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CYTO-MORPHOLOGICAL PATTERN OF PLEURAL EFFUSION AMONG SUDANESE PATIENTS

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ABSTRACT

This is a descriptive study carried out in Omdurman Teaching Hospital during the period from January 2013 to April 2015. The study aimed at assessing the cytomorphological pattern of pleural effusions and screening for malignant changes in patients with accumulated pleural effusions. 50 cytological samples were collected from patients with pleural effusions and processed then microscopically examined. The cytological smears revealed the following findings: Malignant cells "adenocarcinoma type" were found in 3(6%) samples, among which 2(4%) were in females samples and 1(2%) in male sample. However, most of the specimens showed dense inflammatory infiltrate "acute, chronic", which were detected in 40(80%) of the study subjects. Neutrophils and lymphocytes found in 40(80%) samples, histocytes in 39(78%) samples, plasma cells in 5(9%) samples, and eosinophils in only 3(6%) samples. On the basis of this study, cytomorphological assessment of pleural effusions is essential for diagnosis and patients management, nevertheless, immunocytochemistry will improve the diagnostic sensitivity and specificity in this setting.

KEY-WORDS: Pleural Effusion, Exudates, Transudates, Adenocarcinoma, Cytology.

INTRODUCTION

Cytology is the study of cells that either exfoliated from epithelial surfaces, e.g. (vagina, oral mucosa) or cells that removed by physical means from different part of the body, or

cells that obtained from body cavities e.g. (Pleural, Peritoneum, Pericardium). (Culling, 1974).

Numerous sophisticated methods of cytopreparation are available. The selection of a method is largely based on the specimen type. Large variety of cytological specimen can be received in cytology laboratory. These specimen can be collected by different procedures depending on the site of collection. Papanicolaou staining procedure has been the mainstay staining technique used in routine cytological preparation (Bancroft, et al, 1996). Cytology gained an important role in the differential diagnosis of diseases, and with the advent of fine-needle aspiration technique. Cytology has become a vital tool in diagnosis and patient management. Methods of preparation of cytology are rather simple than in histology. The cytological diagnosis is largely based on both alterations of cytoplasmic and nuclear features. Therefore, nucleus, cytoplasm, and the back ground staining are the corner stones of the diagnosis through out the cytological preparation.

The cytological material must be adequate and the cytoprepartory technique must be ideal to achieve a definitive diagnosis. Application of the cytological technique in immunocytochemistry and flow cytometry increased the accuracy in the differentiation of tumors. And aid in the DNA analysis of neoplastic cells and determination of the cell marker in hemopoeitic neoplasm. Cytological data on 1468 patients with histological and clinical histories of breast pathology were evaluated. Cytological sensitivity in breast carcinoma was 92.3% and the specificity was 98.6% (Long, et al, 1992).

Pleural effusion cytopathology has important role in the differential diagnosis of diseases accompaniment the accumulation of fluids in the pleural cavity. Its frequently the diagnostic method of choice and serves as a basis for decision about therapy. The most common diseases associated with the pleural effusions in Sudan are the inflammatory conditions and metastatic malignant cells. Differential diagnosis of pleural effusion through it is cyto-morphologcial significance play an essential role in the management of its etiology. Because the causes of pleural fluid range from benign to malignant, samples of fluid are usually taken. Thoracentesis is performed to obtain a diagnostic sample of pleural fluid and to drain large amounts of fluid for therapeutic purposes. Aspiration of the pleural cavity is a simple and relatively non-invasive to achieve diagnosis. As correct identification of tumors is crucial for therapeutic purposes, it is of great importance to assess the cytomorphology and the cause of pleural effusion accurately. In many instances, the effusion may be the presenting feature of

malignant disease, hence the diagnosis should not delayed as this may increase morbidity and mortality.

MATERIALS AND METHODS

2-1- Study design

This is a descriptive study to assess the cyto-morphological pattern of pleural effusions, using the conventional papanicolaou stain. The study was conducted in Omdurman Teaching Hospital, during the period from January 2013, to April 2015.

