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Cyto-Morphological Pattern of Tuberculous Lymphadenitis among Sudanese Patients

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Abstract: ***This is a descriptive study carried out in Khartoum state hospitals, during the period from January 2013 to April 2014. The study aimed at assessing the cyto-morphological pattern of tuberculous lymphadenitis among Sudanese patients, and screening for cellular changes that accompany this condition. A total of 75 cytological samples (fine needle aspirates) were collected from different lymph nodes. All patients were previously diagnosed with tuberculous lymphadenitis. The samples were prepared, processed, and stained according to the conventional Papanicolaou staining method, then microscopically examined. The study revealed the following findings: The age group of (41-60) were the most observed age which constitute 33(44%), and females 48(64%) were the most frequently observed subject. Western Sudanese tribe 26(34.7%) showed the major population among the study subjects. Caseating Epitheloid Granulomas is the major cytological pattern which found in 35(46.7%) samples, among which 21(28%) were females and 14(18.7%) were males. Followed by Granulomas pattern which found in 22(29.3%) samples, among which 14(18.7%) were females, and 8(10.7%) were males. Necrotizing, and Necrotizing with Suppurative were the least observed pattern with 11(14.7%) and 7(9.3%) respectively. Cervical lymph node 30(40%), was the most lymph node involved, followed by the Inguinal lymph node 19(25.3%), Supraclavicular and Axillary lymph nodes were the least observed with 14(18.7) and 12(16%) respectively. On the basis of this study, fine needle aspiration cytology found to be an essential tool in trending towards making a firm diagnosis in tuberculous lymphadenitis. So we recommend that, fine needle aspiration cytology should be performed for any lymphadenopathy, as well as Z-N stain which will be of great value in cases of inflammatory conditions differentiation. In parallel of cytological and microbiological stains other techniques such as rapid molecular-based diagnostic tests, should be applied in order to improve the diagnostic sensitivity and specificity in this setting.***

Keywords: Tuberculosis, Tuberculous Lymphadenitis, Fine Needle Aspiration, Cytology

1. 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) [1].

Numerous sophisticated methods of cyto-preparation are available, selection of such method is mainly 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 [2]. 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 throughout the cytological preparation.

Fine needle aspiration cytology (FNAC), is an inexpensive, atraumatic technique for obtaining cellular material for cytological examination and diagnosis, it allows a minimally invasive, rapid diagnosis of tissue samples. The advantages of FNAC are innumerable and these include cost effectiveness, rapid reporting and bedside diagnosis, minimal physical and psychological discomfort [3].

Tuberculosis (TB) is a chronic granulomatous infectious disease caused by the bacillus Mycobacterium tuberculosis and It remains a major health problem in low income countries [4]. Infection occurs via aerosol, and inhalation of a few droplets containing M. tuberculosis bacilli. In the year 1993, World Health Organization (WHO) declared TB a global public health emergency. Tuberculosis is the second most common cause of death from infectious disease (after HIV) [5]. The absolute number of tuberculosis cases has been decreasing since 2005 and new cases since 2002.[6] China has achieved particularly dramatic progress, with an 80 percent decline in its TB mortality rate [7]. The distribution of tuberculosis is not uniform across the globe; about 80% of the population in many Asian and African countries test positive in tuberculin tests, while only 5–10% of the U.S. population test positive [8]. The incidence of TB varies with age. In Africa, TB primarily affects adolescents and young adults [9]. However, in countries where TB has gone from high to low incidence, such as the United States, TB is mainly a disease of older people, or of the immuno compromised [10]. Tuberculosis outside the lung is known as Extra-pulmonary tuberculosis and it is usually results from hematogenous dissemination, or sometimes infection directly extends from an adjacent organ. The extra-pulmonary sites commonly involved are: Tuberculous lymphadenitis (scrofula), Miliary Tuberculosis, Genitourinary Tuberculosis, Tuberculosis meningitis, Tuberculosis Peritonitis, Tuberculosis Pericarditis, Tuberculosis of bones and joints, Gastrointestinal Tuberculosis, Tuberculosis of the liver [11].

