Effect of Tamoxifin on Lymphocytes Proliferation in Patients with Breast Cancer:Cytogenic Analysis

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ABSTRACT :

BACKGROUND:

Hormonal agents such as tamoxifen (TAM) and medroxyprogesterone acetate (MPA) are used widely in the treatment of breast cancer . In this context, it is noteworthy to note that there is now much experimental and clinical evidence suggesting that sex hormones can influence immune mechanisms strongly

AIM:

Study the effect of tamoxifin on peripheral blood lymphocytes proliferation in breast cancer patients.

MATERIALS AND METHODS:

Seventy-three patients with breast tumor were included in this study. Sixty-two with malignant breast cancer and 11 with benign breast tumor.The malignant breast tumor: Intraductal carcinoma (IDC) (8 patients),Lobular carcinoma (LC) (5), and infiltrative ductal carcinoma (49) which in turn divided into 11 with Well differentiated ductal carcinoma (WDC), 12 patients with moderately differentiated ductal carcinoma (MDC), 26 patients with Poorly differentiated ductal carcinoma (PDC). All cases were admitted to Al-Yarmouk teaching Hospital, Saddam Medical City, during Dec 1999-Jan 2001. The percentage of estrogen receptor positive patients(ER) recorded 37.0%, while progesterone receptor(PR) positive patients were 51.6%. Peripheral blood lymphocytes were cultured with different concentrations of tamoxifin and were assayed for proliferation using cytogenic analysis assay.

RESULTS :

Results recorded that there were a clear reduction in blastogenic index and mitotic index ,P>0.05. Both concentrations (0.25 and 0.5 mg/ml) of tamoxifen showed clear reduction in BI and MI values.

CONCLUTION:

TAM effect both ER^+ and ER^- patient in slight differences and in both concentrations.

KEY WORDS: tamoxifin, proliferationof lymphocytes, breast cancer, cytogenic analysis, estrogen receptor.

INTRODUCTION:

Lymphocytes transformation assays commonly employ non-specific mitogens such as PHA and conA which primarily stimulate T-cells, as well as pokeweed mitogen (PWM) which stimulate both B and T-cells. Different studies had been carried out to determine the functional status of PBLs by evaluating their response to polyclonal mitogens such as PHA, and they showed different results. Mantovani *et al*, (1989) [(1)], reported that the responsiveness of breast cancer patients PBL to PHA was significantly lower as compared with control response, whereas Scambia *et al* 1988, [(2)] noted that primary breast cancer patients did not differ from controls in their responsiveness to PHA. On the other hand stimulation index of PHA increased gradually as the stages of the disease advanced. The lymphocyte response to PHA was significantly reduced in stage IV in patients with breast cancer of age 50-70 yr women.

Hormonal agents such as tamoxifen (TAM) and medroxyprogesterone acetate (MPA) are used widely in the treatment of breast cancer [(3)]. In this context, it is noteworthy to note that there is now much experimental and clinical evidence suggesting that sex hormones can influence immune mechanisms strongly [(4)].

Furthermore, although a direct effect of these hormones on tumor cells seem to be the major mechanism of the antineoplastic action. The capacity to influence immune mechanisms could possibly explain the response of some receptor negative breast cancers (2).

PATIENTS AND METHOD:

A total of seventy-three patients presented with breast tumer symptoms, were included in this study. The patients were admitted for surgery at , Saddam Medical City, Al-Yarmok teaching

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hospital and Al Arabi private hospital. The patients mentioned were grouped according to their histopathological finding into: benign breast tumor patients (eleven patients) and malignant breast tumors patients (Sixty two patients).

Histopathological finding of all patients were tabulated according to Bloom & Richardson grading system (1957)[5] and as presented in Table (1)

Table 1 : Type of tumor of the specimens under investigation based on bloom & Rechardson (1957)

Group	No. of patients	Type of Tumor
Malignant breast tumors	11	Well differentiated infilterative ductal carcinoma (WDC)
	12	Moderatly differentiated infilterative ductal carcinoma (MDC)
	26	Poorly differentiated infilterative ductal carcinoma (PDC)
	8	In situ ductal carcinoma (IDC)
	5	Infilterative Lobular Carcinom (LC)
Benign breast tumors	4	Fibroadenoma
	6	Fibroadenosis
	1	Epitheliosis

CONTROL

Twenty apparently healthy women were used as a control. Those ladies had similar age range with those of the patients .

Collection of the blood

Two ml of blood withdrawn aseptically from patients under investigation and control subjects were put in hepranized vacutainer tube.

