Skin allergic testing in pulmonary mycotic infection

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SUMMARY

In Pakistan until now the prevalence of fungal infections has not been investigated. The aim of this study was to determine the prevalence of fungi infection by skin allergic testing. In this study all patients were screened with skin prick test using a panel of fungal antigen. Those with positive skin reactions and other investigations were included. Out of 50 patients, 40 showed +ve reaction; 32 were males and 8 females.

This study was conducted at Nishtar Medical College and hospital Multan over a period of two years on pulmonary mycotic infection. In the admitted patients in the Chest ward suffering from various respiratory diseases, different laboratory investigations, radiological investigations and skin allergic testing were performed and 50 patients with repeated sputum positive for fungus were detected. The prevalence was 8.2%. The maximum incidence was in the age group of 21-30 years. From the analysis of initial signs and symptoms and their modifications and investigations including skin prick testing, helped in the diagnosis. Skin Prick test is used as a marker of allergy and lot of allergens are used. On skin prick test 30 patients showed reaction in candida series , 9 aspergillus fumigatus and one niger. Patients having candida albicans infection showed 30 (60%) positive results. In Aspergillus infection 10 (20%) had positive results. Out of 35 patients having candida albicans 17 are living while 18 had died due to associated diseases. Out of 11 cases of aspergillus 9 are living and 2 had died. Out of 4 cases of mucormycosis 2 are living, one died and one case was not traceable. Mean survival of candida, aspergillus and mucor was $16 \pm 13.7$, $25.2 \pm 10.3$ and $21 \pm 12.7$ months respectively.

INTRODUCTION

Skin prick tests were used as a marker of allergy. A number of allergens are used. Blastomycosis, coccidioidomycosis give delayed type hypersensitivity reaction. A positive skin test implies past or recent infection. Cryptococosis skin testing is considered reliable. In various studies like Sundram et. al, Jordan et. al, Smith et. al and Mobeireek et. al the skin testing is considered reliable, convenient and least expensive for the diagnosis of pulmonary mycotic infection. In nocardia skin test antigen has not been developed. No antigen or immune sera available for the diagnosis of cryptococcosis. Diagnosis is confirmed by the culture.

Fungi are simple vegetative structure; they lack chlorophyll and cannot conduct photosynthesis. They are characterized by the formation of filaments or hyphae, which branch and intervene to form dense net of growth. This mat like growth made up of mycelium with its spores constitutes an irregular rudimentary plant called fungus. This is not differentiated into roots, stems or leaves. They are saprophyes as well as parasites, are extremely common and have worldwide distribution, only a few being pathogenic. Mycosis is acquired by inhalation of spores. They cause primary as well as secondary infections during the treatment of bacterial or viral infections. They are now regarded as neither plants, nor animals but are placed in a separate group. They cause damage due to allergic necrosis and not due to toxins.

The fungal infections are superficial as well as deep. The superficial infections involve skin, nail hands and keratin containing structures while the deep infections involves dermis, mouth bones and viscera like lungs.

Pulmonary mycosis is not uncommon disease and it is prevalent all over the world. Islam et al 1962 for the first time reported about histoplasmosis in East Pakistan (now Bangla Desh). A survey was done in Karachi by Noor and Robinson on Histoplasmosis and concluded that this disease exists in the areas of Sind Memon and Moriani, reported 4 cases of pulmonary mycosis. Ahmed et. al reported 11 cases of pulmonary mycosis from Multan.

The genus aspergillus consist of many species of which fumigatus is potential pathogen. The spores are ubiquitous both in urban and rural air. It is world wide in distribution.

In Britain, aspergillosis is the most common and important fungal disease affecting the lungs. The fungi of the genus aspergillus are ubiquitous saprophyes and produce air born spores throughout the year and is also world wide in distribution. Coccidioidomycosis infection is dust borne spores. A unique event recorded in the
history of San Juaqín valley is a dust storm of C-immitis, spread over an area of approximately (87000 km²) in December 1977. This organism has been cultured by Omphalina and moffit in 1900. It commonly occurs in South Western parts of USA, Mexico and Venezuela.

Histoplasmosis has been traced to soil fertilized by chicken manure; to the dust of silo tower and to caves inhabited by bats called cave disease. This disease is present in caves explorers in Venezuela and South Africa. This is a filamentous fungus. Endemic infection of localized area of world occurs by inhalation of spores. Cryptococcosis infection occurs sporadically all over the world.

Blastomyces dermatitides. This infection has been reported form North America. The endemic areas are South Eastern region of USA. It is also present in middle Atlantic south central and Ohio-Mississippi river valley state. There was an outbreak of this disease in Minnesota in 1972.

