

Correlations between Clinical and Laboratory Findings in Patients Investigated for Aspergillosis

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We investigated 120 patients suspected clinically to have pulmonary aspergillosis with different clinical manifestations "aspergilloma (Subgroup A), allergic bronchopulmonary aspergillosis (Subgroup B) and invasive pulmonary aspergillosis (Subgroup C)" and correlated between their clinical and laboratory findings and endogenous specific aflatoxin production. They were subjected to isolation of *Aspergillus* strains, measurement of serum total IgE and specific *Aspergillus* IgG by ELISA and identification of aflatoxin producing *Aspergillus* strains using fluorescence analysis of spectrolin. *Aspergillus* was isolated from 45 patients (37.5%). Subgroup A had a negative statistically non-significant correlation between clinical and laboratory findings as regard total IgE and for *Aspergillus* IgG (only haemoptysis & weight loss had significant correlation with *Aspergillus* IgG). Subgroup B & Subgroup C had positive, statistically significant correlation & negative statistically non significant correlation respectively as regard all clinical findings and both total IgE & serum IgG. This study also showed that 6 *Aspergillus* strains out of 45 (13.3%) produced endogenous aflatoxin. It is concluded that a significant correlation that exists between clinical and serological findings in allergic pulmonary aspergillosis. Aflatoxins may be produced in vivo by strains of *Aspergillus* and may result in manifestations similar to those caused by ingestion of aflatoxin in food.

A *Aspergillus* species are ubiquitous molds. Although more than 200 species have been identified, the majority of human illness is caused by *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger* and less frequently *Aspergillus terreus* and *Aspergillus clavatus* (Bartlett, 2000). *Aspergillus* species cause a broad spectrum of diseases in human ranging from aspergilloma to life-threatening acute invasive disease such as allergic bronchopulmonary aspergillosis (ABPA) and invasive pulmonary aspergillosis (IPA) (Loeffler et al., 2001; Yildirim and Topal, 2004). During the last 20 years, the number of recorded cases of ABPA has raised due to an increasing number of patients undergoing chemotherapy or transplantation, and also increasing numbers of patients with AIDS (Loeffler et al., 2001; Soubani and Chandraskar, 2002).

Aflatoxins are highly toxigenic and carcinogenic agents produced by a few *Aspergillus* species, that are ever-present in

areas of the world with hot humid climate (Gugnani, 2003). They were the first of the mycotoxins to be found as food contaminants and also as animal carcinogen and, therefore, suspected as a cause of cancer in man (Bueno and Oliver, 2004). Several studies have implicated aflatoxins as potential factors in the increased incidence of human gastrointestinal and hepatic neoplasm (Stoloff, 1989; Bruce, 1990). Other studies stated that aflatoxins also impair immunity through inhibition of phagocytosis by macrophages and suppression of cell mediated immunity (Robens & Richard, 1992). Although human and animals are exposed to aflatoxins primarily in diet, considerable evidence that fungal isolates from human lung aspergilloma have the ability to produce aflatoxins. This endogenous production in human bodies was not reported until recently (Mori et al., 1998; Pepelnjak et al., 2004).

The traditional laboratory methods (culture and morphology) should be combined with one or more new diagnostic modalities in

addition to the suspected clinical manifestations to reach rapid appropriate diagnosis of pulmonary aspergillosis (Siew and Brain, 2002). The serologic diagnostic tools (total IgE and Aspergillus IgG) have a considerable value in identification of pulmonary aspergilloma and allergic aspergillosis while its value in identification of invasive pulmonary aspergillosis is limited (Fraser et al., 1999).

The aim of this work was to determine whether or not there is a correlation between clinical and laboratory findings in patients with pulmonary aspergillosis and to detect the endogenous aflatoxin production.

Subjects and Methods

Subjects

This work was carried out in the Microbiology and Immunology Department, Faculty of Medicine, Zagazig University, during the period from January 2003 to April 2004 on 120 patients (79 males & 41 females and their ages ranged from 18 to 69 years with a mean of 39.4 ± 4.2 years) suspected to have pulmonary aspergillosis and attending the outpatient and inpatient sections of Chest Department as well as 20 healthy control individuals.

