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CYTOMORPHOLOGICAL AND IMMUNOHISTOCHEMICAL PATTERNS OF PLEURAL EFFUSIONS IN SUDANESE PATIENTS PRESENTED TO TEACHING HOSPITALS IN KHARTOUM STATE

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ABSTRACT

This is a prospective descriptive cross sectional study. The study is designated as a hospital based study conducted in cytology section. Two hundred and forty four cytological specimens of pleural effusion were collected. Cell blocks and smears were processed and cytological and ICC stains were applied. Haematoxylin and Eosin, Pap stains were used for cytological examination. A panel of six antibodies consisting of cytokeratin 5/6, Calretinin, CA125, CEA, Thyroid transcription factor-1, and CD-10 were used. Patients participated on study population consisted of 136 males and 108 females. There were 244 patients with pleural effusion, 136 males and 108 females. The mean age was 47 with standard deviation 17.4, the minimum age was 1 year and the maximum was 80. According to the age we classified the patients into four groups of ages**.** There is a pivotal role of cytological diagnosis in detection of different pathological conditions of pleural effusions. Immunocytochemistry is an effective method that has considerable importance in improving the diagnostic accuracy of conventional cytology of pleural effusion. Cancer antigen 125 (CA 125) is reliable and effective marker for the identification of malignant cells in pleural effusions especially in suspicious cases Regardless of the histological types of malignancies. CEA was found to be a useful marker in the differentiation of metastatic adenocarcinoma in pleural effusions.TTF-1 was found to be a useful marker in the differentiation of metastatic adenocarcinoma in pleural effusions particularly in case of lung cancer or cancers of gastrointestinal tract.

KEYWORDS: Cancerous cells, Cytomorphological, Effusions, Immunocytochemical.

INTRODUCTION

Effusions are pathological processes characterized by the accumulation of fluid other than blood in excess of the normal amount in the three serosal body cavities, the pleural, peritoneal and pericardial. Effusions are designated according to their location as pleural, peritoneal and pericardial.[1]

Serous effusions accumulate in body cavities with different causes and different degrees of clinical severity i.e. acute or chronic inflammation or cardiac insufficiency and malignancies. Primary or metastatic cancers are the most frequently encountered causes.[2]

Cytological evaluation of pleural effusion to detect the presence of cancerous cells has been done from long time in the diagnosis and final staging of cancers, but this poses difficulties on pathologists. So immunocytochemistry (ICC) is required to elucidate the etiology of the atypical cells. For (ICC) the cellblock technique or paraffin embedding of sediments of fluids is preferable more than conventional smearing technique.[3]

In the pleural effusion the accurate identification of cells as either malignant or benign reactive mesothelial cells is a diagnostic problem in conventional cytological smears. The lower sensivity is due to bland morphological features of cells, overlapping of cells, cell loss, and changes due to different laboratory processing methods.[4]

In the presence of pleural effusion of unknown origin, it is mandatory to define with the highest accuracy the nature of the lesion and differentiate benign lesions from malignant mesothelioma (MM) and cancer metastases[5,6]

MATERIAL AND METHODS

The study design was a prospective descriptive cross sectional one. The study was designated as a hospital based study conducted in cytology section, constructed to evaluate the immunocytochemical and cytological patterns of pleural effusions. It was conducted in certain specialized hospitals in Khartoum state namely (Khartoum teaching hospital (KTH), and Radiation & isotopes center Khartoum (RICK).It was conducted on 244 Sudanese patients admitted to the hospital with pleural effusion. Patients who proved to be free from pleural effusions or those who didn't give their consent were excluded whereas; the included one were those with serous effusions from pleural cavity at any age who have given informed consent.

ETHICAL CONSIDERATIONS

The aim of the study was fully explained to the patients, and their consent to participate was taken. Samples were taken from patients who agreed to participate and after having their informed consent. The results of pleural effusion were revealed and discussed with the patients. It has also been approved by the ethical committee of Omdurman Islamic University (ANNEXURE-1).