Candida albicans is a common commensal of the mouth, alimentary tract and vagina. The superficial infection of the mucous membranes appears as white patches called thrush. Oral candidiasis can occur at any age in the course of debilitating illness. It is a serious opportunistic infection in any condition of impaired cell mediated immunity, and also after the administration of gluco corticoids & cytotoxic drugs. The oral lesion can extend down the alimentary or respiratory tract to produce fatal results. Mucormycosis. Species of mucor, rhizopus and asbidia cause mucormycosis. These genera contain many saprophytic fungal species and some of which are pathogens. Infection is usually acquired through the respiratory tract. The dermatophytes are a group of fungi which cause specific infections of human being and animals by invading only superficial Keratinized areas of the body such as skin, and nails. They do not cause systemic infections. Three genera are recognized like trichophyton Epidermophyton and micro sporum.

In the present study we used skin prick test to identify different types of fungi. This section follows with material and methods and results of study and in the final section the results are discussed.

MATERIALS AND METHODS

Skin testing helps to confirm the significance and relative importance of allergens previously suggested by case history. Bencard recommend the use of prick testing. Prick testing solutions must not be used for intradermal testing.

Using the applicator attached to the vial cap. I placed one drop of the control solution on the skin and in the same way place one drop of each prick test solution at about 3 mm intervals along the arm. The procedure is repeated with each test solution using a fresh prick test instrument. The reaction was examined approximately after fifteen minutes. Reaction was assessed by the degree of erythema and the area of wheal formed. Wheal size was measured with a Bencard skin test reaction gauge. Strength of each reaction was recorded relative to the control on a Bencard skin test reaction chart.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>-</td>
<td>No wheal, erythema absent or less than 1 mm.</td>
</tr>
<tr>
<td>+</td>
<td>Wheal absent or very slight. Erythema present not more than 3 mm diameter.</td>
</tr>
<tr>
<td>++</td>
<td>Wheal not more than 3 mm diameter, with associated erythema.</td>
</tr>
<tr>
<td>+++</td>
<td>Wheal between 3 mm and 5 mm diameter, with erythema.</td>
</tr>
<tr>
<td>++++</td>
<td>Any large size reaction.</td>
</tr>
</tbody>
</table>

The following conditions, where the reactions of SPT may be influenced, have been taken care in this study. Eczema is known to diminish the skin reactivity to histamine but this finding is not consistently observed. It seems reasonable not to perform skin test in areas where there is skin lesion that might interfere with skin reactivity. Patients with chronic renal failure have developed skin reactivity and the texture of skin makes testing difficult.

Some drugs can interfere with the performance of skin test and modulated either the wheal or the flare making the interpretation of the test difficult. Conversely some drugs used in allergic and / or asthmatic patients do not modified the cutaneous responsiveness.

H1 antagonists inhibit the wheal and flare reaction to histamine, allergens and mast cell secretogens. The duration of inhibitory effect appears to be link to the pharmokinetics of the drug and its active metabolites. H1 blockers reduce the activity for up to 24 hours. Cetirizine, loratidine, azelastine, mezolastine and terfenadine are potent drugs blocking the skin reactions for 3 to 10 days. Long-term treatment with classical antihistamines may reduce the inhibitory effect of these drugs on skin tests because tachyphylaxis usually develops. Topical H2 antagonists such as levo cabastine may reduce skin tests H2 antagonists used alone have limited inhibitory activity on skin tests. Kitotifen suppress skin test response for a period of fine days. Tricyclic antidepressants, exert a potent and sustained decrease of skin reactions to histamine. This effect may last for a few weeks. Tranquillizers and antiemetic agents can block skin tests. Topical doxipen hydrochloride has been shown to abolish skin reactivity after 1 to 3 days of therapy and up to 11 days after cessation.

Short term (less than 1 week) administration of corticosteroids at the therapeutic dozes used in patients
Skin allergic testing in pulmonary mycotic infection does not modify the cutaneous reactivity to histamine. Long term corticosteroids therapy does not alter histamine linked vascular reactivity in skin but effects skin mast cells responses and modifies the skin texture making the interpretation difficult in some cases. It has been recently shown that allergens linked skin tests can be accurately carried out in asthmatic patients receiving long term corticosteroids treatment.

Theophylline slightly reduces the reaction. Theses drugs must be stopped before skin testing. Short acting Beta-adrenergic agents inhibits skin reaction in usual dozes used in the treatment of the Asthma. Inhaled cromones do not interfere skin testing.

