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Research Article

Phagocyte Oxidative Dysfunction in Invasive Sino Nasal Aspergillosis

Prankur Verma[1], Kapil Sikka[1]*, Hitesh Verma[1], Alok Thakar[1], Dipendra K Mitra[2], Divya Kamboj[2] and Abhinav Saurabh[2]

[1]Department of ENT, All India Institute of Medical Sciences, New Delhi, India

[2]Department of Transplant Immunology and Immunogenetics, All India Institute of Medical Sciences, New Delhi, India

***Corresponding Author:** Kapil Sikka, Department of ENT, All India Institute of Medical Sciences, New Delhi, India.

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Abstract

Background: Phagocytes play an important role in fungal cell recognition and damage through oxidative and non oxidative methods.

Objectives: This study investigated the phagocyte oxidative function in invasive fungal sinusitis.

Methods: The peripheral blood of the patients was stained with di-hydro-rhodamine and reactive oxygen species production in the phagocytes was measured.

Results: A total of 14 cases were studied out of which 8 and 6 had aspergillosis and mucormycosis respectively. 5 patients, all of whom belonged to the aspergillosis group, showed decreased phagocyte oxidative burst function quantified using Neutrophil Oxidation Index.

Conclusions: Invasive aspergillosis in a patient may point towards an intrinsic derangement in phagocyte oxidative function. Evaluation of phagocytic function should be done in all the cases of invasive aspergillosis.

Keywords: Invasive Aspergillosis; Phagocytic Function; Neutrophil Oxidative Index; Chronic Granulomatous Disease; Innate Immunity

Introduction

Innate immunity against fungal infections consists of neutrophils, mononuclear cells and dendritic cells which are involved in fungal cell recognition and damage [1]. Both mononuclear and polymorphonuclear phagocytes of normal hosts kill the fungi by the generation of oxidative metabolites and cationic peptides [2,3]. Neutrophils express soluble and membrane-bound pattern recognition receptors (PRRs) for binding fungal pathogen associated molecular patterns (PAMPs) [4] that results in activation of signal transduction pathways to elicit a cytotoxic response [5]; which can be oxidative and non oxidative killing. Oxidative mechanisms include Reactive Oxygen Species (ROS) production, mediated by the enzymes nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, nitric oxide synthase and myeloperoxidase, while non oxidative mechanisms include the release of granules containing proteins with antimicrobial and degradative properties, including defensins, lysozyme, lactoferrin, gelatinases, elastase and cathepsin-G [6,7].

Patients with Chronic Granulomatous Disease (CGD) are susceptible to invasive mould infections due to NADPH deficiency rendering neutrophils completely defective in the killing of A. fumigatus hyphae, which normally inhibit Aspergillus conidia germination [4].

The present study investigated the phagocyte oxidative burst function in patients with invasive fungal sinusitis.

Materials and Methods

Study population

The study was conducted at the Department of E.N.T. of our institute. Approval for the study was obtained from the Institute Ethics Committe.

Eligible subjects presenting with invasive fungal infections of the nose, paranasal sinuses, orbits, cavernous sinus, or skull base who underwent microscopic examination of the lesion to confirm the presence of fungal elements were recruited. Patients with known congenital immunodeficiency, hematological malignancy, and those who refused to provide consent to participate in the study were excluded. Ultimately, a total of 14 patients who fulfilled our eligibility criteria were analyzed. All patients received surgical and anti-fungal therapy as per the standard guidelines.

Methodology

Subjects were divided into two groups: those with mucormycosis and those with aspergillosis. All subjects received an explanation regarding the purpose of the study, after which written informed consent was obtained in their vernacular language. The peripheral venous blood of the patients was drawn into a heparinized vial and promptly transported to the Immunology Laboratory for assessment.

In vitro ROS Production Using Di-hydro-rhodamine (DHR)

Di-hydro-rhodamine 123 is an uncharged and non-fluorescent ROS indicator that can passively diffuse across cell membranes where it is oxidized to cationic rhodamine 123 which localizes in the mitochondria and exhibits green fluorescence. The amount of fluorescence is directly proportional to the ROS production.