Sample collection: Samples were taken from patients with pleural effusion to prepare smears and cell blocks for cytology and immunohistochemistry diagnosis. Fresh pleural fluid sample received (firstly submitted for macroscopic examination for physical characteristics. 20 ml of pleural fluid was taken and divided into two parts. The first part of 10 ml was used for conventional smear preparation. The fluid was centrifuged at 2500 rpm for 15 minutes and 10 smears were prepared from the deposits. One smear immediately fixed in 95% ethanol and stained with Papanicolaou's staining technique. The second smear was air dried and stained with May-GrunwaldGiemsa. The remaining smears were fixed in absolute acetone for one hour and then wrapped well in foil and then placed in freezer at – 20. (This necessary to preserve the immunoreactivity for many months before immunocytochemical staining techniques. The second part of 10 ml pleural fluid was used for cell block technique. The 5 ml of ethyl alcohol and 10% formalin in 1:1 proportion was added and fixed for 1 hour. Then mixture was agitated for uniform the fixation of the material. Then the specimens were centrifuged at 3000 rpm for 5 minutes. The supernatant was decanted and sediment drained off by inverting tube over Whatman filter paper. To the sediment 1:1 mixture of ethanol and 10% eosin tinted formalin was added and kept for fixation for 24 hours. Then after discarding the supernatant fixative, the pellet formed was removed with a pointed spatula and placed on top of the lens paper inside the tissue cassette and processed for paraffin wax embedding technique.

Reporting of results for cytological diagnosis: All specimens were signed-out using a descriptive format. All specimens were categorized as negative, suspicious, reactive changes or malignant followed by a description of the cellular abnormality.

Immunohistochemistry Staining Method: Sections (5 µm) of cell blocks from pleural effusions were transferred to an adhesive-coated slide with ploy L-lysine. De-paraffinizied sections were manuallystained after heat-induced epitope retrieval (treat the sectios for 3 minutes at 110^0C in citrate buffer with PH 6.0) using a standard multilink detection kit (Dako detection kit) including endogenous peroxidase block, block of nonspecific binding, horse radish peroxidase, diaminobenzidene as chromogen and haematoxyl in. Sections were immunostained with six primary poly clonal antibodies.Paraffin sections of the formalin- fixed block were immunostained with Dako flex ready to use system of antibodies which has been accepted by leading experts in the field. Firstly sections were stained with Anti-Calretinin (clone: Dak- Calret 1) the staining reaction took place in nucleus and cytoplasm the appropriate positive control used which is appendix. Thirdly sections were stained with C K 5/6 (cloneD5/16 B 4) the staining reaction took place in cytoplasm, the appropriate positive control used which is tonsil. Then sections were immunostained with CEA (clone II-7) the staining reaction took place in membrane and cytoplasm, the appropriate positive control used which is tonsil. Then sections were stained with TTF-1(clone 8G7G3/1, Dako) the staining reaction took place in nucleus, the appropriate positive control used which is thyroid. Finally sections were stained with CD-10**.**

RESULTS

In this prospective study, there were 244 patients with pleural effusion. Of the total number, there were 136 males and 108 females. The mean age was 47 with standard deviation 17.4, the minimum age was 1 year and the maximum was 80 (table 1). According to the age we classified the patients into four groups of ages. The ages of group 0-20 was recorded 14 patients (5.7%), group 21-40 was 89 (36.5), group 41-60 was 85 (34.8%) and group 61-80 was 56 (23%) (table 2). The patients of this study were originated from different tribes, races and places. The majority of these tribes from north were reported in 77 (31.6%), from west 61(25%), center 39 (16%), south 17(7%), 50 (20.5%) patients were documented as unknown tribes (Table 3).The results of cytological diagnosis of pleural effusions were revealed of 244 patients negative in 86 (35.2%), chronic inflammation in 52 (23.2%), acute inflammation in 70 (28.7%) and malignancy in 36 (14.8%) (table 4).

Table: 1. Distribution of gender among study population

Gender	Frequency	Percent
Male	136	55.7%
Female	108	44.3%
Total	244	100.0%

Table: 2. The distribution of study population among age groups

Age groups	Frequency	Percent
0-20	14	5.7%
21-40	89	36.5%
41-60	85	34.8%
61-80	56	23.0%
Total	244	100.0%

Table 3. The residence of the study population

Tribe origin	Frequency	Percentage
Unknown	50	20.5
North	77	31.6
West	61	25.0
Center	39	16.0
South	17	7.0
Total	244	100.0

Table: 4. The cytological diagnosis of the study population

Cytological diagnosis	Frequency	Percentage
Negative	86	35.2%
Chronic inflammation	52	23.2 %
Acute inflammation	70	28.7%
Cancer	36	14.8%
Total	244	100.0%

Table: 5. The relationship between the Cytological diagnosis with the detection of AAFB by Z.N stain.