2-2- Study population

Fifty cytological materials were collected from patients with previously diagnosed as having pleural effusion, and they processed and stained accordingly to pap stain method.

2-3- Samples collection

The cytological specimens were collected by needle aspiration from fifty patients with pleural effusion manifestations and regardless of any other associated diseases. The pleural effusion were aspirated and sent to the laboratory.

2-4- Sample processing

Pleural effusion require concentration of cells prior to transfer to glass slide. Centrifugation is the standard technique, the samples centrifuged for 1500 rpm for 10 minutes. Following centrifugation, the supernatant poured off, and by the aid of a pipette the remnant suspended cells were carefully removed, then a single small drop placed towards the end of a clean, labeled glass slide and spreaded rapidly. Cells in effusion degenerate rapidly. Therefore smear should be prepared and fixed immediately. The smear fixed by 95% ethanol, for 15 minutes. If there is any delay, the specimen should be fixed in 95% ethanol, for 15 minutes, and refrigerated at 4ºC until smear can be prepared. The smear stained by papanicolaou stain in which the nucleus stained by Harries hematoxlyin, while the counter stain will be EA50 and O.G6 to stain the cytoplasm and the smear background. Quality control steps were adopted in all procedures.

2-5- Staining procedure

All samples were stained by papanicolaou staining method, The alcohol fixed smears were hydrated in descending alcohol concentration of 95% through 70% to distilled water for 2 minutes in each stage. For staining the nuclei, the smears were treated with Harri's

Haematoxylin for 5 minutes, rinsed in distilled water and differentiated in 0.5% aqueous hydrochloric acid for 10 seconds. To remove the excess stain particles, and immediately rinsed in distilled water to stop the action of decolourization. Then the smears were blued in alkaline water for 4 seconds, or in running tap water for 10 minutes. And dehydrated in ascending alcohol concentration from 70% through two changes of 95% alcohol for 2 minutes for each change. For the cytoplasmic staining, smears were then treated with O.G6 for 2 minutes, then rinsed in 95% alcohol and treated with EA50 for 3 minutes. Finally, smears were dehydrated in 95% through absolute alcohol, cleared in xylene, and mounted in D.P.X.

2-6- Results interpretation

Smears were ready to be examined by light microscope. For the assessment of different cells appearance which encountered in the smears, including malignant cells. The characteristics of these cells were compared with the illustrated photographs in (Atlas of difficult diagnosis in cytopathology), (Barbara, 2001). All smears were examined by 3 independent investigators.

RESULTS AND DISCUSSION

In this study, cytological changes in pleural effusion were assessed. These changes were assessed among 50 patients with pleural effusion. Their ages ranging from 30 to 72 with a mean age of 52 years old.

As shown in Table 1. Fig 1, the majority of the study population were among the age group 45-60 years old, which constitute 20(40%), followed by the age group 30-45 , 60+ which constitute 18(36%) , 12(24%) respectively.

Table 2. Fig 2, represent the description of the study population by the gender , its apparently that, the majority of the study population were males, constituted 26(52%), while the females constitute 24(48%).

As shown in Table 3. Fig 3, concerning the description of the study population by pathology and gender. The majority of the malignancy cases were found among the females, constituting 2(4%), and only one case among males 1(2%). However, the majority of the inflammatory conditions were detected among males, which constituted 22(44%), while in females were 18(36%). Other pathological conditions were increasingly found among the females which constituted 4(8%), while the males were 3(6%).

Concerning Table 4. Fig 4, which showing the description of the study population by age and gender. The majority of the study population were among the age group 45-60, in which there is similar number of males, 13(26%), and females constitute 11(22%), followed by the age group 30-45 among which females constitute 8(16%), then the age group 60+ they were males comprising 7(14%), followed by the age group 30-45 males constitute 6(12%), and finally the age group 60+ females constitute 5(10%).

As shown in Table 5. Fig 5, malignancy cases among the study population were equal in north, south, and east tribes, which comprises 1(2%) for each, and non in the west tribes. The majority of study population in the inflammatory conditions were reported by the north tribes which constitutes 16(32%), followed by the east, south and west tribes which constitutes 9(18%), 8(16%), 6(14%) respectively.