Phytohaemagglutinin (PHA kindly prepared and supplied by the Iraqi atomic energy commission.Tamoxifin was prepared by dissolving one tablet of tamoxifin (20 mg ;LEIRAS Oy, Finland) in 80 ml of distal water to obtain 0.25 mg/ml conc. and in 40 ml to get 0.5 mg/ml conc.

Culture of peripheral blood lymphocytes and cytogenic analysis were done according to (Shubber ana allak, 1985)(6)

Blastogenic index analysis (BI) was determined by counting 1000 lymphocytes and lymphoblast randomly , and expressed as a percentage of blast cells on the slide (No. of lymphoblast /1000 cells).

Mitotic index analysis (MI) was determined as a ratio of mitotic cells to interphase nuclei in 1000 cells .

Statistical analysis : ANOVA , T test, Chi sq., were included in statistical processes of all experiments.

RESULTS:

Blastogenic index of Lymphocytes treated with PHA are shown in table 1. As shown in this table, there was a clear reduction in blastogenic ability of the peripheral blood lymphocytes in the different types of malignancies. Mitotic index of peripheral blood lymphocytes treated with PHA are shown in table 2 .Results showed a clear reduction in mitotic ability of lymphoblast, in different types of malignancy.

There was highly significant differences between total malignancies and healthy control value (P=0.0001) which might reflect an impairment in lymphocytes function in malignant patients

Table 2: Mean values and standard deviation of BI,MI in different type of malignant and in healthy control.

Types of tumor	BI	MI
IDC	35.1±2.7	1.8±0.9
LC	32.3±3.7	1.5±0.6
MDC	39.9±6.6	1.5±0.8
PDC	36.1±4.4	1.4±1.1
WDC	33.9±2.9	0.4±0.4
Total malignant	36.6±5.8	1.3±0.9
Control	60.6±5.9	3.2±0.4
	T=15.2 P=0.0001	T=8.97 P=0.001

Table 3a and 3b show the mean values and standard deviation of BI , MI by using the concentration 0.25 mg/ml of TAM, one can notice the decrease in BI and MI in almost all the type of malignancies.

When we compared the BI at (conc.0.25) with BI at (Conc.0), a clear significant reduction is noticed in mean values in all types of malignancies (P=0.0001) except in IDC (P=0.102) as shown in table 1.

Table 3a: Mean value and standard deviation of BI using 0.25 mg/ml of TAM.

Types of tumor	Mean value BI Conc.(0)	Mean value BI Conc. (0.25mg/ml)	P values
IDC	35.1±2.7	31.5±0.6	0.102
LC	32.3±3.6	29.3±3.3	0.0001
MDC	39.9±6.6	35.1±5.1	0.0001
PDC	36.1±4.4	31.3±5.3	0.001
WDC	33.9±2.9	28.1±2.5	0.0007
Total malignant	36.6±6.8	31.5±5.1	0.0001
Control	60.62±5.97		
	P=0.0001		

Table 3b: Mean value and standard deviation of MI using 0.25 mg/ml of TAM.

Types of tumor	Mean value MI (Conc. 0)	Mean value MI Conc. (0.25mg/ml)	P values
IDC	1.8±0.9	1.4±0.8	0.099
LC	1.5±0.6	1.4±0.6	0.185
MDC	1.5±0.8	0.8±0.7	0.0003
PDC	1.4±1.1	0.7±0.7	0.004
WDC	0.4±0.4	0.2±0.2	0.146
Total malignant	1.3±0.9	0.8±0.7	0.0001
Control	3.2±0.4		
	P=0.0001		

M_1 values were followed in lymphocytes after addition of 0.25 mg/ml and 0.5 mg/ml of TAM to the reactant mixture. MI in the presence of 0.25 mg/ml of TAM when compared with control group (conc.=0), it showed a significant reduction in MI mean value in MDC and in PDC types (P=0.0003,0.004) respectively (table 4a).

The concentration of TAM at 0.5 mg/ml also showed clear reduction in BI and MI mean values as shown in tables (4 a and b).

When we compared the effect of 0.5 Conc. of TAM with the control (0 Conc.) on BI we found a significant reduction in all types of malignancies (P=0.0001) except in IDC and LC also showed significant differences in MDC and PDC (P=0.0003,0.004) respectively, as shown in table 4a.

The effect of 0.5 mg/ml Concentration of TAM on MI was also showed significant differences in MDC and PDC (P=0.0003,0.004) respectively as shown in table 4b.

Table 4a: Mean values of BI using the Conc. 0.5 mg/ml of TAM in different types of tumor and healthy control.

