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RESEARCH ARTICLE

The cytotoxic effect of some chemotheraptic drugs & functional activity of breast cancer patients peripheral blood lymphocytes.

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Abstract

This study has been done on 30 patients presented with breast cancer they visited the Institute and Hospital of Radiotherapy and Nuclear Medicine-Baghdad after mastectomy for chemotherapy. From each patient, 5 ml of peripheral blood was drawn before taking chemotherapy (day 0) with follow up of each patient. After the first chemotherapy dose in about 21 day a second peripheral blood sample was taken, then after a second chemotherapy dose a third sample was taken in day 42 .In each time the peripheral blood lymphocytes were isolated and washed with media and then put it in the culture medium (RPMI) with the drugs methotrexate , vincrestine , adryamicin , 5 Fluorouracil and Endoxan to evaluate the side effects of the drugs on the peripheral blood lymphocytes. Also, we added the mitogen Concanvalin A (Con A) to compare its effect on lymphocytes with the control groups and then Micro culture Tetrazolium dye assay M.T.T. assay by using ELISA reader on wave length 550 nm. The aqueous solution of the leaves of the vinca rosea was also used for studying the effect on lymphocytes. The results showed the ability of Con A in increasing the cellular activity of peripheral blood lymphocytes. While the aqueous solution of Vinca rosea showed a rapid proliferative effect on Peripheral blood lymphocytes. A control group from 20 healthy women was taken and for each woman 5 ml of peripheral blood was collected and the same step for their blood was processed for comparison with the patient group. The aim of this work is to evaluate the cytotoxic effects of some chemotherapeutic drugs used to treat breast cancer patients on peripheral blood lymphocytes (PBLs). Also to evaluate the effects of the aqueous local extract from ***Vinca rosea*** on patients PBLs.

INTRODUCTION

Breast cancer is the most common female cancer in the world. In the United state it has increase to its current alarming rate of one in eight women. Statistics showed that in the United state alone, about 200000 new cases are diagnosed every year and about 50000 women die annually from this disease (**Davis *et al*., 2004).** The etiology of breast cancer appears to be multifactorial, since both endogenous and exogenous factors are known to increase breast cancer risk **(Baffeta *et al*., 1994).** The immune response and immune surveillance are the most important defense mechanisms against tumor especially of breast cancer **(Feuer *et al*.,1993).** Most immune cells attack tumors by producing proteins that bind to receptors on the target cancer cells. These receptors in turn deliver signals into tumor cells which activate a latent program for cell suicide **(John, 1996**). In recent years, there has been an explosion of life saving treatment advances against breast cancer bringing new hope and excitement. Instead of only one or two options, today there is an overwhelming menu of treatment choices that fight the complex mix of cell in each individual cancer **(Breast Cancer, Org, 2005**). The treatment decisions are, surgery then perhaps radiation, hormonal (anti-estrogen) therapy, and or chemotherapy – can feel overwhelming **(Breast Cancer, Org, 2005).** In Iraq there is an alarming increase in the incidence of breast cancer, with sharp increase in younger age and

since the cases of breast cancer in Iraq is on rise (**AL Hadithi,2000**). The most frequent histology type of breast cancer in Iraq are,infiltrative ductal carcinoma which represents 77.2%, lobular carcinoma 9.8% Comedo carcinoma 1.8% and medulary carcinoma 1.5% and other histological variants of carcinoma .During the years 1976-1985,BC constituted 19.8% of all other types of cancer, in 1989-1991,BC constituted 24%, in 1995-1997 it constituted 30%, and in 2000 constituted 31% of all other malignancies in women **(INCR ,2000).**

Materials and Methods

Subjects

A total of thirty patients who had mastectomy, were included in this study. The patients were admitted to the Nuclear Medicine and Radical Hospital to take a therapy from the period October 2004- June 2005. They were all female, their age range 22-85. Baseline data were obtained from each case. Twenty apparently healthy women were also included as controls. Those ladies had similar age range with those of the patients.

Blood samples and lymphocytes isolation

5 ml venous blood was taken from each patient and Control .They were collected in heparinzed tubes. Lymphocytes were isolated from blood to be ready for use in MTT by Density gradient sedimentation **(Boyum, 1968**). Cell count and viability was done according (**Goldrosen, 1977).** The final lymphocytes concentration/ml= $M^* 10^4$ x dilution factor

M=mean of cells No. per Large square.

10^4= factor of size difference

Dilution factor=10

Viability=[number of viable cells/ number of viablecells+deadcells]x100

The viability accepted for culture should be 95% and above. In our work we committed to these figures, in that we achieved a final concentration of $1\text{-}2x10^6$ cells ml and viability 95% and above.

Micro culture Tetrazolium Assay (M.T.T.)