Concerning other pathological conditions the majority of these conditions were detected among the north tribes which comprises 3(6%), followed by the east and south tribes with equal ratios of 2(4%).

As shown in Table 6. Fig 6, which represent the description of the study population by pathology and age. The distribution of malignancy cases were equal in all age groups which comprises 1(2%). Most inflammatory conditions were among age group 45-60 which constituted 17(34%), followed by the age group 30-45 and 60+ which comprises 14(28%), 9(18%) respectively. Furthermore the majority of other pathological conditions were found among the age group 30-45 which constituting 3(6%), followed by the age group 45-60 and 60+ with equal ratios of 2(4%).

As shown in Table 7. Fig 7, the majority of the inflammatory cells infiltrated were neutrophils and lymphocytes constituting 40(80%), followed by histocytes, plasma cell and eosinophils, constituting 39(78%), 5(9%), 3(6%), respectively.

Table 1 Figure 1: Description of the study population by age

Age	Frequency	Percent	Cumulative percent
30-45	18	36	36
45-60	20	40	40
60+	12	24	100
Total	**50**	**100**	

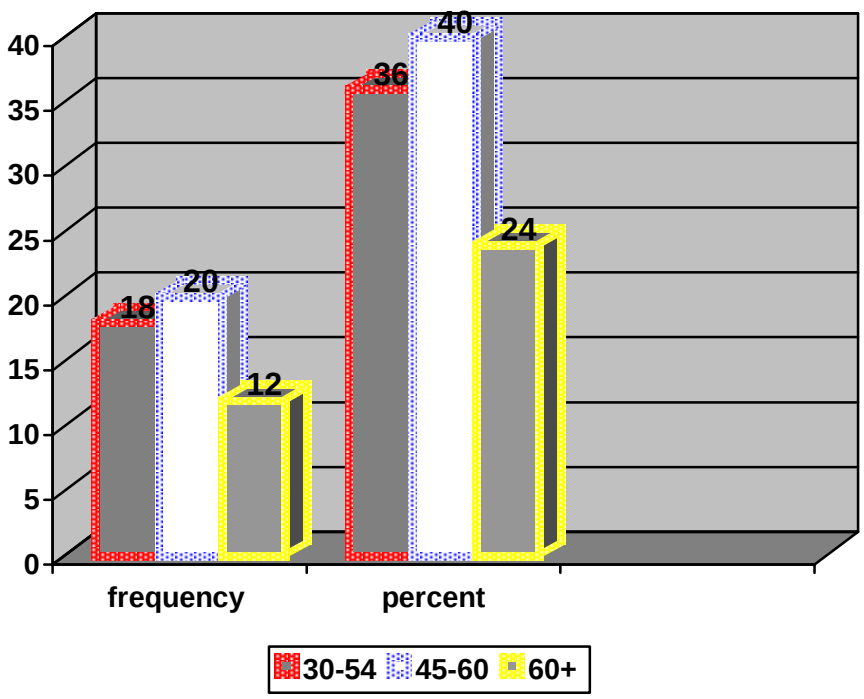


Table 2 Figure 2: Description of the study population by gender

Gender	Frequency	Percent
Male	26	52
Female	24	48
Total	**50**	**100**

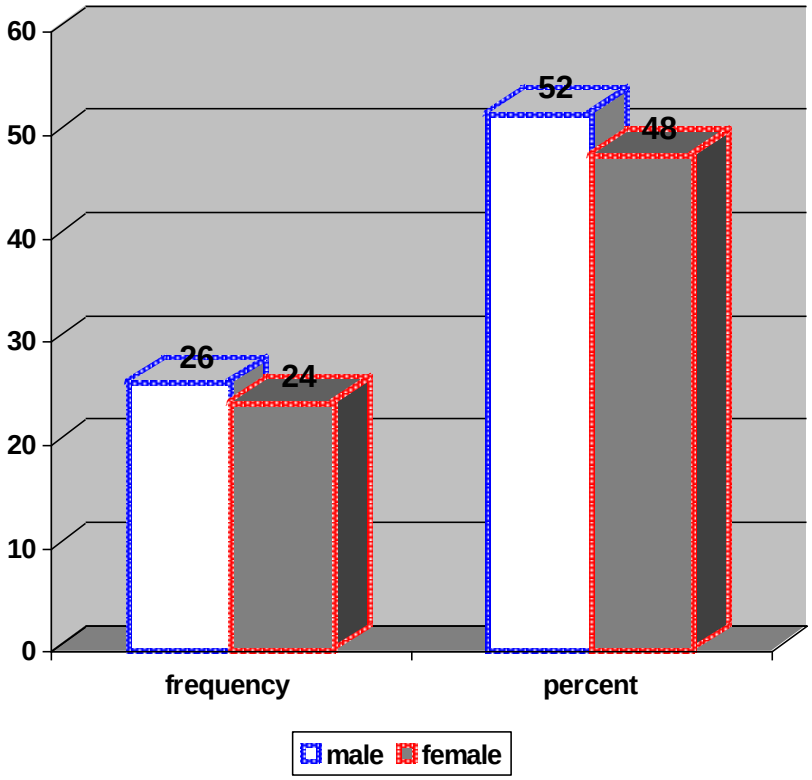


Table 3 Figure 3: Description of the study population by pathology and gender

Gender	Malignancy		Inflammatory conditions		Others*		Total	
	No	%	No	%	No	%	No	%
Male	1	2	22	44	3	6	**26**	**52**
Female	2	4	18	36	4	8	**24**	**48**
Total	**3**	**6**	**40**	**80**	**7**	**14**	**50**	**100**

*others : congestive heart failure, connective tissue diseases (systemic lupus erythematosus, rheumatoid diseases)

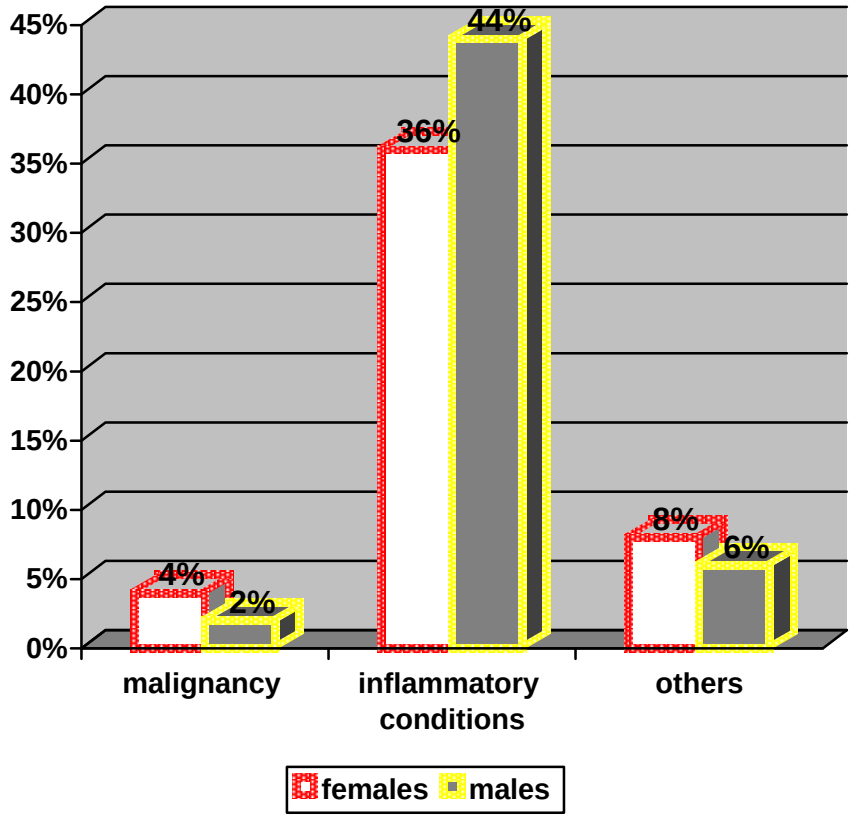


Table 4 Figure 4: Description of the study population by age and gender

Age	Gender				Total	
	Male		Female			
	No	%	No	%	No	%
30-45	6	12	8	16	14	28
45-60	13	26	11	22	24	48
60+	7	14	5	10	12	24
Total	**26**	**52**	**24**	**48**	**50**	**100**