Lymphadenitis is the most common extra-pulmonary manifestation of tuberculosis. It remains both diagnostic and

therapeutic challenge because it mimics other pathologic processes and yields inconsistent physical and laboratory findings. It is also important to differentiate tuberculous from non-tuberculous mycobacterial cervical lymphadenitis because their treatment protocols are vary [12].

2. Materials and methods

2.1 Study design

This is a descriptive study aimed to assess the cyto-morphological pattern of tuberculous lymphadenitis among Sudanese patients, using the conventional Papanicolaou stain. The study was conducted in Khartoum state hospitals, during the period from January 2013, to April 2014.

2.2 Study Population

Seventy Five cytological materials were collected from patients with previously diagnosed as having tuberculous lymphadenitis, the samples were processed and stained accordingly to conventional Papanicolaou staining method.

2.3 Samples Collection

The cytological specimens were collected by fine needle aspiration from different lymph nodes. The samples were aspirated and sent to the cytology laboratory.

2.4 Sample Processing

A 10 mL syringe attached to a 22-gauge needle was used for fine needle aspiration, on a clean glass slide a wet-fixed smears with (95% alcohol for 15 minutes) were prepared from the aspirate for Papanicolaou stain. 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 P
apanicolaou 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 de-colorization. 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 cellular pattern appearance which encountered in the smears. The characteristics of these cells were compared with the illustrated photographs in (Fine Needle Aspiration Cytology, Diagnostic Principles And Dilemmas G.Kocjan 2006). All smears were examined by 3 independent investigators.

3. Results and Discussion

In this study, the cyto-morphological pattern of tuberculous lymphadenitis among Sudanese patients were assessed. These changes were assessed among 75 patients with tuberculous lymphadenitis. Their ages ranging from 30 to 72 with a mean age of 52 years old.

As shown in Fig 1, the majority of the study population were among the age group 41-60 years old, which constitute 33(44%), followed by the age group 60+, then 30-40, which constitute 25(33.3%), 17(22.7%) respectively. Fig 2, represent the description of the study population by the gender, its apparently that, the majority of the study population were females which constitute 48(64%), while the males constitute 27(36%). As shown in Fig 3, the majority of the disease cases were among the western Sudanese tribe which constitute 26(34.7%), followed by eastern, southern, and northern tribes which constitute 20(26.7%), 17(22.7%) and 12(16%) respectively. Table 4. Fig 4, represent the description of the study population by lymph node involvement. Cervical lymph node were the most lymph node among the study population which constitute 30(40%), followed by inguinal lymph node which constitute 19(25.3%), then supraclavicular, axillary lymph nodes which constitute 14(18.7%), 12(16%) respectively. Fig 5, represent the description of the study population by cyto-morphological pattern. Caseating Epitheloid granulomas were the major cytological pattern which constitute 35(46.7%), followed by Granulomas, Necrotizing, and Necrotizing & Suppurative patterns which constitutes 22(29.3%), 11(14.7%), and 7(9.3%) respectively. Fig 6, show the description of the study population by age and gender. The majority of the study population were among the age group 41-60, in which females constitute 25(33.3%), and males 8(10.7%), followed by the age group 60+ among which females constitute 14(18.7%), and males

11(14.7) then the age group 30-40 in which females comprising 9(12%), and males comprising8(10.7%). As shown in. Fig 7, the description of the study population by cyto-morphological pattern and gender. The majority in the CEG pattern were found among the females constituting 21(28%), and the majority in the Granulomas pattern were also females constituting 14(18.7%), the majority in the Necrotizing pattern were found among females which constitute 8(10.7%), The majority in the Necrotizing & Suppurative pattern were found among the females as well, constituting 5(6.7%).