Patients group were further subdivided into three subgroups:

Subgroup A: included 44 cases suspected to have pulmonary aspergilloma (on the basis of clinical findings as cough, expectoration, and haemoptysis together with radiological findings as lung cavity and Mondy's sign).

Subgroup B: included 37 cases suspected to have allergic pulmonary aspergillosis (on the basis of clinical findings as bronchial asthma with recurrent wheeze, low grade fever, malaise, cough and chest pain together with radiological findings as pulmonary infiltrate with or without proximal bronchiectasis).

Subgroup C: included 39 immuno-compromized patients suspected to have invasive pulmonary aspergillosis (on the basis of clinical findings as persistent fever despite broad spectrum antibiotic therapy followed by non productive cough and pleuritic pain together with radiological findings as lung nodule, cavitary and focal infiltrate).

Methods

All patients and controls were subjected to the following:

1- History taking, thorough clinical examination and exclusion of parasitic infestation.

2- Sputum examination: morning sputum samples were collected into sterile capped containers. Then cultivation on Sabouraud's dextrose agar with chloramphenicol; pure colonies were picked up and identified morphologically by the growth characteristics on the plates and microscopically by the "Slide Culture Technique" (Fridman, 1991).

3- Blood eosinophilic count for subgroup B and control group: One ml of blood was collected in EDTA containing tubes for total and differential leucocytic count.

4- Serological examination

- Detection of total IgE by ELISA:

Principle: The assay principle combines an enzyme immunoassay sandwich method with a final fluorescent detection (ELIFA) (According to manufacture of BioMerieux sa.) by mini VIDAS

Procedure: Detection was based on IgE strip and SPR (Solid Phase Receptacle). Strips containing many wells (for samples conjugate, buffer, empty well for reaction and the last well used by the instrument as reading cuvette). SPRs are coated with monoclonal anti IgE and used also by the instrument as tips for pipetting. One new IgE strip & SPR were used for each sample. One hundred μ l serum were pipetted into sample well. All the assay steps were performed automatically by the instrument and completed within 30 minutes. REV (relative fluorescence value) was calculated & results were automatically calculated by the instrument using calibration curves & concentration were expressed in KIU /L according to international standards.

-Detection of Aspergillus IgG by ELISA:

Principle Aspergillus IgG kit is a solid phase sandwich Enzyme Linked Immuno-Sorbent Assay (ELISA) (According to manufacture of KMI Diagnostics).

Procedure: One hundred μ l of diluted serum was pipetted into its wells that were coated by Aspergillus Ag & after incubation for 60 minutes & washing, enzyme conjugate was added. After another incubation for 30 minutes, TMB substrate was added followed by stopping solution. Reading was done on ELISA reader at 450 nm. Results were calculated from calibration curve.

5- Detection of toxigenic strains of *Aspergillus* by ultraviolet lamp (Spectroline): All tested isolates of *Aspergillus flavus* and *Aspergillus fumigatus* were inoculated on coconut cream agar (Dyer & McCammon, 1994) and yeast extract sucrose media

(Farag et al., 1989). Both media were examined in the dark for fluorescence with ultraviolet Lamp by using fluorescence analysis cabinet of Spectroline device (Model Cm-10-USA) (Fente et al., 2001).

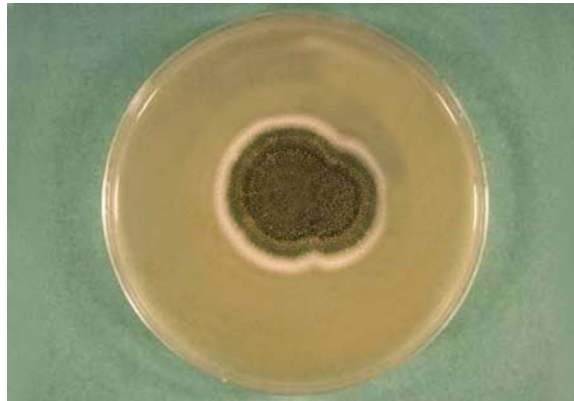


Figure 1. *Aspergillus* growth on Coconut cream agar medium.

