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Identification of Respiratory Fungal Infections using Cytoscreening

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Abstract: *Cytoscreening is a simple and rapid technique that requires a high index of suspicion due to the increased reported cases worldwide especially among the immunocompromised patients. The identification of fungal infection by cytoscreening depends on the finding of the causing agent or its effects (inflammatory cells and cytopathic effects). This study aimed to identify respiratory fungal infections using cytoscreening. Fifty-nine cytological smears were used in this study, 56 of which were sputum samples representing (95.2%) and 3 were BAL representing (4.8%). Samples were smeared directly on clean dry slide and stained by Grocott's Hexamine Silver. Of the total sample size, there were 31 (52.5%) male samples and 28 (47.4%)female samples. The patients' ages ranged between 20- 75 years old with mean of 45.19 years. The age interval 20-59 was 47 representing (79.7%) while the age interval more than 59 years was 12 representing (20.3%). Clinical distribution of patients revealed 31 (52.5%) asthmatic patients from which 12 (38. 7%) were male and 19 (67.8%) female, 15 patients with TB from which 12 (38.7%) were male and 3 (10.7%)female, 12 CF patients from which 7 (28.6%) were male and 5 (17.9%)female and one female cancer patient representing (3.6%) and absent of male. Yeast cells have been in 2 sample representing 3.6% and inflammatory background has been seen in 51 samples (86.4%) while 8 samples (13.6%) had no inflammatory background. The present study concluded that Grocott'sHexamine Silver stain is might help in identification of fungi and its inflammatory background.*

Keywords— cytoscreening; respiratory fungi; Grocott'shexamine silver; fungal elements**.**

1. INTRODUCTION

Respiratory tract infections are globally responsible for one third of infectious diseases associated mortality accounting for 4.3 million annual

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deaths, among these; the fungal infections of the respiratory tract are largely unrecognized [1].

Pulmonary fungal diseases include cryptococcosis, histoplasmosis, blastomycosis, coccidioidomycosis, paracoccidioidomycosis, sporotrichosis, candidiasis, zygomycosis, aspergillosis and pneumonia [2].

Fungi attack mainly the immunocompromised patients because of less virulency of the organism, hence; any factor that attenuates the immune system gives the fungi the opportunity to grow, such as uncontrolled antibiotic use and chronic diseases like diabetes, HIV and cancer can induce fungal infection [3].

Many fungi elicit a range of host reactions from exudative, necrotizing to granulomatous whilst other fungi produce little cellular response to indicate their presence [4]. Fungal infections diagnosis was done by direct microscopy, culture, serological tests and nonculture methods [5].

Wide spectrum of antifungal drugs used for the treatment of fungal infections with different mode of action on fungi [5]. Grocott's hexamine silver, haematoxylin and eosin stains used to stain fungi. A modification applied to Grocott's hexamine silver as to make it suitable for cytological smears and detection of cytological changes caused by the fungi [4]. The delay of fungal culture and the occasional false negative results when using direct microscopy because of the apparent absence of the infectious agent replaced by cytoscreening, which also aids in the confirmation of the microbiological tests as well as in the early diagnosis of the infection cause, which is necessary in order to decide the appropriate treatment. Cytoscreening is a cost effective and non-invasive technique, hence; it is more preferable by the patient.

2. MATERIALS AND METHODS:

2.1 Materials:

Sputum and BAL samples were used in this study.

2.2 Study design

This is a hospital based descriptive retrospective case study, aimed to detect respiratory fungal infections using cytoscreening.

2.3 Study sample:

Deep cough sputum and BAL samples selected from immunocompromised patients, collected in clean, dry and sterile containers.

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3. METHODS:

3.1 Sample processing:

Samples smeared directly on clean dry slide and dry fixed in absolute ethanol.