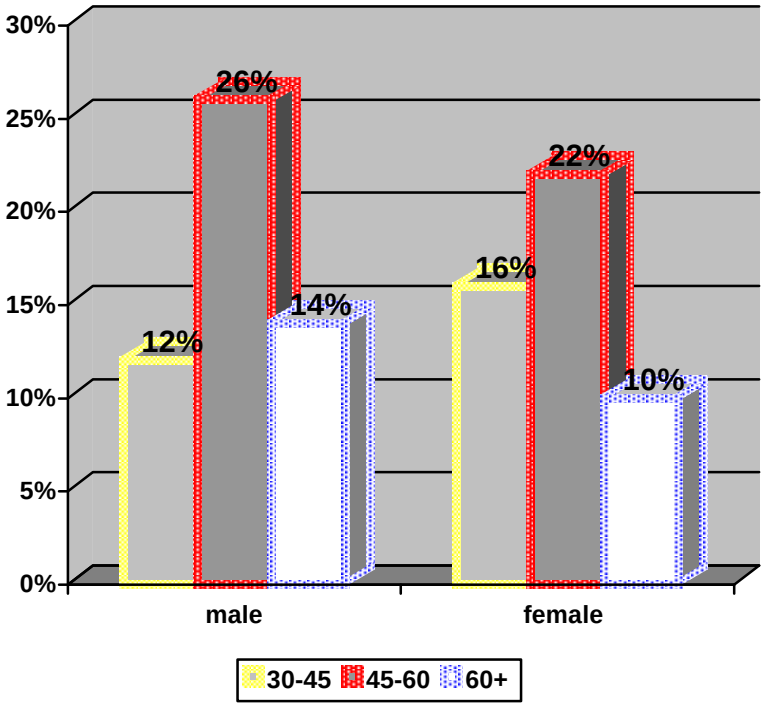


Table 5 Figure 5: Description of the study population by pathology and tribe

Tribe	Malignancy		Inflammatory conditions		Others		Total	
	No	%	No	%	No	%	No	%
North	1	2	16	32	3	6	**20**	**40**
East	1	2	9	18	2	4	**12**	**24**
South	1	2	8	16	2	4	**11**	**22**
West	0	0	7	14	0	0	**7**	**14**

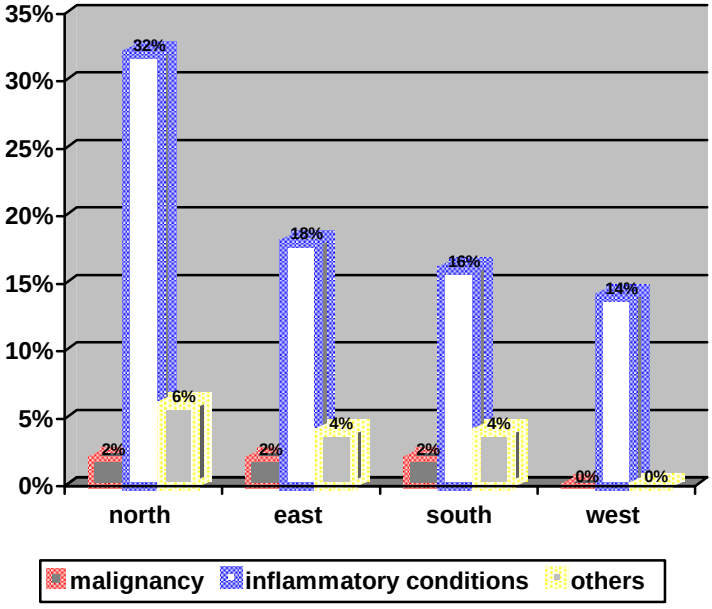


Table 6 Figure 6: Description of the study population by pathology and age

Age	Malignancy		Inflammatory conditions		Others		Total	
	No	%	No	%	No	%	No	%
30-45	1	2	14	28	3	6	**18**	**36**
45-60	1	2	17	34	2	4	**20**	**40**
60+	1	2	9	18	2	4	**12**	**24**
Total	**3**	**6**	**40**	**80**	**7**	**14**	**50**	**100**