Cytological diagnosis	AFB		Total
	positive		negative
Negative	0	86 (35.2%)	86 (35.2%)
Chronic inflammation	39 (16%)	13 (5.3%)	13 (5.3%)
Acute inflammation	1 (0.4%)	69 (28.3%)	69 (28.3%)
Cancer	0	36 (14.7%)	36 (14.7%)
Total	40 (16.4%)	204 (83.6%)	204 (83.6%)

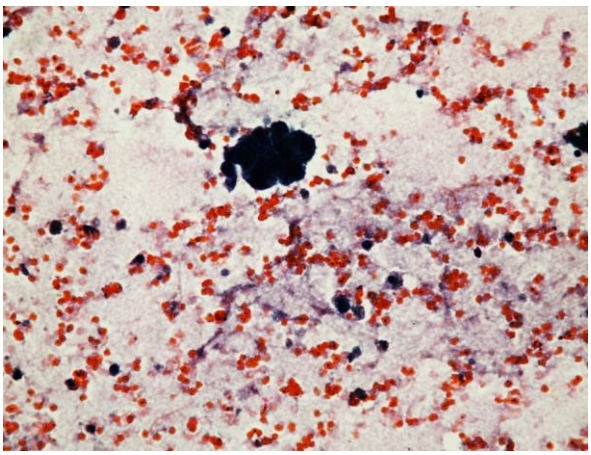

Figure. 1 Lung cancer in pleural effusion.

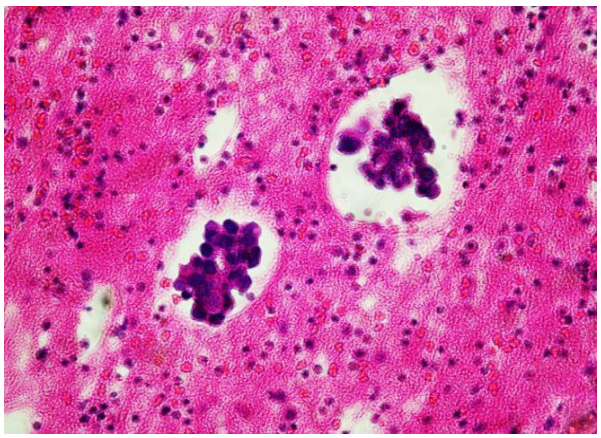

Figure. 2 Lung cancer in pleural effusion (cellblock) (H&E).

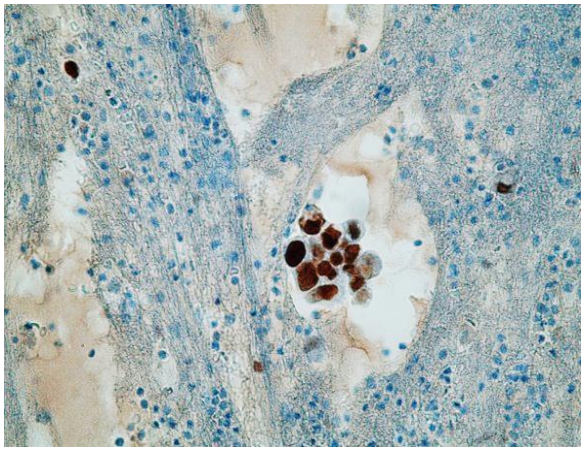

Figure. 3 Lung cancer in pleural effusion (TTF-1).