3.2 Grocott's hexamine silver:

The fungal cell wall contains mucopolysacchariedthat oxidized by chronic acid to release aldehyde groups, which later react with silver nitrate. Silver nitrate was converted to metallic silver black color, which becomes visible on cell wall of fungi [6]. The procedure of this technique as follow, smears were fixed by addition of two drops of absolute ethanol for 5 mins, washed in distilled water and then the smears were flooded with 4% chromic acid for 45 mins for oxidation to release aldehyde groups and washed in DW. 1% sodium metabisuiphite was added for 1 min to bleach the color of chromic acid and washed in DW, after that the working solution of hexamine silver was added for 10 mins then washed with DW, then the silver reduced with 0.1% ferric chloride. 5% sodium thiosulphate was added for 2 mins to remove unreduced silver and washed in DW. 1% light green solution was used as a counter stain for 1 min finally the smears were dried and viewed under oil immersion [6].

3.3 Result interpretation:

The slide with fungal elements stained the inner part of micelle black or pink hyphae with background in pale green was considered as positive.

3.4 Data analysis:

Data were analyzed by SPSS statistics 16 computer program. Frequencies and means were calculated.

3.5 Ethical consideration:

Samples collected after ethical acceptance from Al Shaabi specialized hospital and verbal consent from patients.

4. RESULTS:

Fifty-nine cytological smears were used in this study, 56 of which were sputum samples representing (95.2%) and 3 were BAL representing (4.8%) as indicated in table (4.1). Table (4.2) showed patients' ages ranged between 20-75 years old with mean of 45.19 years. The age interval 10-59 was 47 representing (79.7%) while the interval more than 59 was 12 representing (20.3%). Of the total

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sample size, there were 31 (52.5%) male samples and 28 (47.4%) female samples as indicated in table (4.3).

Clinical distribution of patients revealed 31 (52.5%) asthmatic patients from which 12 (38.7%) were male and 19 (67.8%) females, 15 patients with TB from which 12 (38.7%) were male and 3 (10.7%) females, 12 CF patients from which 7 (28.6%) were male and 5 (17.9%) female and one female cancer patient representing (3.6%) and non male as revealed in table (4.4).

Inflammatory background detected in 51 samples (86.4%) while 8 samples (13.6%) showed no inflammatory background as in table (4.5). From the 56 sputum samples, squamous cells had been isolated in 40 (71.4%) samples, pus cells in 33 (58.9%) samples, columnar cells in 1 (1.8%) sample and yeast cells in 2 (3.6%), while from the three BAL samples, squamous cells had been isolated in 2 (66.7%) samples, pus cells in 3 (100%) samples, columnar cells in 0 (0%) sample and yeast cells in 0 (0%) table (4.6)

Table (4.1): Frequ**Sample**	ency of samples amon**Frequency**	gstudy population**Percentage (%)**
Sputum	56	95.2
BAL	3	4.8
Total	59	100

Table (4.2): Frequency of age among study population

Age group	**Frequency**	**Percentage (%)**
59 and less	47	79.7
More than 59	12	20.3
Total	59	100

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Table (4.3): Distribution of gender among study population		
Gender	**Frequency**	**Percentage (%)**
Male	31	52.5
Female	28	47.4
Total	59	100

Table (4.4): Clinical diagnosis distribution among the gender

Diagnosis	**Male**		**Female**	
	Frequency	**(%)**	**Frequency**	**(%)**
Asthma	12	38.7	19	67.8
TB	12	38.7	3	10.7
CF	7	22.6	5	17.9
Cancer	0	0	1	3.6
Total	31	100	28	100

Table (4.5): Inflammatory background frequency

Background	**Frequency**	**(%)**
Inflammatory background	51	86.4
Non inflammatory background	8	13.6
Total	59	100

Table (4.6): Frequency of isolated cells among study samples

Cells	**Sputum**		**BAL**	
	Frequency	**(%)**	**Frequency**	**(%)**

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Squamous cells	40	71.4	2	66.7
Pus cells	33	58.9	3	100
Columnar cells	1	1.8	0	0
Yeast cells	2	3.6	0	0

5. DISCUSSION

The selection of staining method was primarily based on the sample used. This study involved 59 pulmonary lesion samples, 3.6% of samples reviled fungal infection and its results were incompatible with the findings of Shen and colleagues, and they concluded that most pulmonary fungal elements are isolated from sputum samples rather than BAL [7]. In contrast, another study stated that most fungi are isolated from BAL than sputum samples [8]. .

