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RESEARCH ARTICLE

Serum tumour markers as a diagnostic and prognostic tool in Libyan breast cancer

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Abstract Results from studies on efficacy of carcinoembryonic antigen (CEA), carbohydrate antigen 15.3 (CA 15.3) and thymidine kinase (TK1) as diagnostic and prognostic tools for primary breast cancer (BC) have presented conflicting results, and usefulness of these markers for clinical use in BC remains unclear. The aim of this study is to evaluate potential of concentration of the sera CEA, CA15.3 and TK1 peptides' use as markers in the diagnosis and prognosis of breast lesions of Libyan patients. Serum tumour markers were studied in 20 healthy subjects, 30 patient with benign lesion diseases and 50 patients with histologically confirmed BC diagnosed at the National Cancer Institute (NCI), Misurata, Libya during the period 2005–2009. The concentrations of the BC patients' cutoff points used for diagnostic and prognostic sensitivity were 8.82 ng/ml, 35.57 U/ml and 32.57 U/mg/protein for CEA, CA15.3 and TK1, respectively. Increased CEA (>8.82 ng/ml), CA 15.3 (>35.57 U/ml) and TK1 (>32.57 U/mg/protein) concentrations were found in 62 %, 70 % and 78 % of the BC patients, respectively. For all three tumour markers, increased concentrations correlated increased tumour size and nodal involvement. Significantly higher serum TK1 levels were found in patients with advanced disease ($p<0.0001$) and TK1 levels also correlated with disease-specific survival (DSS, $p<0.07$). The combined data set of the three markers' data from three markers increased the diagnostic sensitivity to 90 %. The serum marker analysis for CEA, CA 15.3, and S-TK1 concentrations is shown to be a useful tool for identification of malignant cases in our BC population and for the prognostic evaluation of patients with primary BC. Increased concentrations of the markers were also observed to be higher in patients with advanced tumours and indicative of the development of distant metastasis.

Keywords Tumour markers · Libyan breast cancer · Diagnosis · Prognosis

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Introduction

Worldwide breast cancer (BC) is still associated with high mortality, accounting for 10–18 % of all cancer death in women [1]. Advanced stage at presentation correlates generally with a poor prognosis; however, stage is not the only prognostic factor. Some tumours are more aggressive and are associated with a poorer prognosis even when detected at an earlier stage; in some populations, up to 20–30 % of the patients with lymph node-positive (LN+) BC are dying of recurrent disease depending on established risk factors and tumour biology [2]. Some data show that mortality risk may even be higher in patients with node-negative grade 3 tumours than the risk demonstrated in some patients with node-positive disease, suggesting that these patients do have a high enough risk to indicate adjuvant chemotherapy [3]. Improvements in adjuvant systemic therapy have made treatments available for some of these aggressive tumours and a relative survival improvement of 15–20 % over the next decade is predicted [4]. However, accurate and reliable prognostic markers are needed to help identify these

aggressive tumours so that these high-risk patients can be treated appropriately.

The routine protocol for the initial diagnosis of BC is that of utilising a triple diagnostic test (fine needle aspiration biopsy (FNAB), mammography and clinical examination), the diagnosis being subject to tissue resection or biopsy for histological confirmation. The sensitivity of FNAB can be quite low especially for well-differentiated BCs [5] and use of tumour markers may improve sensitivity of the diagnosis. In addition, serum molecular diagnostic tools may prove to be a cost-effective, technically simple alternative solution, in addition to being more accessible (due to ease of sampling) for monitoring illness.

Evaluation of serum tumour markers in BC has been studied for many years, but its usefulness clinically remains unclear [6, 7]. Despite this uncertainty, numerous serum tumour markers have been used both clinically and in research for BC (e.g., cancer antigen (CA) 15.3, carcinoembryonic antigen (CEA) and thymidine kinase (TK1)), their concentration levels providing more information about outcome and progression of BC [6, 8–12]. However, serum tumour marker sensitivity in BC and other solid tumours has been reported as being low, which would make their use unsuitable for early diagnosis [13, 14]. Reported correlations between serum tumour marker levels and tumour characteristics have also varied; some groups report a relationship between higher concentrations of serum tumour marker and tumour size, stage or nodal involvement, whereas others fail to find such a relationship [11, 13, 14]. The usefulness of serum tumour markers for clinical prognosis, therefore, remains an issue of debate with a wide range of results and opinions [15–17], all of which emphasises the need for further research into the prognostic value of serum tumour markers to allow definitive conclusions to be drawn [18, 19]. The objective of this study is to evaluate the diagnostic and prognostic potential roles of the serum markers CEA, CA15.3 and TK1 in primary untreated BC in Libyan patients.