The micro culture tetrazolium assay (M.T.T.) was original developed by Mosmann, (1983), to measure the conversion of soluble 1-[4,5-dimethylthiazol-2-YI]-2,5 diphenyl tetrazolium bromide (M.T.T.) to a purple M.T.T. formation precipitate by dehydrogenase enzyme present in the living cells mitochondria (**Hardan, 2000**). Depending on this principle the more active viable cells the more dehydrogenase enzyme is produced, and thus the more formazan is to be produced giving the purple precipitate color (**Mosmann, 1983**). M.T.T. can measure the viability of cell and the drugs cytotoxicity on the living cells, which are exposed to cytotoxic agents. And can also measure the proliferative activity of living cells when exposed to mitogenic substances (**Mosmann, 1983).**

In the current study we used MTT assay on isolated PBL suspensions of patients and control subjects. MTT assay was used to determine the anticancer (Cytotoxic agents) cytotoxicity of the drugs, Vinca Rosa , Adriamycine, Con A, Methotrexate, 5 fluorouracil-5 FU, Endoxan and Vincristine and also to calculate the synergistic cytotoxicity (**Mizutani, 1993).**

Lymphocytes cytotoxicity percentage was measured using the following formula:

Cytotoxicity % =1- [absorbency of experimental well /absorbency of control well] x 100.

Results and Discussion

The range (22-85 years) and the mean age of the patients was 50.4 3.1 years which is higher than results gained by Al Hadithi, (2000) who found that the mean age of patients with breast cancer was 44.6 years with range of (20-85) years **(Al Hadithi, 2000) .** In this study most of patients (19 patients) were in the age group between 40-55 years old and which is in agreement with other studies (INCR, 2000 and Al Hadithi, 2000).

Histological Types:

According to histological types, malignant tumor was divided into ductal carcinoma and lobular carcinoma. Ductal carcinoma constitutes majority of our cases (83.3%) while lobular carcinoma account of (16.7%), this is in agreement with the Iraqi National Cancer Registry (2000) which refer that the most common histopathological types were invasive ductal carcinoma (IDC) (77.2%) comparing with 9.8% for invasive lobular carcinoma (ILC)(Table 1).

Table (1): Distribution of cases according to histological types and proliferative 0f PBLs.

Breast cancer	No.	%
Ductal Carcinoma	25	83.3%
Lobular Carcinoma	5	16.7%
Total	30	100%

Cytotoxicity

The highest proliferative rate was found in Vinca rosea with a proliferative percentage equal to 11% in day 21 and reaches to 37% in day 42. The proliferative rate of Con A in day 21 was equal to 16% while this percent increased slightly in day 42 to 18%. On the other hand, vincrestine account for the highest toxicity which reaches to 14% in day 42 followed by methotrexate with 13%, adryamicin for 9%, 5 FU for 4% and Endoxan for 2% (Table 2).

Table (2): The cytotoxic effects of chemotherapy drugs on PBLs of Breast cancer patients.

Drug +combination of drugs	Cytotoxicity/day 42
Vincrestine	14%
Methotrexate	13%
Adryamicin	9%
5- FU	4%
Endoxan	2%

Peripheral blood lymphocytes activity

To assess the functional activity of patient lymphocytes before having the drugs we checked the proliferation rate at day 0 in patients and control group. In patients group, the proliferative activity of peripheral blood lymphocytes in the drug con A equal to 1.7445 ± 0.148951 and in control group it was 2.263208±0.568806. Statistically there was a significant difference in the optical density in patients and control group (P = 0.017403).The proliferative activity of the control group was higher than that of the patients group (Table 3).

Table (3): The O.D. of the activity of peripheral blood lymphocytes of patients and control groups.

	Patients	Control
Day 0	1.7445 ± 0.148951	2.263208±0.568806
P value	0.017403	

Such a result indicates that there is a possible reduction in functional activity of peripheral blood lymphocytes (PBLs) in patients with breast carcinoma which goes with the fact that cancerous patients suffer from reduction in cell mediated immunity. These results is similar to that obtained by Abed Al-Amir, (2001), who found that PBLs level measured by M.T.T. in general was much lower than PBLs level in healthy control subjects. This could be explained with the fact that peripheral blood lymphocytes (PBLs) can give an idea of the immunological status in patients with cancer (**Abed Al-Amir, 2001)**. Also by comparison between patients groups lymphocytes activities after treatment with Con A with group that did not receive Con A in day 0 , the functional activity of both groups were nearly equal (1.657±0.759) for patients lymphocytes receive Con A and (1.601±1.000) for group who did not receive Con A. Statistically there was no significant difference between both groups (P value = 0.771). While after therapy the picture became different and it was found that there was a significant increase in the functional activity of peripheral lymphocytes measured by optic density in the group that was treated by Con A (1.912±0.577). For the patients lymphocytes that did not treated with Con A, the functional activity nearly remain the same (1.620±0.752). Statistically there was a significant difference between both groups (P = 0.0321)(Table 4).