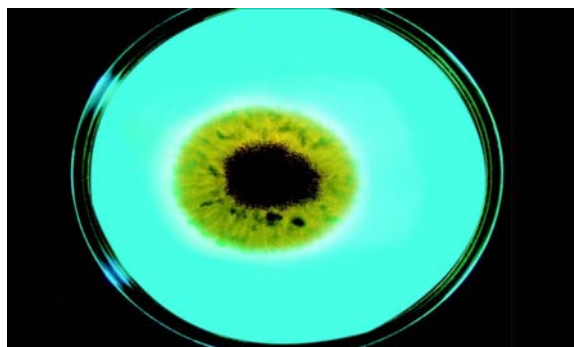


Figure 2. Yellow green fluorescence of aflatoxin produced by aflatoxigenic *Aspergillus* strains observed under UV light of Spectroline device.

Statistical analysis

Data were coded and entered to Epi info file using Epi –info version software computer package according to Dean et al., (1994). Arithmetic Mean (X), standard deviation (SD), probability (P) value, analysis of variance (F-test) and correlation coefficient (r) were used in this study.

Results

One hundred and twenty cases suspected clinically to have pulmonary aspergillosis with different clinical manifestations

(aspergilloma, allergic bronchopulmonary aspergillosis and invasive pulmonary aspergillosis) were investigated. Of these 120 cases only 45 had positive sputum culture of *Aspergillus* with an incidence 37.5%. These 45 cases together with 20 healthy subjects as controls were enrolled in this study.

Aspergillus fumigatus was the most prevalent species (55.5%) followed by *Aspergillus niger* (40%) and only *Aspergillus flavus* (4.5%). Table (1)

Table 1. Sputum culture results & Aspergillus species isolated from sputum of patients with suspected aspergillosis

Patient group	Aspergillus culture				P
	+ve	Asp. Fumigatus (25)	Asp. Niger (18)	Asp. Flavus (2)	
Subgroup A (n=44)	15 (34.1%)	10 (40%)	5 (27.8%)	0 (0%)	0.41
Subgroup B (n=37)	15 (40.5%)	8 (32%)	6 (33.3%)	1 (50%)	0.87
Subgroup C (n=39)	15 (38.5%)	7 (28%)	7 (38.9%)	1 (50%)	0.66
Total (n=120)	45/120 (37.5%)	25/45 (55.5%)	18/45 (40%)	2/45 (4.5%)	

Asp: Aspergillus.

Patient subgroups (A: aspergilloma, B: allergic bronchopulmonary aspergillosis&C: invasive pulmonary aspergillosis).

Table (2) shows that haemoptysis was the predominant clinical finding (80%) in patients with aspergilloma. Cough, expectoration, dyspnea and wheeze were the predominant clinical findings (100%) in patients with suspected allergic aspergillosis and persistent

fever and non-productive cough were the predominant clinical findings in patients with suspected invasive aspergillosis. A significant difference ($P<0.001$) between patients and controls groups as regard to clinical and radiological findings.

Table 2. Clinical and radiological findings in patients with suspected aspergillosis and controls group.

	Patients (45)						Controls (20)		P<
	A*(15)		B (15)		C (15)				
	No	%	No	%	No	%	No	%	
Haemoptysis	12	80%	0	0%	11	73.3%	0	0%	0.001
Cough	9	60%	15	100%	15	100%	0	0%	
Expectoration	9	60%	15	100%	2	13.3%	0	0%	
Dyspnea	10	66.7%	15	100%	5	33.3%	0	0%	
Wheeze	9	60%	15	100%	0	0%	0	0%	
Chest pain	5	33%	12	80%	8	53.3%	0	0%	
Fever	7	46%	5	33.3%	15	100%	0	0%	
Weight loss	11	73.3%	1	6.7%	15	100%	0	0%	
Mondo's sign	15	100%	0	0%	0	0%	0	0%	
Pulmonary Infiltrate	0	0%	10	66.7%	0	0%	0	0%	
Bronchiectasis	0	0%	5	33.3%	0	0%	0	0%	
Lung nodule& Focal infiltrate	0	0%	0	0%	15	100%	0	0%	

*All cases (100%) of subgroup A had history of lung cavity (75% tuberculous).

Patient subgroups (A: aspergilloma, B: allergic bronchopulmonary aspergillosis&C: invasive pulmonary aspergillosis).

