Clinical Role of CEA in Human Mammary Carcinoma

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Abstract

Single measurement of the two-biochemical tumor markers (CA 15-3) and CEA were carried out in serum samples obtained from 40 healthy donors, 22 breast benign patients and 122 breast cancer patients. Mean values of these tumor markers in breast cancer patients were significantly higher ($P<0.05$) than that found in healthy normal or patients with benign breast tumors.

CA15-3 shows the best sensitivity (54%) for detecting preoperative breast cancer patients, than CEA, which gave (44%) sensitivity. Also, CA15-3 gave the highest specificity (100%) for discriminating non-malignant patients while CEA had specificity of (77%).

The cytosolic CEA concentration was determined in the tumor, benign and normal tissue of breast cancer patients. Significant differences between values from the tumor and normal specimens were found. There was no correlation between the preoperative levels of serum CEA and cytosol level of CEA in the patients with carcinoma. Also, CEA in cytosols did not correlate with either stage or histology. It was concluded that the test might provide calculable information for the evaluation and planning of treatment.

الخلاصة

تم إجراء قياس منفرد لاثنين من الدالات الورمية (CA 15-3) و(CEA) من نماذج لمصول ٤٠ شخصا من الأصحاء ، ٢٢ من مرضى مصابين بورم الثدي الحميد ، و ١٢٢ مصابين بسرطان الثدي .وأظهرت النتائج بان (CA 15-3) أعطى نتائج أكثر حساسية وايجابيه من الثاني حيث كانت نسبه الحساسية (٥٤% و٤٤%) على التوالي وكذلك أعطى المستضد (CA 15-3) أكثر خصوصية منCEA بقابليه الفحص لإعطاء نتائج سالبه عندما يكون الأشخاص فعلا غير مصابين بالمرض (١٠٠% و ٧٧% على التوالي).

ووجد بان تركيز (CEA) داخل الخلايا موجود في حالات السرطان والورم الحميد والانسجه الطبيعية ولكن بفرو قات واضحة في حاله السرطان مما في البقية. ولاتوجد أي علاقة بين مستوى تركيز (CEA)في الدم عن تلك التي في خلايا الجسم للمصابين بالسرطان فيما لو قيست قبل العملية كما ولاتوجد علاقة بيت درجه الورم ولا الدراسة النسيجية للنسيج مع تركيز(CEA) في الخلايا وبذلك بينت الدراسة بان هذا الفحص يوفر معلومات محسوبة لتقييم ووضع خطه العلاج،

Introduction

Breast cancer is a disease in which breast cells proliferate abnormally .The diagnosis of breast cancer is established histologically. Breast cancer may present as a breast lump, thickening, or skin change. Non-palpable cancers may be detected by mammography. A biopsy is necessary to confirm the diagnosis and determination the type of cancer present. (1)

When breast cancer cells metastasize from the original tumor and enter the blood stream or lymphatic system, they can form secondary tumors in other parts of the body.

Bilateral cancer is diagnosed when separate primary breast cancers arise in each breast; multifocal breast cancer is diagnosed when breast cancer presents in more than one site in the same breast. Breast cancer is staged from 0 to IV, where 0 is non-invasive tumor, stage I is a small locally invasive tumor without lymph node involvement, stage II is a medium-sized tumor with or without nodal metastases, stage III cancer is a locally advanced cancer, usually with axillary node metastases, and stage IV cancer has already metastasized to distant sites (2). The survival rate is dependent upon the stage at which breast cancer is diagnosed.

The role of breast cancer tumor markers is to enhance the clinic ability to provide more effective management of the disease, CEA was among the earliest circulation tumor markers proposed for evaluation of breast cancer, but its clinical utility was limited by lack of sensitivity and specificity (1,2). In adult life, high circulating levels of CEA are found in a variety of metastatic cancers including breast cancer, in contrast to metastatic breast cancer, CEA level are rarely elevated in sera from patient with primary breast cancer.(1-2) The reasons for this are unknown, but it raises the quest879+ion, does CEA exist in primary tumors and if so what factors effect its concentration.