Materials and methods

Study population

The study population consisted of 20 healthy subjects, 30 patients with benign lesion diseases and 50 untreated patients with BC diagnosed at the National Cancer Institute (NCI), Misurata, Libya between 2005 and 2009. Of the 50 patients with BC, 29 had locoregional disease and 21 advanced disease; in all cases, the diagnosis was confirmed by histological examination. Patients who were disease-free at the time of analysis were followed up afterwards, median follow-up time 28±1.9 months (range, 1–84 months), to verify their BC-free status for this study.

Ethical approval

This study is a part of other BC studies, which got permission from the local ethical committee of NCI of Misurata, Libya. Approval for tumour sample collection for this project was granted by the National Authority for Medical Affairs.

Method

Full tumour data, including stage and grade and other pathological features, were determined by a pathologist during the diagnostic phase and/or after mastectomy and documented using standard classification as recommended by the International Union Against Cancer (UICC) [20]. Serum samples were taken in the outpatient department (OPD) clinic of the NCI of Misurata from January 2005 to October 2009.

TK1 enzyme-linked immunosorbent assay (ELISA) samples were analysed using an ELISA kit for TK1 (Bacon & Tomas, Plica, Alexandria, VA, USA) using the recommended protocol. Briefly, samples, standards, were added to the wells of microtitre plates coated with affinity-purified polyclonal chicken antibodies specific for TK1 peptide and the plates were incubated at 37 °C for 35 min and washed by sys solution four times prior to the addition of a biotinylated polyclonal antibody-specific for TK1. After another cycle of incubation and washes, enzyme-labelled streptavidin was pipetted into the wells, and the plates incubated and washed again. After this final washing, a substrate solution was added and the colour allowed to develop and its intensity measured by a spectrophotometer. Concentrations were calculated from these results, following the manufacturer's recommended methodology, and the TK1 concentration recorded in unit per microgram per protein.

Concentrations of the serum tumour markers CEA and carbohydrate antigen 15.3 (CA 15.3) were measured by an automated sandwich ELISA test system using the manufacturer's recommended kits (ELISA 2010 from Roche Company). CEA and CA 15.3 concentrations were recorded in nanogram per millilitre and unit per millilitre, respectively.

The cutoff points of concentrations taken to distinguish normal (negative) and abnormal (positive) results were CEA 8.82 ng/ml, CA 15.3 35.57 U/ml and TK1 32.57 U/mg/protein, respectively [10, 11]. The cutoff values of concentrations were taken from the upper limit of control normal group.

Statistical analysis

Statistical calculations were performed using the SPSS for Windows, version 15.0. (SPSS, Inc., Chicago, USA), software packages. Frequency tables were analysed using the

Chi-squared test, with likelihood ratio (LR), or Fischer's exact test to assess significance of association between the variables. Comparison of numerical data was done with the *t* test. In addition, ANOVA was also used to test differences between the groups. Univariate survival analysis was performed with the Kaplan–Meier curves and significance determined by log-rank test (KM-LR). For all tests, values with $p<0.05$ were regarded as statistically significant.

Results

Correlation of serum tumour markers with differential diagnosis of BC

All non-malignant cases had low level of at least one of studied serum markers, and most of the histologically malignant cases had elevated serum marker concentration. Only five invasive ductal carcinomas showed concentration below the cutoff levels. All samples with higher values in more than one of the studied markers' concentration were malignant.

The results showed that serum markers were able to support a diagnosis of carcinoma and improve sensitivity, confirming the cytological diagnosis amongst the bulk of definitely benign and malignant cases. However, the method was less powerful in improving sensitivity for detecting carcinoma amongst few cancer cases, especially those malignant cases that showed normal value. In addition, the mean values of the cutoff points of CEA, CA15.3 and TK1 were significantly higher in carcinomas than amongst benign cases ($p<0.0001$).