Table (3) & (4) show a significant difference between patients group and controls group and also between subgroup B and other subgroups as regard total IgE& a significant difference $P<0.001$ between patients group

and controls group and also between subgroup A and subgroup C as regard Aspergillus IgG. Table (5) shows a significant level of blood eosinophils in subgroup B (allergic bronchopulmonary aspergillosis).

Table 3. Total IgE among patients with suspected aspergillosis and controls

Group	Total No.	+ ve cases for IgE		Range(KIU/L)	Mean	P
		No.	%			
Total Patients group	45	23	51.1%	240-901	450	*<0.001
Subgroup A	15	4	26.7%	290-550	360	**<0.001
Subgroup B	15	15	100%	595-901	640	-
Subgroup C	15	4	26.7%	240-420	250	**<0.001
Control group	20	0	0%	40-140	120	-

*P in relation to control group.

** P in relation to subgroup B.

Patient subgroups (A: aspergilloma, B: allergic bronchopulmonary aspergillosis&C: invasive pulmonary aspergillosis)

N.B Normal values of IgE in non-atopic individuals are less than 150 KIU/L.

Table 4. Aspergillus specific level IgG in patients with suspected aspergillosis and controls

Group	Total No.	+ ve cases for IgG		Range(U/mL)	Mean	P
		No.	%			
Total Patient group	45	28	62.2%	12.8-21	17.3	*<0.001
Subgroup A	15	14	93.3%	12.8-21	17.7	-
Subgroup B	15	10	66.7%	13.5-19.7	16.5	**>0.05
Subgroup C	15	4	26.7%	13.9-15.1	12.5	**<0.001
Control group	20	0	0%	2.3-7.8	5.2	-

*P in relation to control group.

** P in relation to subgroup A.

Patient subgroups (A: aspergilloma, B: allergic bronchopulmonary aspergillosis&C: invasive pulmonary aspergillosis)

N.B Normal values of Aspergillus IgG are less than 8 U/ml; borderline values 8-12 U/ml and positive cases more than 12 U/ml.

Table 5. Blood eosinophils in patients with allergic pulmonary aspergellosis and controls.

Group	Eosinophils				P
	Range	Mean	SD	Medium	
Subgroup B (n=15)	304.6-1170	833.33	234.3	795	<0.001
Control group (n=20)	160.2-192.5	167.5	11.7	165	

Patient subgroups B: allergic bronchopulmonary aspergillosis.

Regarding the relationship between major clinical and laboratory findings in all patient subgroups table (6) shows that subgroup A has a negative statistically non significant correlation between clinical and laboratory findings as regard total IgE and for Aspergillus IgG (only haemoptysis & weight

loss have significant correlation with aspergillus IgG). Subgroup B & Subgroup C had Positive, statistically significant correlation & negative statistically non significant correlation respectively as regard all clinical findings and both total IgE & serum IgG.

Table 6. The relationship between major clinical and laboratory findings in all patient subgroups.

