ELISA BASED DIAGNOSTIC TEST FOR ASPERGILLOSIS

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ABSTRACT

Detection of IgE and IgG antibodies in Aspergillosis is of diagnostic significance. The serological methods, such as agglutination, gel diffusion and counter immuno electrophoresis that are commonly used in the laboratories for diagnosis of *Aspergillus* induced infections, are less sensitive and high cross reactivity is often encountered. We carried out work on characterization and identification of diagnostically relevant antigens of *A. fumigatus*. Well characterized antigens were used to develop an ELISA with 92% sensitivity and 89% specificity for detection of specific IgE and IgG in the sera of patients of allergic bronchopulmonary aspergillosis (ABPA), aspergilloma and invasive aspergillosis. Subsequently, a sample kit having "ready to use type" of dry reagents (powder/tableted buffers and lyophilized antigen, conjugate and reference sera) was formulated. The kit was validated with sera from patients of ABPA, related allergic disorders, tuberculosis, post-Kochs cases and thalassemic children receiving repeated blood transfusions. The performance of the kit was found to be satisfactory with coded sera.

KEY WORDS : ELISA, Aspergillosis

INTRODUCTION

The incidence and mortality due to mycotic infections has been observed to be on the rise in past decade (1). Pathogens belonging to fungal genus *Aspergillus* are most ubiquitous and world-wide in distribution. *Aspergillus* induced infections are increasingly recognised as emerging threat to the public health. There are four major species of *Aspergillus* namely *A.fumigatus*, *A.flavus*, *A.niger* and *A.terreus* that cause aspergillosis in humans. However, *A.fumigatus* is the most prevalent and potential pathogen found in about 90% of *Aspergillus* induced manifestations. The allergic disorders caused by inhalation of *A.fumigatus* conidia include allergic bronchopulmonary aspergillosis (ABPA) (2), allergic asthma and hypersensitivity pneumonitis (3). Colonization and saprophytic growth of *A.fumigatus* in pre-existing pulmonary cavities of diverse origin lead to the development of aspergilloma (4). In immunocompromised host

the infection becomes invasive and disseminates from the primary site of infection to other parts of body including gut, liver, kidney, brain etc. Invasive aspergillosis is reported to be associated with a mortality rate of 55% (5). Mortality due to *Aspergillus* infection in bone marrow transplant recipients was observed to be as high as 80% despite appropriate chemotherapy (6). Cerebral aspergillosis (7) presents symptoms of acute meningitis and is always fatal.

Aspergillosis patients may be mistaken as patients of tuberculosis and appropriate treatment is missed (8). Therefore, an early diagnosis of aspergillosis is necessary to prevent irreversible damage caused by *A.fumigatus*.

SERODIAGNOSIS OF ASPERGILLOSIS

Antigens of *A.fumigatus* are known to evoke humoral immune responses in the patients of ABPA and aspergilloma (9-10). The detection of antibodies against *A.fumigatus* in these conditions was suggested as an aid for the diagnosis of aspergillosis (11). Agglutination, double immunodiffusion (12) and

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counter immunoelectrophoresis (13-14) were employed frequently for detection of anti-*Aspergillus* antibodies. However, methods have remained unsatisfactory because they are less sensitive and high cross reactivity is often encountered. A number of investigators used enzyme linked immunosorbent assay (ELISA) for detection of IgG and IgE antibodies in the sera of patients of aspergillosis (15-16). The antigens used in these studies were diverse obtained either from disrupted mycelia of short term cultures or from different stages of the long term autolytic phase of the growth of *A.fumigatus*. Variability in the antigens used led to the variations in the results. Due to the absence of precisely defined antigens of *A.fumigatus*, no WHO reference or international commercial standards were available for specific ELISA for aspergillosis and this restricted the use of ELISA to the R&D laboratories only.