The objective of present study was to evaluate the clinical application of biochemical tumor markers CA 15-3 and CEA in the diagnosis and monitoring of breast cancer. Also, this study investigates the distribution of CEA in breast tumor cytosols and correlates its levels with a variety of biochemical pathologic para-meters.

Materials and methods

Chemmical

All laboratory chemicals and reagents were of annalar grade. Tris (hydroxymethylamino methane) was obtained from BDH.

All buffer solutions were prepared by dissolving the appropriate amount of salts in distilled water and the required pH was adjusted.

The reagents IRMA CA15-3 and CEA kits was provided by Byksangetec Diagostica GmbH & Co. KG/France.

Apparatus:

The apparatus used during this study were, LKB gamma Counter type 1270 Rack, Backman Model-25 spectrophotometer, cooling centrifuge type Hitachi, Pye-Unicam pH meter.

Patients

Three groups of breast cancer patients and one group with benign breast tumors were included in this study. Group 1 contained 38 premenopausal patients with breast cancer. Group 2 consisted of 82 postmenopausal patients with breast cancer. Group 3 consisted of 25 patients with known metastatic (metastatic group). Group 4 comprised 22 patients with benign breast tumors. In addition 4 groups of age matched healthy subjects were also included.

All patients were admitted for treatment to Baghdad Medical city, AL–Husaney, AL-Arabia, and AL-Saddoon Hospitals. They were histologically proven, newly diagnosed and not underwent any type of therapy. Clinical information was recorded at the times were entered into the study by proper investigations and include age,

menopausal status, tumor size, type of carcinoma.

Blood samples

Blood samples for measurement of CEA and CA 15-3 were obtained from healthy donors (40 woman aged 18 to 55 years with no any disease) and all details are explained in the text patients with breast cancer. 5ml blood were withdrawn from every patient one day before mastectomy or biopsy. The diagnosis was confirmed postoperatively by histological examination. All serum were separated and stored frozen (-20 C°) until the determination of biochemical markers was performed.

Collection of specimens and

preparation of tissue homogenate:

The source of tissue in this series of experiments was human breast. The specimens removed surgically from primary adenocarcin-oma of the breast were taken only the central, obviously cancerous portion was used as tumor tissue for extraction.

Normal tissue extract was prepared specimens were taken into the study. from section of breast more than 7cm distant from the visible edges of the tumor. In this way the problem of individual-specific antigenic differences between normal and tumor tissue was overcome. All tissues were placed in labeled clean polystyrene container in normal saline and kept at -20°C until use, some times the specimens were processed immediately. Only pathologically confirmed benign

Cytosol preparation:

Samples were trimned of fat, weight, minced, in four volumes of homogenization buffer, (10% glycerol, 10-mM dithiotheritol, 10 mM Tris, 1.5 mM EDTA). The homogenization was done on an ice bath.

The homogenate was filtered through a nylon mesh sieve to eliminate fibers of connective tissues, then centrifuged at 2000 xg for 10 min at 4°C. The pellet was neglected and the supernatant was centrifuged at 2000 xg for 30 min at 4°C.

After removing the upper layer of fat, an appropriate volume of cytosolic (500-µl) was diluted to 2.5 ml with homogenization buffer and treated with an equal volume of 1.2 mole/liter perchloric acid. After 30 min, the mixture centrifuge at 2000 xg for 10 min and the supernatant was dialyzed for 12 hours against cold distilled water (at 4 c°) [3,4].

CEA was determined in an aliquot of perchloric acid extract by the solid phase sandwich (IRMA)

Protein Assay:

The total protein in the cytosol was measured by using method of Lowry.[5]

Aliquots were taken from the cytosol for each test.