Clinical findings	Laboratory findings			
	IgE		IgG	
	-ve	+ve	-ve	+ve
<u>Haemoptysis</u>				
Group A: -ve	3/11(27.3%)	0/4(0%)	1/1(100%)	2/14(14.3%)
+ve	8/11(72.7%)	4/4(100%)	0/1(0%)	12/14(85.7%)
P	NS		< 0.05	
r	0.16		0.44	
Group C: -ve	3/11(27.3%)	1/4(25%)	3/11(27.3%)	1/4(25%)
+ve	8/11(72.7%)	3/4(75%)	8/11(72.7%)	3/4(75%)
P	NS		NS	
r	0.01		0.01	
<u>Cough</u>				
Group A: -ve	5/11(45.5%)	1/4(25%)	1/1(100%)	5/14(35.7%)
+ve	6/11(54.5%)	3/4(75%)	0/1(0%)	9/14(64.3%)
P	NS		NS	
r	0.14		0.19	
Group B: -ve	-	-	-	-
+ve	-	15/15(100%)	5/5(100%)	10/10(100%)
P	<0.001		< 0.05	
r	0.1		0.43	
Group C: -ve	-	-	-	-
+ve	11/11(100%)	4/4(100%)	11/11(100%)	4/4(100%)
P	NS		NS	
r	0.17		0.17	
<u>Dyspnea</u>				
Group A: -ve	5/11(45.5%)	1/4(25%)	1/1(100%)	5/14(35.7%)
+ve	6/11(54.5%)	3/4(75%)	0/1(0%)	9/14(64.3%)
P	NS		NS	
r	0.14		0.19	
Group B :-ve	-	-	-	-
+ve	-	15/15(100%)	5/5(100%)	10/10(100%)
P	<0.001		< 0.05	
r	0.1		0.43	
<u>Weight loss</u>				
Group A: -ve	4/11(36.4%)	0/4(0%)	1/1(100%)	3/14(21.4%)
+ve	7/11(63.6%)	4/4(100%)	0/1(0%)	11/14(78.6%)
P	NS		< 0.05	
r	0.23		0.32	
<u>Recurrent wheeze</u>				
Group B: -ve	-	-	-	-
+ve	-	15/15(100%)	5/5(100%)	10/10(100%)
P	<0.001		< 0.05	
r	0.1		0.43	
<u>Persistent fever</u>				
Group C: -ve	-	-	-	-
+ve	11/11(100%)	4/4(100%)	11/11(100%)	4/4(100%)
P	NS		NS	
r	0.17		0.17	

Patient subgroups (A: aspergilloma, B: allergic bronchopulmonary aspergillosis&C: invasive pulmonary aspergillosis)

P: Probability -NS: Non significant -S: Significant- HS: Highly significant -r: Kappa coefficient.

Only 6 cases out of 45 (13.3 %) produce aflatoxin and from them 4 are from subgroup

A (aspergilloma) and 5 are *Aspergillus fumigatus* (table 7).

Table 7. The number, type and percentage of *Aspergillus* species which produce aflatoxin (give fluorescence by spectrolin).

	No	%	Type
Subgroup A	4/6	66.6%	<i>Aspergillus fumigatus</i>
Subgroup B	1/6	16.7%	<i>Aspergillus fumigatus</i>
Subgroup C	1/6	16.7%	<i>Aspergillus flavus</i>
Total	*6/45	13.3%	

Patient subgroups (A: aspergilloma, B: allergic bronchopulmonary aspergillosis & C: invasive pulmonary aspergillosis).

*All cases in which *Aspergillus* produce aflatoxins had malaise and vomiting. One case with invasive pulmonary aspergillosis had hepatomegaly, ascites, jaundice and peripheral oedema and this case developed hepatoma later on. Only one case from the four who have aspergilloma has also lung cancer.

Discussion

In the present study sputum culture revealed that *Aspergillus fumigatus* was the most common species isolated (55.5%) followed by *Aspergillus niger* (40%) then *Aspergillus flavus* (4.5 %). This was in agreement with Greenberger, (1998); Ho and Yuen (2000); Soubani and Chandraskar, (2002) who stated that the most common species in pulmonary aspergillosis are *Aspergillus fumigatus*, *Aspergillus niger* and *Aspergillus flavus* and of these the commonest is *Aspergillus fumigatus*. This was in contrast to the results obtained by El-Ahmady et al., (1991) who found that *Aspergillus niger* (35%) prevails more than *Aspergillus fumigatus* (27.5%) in the sputum culture of patients included in their study. The clinical manifestations of pulmonary aspergillosis are dependent not only on the pathogenic properties of the fungus but also on the immune status of the patient and the underlying architecture of the lung in the host (Gugnani, 2003).

This study demonstrated that haemoptysis was the major presenting symptom in patients with aspergilloma, it occurred in (80%) of patients included in this group. This was in agreement with Addrizzo-Harris et al., (1997);