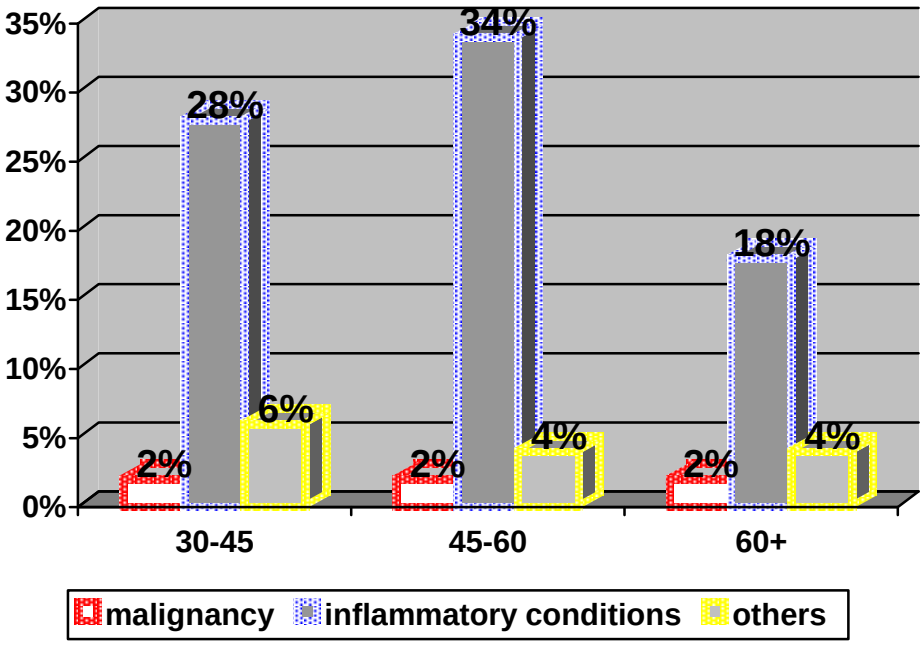
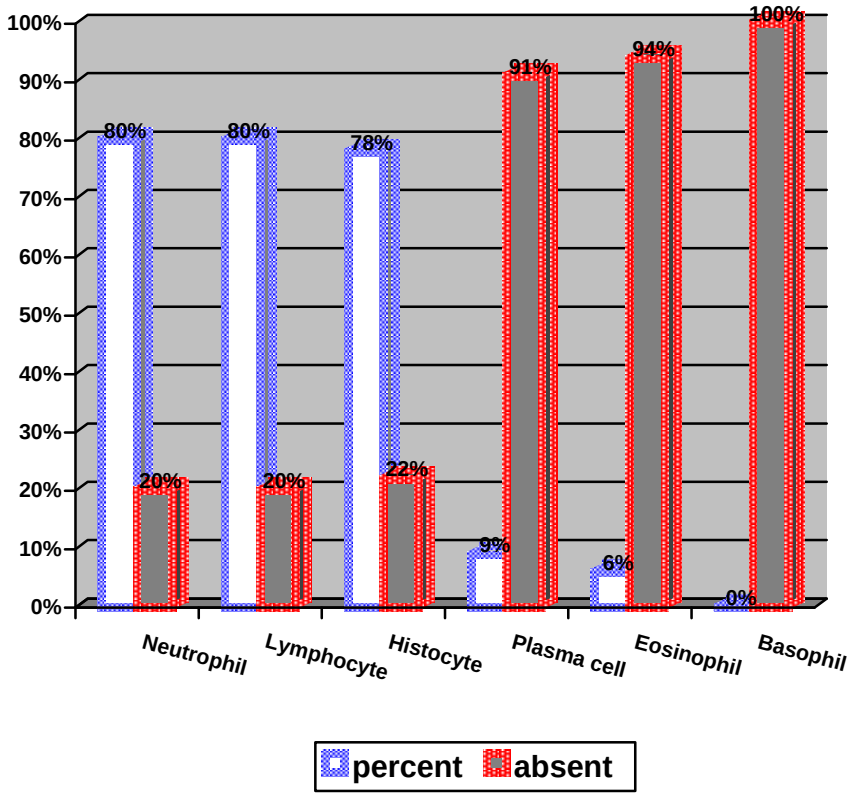