Statistical Analysis

The results of serum and tissues determination of CEA an CA15-3 were analyzed statistically and the values were expreessed as mean ±SD. The levels of significance were determined by Analysis of variance (ANOVA). [6]

Results

Serum Determination of CA15-3 in Breast Cancer

Patients and Controls

Normal Controls:

Low levels of CA_{15-3} were observed in the sera of 40 apparently healthy woman used as a Control (Fig 2-3). The mean CA_{15-3} levels (±SD) in these woman was (15.7±1.24 µ/ml) with an upper normal value of 3 A positive scoring or an abnormal level was indicated by those values of CA15-3 which exceede the 30 µ/ml limited. All normal controls had CA15-3 concentration lower than 30 µ/ml (Fig 2-3), suggesting a test specificity of 100% for the ability of this marker to exclude normal individuals.

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Benign Breast Cases:

Samples of 22 patients with histologically confirmed benign breast cases, none had level of CA15-3 above 30 µ/ml (Fig 1)

The mean level of CA_{15-3} antigen observed in these patients (16.4±1.64 ml) was not significantly different from normal controls (P>0.05) (Table 2-4). Our data are in good agreement with the literature [7,8].

Breast Cancer:

Our data show that the mean serum value ±SD (43.8 ± 5.48 µ/ml) of CA 15-3 for breast cancer patients, is significantly higher than that in normal controls (P < 0.05).

Also the percentage sensitivity of patients with abnormal value of CA 15-3 is 54%, which is quite different from that of patients with benign breast disease (Table1). These results are in agreement with other investigators[9]. The results in Table (2) indicate that the rate of positive scoring of CA 15-3 increased as the grade of breast cancer raised from stage I to IV. These findings are in good agreement with earlier report [10]

The correlation between the histological classification of human malignant mammary carcinomas and percentage sensitivity of CA 15-3 antigen was also examined. As can be seen in table (3) high antigen values are associated with the invasive pure forms of breast malignancy. These results confirmed analogues findings by others[11-14].

In table (4) the percentage of positive scoring of CA 15-3 is presented in relation to menopausal status of breast cancer in women. The postmenopausal patients gave the lowest percentage (44%) of CA15-3 positive scoring, in comparison to the premenopausal patients with (65%) sensitivity.

The post therapeutic patients who had a good prognosis (i.e. good response to therapy) gave the lowest percentage (3.57) of CA15-3 positive scoring, in comparison to postoperative (pretherapeutic) patients with 44% sensitivity or the preoperative patients with 54% sensitivity and the Metastatic group with 100% sensitivity. The later group was at active stage of the disease and had metastases either to the skin, lung, and bone or lymph nodes subsequent to their therapy. The present sensitivity of CA15-3 for preoperative patients agree well with similar percentage of sensitivity observed by others [7,9].

Determination of CEA levels in sera of breast cancer, benign and controls

Normal controls:

Low levels of serum CEA were observed in normal women (n=40) who had a mean value (±SD) 1.57 ± 0.99 ng/ml, (Table5) with the cut off value of 3 ng/ml, and a percentage specificity of 88%. These values are close to those obtained by others [7,12].

Benign Breast Tumor:

Samples of 22 patients with histologically confirmed benign breast disease, five of them had CEA > 3 ng/ml. The other 17 patients gave CEA value 2.25 ±1.5, which is not significantly different from normal controls (P > 0.05) (Table 5). The present studies also show that the mean values of CEA in benign Patients and Control individuals are close to each other.

Breast Cancer:

A mean serum value of CEA (6.8 ± 3.7) for breast cancer patients is shown in Table (5) which is significantly higher than normal controls (P < 0.05).

Also the percentage sensitivity of CEA is 29.5% in preoperative breast cancer (Table6), compared with 22% sensitivity for benign breast patients. The data reported by Heinze. et al[10] , showed

about 41% sensitivity of CEA for breast cancer patients by using a similar cut off limit of normal value.

It seems from the results of table (7) that there is no significant correlation between the increased concentration of serum CEA and the grade and staging of breast cancer patients especially at stages I, II and III, of the disease. However good correlation was observed between CEA sensitivity and patients at stage IV (Metastases), since a positive scoring of CEA was detected in 20 out of 25 cancer (80% sensitivity) these results confirm an earlier study reported by Heinze et. al[10] . Concerning the histological classification of human breast tumors, table 8 indicates high CEA level found invasive pure form of tumors. The percentage of CEA values was distributed as 75%, 54% and 75% for the tumors, medullar carcinoma, infiltrating duct carcinoma, and tubular carcinoma respectively. A lower CEA was observed in other breast malignancies such as infiltrating lobular carcinoma (42%), adenocystic carcinoma (25%) and intraductal noninvasive type (20%).