For assessment of ROS production, the peripheral blood obtained from patients was diluted 1:10 with phosphate buffered saline (PBS) and stained with DHR and incubated at 37 degree Celsius in dark for 30 minutes, along with a control sample drawn from a healthy subject. Polymorphonuclear (PMN) cells were then stimulated in vitro with 50 ng/ml of phorbol 12-myristate 13-acetate (PMA) (Sigma, St. Louis, USA, Cat no. P8139) for 30 minutes and incubated at 37 degree Celsius in dark. Finally, the red cells were lysed using red cell lysis buffer (RCLB), washed with PBS and immediately acquired on a flow cytometer (BD FACS Calibur) to evaluate the ROS production.

Neutrophil Oxidation Index (NOI) was calculated as follows.

NOI = Median Fluorescence Intensity (MFI) of stimulated PMN cells/ Median Fluorescence Intensity (MFI) of unstimulated PMN cells.

Results

Demographic distribution

A total of 14 patients were included among which six had mucormycosis (all of whom had a fulminant or acute invasive course), while eight had aspergillosis (all of whom had a chronic course). Table 1 summarizes the demographics and comorbidities.

Table 1: Characteristics of the patients suffering from invasive fungal sinusitis at presentation.

Characteristics	Mucormycosis patients	Aspergillosis patients
Number	6	8
Comorbidities	5 diabetic	2 diabetic, of them 1 had rheumatoid arthritis
Male : Female	5: 1	5: 3

The phagocyte oxidative burst function

5 out of 14 cases evaluated for ROS production by the phagocytes showed decreased Neutrophil Oxidation Index using Di-hydro-rhodamine test. All of the cases with impaired phagocyte oxidative function belonged to the aspergillosis group. Only one of them had diabetes. Table 2 summarizes the phagocyte oxidative function values of the patients and their corresponding controls.

Discussion

Invasive fungal diseases of Sino nasal and skull base regions are still a group of not so well known and explored conditions occurring in humans and animals. Due to their rarity, these conditions may baffle a clinician into diagnosing and treating them. The present study investigated the oxidative function of the patients' neutrophils using the DHR test.

Five of the total eight aspergillosis cases turned out to have a lower than normal Neutrophil Oxidation Index, which signifies inability of the phagocytes to produce ROS to kill the phagocytosed

organisms, due to a dysfunctional or absent NADPH oxidase enzyme. One such had a conversion from non invasive to invasive disease, and required multiple surgical debridements and long term anti fungals. These patients did not have a history of repeated infection in childhood or a previous instance of fungal disease before, or in their family members, so it is not clear if they were suffering from Chronic Granulomatous Disease (CGD), or had dysfunctional neutrophils due yet unknown reasons. It is however known that in patients of diabetic ketoacidosis, phagocytes are dysfunctional and have impaired chemotaxis and defective intracellular killing by both oxidative and non oxidative mechanism [1], but to the contrary, only one of them was diabetic.

The number of patients was too low to establish prevalence of phagocyte dysfunction, but the fact that only the aspergillosis patient had dysfunctional phagocytes was worth noting. Evaluation of phagocytic function using DHR test is thus imperative in all cases of Aspergillosis.

Kerstin Voelz., *et al*. [8] showed that a defect in phagocytic effector responses causes lethal disease in the zebrafish larval model which shows a similar disease as humans. The role of phagocytes in anti fungal defense is so important that there is a role of using Granulocyte Colony Stimulating Factor, Granulocyte-Macrophage Colony Stimulating Factor and Natural Killer T cell infusion against these deadly diseases with good outcomes [9-12].

Conclusions

Invasive Aspergillosis in a patient may be associated with an intrinsic derangement of phagocyte oxidative function. Further studies investigating the neutrophil dysfunction in aspergillosis patients and their family members are required to elucidate this finding.

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Declaration of Conflicting Interests

The Authors declare that there is no conflict of interest.

Ethics Committee Approval

Approval for the study was obtained from the Institute Ethics Committee of AIIMS, New Delhi.

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