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EVALUATION OF VARIOUS ADJUVANT THERAPEUTIC MODALITIES FOLLOWING SURGERY IN STAGE II BREAST CANCER

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ABSTRACT

120 evaluable patients who had undergone radical mastectomy for stage II breast cancer. Were randomly subjected to one of the adjuvant treatment in the form of chemotherapy (CMF), antiestrogen (Tamoxifen), combined chemohormonal therapy (CMFT) and chemoimmunotherapy (CMF + BCG or CMF + activated lymphocytes by IL-2) with follow up of all patients for 30 months. CMFT therapy was found to be more effective than CMF alone in increasing recurrence free survival. Also chemoimmunotherapy was more benificial than chemohormonal therapy the, latter was due to the fact that NK & lymphocytotoxicity were markedly increased in this group treated by chemoimmunotherapy.

INTRODUCTION

Breast cancer in women is characterized by the variability and inconsistency of its clinical behaviour. Since breast cancer is so often a systemic disease at the time of its initial diagnosis, it appears only logical to attempt systemic treatment as an adjunct to surgery of the primary tumor.

Primary breast cancer is a heterogenous disease with varyng potentials for metastatic relapse and response to adjunctive drug therapy (Carter, 1980).

Breast cancer has been demonstrated to be among the most responsive of adult solid tumors to a variety of chemotherapeutic and hormonal agents (Ahmann, 1984). Adjuvant chemtherapy of breast cancer means the use of cytotoxic drugs after primay therapy. The rationale for adjuvant chemotherapy is to erradicate occult metastatic disease which otherwise

would be fatal. The assessement of adjuvant chemotherapy must balance efficacy against toxicity.

Bonadonna et al., (1976) advocated the prolonged use of cyclic combination chemotherapy with cyclophosphamide, methotrexate and fluorouracil (CMF) folowing radical surgery for stage II breast cancer, their preliminary results showed a significant advantage in increasing disease free interval in these patients. Pearson, et. al. (1989) reported that tamoxifen in postmenopausal women with stage II breast cancer had a significantly lower recurrence rate than those treated with CMG alone. Several studies have shown a correlation between certain immunologic parameters and prognosis in breast cancer, local treatment consisting of either surgery or radiotherapy, is necessary for eradication of the tumor, but both of these treatments are to a certain extent immunosuppressive, for this reason, various immunomodulating agents have been studied in the treatment of breast cancer (Klefstrom et. al., 1985).

The activity of Natural Killer cells (NK) decline as the tumor progress & increases by reduction of the tumor mass & can be enhanced by IL-2 (interleuking-2) activation in peripheral culture (LAK) lymphokine activated killer cells (Thompson et. al., 1988), Cohen et al (1982). Blumenschein et. al., (1980). Samaan et. al., (1978), and Hortobagyi et. al., (1979), advocated the use of chemoimmunotherapy containing BCG for patients with operable breast cancer after radical mastectomy.

Rosenberg, 1986 demonstrated that activated killer cells can be safely administered as adoptive immunotherapy of human cancer. Pinsky, et. al., (1977) suggested that prolonged administration of non specific immunotherapy added to combination chemotherapy prolong the duration of chemotherapy induced remission and survival of treated patients.

The aim of this study is to evaluate adjuvant therapeutic modalities in patients undergoing radical mastectomy as regard recurrence rate & disease free survival.

PATIENTS AND MATHODS

120 women who underwent radical mastectomy for stage II breast cancer (infilterating duct carrinoma), the number of L.N. affection more than 3. Patients underwent physical examination, chest radiography, bone survey, abdominal ultrasonography to rule out distant spread of disease. Normal haemograms, normal blood chemistry (Liver function including SGOT, SGPT and alkaline phosphatase, kidney function including creatinine & blood

urea) to rule out liver & kidney diseases which contraindicate some therapy. Immunological assays also were done in all patients before & after treatment. Including effect of pHA on peripheral blood & number of lymphocytes OKT3.

Tamoxifen tablets, 10 mg was given twice daily for one year in the thrid group (20 patients). CMF regimen (cyclophosphamide 600 mg/^{2} IV day 1&8, methotrexate 40 mg/m^2 / IV day 1&8, fluorouracil 500. mg/m^2 / IV day 1&8) and this course was repeated every 4 weeks depending on the patient's tolerance and haemopoietic reserve for one year for the second group (20 patients). The fourth group receive (CMFT regimen for one year also. The fifth group (20 patient had received BCG immunotherapy every month intradermally with CMF for one year. (CMF-BCG regimen). The sixth group has received CMF-lymphocyte (CMF-LAK) for one year also in which 10^9 lymphokine activated cells by IL-2 (interleuking-2) were injected in the peripheral blood three times daily for one week repeated with every course of chemotherapy CMF for one year. The first group receive no treatment (20 patients) as a control .