There was no positive scoring of CEA levels in post therapeutic breast cancer patients (Table 6). However, about 12% of all postoperative breast cancer patients gave abnormal high CEA level compared to 29.5 sensitivity of CEA for preoperative breast cancer patient and 100% positive scoring of CEA level for metastatic group. This result is similar to that reported in the literature [9,11].

The serum level of CEA was markedly affected by menopausal status. High rate of positive scoring was found in premenopausal 52% table (9). Compared with 19% sensitivity for postmenopausa

Determination of cytosolic CEA in breast cancer and normal specimens

Normal Specimens:

Cytosolic CEA was assayed in 32 specimens of normal individuals The normal level of this glycoprotein is presented in table (10) indicating a mean value of 2.2 ± 0.81 ng/mg cytosol protein (cp) and range of 0-3.7 ng CEA/mg protein. The specificity of CEA in cytosol for excluding normal individuals was 93%, by regarding the normal/abnormal cutoff value of 3.0 ng/mg cytosol protein. At present no report is available in the literature on the cutoff value of CEA in cytosol.

Benign Breast Tumor:

When the assay was performed on 12 patients with benign breast tumors, supernormal levels (concentration > 3ng/mg cytosol protein) of CEA were observed in 4 of these patients, suggesting a specificity of 67% (Table11) and mean value of 2.8 ± 0.05 ng/mg cytosol protein. (Table10) which is not significantly different from that of normal ($p>0.05$). There have been no previous reports on the CEA level in cytosol with breast benign tumors.

Breast Cancer Tissue:

The distribution of CEA in breast carcinoma tissue is shown in table (6) (11). CEA concentration > 3 ng/mg protein was found in 51/62 primary carcinoma.

The percentage sensitivity of CEA for cancer specimens is about 82% (Table11), compared with 6% for normal specimens. The mean value of CEA in cancer specimens is 17.25±3.4 which is significantly higher ($P<0.05$) than in benign and normal (Table 2-14). One female case shows very high levels of CEA (117 ng/mg protein) died within 3 months after surgery.

It seems from the results that there is no significant correlation between preoperative level of serum CEA and cytosol level of CEA. Also cytosolic CEA didn't correlate with either carcinoma stage or histological classification.

Most research on CEA has concentrated on measurement of this

glycoprotein in sera from patients with cancer. However, a logical first step in the study of tumor markers would be examination of the tumor tissue for the presence of particular marker of interest. Primary breast cancer in contrast to metastatic disease rarely causes elevation of circulating levels of CEA. Our work shows that this is not due to the absence of CEA from primary tumors. It could however relate to the tumor bulk which may not be sufficiently large to produce elevate serum level at the localized stage, The prognostic value of CEA in the tissue is not yet defined. Some authors reported a correlation between presence of CEA in the tissue and a worse prognosis[12]; other authors found such a relationship only in tumors of 3 cm or smaller [13,14]; others deny any relation between CEA and prognosis [15] or do not take into account this problem [16,17,,18]. Furthermore, CEA in the tissue does not appear to be related to the degree of differentiation of tissue [16,18].

Analysis of CEA in the cytosol may prove to be helpful in evaluating plasma CEA as marker for recurrence subsequently in tumors with low CEA cytosol values must produce bulky metastases before blood CEA concentration increases measurably. Also, the clinical utility of the tests may lie in providing additional information on the tumor proliferation rate in term of planning the treatment for woman with early stage breast cancer.

Discussion

The comparative study was carried out in an attempt to overcome the limitation of specificity and, sensitivity of single tumor marker determination. Hence a combination of two Markers that could complement each other in function, were used.

