed to investigate the possible alterations of immune state of breast and uterus cancer patients before and after complete doses of radiotherapy and to estimate the degree of some cytological abnormalities in their lymphocytes .

Materials and Methods

Twenty five patients with breast and uterus cancer were follow up at the Institute of Radiology and Nuclear Medicine , Baghdad, before and after radiotherapy . The range of patients ages are 40-55 years .

Blood samples were collected from patients , before and after irradiation by vein puncture . in sterile heparinized vials and transferred to laboratory with ice container . Blood samples were divided in two groups . First one for haematolgical and immunological and the second for cytological investigations .

Cytological investigation including lymphocytes culture were done as follows :

0.5 ml of peripheral blood was added to 4ml of RPMI 1640. 1 ml of bovine serum and 0.1 ml pf PHA (phytohaemagglutinin) . Tubes were incubated for 44 hours at 37 c . 3 ug of cytochalasin B was added . the tubes were incubated for 28 hours . The cells suspentions were centerfuged at 150 g for 10 minutes , the supernatant was removed and the cell pellet was suspended in hypotonic solution of 0.1 M potassium chloride . The cultures were left at room temperature for 3 minutes to stand . Another centerfugations were done for samples . supernatant was removed and replaced by fixative of 3:1 methanol / acetic acid . this operation was repeated four times then the supernatant removed at the end of the last wash . The pellet was resuspended in 0.5 ml of fixative . spread on slides and stained with Giemsa stains .

Microscopical examination of slides were done to show the various forms of lymphocytes (mono . bi . tri and tetranuclear) . Calculation of micronuclei were observed in 500 binuclear lymphocytes (9).

Statistical analysis were done by T students test (10) .

Results

Peripheral blood lymphocytes of breast and uterus cancer patients were cheacked before and after the course of irradiation treatment .

The total white blood cell count was decreased in patients with carcinoma of the breast and uterus treated by radiotherapy . There are a significant difference (p> 0.01) between the total blood cells before and after irradiation table (1).

The average number of lymphocytes in the peripheral blood of patients was (23.56 + 3,3) . Complete therapy with irradiation caused a significant decreased (p> 0.01) in their numbers table (1) .

The study also reveals anoticeable variation in some cellular immunity parameters through and after radiotherapy. Rosette formation T lymphocytes showed noticeable changes in their percentage . Total and active rosette T lymphocytes were decreased after irradiation . they reached 57.4 and 48.08 respectively . The differences of active and total rosette formation T lymphocytes were significant (p > 0.01) between pre and post radiotherapy table (2) .

Phagocytosis were also investigated . The nitroblue tetrazolium reduction percentage of polymorphonuclear cells showed anoticeable changes . The NBT positive cells of post irradiation patients was highly (9.04) than that of preirradiation (6.2) (p>0.01). table (3).
Cytogenetic investigations find out structural changes in lymphocytes including the appearance of many nuclei in their perikarya. The binuclear cells are the most form of lymphocytes appear after irradiation table (4).

The numbers of micronuclei in different forms of lymphocytes were increased after irradiation. the percentage of micronuclei in binuclear lymphocytes was significantly increased (p > 0.01) in patients after complete course of radiotherapy table (5).

Discussion

Different kinds of irradiation were widely used in treatment of carcinoma . this treatment was known to have cytological. physiological and immunological effect on patients state (4.11). Lymphocytes in thymus gland , bone marrow or peripheral blood are very sensitive to radiotherapy . this sensitivity depends on the dose of irradiaton and patients state (7) .

The present study reveals quality and quantity variations in white blood cells after the exposure of patients to radiotherapy . white blood

cells and lymphocytes count were decreased after these exposure . Products of tissue damage by irradiation may cause release of cortisol from adrenal cortex or it may cause adirect damage to lymphocytes and stem cells and product of lymphopenia (12) .

The appearance of different forms of lymphocytes may be due to the inhibition of mitotic activity of lymphocytes so they appear in bi . tri or tetra nuclear cells .

Immunological System of cancer patients were effected with radiotherapy , rosette forming lymphocytes was significantly decreased after the complete treatment with radiation . The decline in rosette forming lymphocytes indicated the exertion of T lymphocytes and temporary inhibition of cellular immunity . Many authors observed a drop in T and B cells post radiotherapy including alteration in their function (3.13) It has been suggested that suppression in mitogenic responses after irradiation might related to some immune factors release from some inhibitory cells (14) .

