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Original Research Article

The Utilization of CA15-3, CEA, EMA, and NSE Tumour Markers in the Diagnosis of Malignant Serous Effusions

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ABSTRACT

This is a descriptive study carried out in Khartoum State hospitals during the period from October 2008 to April 2011.One hundred seventy eight blood, and effusion, samples were collected from patients with accumulated effusion. Three milliliter of blood and twenty milliliter of serous effusion samples were prepared according to the Conventional Pap Smear, Enzyme-linked Immune-sorbent Assay ELISA. Malignant serous effusions were observed in 121 (62%) samples, among which 75 (62%) were in females samples and 46 (38%) were in males samples. The calculated means for all markers levels in effusion and blood samples between benign and malignant individuals showed statistical significant differences with P-value= (0.000). Except NSE blood samples level which showed no statistical significant difference with P-value= (0.665). High Pearson's Correlation was observed between CEA effusion level and CA15-3 blood level with r=0.867.

Key words: Effusion, Malignant, CA15-3, EMA, NSE, CEA.

INTRODUCTION

Serous effusion is a condition of excess accumulation of fluids in serous cavities due to different underlying pathological conditions, which vary from inflammatory conditions to primary or secondary malignancies. Some reports suggest that as many as 50% of the patients with lung cancers or breast cancers will develop pleural effusion. [1] The commonest primary malignant tumors causing metastases to the serous cavities are adenocarcinoma of the breast, lung, ovary, stomach, large intestine, pancreas, thyroid, kidney, sarcoma and malignant thymomas. [2] In Sudan the most common aetiology of accumulation of serous effusions are inflammatory conditions and metastatic malignancies, while primary malignant mesothelioma is very rare condition, few cases are reported in areas with endemic exposure to asbestos. After aspiration of effusion a set of laboratory investigations should be conducted to identify the nature of the effusion constituents, and it is chemical composition. Effusion sample must be submitted to cytopathology lab to identify its cellular component, which contributes to the identification of malignant cells involving the serous cavities, and is usually made by conventional Papnicolaou (Pap) staining technique. Ancillary techniques such as image analysis and

flow cytometry have proved useful in the distribution of benign and malignant fluids but, they are not readily available in most laboratories in the Sudan. Immunocytochemistry (ICC) is probably the most frequently used ancillary technique applied to effusion diagnosis; it can provide reliable insights into various diagnostic dilemmas in effusion cytology. So far many antibodies have been used in serous effusions to enhance the diagnosis with varying degree of efficacy. [3] The serous effusions represent a common and challenging diagnostic problem with diverse and non similar aetiology. Current methods which applied in this task are either insufficient or invasive. Immunocytochemistry plays an important role in diagnosis and patients management through its ability in determining the nature of the cells that encountered in the effusions and the discrimination between reactive and neoplastic cells that underlying the disease. In certain conditions benign serous effusions, produce reactive mesothelial cells, mimicking the morphology of the neoplastic cells, which increase the difficulties of diagnosing of metastatic carcinomas which is often associated with the reactive mesothelial hyperplasia, and the morphological variations of the mesothelial cells offer great potential for negative diagnosis, and false positive diagnosis which can have a real disastrous consequences for patients. [4] The use of immunocytochemistry (ICC), attempts to add useful diagnostic criteria especially in the differentiation between benign and malignant effusions. [5]