Table (4): The Con A proliferation rate induction of PBLs of patients group before & after chemotherapy.

	With Con A	Without Con A	P value
Day 0	1.657±0.759	1.601±1.000	0.771
Day 42	1.912±0.577	1.620±0.752	0.0321

In comparison between the proliferative rate of patients lymphocytes with that of the control group after exposing those cells to Con A the results indicated that the proliferative effect of Con A at day 21 was equal to 16% and this percent increased slightly at day 42 to 18% while for the PBL patients of group that did not receive Con A , the proliferative rate was not exceed 1%.

Chemotherapy drugs cytotoxicity

Chemotherapy drugs had an effect on the lymphocyte counts in breast cancer patients, recent reports suggested that the number of circulating peripheral lymphocytes may be useful as prognostic index in patients with breast cancer **(Krant *et al*., 1995).**

The results showed that methotrexate, 5-FU and adryamicin cause a reduction in the total peripheral blood lymphocytes where the O.D. of the methotrexate pretreatment group were 1.806±0.835 at 0 day and decreased to 1.575 in day 42 and 1.478±0.697 at day 0 and decreased to 1.417±0.820 in day 42 with 5-FU drug while the O.D. of the adryamicin pretreatment group were 1.958±0.856 then decreased to 1.786±0.620 in 40 days (Tables 5,6,7). Methotrexate (MTX) is a folate antagonist first developed for the treatment of malignancies and, subsequently, used in non-neoplastic diseases as an anti-inflammatory and/or immunosuppressive drug (**Farber *et al* .,1999).**

Table (5): O.D (measured by 550 nm) of lymphocytes treated with methotrexate after 3 successive doses of chemotherapy .

Day	Methotrexate Mean OD±SD
Day 0	1.806±0.835
Day 21	1.685±0.799
Day 42	1.575±0.729
P value	0.1684

Table (6): O.D.(550nm) of lymphocytes treated with 5-FU after 3 successive doses of chemotherapy .

Day	5-FU Mean OD±SD
Day 0	1.478 ± 0.697
Day 21	1.499 ± 0.894
Day 42	1.417 ± 0.820
P value	0.7562

Table(7): O.D.(550nm) of lymphocytes treated with adriamycin after 3 Successive doses of chemotherapy .

Day	Adriamycin Mean OD±SD
Day 0	1.958±0.856
Day 21	1.885±0.751
Day 42	1.786±0.620
P value	0.5536

Methotrexate cause a reduction in the total peripheral blood lymphocytes treatment can induce apoptosis and clonal deletion of activated T cells. Methotrexate exerts antiproliferative properties by inhibition of dihydrofolate reductase and other folate-dependent enzymes (**Laurent *et al*. ,1998).**

The cytotoxicity percentage of the drug 5-FU was equal to 4% which may be due to the fact that 5-FU functions by inhibiting DNA and RNA metabolism dividing cells .Since normal cells are also dividing ,they may be killed also by the treatment. The death of normal cells produces many of the side effects experienced by patients receiving 5-FU and this may lead to many side effects of this drugs like increases tendency to bruise, drop in bone marrow function-possibly leading to anemia (**Daher *et al*.,1994**). On the other hand, the cytotoxic rate of the drug adryamicin was equal to 9% at day 42. The mode of action of this drug is to interact primarily with topoisomerase II deform DNA structure of cancer cells by inducing formation of topoisomerase-DNA complexes and disrupting adjacent DNA base pairs to cause single and double strand breaks **(Ozgen , 2000).** The main biochemical effect of the drug is concerned with inhibition of nucleic acid synthesis. The drug is concentrated rapidly in cell nuclei and high-affinity binding to DNA has been demonstrated in vitro . the drug is believed to intercalate into DNA (Literally , insert between the double helical strands of DNA). The cytotoxic effect of adriamycin is maximal during the DNAA synthetic phase (S), but it has some activity during other phases of the cell cycle (**John, 1980**).

On the other hand, the optical density of endoxan at day 0 was equal to 1.623 and it increases to 1.646 in day 21 then return to 1.730 at day 42(Table 8). The cytotoxic rate of endoxan was equal to 2% at day 42. This goes with the fact that endoxan may lead to different degree of myelosuppression involving leukocytopeniam thrombocytopenia and anemia (**Meyer 1980).**

For ***Vinca Rosea***, the functional activity of patient peripheral blood lymphocytes increased from 1.363 at day 0 to1.526 at day 21 then it reaches at day 42 to 1.826 and this indicates that there is a steady increase in the number of lymphocytes and this mean that this aqueous solution had only proliferative role with no cytotoxicity. Statistically there was significant difference between optical density of lymphocytes in different times (day 0,day 21 and day42) (Table 9).