The mean value of CEA was 95.1±90.4 ng/ml in cancer cases and 9.7±1.9 ng/ml in benign lesions. The mean value of CA15.3 was 160.7 ±115.3 U/ml in BC and 26.8±10.3 U/ml in benign lesions. The mean value of TK1 was 72.7± 41.3 U/ml in BC and 25.02±10.4 U/ml in benign lesions.

CEA in carcinomas ranged between 0 and 304.5 ng/ml and, in benign cases, between 0 and 40 ng/ml. When using 8.82 ng/ml as the cutoff value, the CEA serum concentration value distinguished between benign and malignant cases at a high level of significance ($p<0.0001$, *T* test). At this cutoff point, it was possible to distinguish malignant from benign diseases with sensitivity of 62 %, specificity of 63.3 % and efficiency of 62.5 %. Concentration value of CA15.3 in carcinomas ranged from 10 to 415.5 U/ml and, in benign cases, from 17.0 to 55.0 U/ml. At the cutoff point of 35.57 U/ml, sensitivity was 70.0 %, specificity 80.0 % and efficiency 73.8 %. Concentration value of TK1 in carcinomas ranged from 18 to 160.5 U/mg/protein, and in benign cases, from 10.0 to 48.5 U/mg/protein. At the cutoff point of 32.57 U/mg/protein, sensitivity was 78.0 %, specificity 80.0 % and efficiency 77.5 %. All studied markers levels show a high degree of significance when comparing benign with malignant cases. In summary, there are abnormally increased CEA (>8.82 ng/ml), CA 15.3 (>35.57 U/ml) and TK1 (>32.57 U/mg/protein), concentrations that were supported by the presence of carcinoma in 62 %, 70 % and 78 % of the patients, respectively, when the interpretation was based on one marker. From three combined makers, the diagnosis of carcinoma was supported in 90 % of samples, and the specificity reached up to 100 % (see Figs. 1, 2 and 3).