Daly and kavanagh, (2001); Fajraoui et al., (2001); Soubani and Chandraskar, (2002); Lee, et al., (2004). This could be explained by local invasion of blood vessels lining the cavity; endotoxin released by the fungus with haemolytic properties and mechanical friction of aspergilloma with the cavity wall blood vessels (Soubani and Chandraskar, 2002). In our study the most common predisposing factor for aspergilloma was tuberculous lung cavity (75%). This is in accordance with Kawamura et al., (2000); Lee et al., (2004) who reported that similar percentages (72% & 81%) respectively from a series of patients with aspergilloma who had pulmonary tuberculosis as the underlying disease. In the present study, all patients with aspergilloma (100 %) showed upper lobe mobile intracavitary mass with an air crescent sign (Mondo's sign). This was in agreement with Daly and Kavanagh, (2001); Soubani and Chandraskar, (2002); Kohno et al., (2003); Lee et al., (2004) and in disagreement with percentage reported by Kawamura et al., (2000) (67%). In our study recurrent wheeze, cough, expectoration and dyspnea were the predominant clinical findings (100%) in patients with ABPA. This was in agreement

with many authors as Daly and Kavanagh, (2001); Sandhya and Jnl, (2002). In the present study, (66.6%) of patients with allergic aspergillosis showed pulmonary infiltrates in radiological finding and (33.3%) only showed central bronchiectasis. These findings were in agreement with Fraser et al., (1999); Steven and Ceorge, (1998) who found similar results.

Invasive pulmonary aspergillosis (IPA) has been a significant cause of life threatening opportunistic infections in immuno-compromized hosts and the reported mortality rate varies from 50% to nearly 100% (Bernabe et al., 2000). So, early diagnosis of IPA is important to start adequate therapy that may improve the clinical outcome and mortality rate (Caillot et al., 1997).

In this study persistent fever not responding to antibiotics, non-productive cough, haemoptysis and weight loss were the major presenting symptoms in patients with invasive pulmonary aspergillosis. This was in agreement with Bernabe et al., (2000); Daly and Kavanagh, (2001) and Soubani and Chandraskar, (2002).

In the present study all patients with IPA (100%) had lung nodule and focal infiltrate. Daly and Kavanagh, (2001); Soubani and Chandrasekar, (2002) found that these radiological findings are relatively specific for IPA.

The serological diagnostic tools (total IgE & Aspergillus IgG) have a considerable value in identification of pulmonary aspergilloma and allergic aspergillosis while its value in identification of IPA is limited (Fraser et al., 1999). In the present study in patients with aspergilloma, elevated level of total IgE was detected in only (26.7%) of cases. This was in accordance with Boon et al., (1991) who reported that total IgE was detected in 32% of his series of patients with aspergilloma. However, Sandhya and Jnl, (2002) reported that total IgE is not a consistent finding in aspergilloma. In our patients with allergic

aspergillosis elevated level of total IgE was detected in (100%) of cases. This was in agreement with ElAhmady et al, (1991); Steven and Geoarge (1998) and Fraser et al., (1999) who reported that almost all patients with allergic aspergillosis have elevated level of total IgE. In this study (26.7%) of patients with IPA showed elevated total serum IgE. This was in agreement with Siew and Brain (2002) who reported that 21% of cases of IPA had elevated total serum IgE. In patient with allergic aspergillosis, the mean value of total IgE was higher than that of other groups with highly significant difference ($P < 0.001$). These results were in agreement with Steven and Geoarge (1998); Fraser et al., (1999).

Aspergillus IgG was detected in (93.3%) of our patients with aspergilloma. This was in agreement with Daly and Kavanagh, (2001); Michael et al., (2001), Soubani and Chandraskar, (2002), and Kohno et al., (2003) and in disagreement with that obtained by Kawamura et al., (2000) and Uffredi et al., (2003) (70% & 78% of patients respectively). This may be explained by Soubani and Chandraskar, (2002) who stated that some of the patients with aspergilloma might have false negative results to Aspergillus IgG due to corticosteroid therapy.

In this study, (66.7%) of cases with allergic aspergillosis had high level of Aspergillus IgG. Steven and Ceorge, (1998); Soubani and Chandraskar, (2002) stated that patients with ABPA had significant high level of Aspergillus IgG. In this study only 26.7% patients with IPA, showed elevated Aspergillus IgG antibodies. This is in agreement with Siew and Brain, (2002) who reported that 28.7% of cases with IPA had elevated Aspergillus IgG. In contrast to these results Michael et al., (2001) found that in serum samples of patients suffering from IPA, 64% were positive for Aspergillus IgG. This may be explained by the high frequency of false positive results, which may be due to cross-reactivity with other fungi or other

unidentified serum components (Swanink et al., 1997).