The cytogenetic alterations of lymphocytes occurs after exposure to irradiation. Micronuclei were increased significantly post radiotherapy especialy in binuclear lymphocytes. Inducing of micronuclei formation in peripheral blood lymphocytes of humans were used as a biological indicator of exposure to ionizing radiation . this corresponding with the sensitivity of lymphocytes to detect genetic damage (5).Forethermore , the exposure to irradiation source for several times cause frequence of chromosomal aberrations in somatic cells and germ cells which corresponding with genetic risk to later generations (15). Many Authors observed increasing in chromosomal aberrations including micronuclei of lymphocytes (8.16). The cytogenetic changes of lymphocytes increased at all period of radiotherapy and the degree of increasing was highly at the end of radiotherapy .

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Table (1). White blood cells (w.b.c.) and lymphocytes count of uterus and breast cancer patients after radiotherapy .

Patients No.	Pre radiotherapy		Post radiotherapy	
	W.B.C.	Ly %	W.B.C.	Ly %
1.	5000	22	4400	20
2.	5800	26	5200	24
3.	5200	30	5400	28
4.	4800	30	4400	26
5.	5600	26	5000	24
6.	5000	23	5600	22
7.	6400	22	6000	20
8.	6200	28	5200	28
9.	4600	22	4000	21
10.	4200	20	3800	16
11.	4800	26	4200	20
12.	5400	25	4800	24
13.	4800	22	5000	22
14.	4400	18	4600	18
15.	5000	25	4200	24
16.	6000	20	5400	18
17.	6200	22	4800	20
18.	5200	24	5000	22
19.	4800	25	4000	26
20.	4200	20	3600	18
21	5000	22	5000	20
22.	5200	21	`4400	18
23.	4800	25	4800	21
24.	6000	26	4600	17
25.	4800	19	4000	20
Mean +S.D.	5176+664	23.56+3.3	4696+646.5	21.42+3.4

Table (2) . Lymphocytes rosette formation percentage of uterus & breast cancer patients after radiotherapy.

Patients No.	Pre radiotherapy		Post radiotherapy	
	Total E-rosette %	Active E-rosette %	Total E-rosette %	Active E-rosette %
1.	68	59	53	40
2.	72	60	54	44
3.	70	58	50	50
4.	73	60	54	48
5.	70	60	60	46
6.	68	62	56	50
7.	73	64	62	50
8.	68	62	62	48
9.	65	58	58	51
10.	66	57	57	48
11.	68	60	60	52
12.	66	57	58	50
13.	64	56	56	50
14.	64	52	57	48
15.	65	52	60	52
16.	72	60	62	50
17.	68	58	55	46
18.	64	56	58	44
19.	62	52	54	44
20.	70	54	62	50
21.	71	58	57	47
22.	65	60	50	50
23.	68	61	62	44
24.	70	52	59	49
25.	65	55	61	50
Mean +S.D.	67.72+3.27	57.72+3.42	57.48+3.43	48.04+3.15

Table (3). Percentage of NBT positive polymorphonuclear cells of cancer patients pre and post irradiation.

Pat. No.	NBT% Healthy(control)	NBT% Pre irradiation	NBT% Post irradiatin
1.	12	5	8
2.	10	8	109
3.	9	6	9
4.	12	5	8
5.	13	4	10
6.	9	6	11
7.	12	8	10
8.	14	9	8
9.	10	5	7
10.	9	4	9
11.	10	7	8
12.	9	4	9
13.	10	7	8
14.	8	8	9
15.	9	6	10
16.	10	9	9
17.	12	4	10
18.	10	8	12
19.	11	6	8
20.	9	5	10
21.	11	6	9
22.	8	5	8
23.	9	7	10
24.	8	6	8
25.	11	6	9
M- S.D	10.44± 1.58	6.2± 1.4	9.04 ± 1.15

Table (4). Effect of radiotherapy on lymphocytes divission of uterus & breast cancer patients.