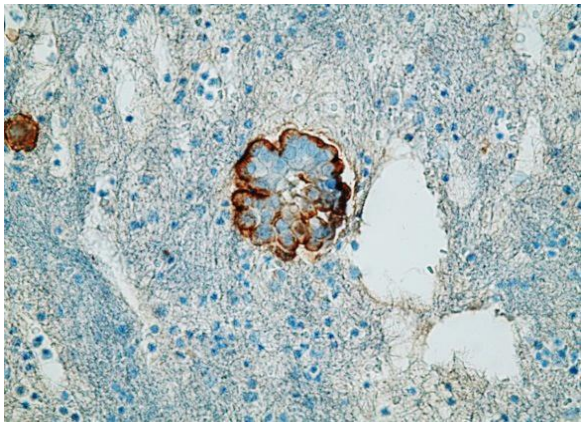

Figure. 4 Lung cancer in pleural effusion CA125.

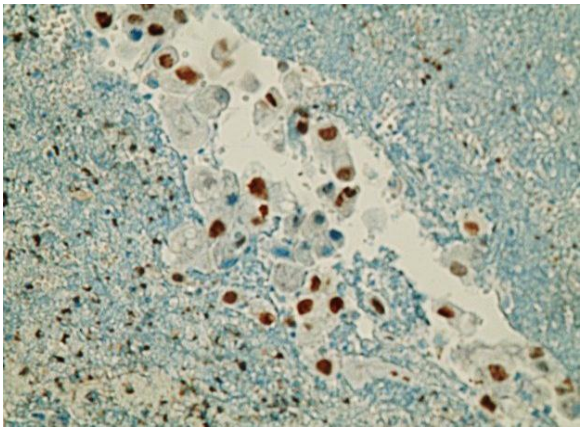

Figure. 5 Gastric adenocarcinoma TTF-1.

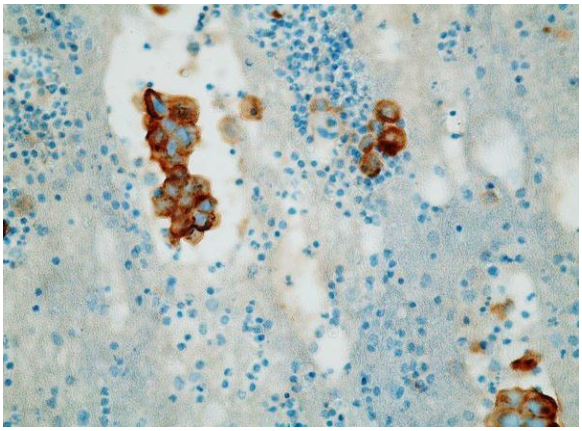

Figure. 6 Gastric Adenocarcinoma CA125.

DISCUSSION

In this prospective study of 244 patients with pleural effusion. The included patients were 136 males and 108 females (table 1). The mean age was 47 with standard deviation 17.4, the minimum age was 1 year and the maximum was 80 (table 2). The results of cytological diagnosis of pleural effusions were revealed of 244 patients negative in 86 (35.2%), chronic inflammation in 52 (23.2%), acute inflammation in 70 (28.7%) and malignancy in 36 (14.8%) (table 4).This finding was consistent with etiologies of pleural effusions reported consecutively by Sahn SA(2006) as congestive heart failure, bacterial pneumonia and malignancies.[7] ZN stain for AAFB was detected positive in 40 (16.4%) cases, 39 of chronic inflammation and one in acute inflammation and negative in 204 (83.6) cases of the patients (table 5) with significant P.value= 0.000**.** This result is in agreement with the results of Ferrer (1997) who found that the frequency of pleural effusion in TB patients was approximately 31%.[8]

According to the age and cytological diagnosis, the elderly patients showed highest incidence of malignancies 18 cases in range of 41-60 years and 13 cases in range of 61-80 years while the incidence of malignancies reported only in five below 40 years. The difference of cytological diagnosis concerning range of age was statically significant at value of probability =0.024. This result is in agreement with the results of Beers and Abramo (2007) who reported that pleural effusions usually occur in adults. But they documented that the incidence of pleural effusions appear to be increasing in children specially in underlying pneumonia.[9]

The association between gender and cytological diagnosis recorded statistically insignificant difference between males and females with p.value = 0.687 and this finding also consistent with that of Heffner JE (2008) who documented that in general the incidence of pleural effusion is equal between two sexes, but Heffner JE (2008) reported that the malignant pleural effusions are more frequently in women certainly with breast and gynecological malignancies.[10]