RESULTS

In this study, the 120 patients included 72 patients were premenopausal and 48 patients were postmenopausal. There has been 30 recurrence, 10 in the control group, 6 in CMF group, 5 in Tamoxifen group, 4 in CMF-BCG group, 3 in CMFT group & the least incidence of recurrence was in the CMF LAK group (Table 2 & 3).

Table (2 & 3) show the recurrence rate in premenopausal women treated by adjuvant hormonal therapy much more than the postmenopusal ones 50% & 14% respectively whereas adjuvant chemotheropy in premenopausal women showed better response rather than postmenopausal women as the recurrence rate in both groups were 19% & 75% respectively table (2 & 3). The most benificial adjuvant therapy after radical mastectomy was the adjuvant chemoimmunotherapy for both pre & post meno pausal women expecially in the premenopausal ones as the chemotherapy alone has better response in the preemenopausal women table (2 & 3). The adjuvant chemohormonal therapy was better than chemotherapy or hormonal therapy alone & also better response in the postmenopausal women as the recurrence rate was 10% & 20% respectively table (2&3). As regard the type of chemoimmunotherapy CMF+LAK was more benificial than CMF+BCG as regard the recurrence rate & also better in premenopausal than postmenopausal ones. Table (2 & 3). The statistical analyses of this study were carried out on proportion of recurrence rate

and were subjected to One Way Anova test using computer .

The T- Cell rate of circulating lymphocytes in patients with cancer were low. These patients were divided into 5 groups according to the type of treatment. Group II who received adjuvant chemotherapy alone the T- cell rate was 70.20 ± 9.24% (mean + S.D.) before treatment become 66.78 ± 9.12 indicate reduction of the number of lymphocytes by the effect of chemotherqpeutic drugs but still insignificant reduction (P>0,05). In the group received hormonal therapy alone showed change of T. cell rate from 70.5 ± 12.28 to 64.12 ± 10.8 which also indicate insignificant reduction (P>0,05). In the group received chemohormonal therapy the total T- cell rate was significantly increased from 64 ± 8.6 to 79.12 ± 5.8 (P < .001). The most beneficial effect of adiuvant postoperative therapy was by chemoimmunotheropy, the T- cell rate in CMF-LAK increased from 70.12 ± 9.18 to 97.18 ± 1.7 which show very significant results (P < 0.001) (Table 4&6).

Following CMF-BCG & CMF-LAK the T cell rate returned to the normal or higher ranges regardless to their initial values before therapy.

Proliferative response by PHA stimulated PBL from patients before and after treatment was evaluated. PBL from age matched healthy ones were used as a control, whose proliferative response to PHA were initially medium. The proliferative response markedly deteriorated in most of patients as regard of the control & insignificantly decreased in the groups received chemotherapy & hormonal therapy from 24.3 ± 6.84 to 22.8 ± 6.22 & from 26.64 ± 7.28 to 23.28 ± 7.24 respectively (P > 0.05) .

In the groups who received chemoimmunotherapy by CMF-BCG & CMF-LAK showed marked & very significant increase in the proliferative index by 28.22 ± 7.02 to 66.75 ± 9.28 & from 23.16 ± 5.65 to 68.23 ± 15.16 respectively (P < 0.001) (Table 5 & 7) .

In comparison of T- cell rate in between different groups there is significant rising of T- cell rate in the groups receive chemoimmunotherapy and chemohormonal therapy in relation to the group who receive chemotherapy alone (Table 8) (P < 0.001).

As regard of PHA stimulation index there is significant rising (P < 0.001) of rate in the groups receive chemoimmunotherapy (Table 9).

In this study, it was found that patients received CMF-LAK has the longest disease free interval (24 months) followed by those received

CMFT (18 months) CMF-BCG (17 months). Tamoxifen (16 months) and CMF (14 months) while patients not received adjuvant treatment has the lowest disease free interval. The correlation between disease free interval and adjuvant treatment was statistically insignificant (P > 0.05, Table 10).

Table 11 represent the overall recurrence in each group after adjuvant treatment.

DISCUSSION

Breast cancer has been demonstrated to be among the most responsive adult solid tumor to a variety of chemotherapeutic and hormonal agents (Ahmann 1984). Immunotherapy is one modality and preliminary results from seveval centers are encouraging, the investigations of Israel (1976) as weel as Pinsky et al (1977) using BCG with combination chemotherapy as CMF suggest that non specific immunotherapy prolong disease free interval and survival of responding patients. More recently the generation of cells with antitumor activity by short-term activation of lymphoid cells in culture with interleukin-2 to become antitumorcytotoxic cells by in vitro exposure to lymphokines, has been extensively studied (Rosenberg et. al., 1986) .