CA15-3 showed the best sensitivity (54%) for detecting preoperative breast cancer patients (Table12) and CEA gave 29.5% sensitivities. However the low specificity (77%) of CEA for discriminating benign from cancerous patients, as well as its general elevation in other type of cancer puts some doubt on clinical detecting breast cancer patients. Therefore, a combination of CEA and CA 15-3 (with 100% specificity), though not tested as such but it can be better to be choice as a diagnostic tool for preoperative patients with breast cancer.

According to the initial determination, the groups free of metastases showed normal CEA serum level below 3ng/ml in 78.68%, where as only 29.5% were in the intermediate pathological range (exceeding 3ng/ml). A significantly different distribution ($P< 0.05$) was found in the patient group with metastases. Thus it may be concluded that CEA test recognized all cases with proven metastases, but does not recognize all cases without metastases.

Owing to lack of a understanding of the pathophysiological behavior of the CEA produced by malignant tumors, no body can explain why high CEA – concentration in tumors are not in all cases to be found in the serum. A reason for this finding may be, that the CEA may be masked by naturally occuring substances after leaving the tumor, giving rise to negative or unreasonably low results.

From 53 follow - up cases with 5 –15 serial determinations over 3–12 months, 25 cases had constantly normal CEA levels or values not exceeding 3ng/ml. All these patients were free of metastases, This associate with a regression of the disease.

From the 28 cases (52.8%) with increased CEA levels, 25 had proven metastases. In 3 further patients, no metastases were found; one of these patients had CEA levels between 14 and 40 ng/ml over a period of 12 months, the other patient had rather constant levels between 7 and 13 ng/ml over an observation period of 10 months. The patients with metastases and elevated CEA levels in direction to higher values. Most of these patients developed clinical

progression of disease during the observation period.

In conclusion, the present results lead to the following:

1. In mastectomized patients without metastases, CEA serum levels remain within the normal or low pathological range.
2. Most of mastectomized patient with metastatic breast cancer shows slightly to obviously pathological serum CEA levels. The percentage of pathological values is less with isolated lymph node involvement and gradually from skin to liver, lung, bone up to significantly higher percentage with multiple organ manifestation.
3. Follow – up studies during drug or radio therapy show a correlation of increasing CEA concentration with progression, decreasing values with remission and persistent or slightly fluctuating levels with stationary disease.
4. The CEA test may not be recommended for screening of breast cancer, but as a valuable adjuvant of monitoring metastatic disease and the response to therapy.

The reported results are essentially consistent with the finding of several other investigators [7-12].