Nishtar Hospital Multan is a teaching hospital for the undergraduate as well as postgraduate medical students and has a wide catchments area covering civil divisions of Multan, Bahawalpur and Dera Ghazi Khan, Northern part of Sind, Baluchistan and a part of NWFP including Dera Ismail Khan and Waziristan. The cases of pulmonary mycosis were isolated from respiratory diseases in the department of chest diseases and Tuberculosis. The study was conducted over a period of 2 years. During this period 610 patients suffering from various respiratory diseases were admitted and 50 patients with positive sputum for fungus were isolated in this study. Cases negative for fungus in the sputum were excluded. The Assessment of all these patients was made through history, clinical examination, X-Rays and other relevant investigations like urine, complete blood, blood sugar, tuberculin test, sputum smear for AFB, sputum culture for AFB, bronchoscopy & pleural fluid cytology. Apart from sputum culture examination on sabourauds glucose Agar and broth media the skin allergic tests for various fungi was done.

Skin prick test was performed using allergens like candida, aspergillus and mucor available in a commercial kit. These tests were used in all cases. 0.1 ml of antigen was given after intra-dermal injection. The sites were examined after 15 minutes and size of the wheal was recorded. In general 3 mm reaction can be considered positive, whereas 1-2 mm was negative.

RESULTS

The skin prick test was performed on fifty identified patients. 40 out of these 50 patients showed positive reaction. The results are listed in table 1.

<table>
<thead>
<tr>
<th>Type of fungus</th>
<th>No. of cases</th>
<th>Positive reaction</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida Albicans</td>
<td>35</td>
<td>30</td>
<td>86</td>
</tr>
<tr>
<td>Aspergillus</td>
<td>11</td>
<td>9</td>
<td>82</td>
</tr>
<tr>
<td>Mucor</td>
<td>4</td>
<td>1</td>
<td>25</td>
</tr>
</tbody>
</table>

In the present study the Bencard sera was used. It gave positive results in 40 (80%) cases. Patients having candida albicans infection showed 30 (60%) positive results; in aspergillosis infection 10 (20%) had positive results. In this study, 40 out of 50 were positive reactors in Pakistan. This result is almost in line with findings of Sundaram et. al12 who had 21 positive out of 25 patients. Male patients were predominant in Candida Albicans while in the Aspergillus females were more as compared to male Table 2.

Distribution of confirmed Skin Positive Test(SPT) by sex is given in Table 2.

<table>
<thead>
<tr>
<th>Types of fungus</th>
<th>No. of cases showing positive Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida Albicans</td>
<td>Male 27 female 3</td>
</tr>
<tr>
<td>Aspergillus</td>
<td>Male 5 female 4</td>
</tr>
<tr>
<td>Mucor</td>
<td>Male 0 female 1</td>
</tr>
</tbody>
</table>

DISCUSSION

Skin prick test may be used as a marker of allergy. A number of allergens are used which are obtained from commercial firms. The sites are examined after 10-20 minutes. Size of wheal is recorded. In general 3 mm reaction can be considered positive whereas 1-2 mm is probably positive. The clinical significance of the reaction is finally confirmed by sputum culture.

Blastomycosis gives a delayed type of hypersensitivity comparable to coccidiodin, histoplasmin and tuberculin; sometimes the test is negative in severe disseminated disease. Serodiagnostic tests are at the moment insufficiently sensitive for reliable diagnosis. The intradermal histoplasmin test is useful as an indication of past or present infection. The test resembles the tuberculin hypersensitivity test and becomes positive about 4-8 weeks after infection. Occasionally negative results are obtained during active disease.

Coccidiodin comparable to (tuberculin) gives a delayed type positive reaction when injected intra dermally into patients who have developed hypersensitivity to the products of infection. The test becomes positive about a month after infection. A positive test implies past or recent infection.

A survey of histoplasmosis sensitivity test was carried out in Karachi by Noor and Robinson et al in a hospital population free of active pulmonary disease and in a group of farm workers. No positive reactor was found in the hospital group and only 2 positive reactors were detected in the farmers. In Smith et. al study, Sera from
44 patients with clinically suspected pulmonary aspergillosis were examined for antibodies to aspergillus fumigatus using an enzyme-linked immunosorbent assay and counter immuno electrophoresis. Out of 44, 15 were considered to contain aspergillus antibodies. The serum of 1 patient with invasive pulmonary aspergillosis was negative in both tests. Protein enriched antigens derived by ammonium sulphate precipitate of crude hyphae homogenates is used in Elisa (but not CIE). However, some patients with invasive aspergillosis can be antibody-negative even with sensitive test such as Alisa.

In our study 40 out of 50 showed positive reaction. Whereas Sundaram et al \(^1\) conducted a study in which Sera from 25 suspected cases of pulmonary aspergillosis were tested against antigen prepared from 4 strains of aspergillus flavus using the ouchterlong double diffusion and immuno-electro phoresis techniques. Out of 25 sera 18 reacted positively with antigen of aspergillus flavus and 2 with both these species.