The results of this study after 30 months of follow up showed that tamoxifen added to CMF was associated with a significant lower recurrence rate and CMF alone in women who had undergone radical mastectomy for stage II primary breast cancer, this may be due to heterogenicity of the breast cancer. CMFT is mor benificial than CMF in postmenopausal patients, it seems possible that the benifit seen from CMFT in postmenopausal patients in this study may be due to tamoxifen alone, this is similiar to that reported by Pearson et. al., (1989).

It is apparent that the interaction of chemotherapy and endocine tratment was favourable in this study and that there was at least an additive effect of the two modalities via different mechanism and finally the maximum effective dose of each component can be administrated without manifestation of cumulative toxicity.

Adjuvant CMF therapy alone was significantly improved the recurrence free survival, but the beneficial effects appeared to be confined to premenopausal women and this may be due to the chemical casteration effect wh ich may be responsible for the therapeutic efficacy of CMF in premenopausal women, this is confirmed by Bonadonna et. al., (1980). In this study the response to adjuvant hormonal therapy in the form of tamoxifen in terms of recurrence free survival and least rate of failure is more prevalent in postmeno-

pausal patients. This means that tamoxifen treatment benfited primay postmenopausal women this is comparative to that reported by Pearson et. al., (1989) .

The recurrence rate was less in patient whom received chemimmunotherapy & more better in patient received CMF-LAK than CMF-BCG and also the disease free survival.

The ability of LAK cells to Kill tumor cells evidenced by absence of development of new lesions indicate the profound effect of immunotherapy on microdeposits which may appear later, or by immunorestoration as another vehicle to prolonged disease free interval & diminish the rate & number of recurrence as advised by Cohen et. al., (1982). Also surgery for cancer becomes the first step in immunotherapy because can favourable affect the host-tumor relationship and even cure the patient with subclinical distant metastases due to removal of the immunosuppressive factors and tumor associated antigens which decrease defenses and facilitate growth of micrometastases.

It is attempting to postulate that, in addition to their carcinogenic or cocarcinogenic activity, sex hormones have a direct or an indirect modulatory influence on immunological functions and may facilitate or enhance tumor promotion. It has indeed been reported that ceratin expression of the immune system can be regulated by sex hormones (Luster et. al., 1984). Morever OKT 8 + cytotoxic suppressor cells have been shown to express receptors for oestrogen (Cohen et. al., 1983). However, in view of a dual role of oestrogen in both carcinogenesis and immunomodulation was studied by Yron et. al., (1986).

Followin cultured lymphocyte cell administration, T- cell rate markedly increased to the normal or even higher than normal with probabilities of <0.001 in chemoimmunotherapy. The increase of OKT3 cells indicates the increase of substantial level of cytotoxicity against malignant cells. The same results were also reported by Kurnick et. al., (1986). So we can conclude that OKT3 is a sensitive parameter that can preclude the fate and prognosis of cases studied.

The T- cell rate increased as a result of induction of endogenous IL-2, production of macrophage activating factors, colony stimulatin factor and lymphotoxin by the administrated cultured lymphocytes (Thompson et. al., 1986). Also release of gamma interferon from these activated infused cells participated in this response (Itoh et. al., 1985). Not only the increase in the T-cell rate but also IL-2 promote long term proliferation of antigen specific

effector T-lymphocytes and induction of cytotoxic T-lymphocyte activity (Fukuhara et. al., 1985).

The proliferative response (Stimulation index markedly deteriorated in cancer patients & patients who receive chemotherapy or hormonal therapy alone. Marked improvement of proliferative response was detected after chemoimmunotherapy.

At least two non-mutually exclusive mechanisms could attribute the suppression of responsiveness to PHA by peripheral blood lymphocytes of patients with cancer: (1) the involvement of suppressor T-cell acting directly or via soluble mediators (2) A reduction in frequency of T-Helper cells or a functional defect of such cells (Yron 1986).

In conclusion, adjuvant treatment is needed to increase the overall cure rate and not just delay the appearence of detectable metastasis. Also immunorestoration favour the delay of metestastatic potential of residual micrometastasis. There is considerable doubt whether there is a distinction between these two criteria in the case of human breast cancer as ther is now a good evidence that year by year after curative treatment, the patient die from the disease at a constant rate. It is very likely in the case of breast cancer cure merely means that the clinical appearence of metastases is delayed to the extent that the patient may unlikely die of old age or intercurrent diseases.

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Table (1) : Groups received adjuvant therapy after radical mastectomy.