MATERIALS AND METHODS

This is a descriptive study aimed to assess the utilization of CEA, CA15-3, NSE, and EMA tumor markers in the diagnosis of malignant serous effusions. The study was conducted in Khartoum state hospitals, in the period from September 2008, to September 2011. One hundred and seventy eight cytological smears, blood and effusion materials were collected from patients previously diagnosed as having serous effusion. The cytological smear samples were processed and stained according to (Pap) staining method. Demonstration of tumor markers levels in blood and effusion samples were performed by a non-competitive biotin-avid in based sandwich ELISA assay (Fujirebio Diagnostics).The serous effusions specimens were collected by needle aspiration from the patients, and then it had been delivered to the laboratory. By 5ml syringe 3 ml of venous blood were also collected from the same patients. The cut-off levels of CEA, CA15-3, NSE, and EMA for differentiation of benign and malignant serous effusions were (3.95ng/ml and 5.60ng/ml), (28.50ng/ml and 20.11ng/ml), (11.20ng/ml and 6.30ng/ml) and (7.80ng/ml and 10.25ng/ml) in blood and serous effusions respectively. The primary antibody was diluted in 0.1M Bicarbonate buffer, pH 9.2, then 100ul were added to each well of the microtiter plate, then the antibody coated plate was covered with plastic wrap and incubate at RT in a humid chamber for two hours, then the plate was empted, and washed three times with Phosphate Buffer Saline (PBS). The unoccupied sites were blocked with 100ul of blocking buffer containing 100mM Phosphate Buffer Saline (PBS), for 30 minutes at room temperature, then the plate was empted and washed 3 times with the washing buffer. The antigen solution was diluted in antigen buffer (100mM Phosphate buffer, 150mM sodium chloride NaCl), then added to the plate in a volume 100ul per well, then the plate was incubated at room temperature for 54-60 minutes, then the plate was empted again and washed 3 times in washing buffer, 100ul of appropriately diluted enzyme-labeled antibody was added to each well and incubated at room temperature for 30 minutes, then the plate was empted again

and washed 3 times in washing buffer. The colour development system (substrate solution) was added, and then the absorbencies were measured at appropriate wavelength.

Statistical analysis: Analysis was performed using statistical software SPSS version 18 (Statistical package for the Social Sciences). Preliminary analyses were done such as; descriptive statistics, frequencies, cross tabulation, and T-test. Due to the lack of normal distribution among variables, non-parametric test was used for analysis of data. Mann-Whitney test was used to analyze the difference between the study populations. Correlation between tumor markers levels in blood and serous effusions was detected by Pearson's test.

Ethical Considerations: The proposal of this study was approved by the research council of Sudan University of Science and Technology - College of Medical Laboratory Science. The aims and benefits of this study were explained to the participants. Informed consents were obtained from all members who involved in this study. Health education was provided each participant.

Method of data collection: Data concerning patients involved in this study such as age, sex, and the results of effusion diagnosis were collected by check list method.

RESULTS

Two groups of individuals were classified according to the diagnostic yields of their effusions, the majority of the study populations were with malignant effusion which constituted 121 (68%), and the other group were with non malignant (benign) effusion which constituted 57 (32%), as shown in table (1).

Table (2) shows the distribution of the study population by malignancy and gender. The majority among patients with malignant effusion were female which constituted 75 (62%), while males constituted 46 (38%). In the patients with benign effusion the majority were female which constituted 32 (56.1%), followed by male which constituted 25 (43.9%).

Table (1): Distribution of the study population by malignancy

Effusion Type	Frequency	Percent
Malignant	121	68.0%
Non-malignant	57	32.0%
Total	178	100.0

Table (2): Distribution of the study population by malignancy and gender

Gender	Malignant		Non-malignant		Total	
	No.	%	No.	%	No.	%
Male	46	38.0%	25	43.9%	71	39.9%
Female	75	62.0%	32	56.1%	107	60.1%
Total	121	68.0%	57	32.0%	178	100.0%

Table (3): Distribution of the study population by malignancy and age

Age	Malignant		Non-malignant		Total	
	No.	%	No.	%	No.	%
20-35	14	11.6%	10	17.5%	24	13.5%
36-50	44	36.4%	20	35.1%	64	36.0%
51-65	42	34.7%	22	38.6%	64	36.0%
66+	21	17.4%	5	8.8%	26	14.6%
Total	121	68.0%	57	32.0%	178	100.0%

Table (4): Distribution of the study population by malignancy and effusion site

Effusion site	Malignant		Non-malignant		Total	
	No.	%	No.	%	No.	%
Pleural	109	90.1%	29	50.9%	138	77.5%
Peritoneal	12	9.9%	21	36.8%	33	18.5%
Pericardial	0	0.0%	7	3.9%	7	3.9%
Total	121	68.0%	57	32.0%	178	100.0%