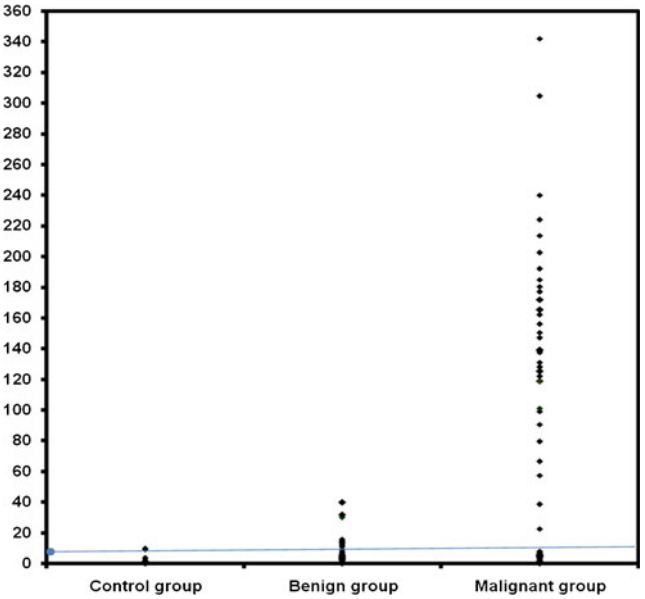

Fig. 1 Cutoff value of CEA in three study groups

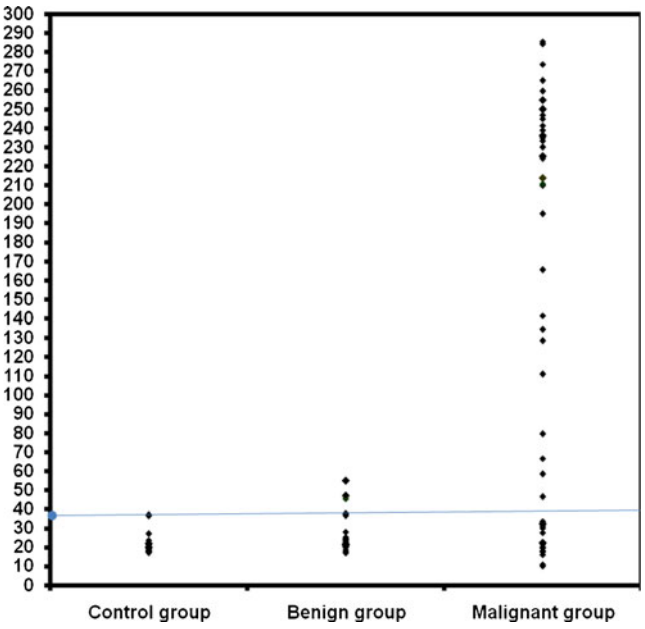

Fig. 2 Cutoff value of CA15.3 in three study groups

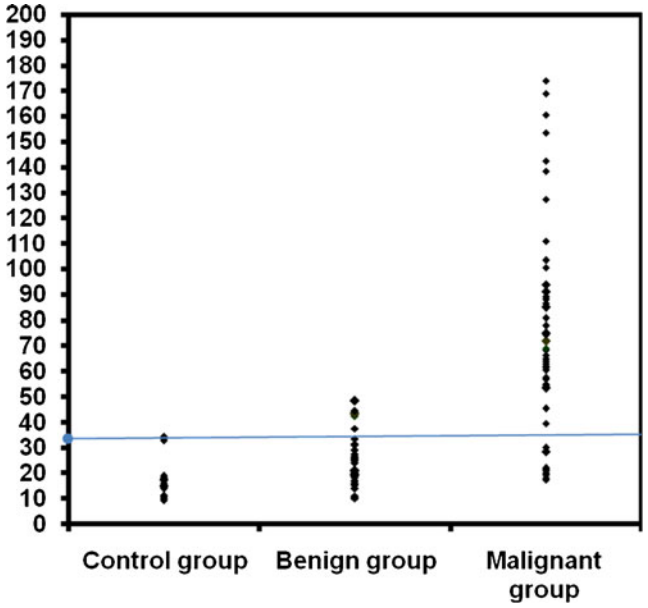

Fig. 3 Cutoff value of TK1 in three study groups

Correlation of serum tumour markers with clinico-pathological features

Table 1 shows a summary of tumour characteristics correlated to serum tumour markers CEA, CA 15.3 and TK1 concentrations. The most common presentations were infiltrating ductal carcinoma (72 %), grade 3 (48 %) tumours, tumour size greater than 5 cm (60 %), positive lymph node involvement (73.2 %), but with no systemic metastasis (58 %).

The serum concentrations were clearly related to tumour size and nodal involvement, with significantly higher concentrations in larger tumours and in those with nodal involvement. CA 15.3 shows the weakest correlation with all studied features (Table 1).

Significantly higher concentrations of CEA and CA 15.3 were found in stage T3 than stage T1 ($p<0.027$ and $p<0.01$, respectively) and in stage T4 than stage T2 ($p<0.021$ and $p<0.008$, respectively). The higher concentrations of these tumour markers recorded for stage T4 compared to stage T3 failed to reach statistically significant levels.

It is notable that, although not statistically significant, CEA serum levels in node-positive patients increased with the number of nodes involved (data not presented). CEA, CA15.3 and TK1 levels also varied with histological type, the highest concentrations being in IDC and the lowest in lobular carcinomas, although these differences were not statistically significant nor were the higher concentrations found in undifferentiated tumours; (Table 1). There are higher concentrations in undifferentiated tumours but also without statistical significance. Other factors, such as, age and menopausal status, were not related to any serum markers.