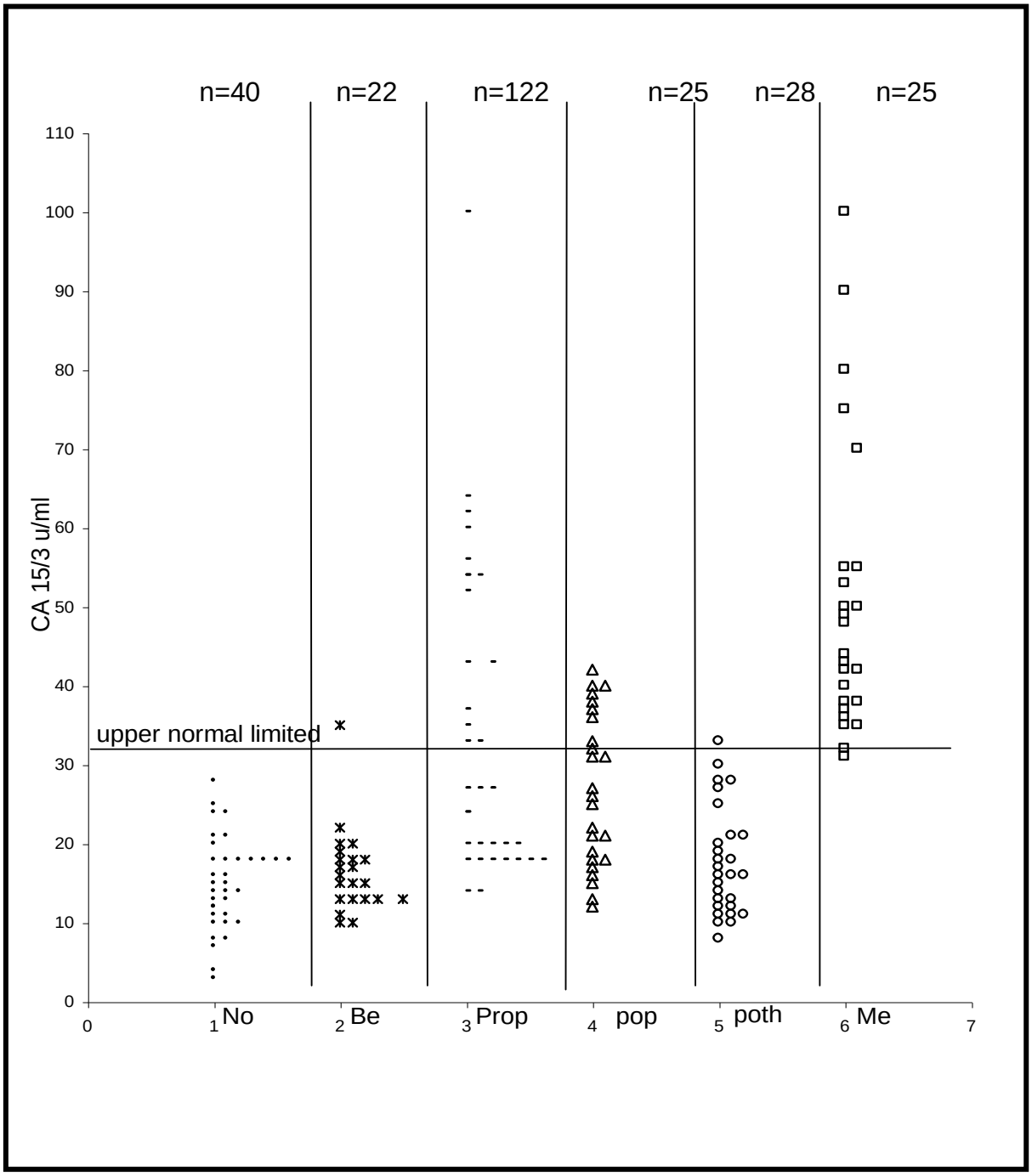

Fig. (1): Distribution of CA 15-3 value over different groups of patients (n)=Numberofpatients,(No)=Normal,(Be)=Benin, (prop)=preoperative, (pop)=postoperative (poth)=posttherapy, (Me)=Methastatic

(Table -1) Incidence of elevated serum CA 15-3 in-patients with benign and malignant breast tumors (All details are explained in the text).

Cases	*No. patients*	*No. of elevated CA 15-3 level*	*% Patients with elevated CA15-3 level*
Benign breast tumors	22	0	0%
Breast cancers collectively	200	102	51%
Preoperative	122	65	54%
Post operative	25	11	44%
Post therapeutic	28	1	3.57%
Metastatic group	25	25	100%

(Table 2)Correlation of elevated CA 15-3 levels (% sensitivity) with the TNM stage of breast cancer (All details are explained in the text).

Disease stage	*No. patient*	*No. elevated CA_{15-3} level*	*%*
I	10	2	20
II	40	12	30
III	47	22	46.8
IV	25	25	100

(Table 3)Incidence of elevated CA15-3 level inpatients with malignant breast tumor of different histology

(All details are explained in the text).