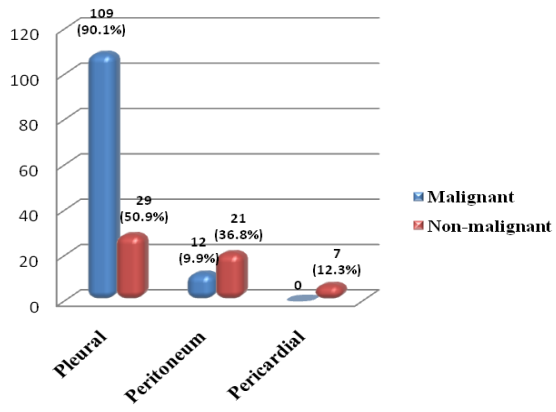

Figure (1): Distribution of the study population by malignancy and effusion sit

Tables (3) represent the distribution of the study population by malignancy and age. In patients with malignant effusion the majority of the study population were among the age group 36-50 years old which constituted

44 (36.4%), followed by the age group 51-65, 66+, and 20-35 which constituted 42 (34.7%), 21 (17.4%) and 14 (11.6%) respectively. Whilst in patients with benign effusion the majority of the study population were among the age group 51-65 years old which constituted 22 (38.6%), followed by the age group 36-50, 20-35, and 66+ which constituted 20 (35.1%), 10 (17.5%), and 5 (8.8%) respectively.

Table (5): levels of tumor markers in blood and effusion in the study population (mean ± standard error of mean SEM)

Tumor Marker		Malignant (n = 121)	Non-malignant (n = 57)
CEA	Serum	7.5093 ± .35756	3.9423 ± .27717*
	Effusion	21.5399 ±. 85919	8.9284 ± .99579*
NSE	Serum	10.2539 ± .20769	10.0777 ± .39466**
	Effusion	25.0279 ± 1.37462	11.8811 ± .97638*
EMA	Serum	64.3229 ± 2.93170	8.3567 ± .57782*
	Effusion	46.8156 ± 1.81796	16.3249 ± 1.22660*
CA15-3	Serum	71.8374 ± 2.39960	30.6749 ± 2.47532*
	Effusion	146.7860 ±4.47907	27.3818 ± 4.71131*

*P-value = 0.000 **P-value = 0.665

Table (6): Pearson's Correlation (r) of tumour markers in blood and effusion samples

Tumour Markers	CEA Blood.		CEA Eff.		NSE Blood.		NSE Eff.		EMA Blood.		EMA Eff.		CA15-3 Blood.		CA15-3 Eff.	
	r	sig	r	sig	r	sig	r	sig	r	sig	r	sig	r	sig	r	sig
CEA. Blood.	1		.677**	.000	.273**	.000	.724**	.000	.729**	.000	.632**	.000	.685**	.000	.519**	.000
CEA. Effusion.	.677**	.000	1		.061	.422	.667**	.000	**.824****	**.000**	.768**	.000	**.867****	**.000**	.703**	.000
NSE. Blood	.273**	.000	.061	.422	1		.361**	.000	.207**	.005	.185*	.013	.088	.242	.023	.762
NSE. Effusion.	.724**	.000	.667**	.000	.361**	.000	1		.690**	.000	.630**	.000	.630**	.000	.509**	.000
EMA. Blood.	.729**	.000	**.824****	**.000**	.207**	.000	.690**	.000	1		.799**	.000	**.855****	**.000**	.761**	.000
EMA. Effusion.	.632**	.000	.768**	.000	.185*	.013	.630**	.000	.799**	.000	1		.778**	.000	.735**	.000
CA 15-3. Blood.	.685**	.000	**.867****	**.000**	.088	.242	.630**	.000	**.855****	**.000**	.778**	.000	1		.739**	.000
CA15-3. Effusion.	.519**	.000	.703**	.000	.023	.762	.509**	.000	.761**	.000	.735**	.000	.739**	.000	1	