The study population ages ranged from 20-75 years with a mean of 45.19, most of the study population ages were less than 59 years, which is inconsistent with study concluded that more frequent inpatients above 65 years old [9].

This study revealed that males were affected by pulmonary lesions more than females, these finding consolidated by study of Chen and colleagues; they stated that the pulmonary invasive lesions were more common among male than female [9]. In addition, another study represented homogenous result with this study [10].

This study resulted in that the pulmonary lesions were most common among asthmatic patients while the cancerous patients were less frequent; these outcomes disagreed with study showed that the pulmonary lesions were common among chronic obstructive pulmonary disease while TB was the least common [7].

The present study deduced that cytoscreening help in identification of inflammatory background, which was compatible with study, inferred that cytology is a useful tool for identification of unusual fungi with characteristic microscopic morphology in cervical smears [11].

6. CONCLUSION:

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The present study concludes that fungal infection is less frequent among respiratory lesions f Sudanese patients and Grocott's Hexamine Silver stain help in identification of fungi and its inflammatory background.

REFERENCES:

1- Chowdhary, A., Agarwal, K. and Meis, J.F. (2016). Filamentous fungai in respiratory infections. What lies beyond Aspergillosis and Mucomycosis, Canada. PLoS pathogens, 12(4).

2- Edmund,S.C and Barbara,S.D. (2009). Cytology diagnostic principles and clinical correlates. 3th edition. Philadelphia: Elsevier: 65- 104.

3- healthcommunities.com, (2000). Fungal Infection Risk Factors. (Online) available at http://www.healthcommunities.com. (accessed 31 Aug 2000).

4- Bancroft, J.D., Suvarna, S.K. and Layton, C. (2013). Theory and Practice of Histological Techniques. 7th edition. London: Churchill Livingstone: 291-315.

5- Subhash ,C.P. (2012). Textbook of Microbiology and Immunology. 2th edition. India: ELSEVIER: 603-607.

6- Vijai, K.G., Maria, G.T., Manimaran, A., Anthonia, O. and Kevin, M. T.(2013). Laboratory Protocols in Fungal Biology. 1th edition. London: Springer: 237-258.

7- Shen, Y., Zhang, X., Lin, W., Wan, C., Li, Q. and Jiang, Y. (2017). Liquid- based cytopathology test as a novel method to identify Aspergillus in patients with pulmonary aspergillosis. Scientific reports: 7: 7528.

8- Danés, C., González-Martín, J., Pumarola, T., Rañó, A., Benito, N., Torres, A., Moreno, A., Rovira, M.,and de la Bellacasa, J. P. (2002). Pulmonary Infiltrates in Immunosuppressed Patients: Analysis of a Diagnostic Protocol. American Society for Microbiology, 40(6): 2134– 2140.

9- Chen, C. Y., Sheng, W. H., Tien, F. M., Lee, P, C., Huang, S. Y., Tang, J. L., Tsay, W., Tien, H. F. and Hsueh, P. R. (2018). Clinical characteristics and treatment outcomes of pulmonary invasive fungal infection among adult patients with hematological malignancy in a medical centre in Taiwan, 2008-2013. Journal of Microbiology,Immunology and Infection.

ISSN (E): 2181-4570 ResearchBib Impact Factor: 6,4 / 2023 SJIF 2024 = 5.073/Volume-2, Issue-3

10- Simoni M, Cai G-H, Norback D, Annesi-Maesano I, Lavaud F, Sigsgaard T, Wieslander G, Nystad W, Canciani M, Viegi G, Sestini P. (2011). Total viable molds and fungal DNA in classrooms and association with respiratory health and pulmonary function of European schoolchildren. Pediatr Allergy Immunol: 22(8): 843–852.

11- Gupta, S., Sodhani, P. and Jain, S. (2003). Macroconidia ofFusarium Species. Acta cytological, 47(1): 41-44