Histological classification of breast cancer	*No. patients*	*No. elevated CA 15-3 level*	*% patients with elevated serum CA15-3 level*
A- Noninvasive			
Intraductal or comedo carcinoma	5	2	40
Lobular carcinoma in situ	-	-	-
B- Invasive pure form			
Infiltrating duct	65	36	55.38
Medullary	3	3	100
Infiltration lobular	19	2	10.5
Adenocystic	4	1	25
Tubular	4	2	50
Papillary	-	-	-
mucinous	1	0	-
Carcino-sarcoma(ex. Cystosar - coma phylloides)	1	1	100
C- Paget's disease	-	-	-
Mixed histologies	-	-	-

(Table 4) Positivity of CA15-3 in relation to menopausal status

(All details are explained in the text)

Menopausal state	*No. patients*	*No. elevated CA 15-3 level*	*% patients with elevated CA15-3*
premenopausal	38	25	65.7
postmenopausal	84	37	44

Table (5) Serum determination of CEA in cancer patients and controls

(All details are explained in the text).

Woman clinically diagnosed	***No. of cases***	***Serum level of CEA ng/ml***		
		Mean	***±SD***	***F value***
Serum control	40	1.57	0.99	
Benign breast tumors	22	2.25	1.5	P>.05 N.S
Breast cancers (collectively)	200	10.5	2.63	P<.05
Preoperative	122	6.8	3.7	P<.05
Postoperative	25	2.28	1.57	P>.05
Post therapeutic	28	2.09	1.8	P>.05
Metastatic group	25	46.24	5.84	P<.05

Table (6) Incidence of elevated levels of serum CEA in malignant and benign patients (All details are explained in the text).

Cases	*No. patients*	*No. elevated CEA level*	*% Patient with elevated CEA*
Benign breast tumor	22	5	22.72
Breast cancer (collectively) Preoperative	200	67	33.5
Postoperative	122	36	29.5
Post therapeutic	25	3	12
Metastatic group	28	3	10.7
	25	25	100

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Table (7) Incidence of elevated levels of serum CEA (% sensitivity) in breast cancer patients according to TNM staging (All details are explained in the text)

Disease stage	*No. patients*	*No. elevated CEA level*	*% Patient with elevated CEA*
I	10	6	60
II	40	14	35
III	47	26	55
IV	25	20	80

Table (8)Incidence of elevated CEA level in patients with malignant breast tumors of different histology (All details are explained in the text).

Histological classification of breast cancer	*number Of patients*	*number of elevated CEA level*	*% patients with elevated serum CEA*
A- Noninvasive			
Intraductal or comedo carcinoma	5	1	20
Lobular carcinoma in situ	-	-	-
B- Invasive pure forms			
Infiltrating duct	65	35	54
Medullary	4	3	75
Infiltrating	19	8	42
Mucinous	1	0	-
Adenocystic	4	1	25
Tubular	4	3	75
Papillary	-	-	-
Carcino-sarcoma (ex. Cystosarcoma phylloides)	1	1	100
C- Paget's disease	-	-	-
Mixed histologies	-	-	-

Table (9) Positivity of CEA in relation to menopausal status (All details are explained in the text)

Menopausal state	*Number of patients*	*Number of elevated CEA level*	*% patient with elevated CEA*
Premenopausal	38	20	52.6
postmenopausal	84	16	19

Table (10) Cytosolic CEA determination in cancer, benign, and normal, human breast tissue (All details are explained in the text)

Source of tissue	*No. sample*	*Cytosol level of CEA (ng/mg protein)*		
		Mean	*±SD*	*F value*
Normal	32	2.2	0.08	
Benign	12	2.8	0.05	P>0.05 N.S
Primary carcinomas	62	17.25	3.4	P<.05

Table (11) Sensitivities and specificity's of CEA in normal, benign, and human breast carcinoma tissue.
(All details are explained in the text)

Source of tissue	*No. specimens*	*No. Positive cases*	*% Positive*	*No. Negative cases*	*% Negative*
Normal	32	2	6	30	93.75
Benign	12	4	33	8	66.6
Primary carcinoma	62	51	82	11	17.7

Positive > 3ng CEA/mg protein

Negative < 3ng CEA/ mg protein

Table (12) Comparison of the sensitivity and specificity of the two marker
(All details are explained in the text)

Tumor marker	*% sensitivity for preoperative detection of breast cancer patient*	*% specificity for discriminating normal and benign patient*	
		Normal	*benign*
CA 15-3	54	100	100
CEA	29.5	73	77

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