Table 1 CEA, CA 15.3 and TK1 serum concentrations in relation to the main clinico-pathological features in Libyan patients with primary BC

Parameter	Number of patients	CEA		CA 15.3		TK1	
		%>8.82 ng/ml	*p* value	%> 35.57 U/ml	*p* value	%>32.57 U/mg/protein	*p* value
Stage 1	7	2 (28.6 %)	0.2	5 (71.4 %)	0.4	2 (28.6 %)	0.0001
Stage 2	7	5 (71.4 %)		5 (71.4 %)		4 (57.1 %)	
Stage 3	15	9 (60.0 %)		8 (53.3 %)		12 (80.0 %)	
Stage 4	21	15 (71.4 %)		17 (81.0 %)		21 (100.0 %)	
$T\leq 5$ cm	11	9 (47.4 %)	0.01	8 (53.3 %)	0.1	3 (27.3 %)	0.002
$T>5$ cm	39	25 (80.6 %)		26 (74.3 %)		31 (79.5 %)	
LN−	9	3 (33.3 %)	0.05	6 (66.7 %)	0.5	3 (33.3 %)	0.002
LN+	41	28 (68.3 %)		29 (70.7 %)		36 (87.8 %)	
IDC	36	23 (63.9 %)	0.7	27 (75.0 %)	0.4	31 (86.1 %)	0.06
Lobular	6	4 (66.7 %)		3 (50.0 %)		4 (66.7 %)	
Others	8	4 (50.0 %)		5 (62.5 %)		4 (50.0 %)	
Grade I	7	2 (28.6 %)	0.1	5 (71.4 %)	0.7	4 (57.1 %)	0.3
Grade II	19	13 (68.4 %)		12 (63.2 %)		15 (78.9 %)	
Grade III	24	16 (66.7 %)		18 (75 %)		20 (83.3 %)	
M−	29	16 (55.2 %)	0.2	18 (62.1 %)	0.1	18 (62.1 %)	0.001
M+	21	15 (71.4 %)		17 (81.0 %)		21 (100.0 %)	
Pre-menopausal	28	18 (64.3 %)	0.5	20 (71.4 %)	0.5	23 (82.1 %)	0.32
Post-menopausal	22	13 (59.1 %)		35 (70.0 %)		16 (72.7 %)	

T tumour size, *LN* lymph node involvement, *M* systemic metastasis, *IDC* invasive ductal carcinoma

Correlation of serum tumour markers with disease outcome

Univariate (Kaplan–Meier) survival analysis was used to test the value of serum tumour markers as a predictor of disease-specific survival (DSS). The previous cutoff values of different serum markers (TK1<or≥32.57, CA15.3<or≥35.57, and CEA<or≥8.82) were used as a discriminator between aggressive and non-aggressive types of BC. These serum markers at these cutoff values were shown to be a predictor of DSS, although the correlations were not significant (log rank; p=0.07, p=0.1, and p=0.3 for TK1, CA15.3 and CEA markers, respectively) (Fig. 4). For example, at 5 years, 8 % of patients with lower TK1 level were dead due to the disease, as compared to 50 % of the patients with higher levels of TK1 (Fig. 4a, p=0.07). The other serum marker showed the same tendency in that BC patients with elevated serum marker levels had a shorter DSS than those patients with normal levels and the correlation between them, also here, is less significant (Fig. 4b and c).

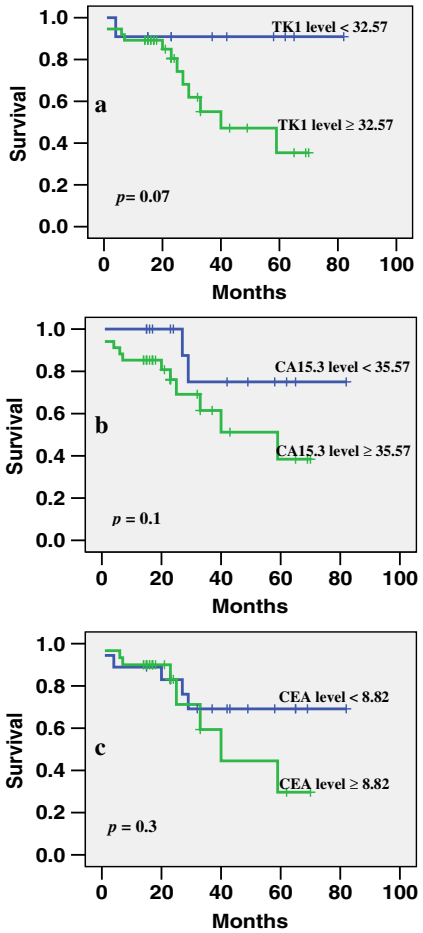

Fig. 4 Survival curves for 50 Libyan female patients with BC divided by TK1, CA15.3 and CEA cutoff points of 32.57 U/mg/protein, 35.57 U/ml and 8.82 ng/ml (**a**, **b** and **c**, respectively). The differences between the curves are obvious although the correlation is not significant, particularly, in case of CEA outcome

