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A Study of Pulmonary Aspergillosis

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***Abstract**: The aim of this study is to know the prevalence of Pulmonary Aspergillosis in chronic lung diseased patients, its age wise , area wise distribution and the predominant lung conditions affected. Prevalence was studied by Direct Microscopy by LCB , Fungal Culture on SDA and Serology by IHA.*
***Material & Methods**: 125 samples of sputum from patients suffering from chronic lung disease on 2 consecutive days was collected and tested with Direct Microscopy and Fungal Culture. Serum samples were collected from these patients and subjected to Indirect Haemagglutination for Aspergilollosis.*
***Results**: Out of 125 samples tested 28% were positive by Direct Microscopy ,37.6 % were Culture positive and 50 % were Serologically positive. The most predominant growth was Aspergillus flavus (20.8%] followed by Aspergillus niger [12 %] and Aspergillus fumigates [4.8%].*
***Conclusions**: As Direct Microscopy fails to identify most of the cases ,culture always should be done in conjuction with Microscopy .Serology is an additIonal diagnostic tool to know the invasiveness of Aspergillus.*
***Keywords**: Pulmonary Aspergillosis ,Pulmonary tuberculosis ,Sabourauds dextrose agar, Czapex dox agar, Indirect Heamagglutination.*

I. Introduction

Occurrence of Aspergillus infection in association with chronic lung disease like pulmonary tuberculosis ,lung abscess ,bronchopneumonia with residual lung cavity ,asthma and lung malignancy is documented. [Cordonier .C.et.al 1986]. In the last 2 decades fungal infections have become a primary cause of respiratory tract infections. The exact incidence is not known. The present study was conducted to know the incidence of Aspergillosis in chronic lung diseased patients by isolation and charecterisation as well as by the serological study of anti aspergillus antibodies in the sera of patients with chronic lung diseases.

II. Aims & Objectives

To know the prevalence ,age and sex wise distribution ,urban and rural distribution of Aspergillus species in chronic lung diseased patients. To know the most common species and also the most predominant clinical condition of chronic lung disease which is effected by Apergillus. To correlate Direct Microscopy ,Fungal culture and IHA for diagnosis of Aspergillus species

III. Material & Methods

From 125 chronic lung diseased patients attending The Fever General Hospital, Guntur 2 spontaneously expectorated early morning sputum samples were collected in a wide mouthed sterile container and transported to the laboratory in the Department of Microbiology ,Guntur Medical College under aspetic conditions for processing and identification of Aspergillus species by Direct Microscopy and Fungal Culture to confirm the diagnosis. All samples were exmined by 10% KOH mount. The appearance of dichotomously branched septate hyphae in direct microscopy was taken as positive. [Text book of practical Medical Microbiology by Mackie and Macartney,14th edition.] All samples were inoculated onto Sabourads dextrose agar medium and Czapek dox agar medium in duplicate .The tubes were incubated at 25c and the other at 37c for a period of 6-7 days. SDA had an additional constituent of chloramphenical [0.05mg/ml] [Text book of Medical Mycology ,EMMONS ,3rd edition ,Text book of Medical Mycology Jagadish Chander 2nd edition.]

Growth was observed after every 2 days and growth was observed by Lactophenol Cotton Blue tease mount. Slide culture was done as per guidelines to confirm the Morphology. Positive cultures were repeated to confirm pathogenicity. Serum samples were collected from these patients and Serological diagnosis was done with The haemagglutination test kit ,Aspergillosis Fumouze manufactured by Medi span ,France [lot code :7364] .The results are recorded and the test is performed according to the instructions given in the kit protocol.

Principle: Aspergillosis fumouze is based on indirect haemagglutination [IHA]. Sensitised red blood cells are composed of sheep red blood cells coated with an Aspergillus fumigatus antigen. Specific serum antibodies are revealed by an agglutination of the sensitized red blood cells : A reddish brown film can be observed in the well. In the absence of specific antibodies ,these red blood cells deposit ,forming a ring in well bottom.

The unsensitised red blood cells ensure the reaction specificity and allow the elimination of interference due to natural anti sheep agglutinins [forssman hetero antibodies ,infectious mononucleosis antibodies.]

Interpretation of results:
Titre <1:320 - nonsignificant reaction [probable absence of deep seated Aspergillosis]
Titre =1 :320 - equivocal reaction
Titre >1:320 - significant reaction in favour of deep seated Aspergillosis.

IV. Results

Out of 125 samples tested 28 %[35] were Direct Microscopy Positive ,37.6 % [47] were culture positive and 34.6 % were IHA positive. The highest positivity was detected in patients with COPD [32%] followed by patients with Pulmonary tuberculosis [28%] and then with Fibrocavity of lung [16%] and Chronic bronchitis with HIV seropositivity patients [16%]. Aspergillus flavus was the predominant species [20.8%] followed by Aspergillus niger [12%] and Aspergillus fumigates [4.8%] . Positivity was highest in age group of 40-50 years [31.9 %] males [76.9 %] living in rural areas [70.21 %].

Table 1: Distribution of total samples and their culture isolates

S.no	Clinical condition	No. of samples	Percentage	Aspergillus flavus	Aspergillus fumigatus	Aspergillus niger	total
1	Pulmonary tuberculosis	35	28 %	6	1	6	13
2	Chronic obstructive pulmonary disease [COPD]	40	32 %	10	1	4	15
3	Chronic bronchitis [HIV seropositive]	15	16%	1	2	3	6
4	Fibrocavity of lung	20	16 %	8	2	1	11
5	Carcinoma of lung	10	8%	Nil	Nil	1	1
6	Collapse of lung	5	4 %	1	nil	nil	1
	Total	125	100%	26 [20.8%]	6[4.8%]	15[12%]	47[37.6%]

Table 2 : Direct Microscopy and Fungal Culture results

S.no	Microscopy	Culture		
		Positive	Negative	Total
1	Positive	29	6	35
2	Negative	18	72	90
3	Total	47	78	125

Table 3 : Indirect haemagglutination results

s.no	Seropositivity Titre >640	Seronegative Titre <320	Equivocal Titre 320 -640
1	34.65%	50%	15.35%

Table 4 : Age wise distribution of Culture positives

Age in years	No of samples		Culture positives	
	No	%	No	%
20-30 yrs	25	20%	10	21.27%
30-40 yrs	28	22.4%	10	21.27%
40-50 yrs	41	32.8%	15	32%
>60	14	11.2%	3	6.38%
Total	125	100%	47	100%

Table 5: Sex wise distribution of Culture positives

Sex	Cases	Culture positivity
Males	91	36[77%]
Females	34	11[23%]
Total	125	47

Table 6 : Area wise distribution of Culture positives

Area	Cases	Positives
Rural	93	33[70.21%]
Urban	32	14[30%]
Total	125	47

V. Conclusions

Aspergillus flavus is the most common isolate. Males [30-40 yrs] living in rural areas are the predominant group affected. Patients with COPD [32%] and pulmonary tuberculosis [28%] were at high risk. Serological study must be taken up along with along with Microscopy and Culture for laboratory confirmation of Pulmonary Aspergillosis. This is essential to reduce the morbidity and mortality of patients as they can have effective treatment of the pre-existing condition along with antifungals.

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