Types of tumor	Mean value BI Conc. -0	Mean value BI Conc. -0.5mg/ml	P values
IDC	35.1±2.7	26.5±3.1	0.096
LC	32.3±3.6	29.0±4.9	0.02
MDC	39.9±6.6	31.4±5.7	0.0001
PDC	36.1±4.4	28.9±6.8	0.0001
WDC	33.9±2.9	25.3±2.9	0.0002
Total malignant	36.6±5.8	28.9±5.2	0.0001
Control	60.6±5.9		
	P=0.0001		

Table 4b:Mean values of MI using the Conc. 0.5 mg/ml of TAM in different types of tumor and healthy control.

Types of tumor	Mean value MI Conc. -0	Mean value MI Conc. -0.5	P values
IDC	1.8±0.9	0.6±0.8	0.129
LC	1.5±0.6	1.3±0.9	0.558
MDC	1.5±0.8	0.8±0.7	0.002
PDC	1.4±1.1	0.4±0.5	0.003
WDC	0.4±0.4	0.1±0.1	0.168
Total malignant	1.3±0.9	0.6±0.7	0.0001
Control	3.2±0.4		
	P=0.0001		

When we compared the 0.25 and 0.5 Conc. It was noticed that there was no significant differences between types of malignancies except in MDC and WDC as far as is concerned with other type of malignancies (P=0.008), PDC showed MI/ value different from other types malignancies P=0.03 this may reflect that the different Conc. had almost the same effect on Immune function and probably unrelated to the type of malignancy Table 5and 6.

Table 5: Comparison between BI in 0.25 mg/ml and 0.5 mg/ml of TAM.

Types of tumor	Mean value BI Conc. -0.25	Mean value BI Conc. -0.5mg/ml	P values
IDC	31.48±0.59	26.47±3.13	0.136
LC	29.32±3.28	29.05±4.95	0.798
MDC	35.10±5.09	31.45±5.67	0.008*
PDC	31.35±5.29	28.96±6.81	0.103
WDC	28.05±2.47	25.28±2.89	0.01*
Total malignant	31.56±5.12	28.86±5.25	0.0001*

Table 6 :Comparison between MI in 0.25 mg/ml and 0.5 mg/ml of TAM.

Types of tumor	Mean value MI Conc.-0.25 mg/ml	Mean value MI Conc.-0.5 mg/ml	P values
IDC	1.4±0.8	0.6±0.2	0.17
LC	1.4±0.6	1.3±0.9	0.97
MDC	0.8±0.7	0.8±0.7	0.92
PDC	0.7±0.7	0.4±0.5	0.03*
WDC	0.2±0.2	0.1±0.1	0.733
Total malignant	0.8±0.7	0.6±0.7	0.060

DISCUSSION:

Lymphocytes functions was assessed by the in vitro stimulation with different mitogens and the response was assessed by MI and BI. The results of this study revealed reduction in BI, MI in different types of malignancies .

In this study adding TAM in different concentration significantly reduce the BI and MI and this reduction did not related to the concentration of the dose by which the blood were treated, this may reflect that the reduction by TAM were not dose related. This result agreed with that of Levin *et al*, 1997(7) and disagreed with that of Scambia *et al*, 1988(2) who concluded that TAM does not increase the well known immunosuppressive action of chemotherapy. In our study TAM effected the BI and MI in both patients with ER+ as well as ER-(data not shown), as there is no significant differences between the two patients except in PDC and MDC.

AS we know TAM is an anti-tumoral agent over rides its value as an antiestrogen. In addition to several effects not mediated by estrogen receptor (ER) mechanism [8] , TAM may woke agonist/antagonist estrogenic actions simultaneously and/or successively through changes in ER conformation and reactivity with related molecules [9]. In different studies, investigator concluded that there was an overall advantage from TAM therapy that was independent of menopausal , nodal or ER status [10].

This may indicate that TAM might exert its antitumor effect through a variety of biologic mechanisms unrelated to its binding to tumor ER^+ [11]. Laboratory investigations had demonstrated that TAM might be effective in inhibiting the proliferation of hormone-independent breast cancer cells by altering production of growth factor such as transforming growth factor beta [12], by stimulating natural killer cell activity and consequently the risk for tumor necrosis [13] and by decreasing insuline like growth factor I, in which modifies the regulation of cancer cell kinetics [14]. Information demonstrating that breast cancer growth and metastasis are related to angiogenesis [11]. And that non corticosteroidal antiestrogen like TAM inhibit angiogenesisi provided evidence to support the belief that the antitumor effect of antiestrogens is not entirely related to the inhibition of ER mediated action. The previous facts may explain the results of this stud

in which TAM effects both ER+ and ER^- patients.

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