The expression of CA125 was positive in 36 cases (14.8%) of study group and this positive reactivity was shown only in malignancies which comprises 30 (83.33%) of adenocarcinoma and 6 (16.66%) of malignant mesothelioma this result is in agreement with Choi WI, et al (2013) who documented that the expression of CA 125 is significantly higher in the malignant group than the all benign groups. They also reported that there was no significant difference of expression of CA 125 between histological types of malignancies.[11]

The antibody of CEA was detected in 30 (83.3%) out of 36 cases of malignancies, with 100% of specificity and sensitivity for adenocarcinomas types. All this malignancies are of adenocarcinoma type from different organs of the body this result was in consistent with King J E, et al (2006) and Takeshi M et al (2007) who they documented that the CEA was sensitive and specific for adenocarcinoma with 97%.[12] 56 But this result was in contrast with Murugan P etal (2009) who reported that the CEA and Vimentin had unsatisfactory value as individual diagnostic markers.[13]

Positive reaction of CK5/6 was detected in 7 (19.44%) out of 36 cases of malignant smears of pleural effusion 6 (16.66%) of them were diagnosed as malignant mesothelioma and one (2.77%) as renal clear cell carcinoma. The specicicity of Ck5/6= 85.7% and sensitivity = 100% for malignant mesothelioma. All of the remaining malignant pleural effusions were adenocarcinomas of different sites that showed negative reactivity. The statistical difference between the groups of cytological diagnosis by using CK5/6 expression was significant with P.value =0.000 and this finding is consistent with the results of P.W shield, K. Koivurinne (2008) who reported that the calretinin and CK5/6 are useful markers for malignant mesothelioma in effusion specimens with percentage of positive detectection of these markers 97% of both.[14]

Positive reaction of Calretinin was detected in 6 (16.66%) cases of malignant smears of pleural effusion 6 of these maliganancies were diagnosed as malignant mesothelioma. The all remaining malignant pleural effusions of adenocarcinoma were shown with negative immunostainingreactivivty. The statistical difference between the groups of cytological diagnosis by using Calretinin expression was significant with P.value =0.000 and this finding are consistent with results of P.W shield, K. Koivurinne (2008). They reported that the The calretinin and CK5/6 are useful markers for malignant mesothelioma in effusion specimens with percentage of positive detectection of these markers 97% of both.[14]

The expression of TTF-1 was positive in 14 (38.88%) out of 36 of malignant cases and negative in 22 (61.1%) remaining cases of malignancies. The difference in expression between cytological diagnosis is statistically significant at P. value =0.000. The Immunocytochemical reactions of TTF-1 were shown positive in 14 cases of adenocarcinoma (9 lung origins, 4 GIT and 2 liver). The negative reaction was found in 16 cases of adenocarcinoma beside all the 6 cases of malignant mesothelioma. This result is in agreement with finding of Takashi M et al, 2007 who found that TTF-1 was sensitive for adenocarcinoma with 92.4% and specific with 100%. They also found that TTF-1 is negative for Malignant mesothelioma.[15]

The expression of CD-10 documented positive in 1 (2.77%) out of 36 of malignancy and negative in 35 remaining cases of malignancies, also did not expressed in the other cytological diagnosis (table 13). The difference in expression between cytological diagnosis is statistically insignificant at P. value =0.496. The reactivity of CD-10 was shown positive in one case only of Adenocarcinoma of renal cell carcinoma while negative in remaining cases. This result was agree with Dabb D et al 2006 who documented that CD-10 antibody is useful for the identification of Burkitt's lymphoma, follicular lymphoma except grade III, precursor B-cell acute lymphoblastic leukemia, and clear cell renal cell carcinoma.[16]

CONCLUSIONS

There is a pivotal role of cytological diagnosis in detection of different pathological conditions of pleural effusions and immunocytochemistry is an effective method that has considerable importance in improving the diagnostic accuracy of conventional cytology of pleural effusion.

RECOMMENDATIONS

All laboratory investigations should be applied for pleural fluid samples from pleural effusions of unknown cause including cytological, Immunocytochemical, microbiological and chemical tests. Cytological examination of pleural effusion should be considered as primary monitoring tools for diagnosis of different diseases.

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