Radioimmunoassay used to analyse patients for antigenemia showed 74% sensitivity and 90% specificity. The ELISA was found to be more than 95% sensitive and specific when used to measure the *A.fumigatus* antigen in sera or urine. Although antigen detection based methods were considered to hold great promise, these methods have not been established and made available for early diagnosis of aspergillosis.

SPECIFIC ELISA FOR ASPERGILLOSIS

The development of an ideal assay system for diagnosis of aspergillosis was not possible till recently due to antigenic complexity of *A.fumigatus* and non-availability of reference standards. The work on purification and characterisation of antigens of *A.fumigatus* was undertaken in our lab at CBT and immunodominant antigens of diagnostic relevance were identified (16). A sensitive and specific ELISA was developed using well characterised antigens of *A.fumigatus* for detection of specific IgG and IgE antibodies in the sera of patients of ABPA and IgG antibodies in aspergilloma. The ELISA was simplified and assay conditions were optimised so as to develop a 4 hr test. Subsequently a sample kit was formulated for diagnosis of aspergillosis. The kit was validated with sera samples of ABPA, related allergies, tuberculosis post-tuberculosis cases and multitransfused thalassemic children. A total of 162 proven cases of aspergillosis and 50 appar-

ently healthy controls were analysed for evaluating kit. In patients, the ELISA OD ranged from 0.25 - 2.10, Mean $\pm$ SD being 0.76 ± 0.48 . Mean $\pm$ SD OD in controls was 0.12 ± 0.08 , range being from 0.026 - 0.28 (Fig. 1). Controls to test ratio 1:3 was considered to be of diagnostic significance. The sensitivity and specificity of the test was found to be 92% and 89% respectively. Links were established with hospitals, research institutes and R&D units of industries to demonstrate the performance of test using coded sera. Results were found to be satisfactory. The test is now being used in the laboratory routinely for analysis and diagnosis of aspergillosis in patients referred to us by hospitals and clinical centres located in various parts of our country. More than 1400 patients have been screened so far and approximately 18% of them were found to be positive for anti-*A.fumigatus* antibodies.

It may be difficult to detect antibodies in immunocompromised host with invasive aspergillosis. Therefore, ELISAs for detection of antigenemia (17-18) and ultrasensitive methods based on luminescent immunoassay have been described (10,19).

Fig. 1 : Distribution of ELISA absorbance 490 nm in controls and patients of ABPA

AN ULTRASENSITIVE LUMINESCENT IMMUNOASSAY (LIA)

The use of LIA for the detection of IgG in biological fluids has been a comparatively recent approach. In this assay system hydrogen peroxide acts on the peroxidase component of conjugate to liberate oxygen radical which in turn oxidises luminol to produce aminophthalic acid and nitrogen with simultaneous emission of light photons (Fig. 2).

Fig. 2 : Schematic representation of LIA.

The photons thus produced are then detected by a luminometer.

The LIA was evaluated for its potential use in the diagnosis of ABPA. When compared with chromogenic ELISA, the LIA was found to be more sensitive. The LIA could detect up to 0.19 ng of human IgG in the samples. The detectability limit of ELISA was 1.48 ng. The ELISA absorbance or LIA emission of diagnostic significance was obtained at a dilution of 1:400 by ELISA and 1:2400 by LIA in an ABPA patient (Fig. 3). The analysis of HIV seropositive thalassemic children by ELISA and LIA revealed that the LIA had better discriminatory value (Table 1). These observations indicated its possible application in the screening of immunocompromised patients.

Fig. 3 : ELISA absorbance 490 nm (o—o) and LIA emission of light (●—●) with various dilutions of serum of an ABPA patient.

TABLE . Incidence of Specific IgG to *A. Fumigatus* in Multitransfused HIV Infected Children

Group	Total No. of samples	Positive by ELISA		Positive by LIA	
		No.	Percent	No.	Percent
MT-HIV seropositive	31	8	25.8	10	32.2
MT-HIV seronegative	33	1	3.03	1	3.03
Control	25	0	0	0	0

No. = Number

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