Table (8): O.D (550 nm) of lymphocytes treated with Endoxan after 3 successive doses of chemotherapy.

Day	Endoxan Mean OD±SD
Day 0	1.623±0.639
Day 21	1.646 ±0.826
Day 42	1.730 ±0.683
P value	0.8806

Table (9) O.D (550 mn) lymphocytes treated with Vinca rosea after 3 successive doses of chemotherapy.

Day	Vinca rosea Mean O.D. ± S.D
Day 0	1.363±0.931
Day 21	1.526±0.816
Day 42	1.826±0.630
P value	0.04850

If we compare this result with that of **Vincrestine** we can find that Vincrestine cause transient increase in the functional activity of patient peripheral blood lymphocytes from 1.671 at day 0 to 1.705 at day 21 and then decrease to 1.442 at day 42 which is less than that of the day 0 and by application of the cytotoxicity of the Vincristine it was found to be 14% and this goes with the fact that vincristine inhibit cell division during early mitosis. Vincrestine works to keep the cancer cells from dividing , it does selectively inhibit cancer cell division . It can halt the division of some healthy cells **(Jordan et al.,1991**).

References

1. Abd ALAmir, A.(2001) .Immunological study of Head and Neck Cancers in Iraqi patients P.101-109.
2. AL Hadithi, R.H.and AL Hassany, A.d.(2000). Breast cancer pathological andImmunohistochemical study :M.Sc.thesis ,AL-Na- harain University.
3. American cancer society,2005.

4. Baffeta , P.and Perkia, D. M. (1994). Cancer in developing countries .Int. J.Cancer 44:81-90.
5. Boyum ,A. (1968). Isolation of mononuclear cells and granulocytes From human blood .Scond .J.clin. lab.invest(1968)21 (suppl.97):77-89.
6. Breast cancer ,(2005). Org , Anonprofit organization for breast cancer education , [Internet].
7. Daher,A. (1994). Metabolism of pyramiding Analogues and their Nucleos- ides.in Anticancer Drugs :Antimetabolite Metabolism and Natural Anticancer Agents ,pergamon press,55-94.
8. Davis, D.L .; Wine,Beer,Tea,tea may slow breast cancer .(2004) .American Physiological Society ,Annual meeting, (internet).
9. Farber ,S. R.;Toch, E. and Sears, D.(1999). Pinkel Advances in Chemotherapy of cancer in man .In Advances in cancer Research .H.A. Greenstein J. P. editor. Academic Press, New York .2-73.
10. Feuer, E. J.;Wun, L.M.;Boring, C.C. and et al.(1993). :the life time risk of develo- Ping breast cancer .Journal of the National Cancer Inst- itute;85(11):892-897.
11. Goldmen ,J. and Tarig, M.(1999). understanding Leukemia and Related Cancer, 3-45. Black well science ,UK.
12. Goldrosen,M.H.;Lutz,M ,and Holyoke, d.J.(1977). immunol.Method14,15- 17.
13. Hardan,A.R.(2000). Immune phenotype of lymphoma in Iraq. PhD. Thesis,Colleg of Medicine,AL-Nahrain University
14. Iraqi National Cancer Registry. (2000).
15. John, F.and Smyth. (1980). Basic Principles of cancer chemotherapy : Imperial Cancer Research Fund, Professor of Medical Oncology, University of Edinburgh P:71-73.
16. John Reed, M. D.(1996). Dimmue responses to breast cancer the , Institute.
17. Jorden, M. A.; Thrower,D.and Wilson,L. (1991). Mechanism of Inhibition Of cell proliferation by Vinca alkaloid Cancer Res , 51: 2212.
18. Krant, S.W.(1995). Prognostic classification of breast carcinoma in Situ. Lancet.
19. Laurent ,Genestier, and et al. (1998). Immunosuppressive properties of Methotrexate: Apoptosis and clonal Deletion of Activate Peripheral T cells ,J.Clin. Invest. Volume 102: Number 2, 322-328.
20. Meyer,K.K.(1980). Radiation-induced lymphocytes immune deficiency .Arch.Surg.101:114-121.
21. Mizutani , Y.;Fukumuoto, M.,;Bonavida, B. and Yashida, O. (1993). Enha- ncement of sensitivity of urinary bladder tumour cells to Cisplatin by cmyc antisense Oligonucleotide cancer .74:2546-53.
22. Mosmann,T.(1983). Rapid colorimetric assay for cellular growth and Survival application to proliferation and cytotoxicity Assay .J.Immunol.Meth.,65,55-63.
23. Ozgen,u.;Sevasan,S.;Stout,M.and Bucks.(2000). Further elucidation of mechanism of resistanceto Vincrestine in myeloid cells ,14(1):47-51.