As shown in. Fig 8, which represent the description of the study population by cyto-morphological pattern and age. The majority in the CEG pattern were found among the age

group 41-60 which constitute 17(22.7%), and the majority in the Granulomas pattern were among the age group 60+ which constitute 10(13.3%), the majority in the Necrotizing pattern were found among the age group 41-60 which constitute 5(6.7%), The majority in the Necrotizing & Suppurative pattern were found among the age group 41-60 which constitute 3(4%). As shown in. Fig 9, which describe the study population by cyto-morphological pattern and tribe. The majority in the CEG pattern were found among the western tribe which constitute 16(21.3%), and the majority in the Granulomas pattern were found among eastern tribe which constitute 9(12%), the majority in the Necrotizing pattern were found equally among western, southern, and eastern tribes which constitute 3(4%), The majority in the Necrotizing & Suppurative pattern were found among western tribe which constitute 3(4%).

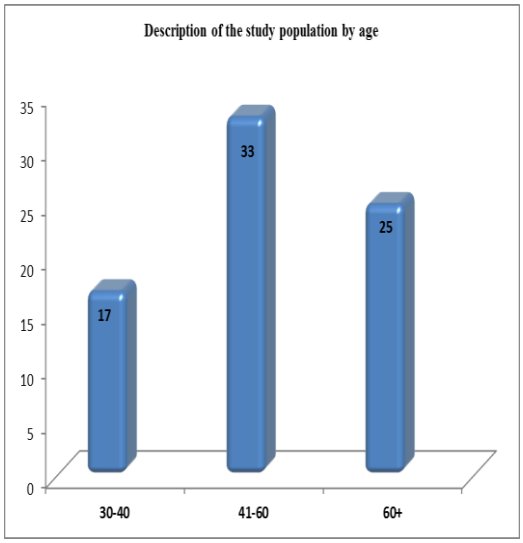

Figure 1: Description of the study population b age

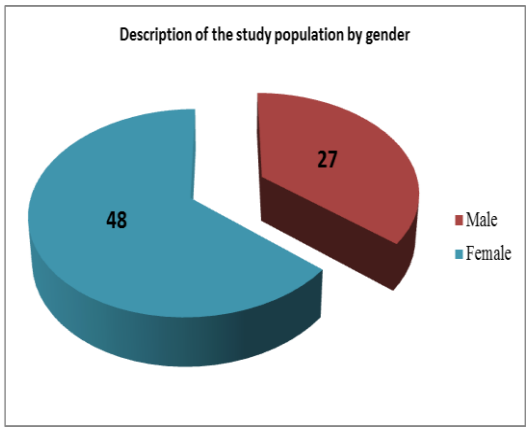

Figure 2: Description of the study population by gender:

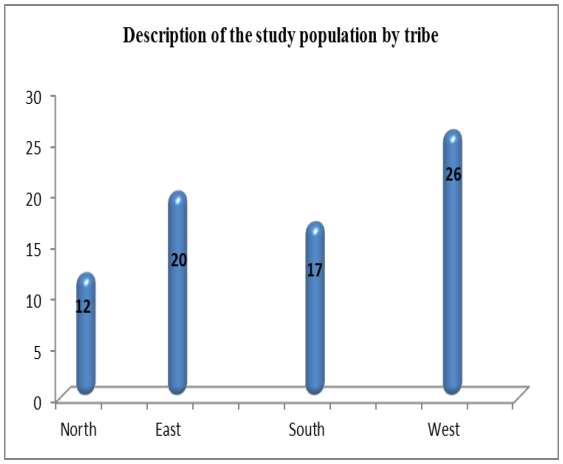

Figure 3: Description of the study population by tribe.