**Correlation is significant at the level 0.01 (2-tailed).
*Correlation is significant at the level 0.05 (2-tailed).

Table (4) and Fig (1) represent the distribution of the study population by malignancy and effusion site. The majority among the patients with malignant effusion were pleural effusion which constituted 109 (90.1%), followed by peritoneal effusion which constituted 12 (9.9%), whereas, no pericardial effusion was received among this group. Pleural effusion was also the major one among patients with benign effusion which constituted 29 (50.9%), followed by peritoneal, and pericardial effusion which constituted 21 (36.8%), and 7 (3.9%) respectively. Table (5) shows the levels of tumor markers in blood and effusion samples among the study population. The level of all studied markers in both blood and effusion fluid were significantly higher in malignant condition compared to benign ones except NSE. All tumor markers mean exhibited results of P-value = (0.000), except the means of NSE blood samples level in malignant and benign cases which showed P-value = (.665), hence, no statistical significant difference between them. Table (6) represents Pearson's Correlation between the different tumor markers levels in blood and effusion samples. A high correlation were observed between CEA level in effusion samples and CA15-3 level in blood samples with (r=0.867). Followed by, EMA level in blood samples and CA15-3 level in blood samples, then CEA level in effusion samples and EMA level in blood samples, with (r=0.855 and r=0.824) respectively. A relatively good

association were observed between EMA level in blood and effusion samples with (r=0.799), and CA15-3 level in blood and effusion samples with (r=0.739). Weak association were observed between CEA level in blood and effusion samples with (r=0.677), very weak correlation were observed between NSE level in blood and effusion samples with (r=0.361).

DISCUSSION

This study validates the utilization of CA15-3, CEA, NSE and EMA tumor markers in the diagnosis of malignant serous effusions. Out of 178 (100%) patients with accumulated serous effusion, malignant effusions due to metastatic malignant cells were detected in 121 (62%) patients. These findings supports a number of studies [6,7] which reported that malignant serous effusion commonly occurs as a secondary manifestation due to the metastatic involvements of malignant cells from diverse body organs to different body cavities. The present results showed that the accumulation of malignant serous effusion was detected mainly in the pleural cavity 109 (90.1%), whilst females 75 (62%) comprised the major population among the patients with malignant effusion. These results support the studies [8,9] which elucidate the increased incidence of breast cancer in females and its impact in the accumulation of malignant serous effusion especially in the pleural cavity.

Although, all tumor markers levels in blood and effusion samples showed significant differences, it could not be observed any significant difference between the blood samples of NSE level of malignant and benign individuals which indicated the futility of the utilization of NSE blood samples in the differentiation between benign and malignant serous effusion. This finding supports the study by [10] which did not observe any differences between blood NSE levels in malignant and benign conditions. In contrary a study [11] reported a much higher differences of blood NSE level between benign and malignant individuals. This diversity can be attributed to the different pathological conditions underlying the causes of malignant effusion. During this study a high correlation between CEA effusion samples level and CA15-3 blood samples level was observed. This finding supports the studies [12,13] which reported that (CEA and CA15-3) are among the best tumor markers for differential diagnosis of malignant serous effusions in accordance to their high correlation in their levels in effusion and blood samples respectively.

CONCLUSIONS AND RECOMMENDATIONS

On the basis of this study and review of other studies, it could be concluded that:

1. Malignant serous effusion was frequently observed as a secondary manifestation of a metastatic cancer from different body organs.
2. Analysis of EMA and CA15-3 levels in blood and effusion samples found to be useful diagnostic tool in the differentiation of malignant serous effusions from the other pathological conditions underlying the accumulation of effusions.
3. NSE had the minimal role among the markers used in the differentiation between benign and malignant cells present in the effusions.
4. It is recommended that EMA and CA15-3 tumor markers routinely used in the laboratory to differentiate between malignant and benign serous effusion.
5. Validated panels of tumor markers are necessary to be used because no single antibody proves or rules out the malignancy.
6. It is recommend that additional ancillary techniques such as flow cytometry, automated image

morphometry should be incorporated in the diagnosis of doubtful effusions so as to help solving the everlasting dilemma of the malignant effusions.

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