Group	Adjunant treatment	No. of cases
1. Group I	No treatment (control)	20
2. Group II	CMF	20
3. Group III	Tamoxifen (T)	20
4. Group IV	CMF + T	20
5. Group V	CMF + BCG	20
6. Group VI	CMF + LAK	20

Table (2) : Represent the correlation between adjuvant treatment and recurrence in premenopausal patients.

Treatment Group	No. Postmenopausao	No. Recurrence	%
I Group (no treatment)	12	6	50
II Group (CMF)	16	3	19
III Group (T)	6	3	50
IV Group (CMF)	10	2	20
V Group (CMF - BCG)	16	3	18.75
VI Group (CMF - LAK)	12	1	8.25

Table (3) : Represent the correlation between adjuvant treatment and recurrence in postmenopausal patients.

Treatment Group	No. Postmenopausao	No. Recurrence	%
I Group (no treatment)	8	4	50
II Group (CMF)	4	3	75
III Group (T)	14	2	17
IV Group (CMF)	10	1	10
V Group (CMF - BCG)	4	1	25
VI Group (CMF - LAK)	8	1	12.5

Table (4) : Effect of treatment on OKT3 in PBL in cancer patient before and after treatment.

	Group II		Group III		Group IV		Group V		Group VI	
	Before	After	Before	After	Before	After	Before	After	Before	After
Mean	70.20	66.78	70.5	64.12	64.00	79.12	71.18	84.22	70.12	79.18
SD	9.24	9.12	12.28	10.80	8.6	5.80	10.20	2.25	9.18	1.7

Table (5) : Effect of PHA on PBL of breast cancer patients before and after treatment in 120 patients.

	Group II		Group III		Group IV		Group V		Group VI	
	Before	After	Before	After	Before	After	Before	After	Before	After
Mean	24.30	22.80	26.64	23.28	25.28	32.26	28.22	66.75	23.16	68.32
SD	6.84	6.22	7.28	7.24	7.60	12.20	7.02	9.28	5.65	15.16

Table (6) :

		Group II	Group III	Group IV	Group V	Group VI
Before	X1	70.2	70.5	64	71.18	70.12
	sd1	9.24	12.28	8.6	10.2	9.18
	n1	16	6	10	16	12
After	X2	66.78	64.12	79.12	84.22	79.18
	sd2	9.12	10.8	5.8	2.25	1.7
	n2	16	6	10	16	12
	t	1.053705	0.95562	-4.6094	-4.9937	-10.04
	p	> 0.05	> 0.05	<0.001	< 0.001	< 0.0001

Table (7) :

		Group II	Group III	Group IV	Group V	Group VI
Before	X1	24.3	26.64	25.28	28.22	23.16
	sd1	6.84	7.28	7.6	7.02	5.65
	n1	16	6	10	16	12
After	X2	22.8	23.28	32.26	66.75	68.23
	sd2	6.22	7.24	12.2	9.28	15.16
	n2	16	6	10	16	12
	t	0.64898	0.80161	-1.5356	-13.245	-9.6502
	p	> 0.05	> 0.05	>0.05	< 0.001	< 0.001

Table (8) :

AF. 2	X1	66.78	66.78	66.78	66.78
	sd1	9.12	9.12	9.12	9.12
	n1	16	16	16	16
AF. 3,4,5.	X2	64.12	79.12	84.22	97.18
	sd2	10.8	5.8	2.25	1.7
	n2	6	10	16	12
	t	0.53598	-4.2171	-7.4265	-13.035
	p	> 0.05	<0.001	< 0.001	< 0.001

Table (9) :

AF. 2	X1	22.8	22.8	22.8	22.8
	sd1	6.22	6.22	6.22	6.22
	n1	16	16	16	16
AF. 3,4,5.	X2	23.28	32.26	66.75	68.23
	sd2	7.24	12.2	9.28	15.16
	n2	6	10	16	12
t		- 0.1437	-2.2743	-15.736	-9.7817
p		> 0.05	<0.05	< 0.001	< 0.001

Table (10) : Represent correlation between adjuvant treatment and disease free interval.

Treatment Group	Dis. free interval (months)
I Group (no treatment)	12
II Group (CMF)	14
III Group (T)	16
IV Group (CMF)	18
V Group (CMF - BCG)	17
VI Group (CMF - LAK)	24

Table (11) : Over all recurrence in each group after adjuvant treatment.

Treatment Group	Over all recurrence	%
I Group (no treatment)	10	50%
II Group (CMF)	6	30%
III Group (T)	5	25%
IV Group (CMF)	3	15%
V Group (CMF - BCG)	4	20%
VI Group (CMF - LAK)	2	10%