Table 4: Description of the study population by the lymph node involvement

lymph node involvement	Frequency	Percent %
Cervical	30	40%
Inguinal	19	25.3%
Supraclavicular	14	18.7%
Axillary	12	16%
Total	75	100%

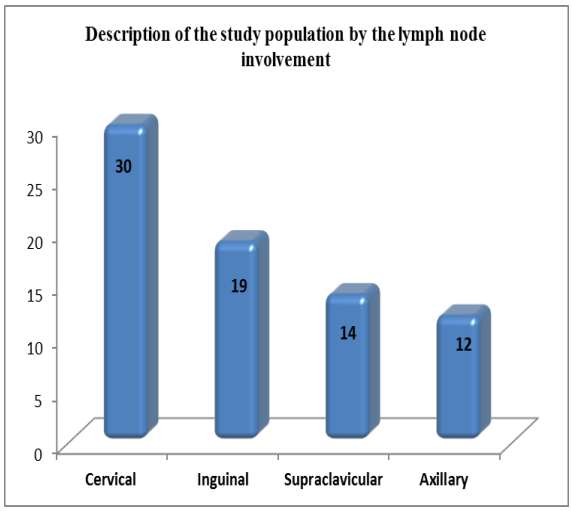

Figure 4: Description of the study population by the lymph node involvement

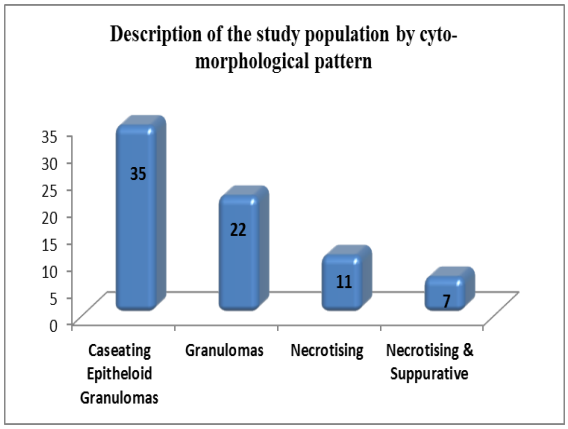

Figure 5: Description of the study population by cyto-morphological pattern

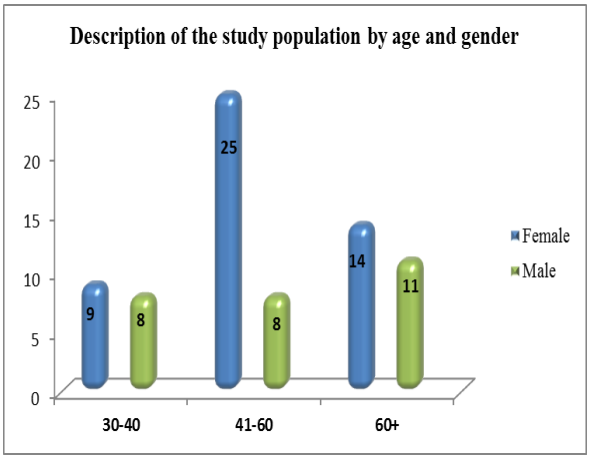

Figure 6: Description of the study population by age and gender

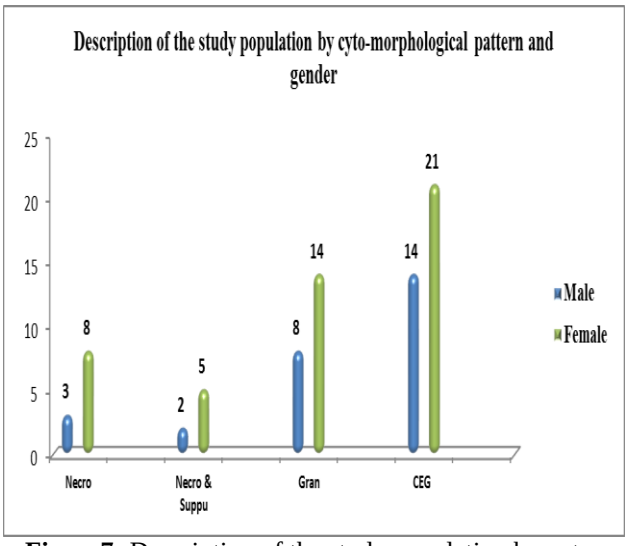

Figure7: Description of the study population by cyto-morphological pattern and gender:

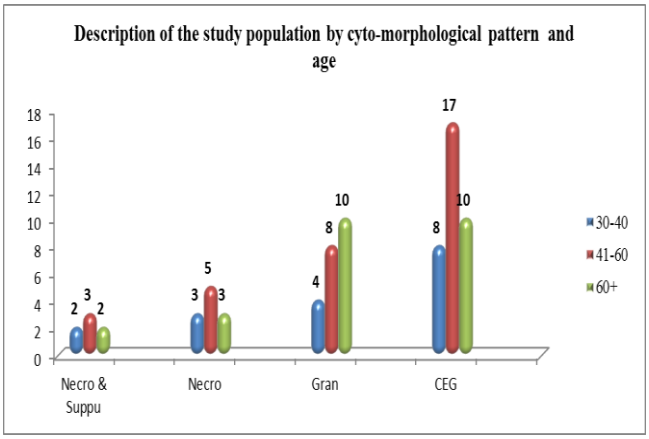

Figure 8: Description of the study population by cyto-morphological pattern and age

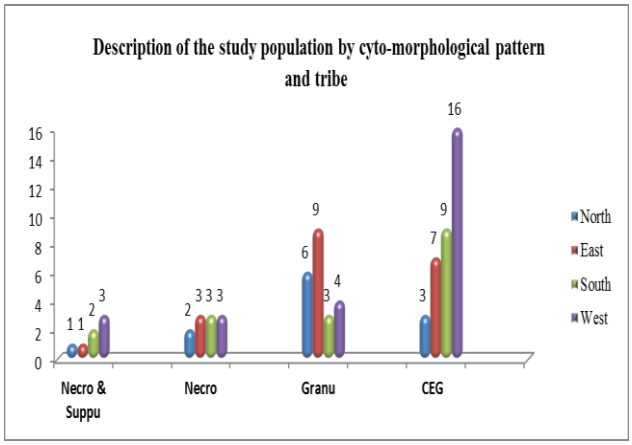

Figure 9: Description of the study population by cyto-morphological pattern and tribe:

4. Discussions

This study assessed the cyto-morphological pattern of tuberculous lymphadenitis among Sudanese patients. Out of 75(100%) patients with tuberculous lymphadenitis, females and age group of 41-60 were the major subject among the study population, this results support the finding of V Koo et al [13]. The study finding showed that the western Sudanese tribe was the most frequently affected by tuberculosis lymph adenitis and we assume this result is due to environmental and social factors which result in the high incident of tuberculosis among western tribe individuals. Cervical lymph node were the most common lymph node involved 30(40%),these findings supports a number of studies[14,15,16], who reported that ; cervical lymph node is the commonest in most frequently involved lymph node in tuberculous lymphadenitis. Our results show that Caseating Epitheloid Granulomas pattern is the major cytological pattern observed among the study population with 35(46.7%), these results support many studies by Rajesh et al**.** and Malakar et al [17,18], who reported that, Caseating Epitheloid Granulomas is the major and the most observed cyto-morphological pattern seen in tuberculous lymphadenitis followed by Granulomas pattern**.**

5. Conclusions and Recommendations

On the basis of this study and review of other studies, we conclude that, FNAC is essential tool in trending towards making a firm diagnosis in tuberculous lymphadenitis. Caseating Epitheloid Granulomas pattern were more frequently observed among the study population. The most encountered lymph node in tuberculous lymphadenitis were cervical lymph node. And the most affected individuals were observed among western Sudanese tribe. We recommend that, FNAC should be performed for any lymph node adenopatheis, as well as Z-N stain which will be of great value in cases of inflammatory conditions differentiation. In parallel of cytological stains other techniques e.g. rapid molecular-based diagnostic tests, should be applied in order to improve the diagnostic sensitivity and specificity in this setting

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