Discussion

The low abnormal levels of CEA, CA15.3 and serum TK1 found in early local BC suggest that these serum markers may not be useful as diagnostic markers in the early stages of BC due to their low sensitivity, particularly when evaluated separately. However, when the three serum tumour markers were evaluated simultaneously in patients with BC, the sensitivity of diagnosis improved up to 90 %. Multiple serum markers represent a reproducible, cheap and quick/easy test which can usefully be used to support the triple test or even the core needle biopsy investigation. Core needle biopsy, in experienced hands, is good a method to distinguish between benign and malignant lesions, and where possible, to prove obvious malignancy histologically or cytologically, use of multiple serum markers may seem to be unnecessary. However, core biopsy, like FNAB, may give false-positive or false-negative results and so a supporting test would add confidence to the results of the core biopsy. In serum markers that are very fast, a repeated core biopsy, in some cases, may lead to delay in the final diagnosis and treatment.

Determination of prognosis is an important factor in decisions of current therapy of primary BC [21, 22]. Prognostic factors are not only used to help selection of patients with aggressive disease that may benefit from adjuvant therapy but also avoid over-treatment of patients with indolent disease. The main useful prognostic factors are lymph node involvement, tumour size, lymphatic and vascular invasion, histological grade, nuclear grade and sex steroid receptor status [1, 19, 21].

Many biological parameters, such as, cDNA microarray, p53, c-erbB-2, cathepsin D and urokinase, have been evaluated as prognostic factors but with varying success [23–27]; this plus the problem of cost effectiveness associated of these novel molecular factors do not make them practical or reliable enough for routine clinical use. Serum marker concentrations, however, are more reproducible, cheaper and have been reported as having prognostic value [28]. Abnormal serum concentrations of CEA, CA 15.3 or TK1 are associated with advanced tumours. Significantly higher CEA and TK1 serum levels were found in patients with nodal involvement or in patients with larger tumours and in patients with metastases. These results are consistent with several pervious published studies [12, 27, 29, 30].

In patients with primary loco-regional BC, we found a similar relationship between abnormal tumour markers and tumour extent, with significantly higher values in patients with larger tumours or in those with nodal invasion. These results are also in line with the majority of published

research studies [23, 29, 31]; however, there are also few discrepancies [23, 32, 33] which may be the result of using different cutoff values.

The relationship between serum tumour markers and tumour size or nodal involvement seems to directly mirror the number of active malignant cells and their migration into the circulation. The most frequently reported cutoff value for CEA was 5 ng/ml, which primarily was used to detect gastrointestinal cancer [30, 34–36]. Even higher CEA serum concentrations (>5 ng/ml up to 8 ng/ml) have been reported for smokers and in cases of liver or renal disease [30, 35]. The cutoff point for this study was taken at a level matching the highest concentration detected in the control group, (8.82 ng/ml).

The relationship between tumour markers and well-known prognostic factors suggests that they are of potential prognostic value. The results presented in this study clearly showed that both tumour markers, CA15.3 and CEA, are weak prognostic factors in univariate analysis. These results are in line with several other studies [14, 18, 21, 37]; however, conversely, several other research groups have reported the absence of prognostic value especially in multivariate analysis [12, 23, 31, 33, 38, 39]. Possible explanations for the apparent discrepancy may be related to factors, such as, heterogeneity of studied groups in the number of patients and length of follow-up.

In summary, combined data of TK1, CA 15.3 and CEA serum concentrations appear to act as a useful as diagnostic indicator in the Libyan BC population. Determination of TK1 and/or CEA in primary BC is indicative of large tumour size and nodal involvement, and elevated pre-treatment. TK1 may be of value in identifying a sub-group of patients with high risk of metastases.

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Conflicts of interest None

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