Pats. No.	Cells exa.	Pre radiotherapy				Post radiotherapy			
		Mono nu. cells	Bi nu. cells	Tri nu. cells	Tetra nu. cells	Mono nu. cells	Bi nu. cells	Tri nu. cells	Tetr nu. cells
1.	500	496	4	2	0	469	24	4	2
2.		492	3	4	1	462	22	14	3
3.	=	498	0	1	1	480	14	5	1
4.		496	2	2	0	473	15	12	0
5.		498	0	2	0	483	8	8	1
6.	=	498	2	0	0	480	12	6	2
7.	=	500	0	0	0	470	12	17	1
8.	=	499	1	0	0	488	9	3	0
9.	=	497	2	1	0	477	13	10	0
10.	=	498	2	0	0	477	18	3	2
11.	=	498	0	1	1	474	14	10	2
12.	=	499	1	1	0	473	16	11	0
13.		498	1	2	0	474	18	8	0
14.		498	0	0	1	478	12	9	1
15.	=	495	1	1	0	476	14	12	0
16.	=	500	0	0	0	487	10	2	1
17.	=	497	2	1	0	471	17	11	1
18.	=	499	1	0	0	481	14	4	1
19.	=	498	1	1	0	478	20	2	0
20.	=	497	1	1	1	473	21	5	1
21.	=	499	1	0	0	476	12	12	0
22.	=	497	2	1	0	470	20	9	1
23.		498	1	1	0	477	18	5	0
24.	=	497	1	1	0	470	12	8	0
25.	=	499	1	0	0	479	14	6	1
M.S.D		497.6 ±1.6	1.24 ±1.1	0.92± 1.1	0.2± 0.43	475.8 ±7.1	15.16 ±4.36	7.84± 4.29	0.84± 0.87

Table (5). Formation of micronuclei through binuclear lymphocytes of the uterus & breast cancer patients .

Pats. No.	Cells exa.	Mn. Pre radiotherapy 0	1	2	3	Mn. Post radiotherapy 0	1	2	3
1	500	496	3	1	0	489	6	4	1
2.		497	2	1	0	482	9	8	1
3.	-	496	2	2	0	487	8	4	1
4.	=	498	2	0	0	486	8	6	0
5	-	497	2	1	0	486	10	3	1
6		500	0	0	0	487	9	3	1
7.	-	496	3	1	0	477	16	6	1
8	=	495	3	2	0	480	16	4	0
9.	-	498	1	1	0	472	20	7	1
10	=	500	0	0	0	481	14	5	0
11.		499	0	0	1	489	6	5	0
12	-	498	1	1	0	479	16	4	1
13	-	499	1	0	0	480	12	6	2
14.	-	498	2	0	0	478	18	3	1
15	=	498	1	0	1	478	18	3	1
16.	-	498	1	1	0	480	14	6	0
17	-	496	2	2	0	475	18	6	1
18.		499	0	1	0	488	6	5	1
19.	=	499	1	0	0	485	11	4	0
20	=	499	1	0	0	489	7	4	0
21.	-	496	3	0	1	480	14	5	1
22.	=	497	2	1	0	479	16	4	1
23.	-	498	1	1	0	483	11	6	0
24.	-	496	3	0	1	487	10	3	0
25		497	2	1	0	479	11	9	1
M+S .D.	=	497.6 1.4	1.56+ 0.9	0.68 -0.7	0.16 +0.3	482.6 5.16	12.16 +4.67	4.88+ 1.44	0.68+0.58

بعض النواحي المناعية والخلوية لمرضى سرطان الثدي والرحم بعد المعالجة بالاشعاع

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الخلاصة

درس دور المعالجة بالاشعاع في الجوانب المناعية والخلوية لمرضى سرطان الثدي والـــرحم باستخدام تقنية التشكل الزهري الثاني للخلايا اللمفاوية ،حساب عدد كريات الدم البيض والخلايا اللمفاوية وتكون وتوزيع النوى الصغيرة فيها كمعايير للتاثير .

انخفـــض عـــدد الخلايا اللمفاوية وكريات الدم البيض في الدم المحيطي بتأثير العلاج الاشـــعاعي ، كما انخفضت بشكل ملحوظ نسب الخلايا اللمفاوية المكونة للتشكل الزهري الثانـــي في حين ازدادت نسب الخلايا متعددة الفصوص الموجبة لصبغة NBT باستخدام العلاج الاشعاعي .

لوحظت الخلايا اللمفاوية بأشكال عديدة وأظهرت تكون النوى الصغيرة micronuclei ازداد عـــدد تكـــون النوى الصغيرة بشكل معنوي باستخدام العلاج الاشعاعي ولاسيما في الخلايا اللمفاوية ثنائية النوى موازنة بما عليه قبل العلاج الاشعاعي .