In the present study, patient with allergic aspergillosis showed significant high degree of eosinophilia. These results were in agreement with Steven and Geoarge (1998) and lee et al., (2004).

In the present study patients with pulmonary aspergilloma showed a low correlation between clinical and laboratory findings as regard to total IgE (all clinical findings show no statistically significant correlation with total IgE). This was in agreement with Sandhya and Jrnl (2002) who reported that total IgE is not a significant finding in aspergilloma and has a low correlation with the clinical manifestations of the disease. On the other hand our patients with pulmonary aspergilloma showed moderate correlation between clinical and laboratory findings as regard Aspergillus IgG (only haemoptysis and weight loss have statistically significant correlation with Aspergillus IgG) and this was in agreement with Michael et al. (2001).

In this study patients with allergic aspergillosis showed a high correlation between clinical and laboratory findings as regard total IgE & Aspergillus IgG. This was in agreement with Fraser et al. (1999) and Usha et al., (2002). In our study patients with IPA show low correlation between clinical and laboratory findings as regard both total IgE and Aspergillus IgG, this is in agreement with Siew and Brain (2002). From the above it shows that establishing the diagnosis of patients with IPA continues to be a challenging problem for the clinician, since the clinical symptoms of the disease are not pathognomic (Grillot et al., 1994). Moreover Aspergillus isolation from the respiratory tract may represent transient exposure to inhaled conidia (kurup and kumar, 1991). In addition, serology is not of great value in the diagnosis of IPA as stated before by Soubani and Cbandrasekar (2002).

Various studies on aflatoxins revolved around the effect of exogenous toxin in man; in this study we tried to detect endogenous production of aflatoxin and if there was any effect of its production on human body through examining different isolated Aspergillus strains for aflatoxin production. From these samples only 5 strains of our isolated *Aspergillus fumigatus* strains (4 from patients with Aspergilloma and one from a patient with ABPA) and only one strain of *Aspergillus flavus* (isolated from a patient with IPA) produced aflatoxin. Isolated toxigenic strains of Aspergillus from human were reported by few authors as Mori et al., (1998); Pepeljnjak et al., (2004).

In this study 5 out of 6 patients in whom Aspergillus was suspected to produce aflatoxins had only malaise and vomiting. These symptoms might be related to endogenous aflatoxins production but only one patient with IPA (he developed hepatoma later on) had hepatomegaly, ascites, jaundice and peripheral oedema in addition to malaise and vomiting and these are the clinical features of aflatoxicosis that were reported by Denning (1987) as community outbreak of poisoning and such condition is rare. Individual cases probably occur but remain unrecognized, very difficult to be diagnosed and may require measurement of serum or hepatic aflatoxin levels. A correlation between hepatoma and aflatoxin exposure does exist, as demonstrated by Peer et al., (1987). Also in a study conducted in Egypt by Abdel-Ghaffar et al., (1991) on 28 patients with hepatocellular carcinoma, 6 liver biopsies, 5 blood samples and 6 urine samples were positive for aflatoxin. In another study done in Sharkia Governorate in Egypt, Hifnawy et al., (2004) found that the incidence of hepatocellular carcinoma is strongly associated with food contamination of aflatoxin on one hand and with hepatitis C virus on the other hand. At the same time one patient with aspergilloma in our study had

lung cancer and it is not known whether this is an accidental association with the aspergilloma or it developed secondary to endogenous aflatoxin production. Several reports of association between aspergilloma and lung cancer do exist Hashiguchi et al., (1999) and Tomioka et al., (1999) but none of them had an adequate explanation for such association.

We recommend that as ABPA presented in a considerable incidence in our community so physicians should be aware of ABPA manifestations to identify patients before large areas of bronchiectasis have been occurred. The high correlation that exists between clinical and serological findings in patients with ABPA may allow the physicians to start specific treatment of these cases on clinical bases without waiting for serological results. Recent diagnostic trends should be applied for better and rapid detection of IPA as it is a fatal disease and empiric therapy should be started as soon as there is a clinical suspicion of IPA while a work up is underway. Further studies for detection of endogenous aflatoxin production and subsequent effects on the human body are recommended.

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