Table 7 Figure 7: Description of the study population by inflammatory cells:

Inflammatory cell	Present		Absent	
	No	%	No	%
Neutrophil	40	80	10	20
Lymphocyte	40	80	10	20
Histocyte	39	78	11	22
Plasma cell	5	9	45	91
Eosinophil	3	6	47	94
Basophil	0	0	50	100



DISCUSSIONS

This study assessed the cyto-morphological pattern of pleural effusions in patients with pleural effusions manifestations.

Of the 50(100%) patients with accumulated pleural effusions, metastatic malignant cells were detected among 3(6%). These findings supporting a number of studies by Hsu. (1987), and Bower. (1997), who reported that, pleural cavity is the commonest site for metastatic cancers from different body organs.

However, all these cancers were of adenocarcinoma type. These results supporting many studies by, Smith, et al.(1989) and John. (1995), who reported that, the most commonest tumors shows involvement with the pleural serous membrane are the metastatic adenocarcinomas of breast , ovary and gastro intestinal tract "GIT".

Our results show inflammatory infiltrate in 40(80%) of the study subjects, these results supporting the study by Koss. (1992), who reported that, major pathological involvements with the accumulation of fluid in the pleural cavity were the inflammatory conditions. The findings is furthermore supporting the study by Guzman, et al. (1991) who found that, pleural effusions were exudative in 125(76%) of the study subjects.

Among these cellular inflammatory infiltrate, neutrophils and lymphocytes were the most predominant cells, found in 40(80%) samples from the total study subjects.

The presence of these cells in such high number emphasizing the involvement of the inflammatory conditions with the fluid accumulated on in the pleura. These results is supporting the results by Goodman, et al (1997), who elucidated from reviewing of 210 samples of pleural effusion that, the presence of lymphocytes and neutrophils in 160 , 145 samples respectively were due to the inflammatory processes that caused the effusion.

Histocytes were found in 39(78%) samples, these findings is supporting the study by Orles, et al, (2000), Spriggs. (1989) they showed that, histocytes were among the predominance cells infiltrate in pleural effusions associated inflammatory conditions.

The appearance of the polymorph nuclear neutrophils and histocytes in pleural effusions indicate an inflammatory process, as well as the possibility of malignancy, This out come is supporting the study by Jean, et al (1990) who examined 262 pleural effusions, and showed that neutrophils and histocytes were present in benign effusions as well as in malignant ones.

The study showed little variations between the numbers of males and females with pleural effusions manifestations, males were 26(52%), females were 24(48%). These results is supporting a numbers of studies, Naylor.(1990) and Boddington, et al.(1971) who showed that there were no significant relations between the plural effusions and gender, since the effusion were found in almost similar percentage between males and females . The result is furthermore supporting the study by Gad, et al (1975) who confirmed that lung carcinoma, malignant mesothelioma (MM), breast and ovary adenocarcinomas accounts for relatively large numbers of cases among both sexes.

CONCLUSIONS AND RECOMMENDATIONS

On the basis of this study and review of other studies, we conclude that, effusion cytopathology is essential tool in trending towards making a firm diagnosis.

Malignant cells with characteristics of adenocarcinoma features were more frequently observed. The most encountered inflammatory cells infiltrates in the pleural effusion were neutrophils, lymphocytes, histocytes and plasma cells.

We recommend cytological investigations should be performed for any pleural effusion, as well as Z-N stain which will be of great value in cases of inflammatory conditions. In parallel of cytological stains other techniques e.g. immunocytochemistry, should be applied in order to improve the diagnostic sensitivity and specificity in this setting.

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