In our study histoplasmosis and blastomycosis were not detected. False – positive serological tests for histoplasmosis and blastomycosis are common in population from endemic areas. In the study of Jordan et. al\(^2\), 263 patients whose sera were examined 29 had histoplasmosis and 41 had false-positive results. A positive fungal serology is not useful in suggesting the presence of a pulmonary disease in patients from an endemic areas suspected of having a pulmonary mycosis. In Smith et al\(^3\) study the serological diagnosis of fungal disease in 44 (88%) patients suffering from aspergillosis, 15(30%) showed antibodies. In addition out of 8, 4(50%) having candida infection showed positive reaction. Humphrey et al\(^4\) has reported that in 58 hospital patients who were examined with antigen to candida. In 11 cases of candidiasis, fungal antibodies were positive.

Early diagnosis can be made in pulmonary opportunistic infection by using polymerase chain reaction and beta-glucan. In a study 58 patients with hematological neoplasm accompanied by severe pulmonary infections of pneumocystic carinii, and cytomegalovirus pneumonia was made by PCR by sputum sample for pulmonary mycosis by measuring blood betaglucan level. PCR and betaglucan method can effectively enable the clinicians to diagnose pulmonary opportunistic infection in the early stage. The underlying disease state can influence the clinical outcomes of the patients in terminal pulmonary infection caused by CMV or mucor suggesting that prevention and early diagnostic measure for these infections remain to be established Jojima\(^5\).

Comparison between Wako-WB003 and fungitec G tests for detection of (1-3) beta glucan measurement by the kinetic turbidimetric limulus test for the clinical diagnosis of mycotic infections can be used Mori et al\(^6\).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Total</th>
<th>No. of Cases Reactors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noor &amp; Robinson 1965</td>
<td>69</td>
<td>2</td>
</tr>
<tr>
<td>Sundaram et al 1981</td>
<td>25</td>
<td>21</td>
</tr>
<tr>
<td>Smith et al 1984</td>
<td>44</td>
<td>15</td>
</tr>
<tr>
<td>Humphrey &amp; Weiner et al 1983</td>
<td>58</td>
<td>11</td>
</tr>
<tr>
<td>Al-Mobeireek et al 2001</td>
<td>62</td>
<td>62</td>
</tr>
<tr>
<td>Present study</td>
<td>50</td>
<td>40</td>
</tr>
</tbody>
</table>

The papers on skin test, from the European Academy of allergy and clinical immunology and the United States joint council of allergy. Asthma and immunology agree that when properly performed, prick puncture tests are generally considered to be the most convenient and least expensive screening method for detecting allergic reactions in most patient. However, until the diagnostic efficacy of prick puncture tests is fully established with standardized allergens and methods, negative prick puncture tests may be confirmed by more sensitive intradermal techniques especially for drugs and venoms. Even after false – positive and false – negative have been eliminated, the proper interpretation of results requires a thorough knowledge of the history and physical findings. A positive skin test alone does not indicate a definite clinical sensitivity to an allergen. Skin test represents the primary and most effective diagnostic method. A positive skin test with a history suggestive of clinical sensitivity strongly incriminates the allergen as a cause of the disease. Conversely a negative skin test with a negative history favours a non-allergic disorder. Interpretation of skin tests that do not correlate with the clinical history is more difficult

A panfungal PCR assay which detects the small – subunit rRNA gene sequence of the two major fungal organism groups was used to test whole-blood specimens obtained from a series of blood or bone marrow transplant recipients. The 580 bp PCR product is identified after amplification by panfungal primers and hybridization to a 245 – bp digoxigenin labeled probe. The lower limit of detection of the assay was approximately four organisms per milliliter of blood\(^10\). In a study multiple whole blood specimens from five patients without fungal infection or colonization had negative PCR results. Blood from 3 patients without pulmonary aspergillosis had positive PCR results. Out of these 3 patients one patient blood specimen obtained in the week prior to the diagnosis of infection by bronchoalveolar lavage fluid Culture result was positive by PCR and blood specimens obtained from two patients 1 to 2 days after lung biopsy which were sterile by culture were positive by PCR. The panfungal PCR assay can detect multiple fungal genera and may be used as an adjunct to conventional methods for the
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detection of fungal infection or for describing the natural history of fungal infection further studies are needed to define the sensitivity and specificity of the assay for the diagnosis of fungal infection prior to the existence of other clinical or laboratory indications of invasive fungal infection. At present, amplification methods for the introduction of diagnostic amplification techniques into a clinical laboratory implies a level of proficiency for excluding false – positive and false – negative results Leven et. al. Immuno diffusion serology a technique that identifies specific circulating antibodies is suggestive of an active infection. Marine animals largely has aspergillosis Reidarson et. al.

REFERENCES

1. Sundaram S; Kalyanasundaram, R; Rangaswamy V. “Serological diagnosis of pulmonary Aspergillosis”, Mycopathologia; 1981 Aug 7; 75(2) : 93-9”.