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Fungal Contaminations of Blood Stains and their Secretory Substances

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KAN LEKELERİNDEKİ MANTAR BULAŞIKLIĞI VE BUNLARIN SALGI MADDELERİ

Özet

Mikro-organizmalarla bulaşmış kan lekelerindeki ABH antijenlerinin gerçek yapılarının gösterilebilmesini zorlaştırır. Bu araştırmanın amacı, kan lekelerinde çoğalan mantarların ayırımı ve kan grubu antijenlerinin kaybına/değişmesine yol açan sorumlu faktörlerin meydana çıkarılmasıdır.

Bulaşık kan lekelerinde, 3 *Aspergillus* türü ve 1 *Penicillium* türü mantar bulundu. Bunların kan lekesi üzerindeki salgılarında, mantarlara ait değişik yapıda çeşitli enzimler tesbit edildi. Bu enzimlerin, kan lekelerinin antijenik yapılarının incelenmesinde güçlük çıkarması mümkündür.

Summary

Blood stains contaminated with micro-organism fail to show the true nature of their ABH antigen. The present investigation is aimed to identify fungi infesting blood stains and their secretory substances, to determine the factor responsible for löss/alteration of blood group antigens.

In contaminated blood stains, three species of *Aspergillus* and one of the *Penicillium* have been identified. Several enzymes of different nature are detected in their exudate over the stains. These enzymes may be responsible for such indifferent results.

Keywords : *Contaminated blood stains - Fungi - Secretory substances - Blood group antigens*

INTRODUCTION

Blood stains brought to the laboratory for determination of blood groups are often found contaminated with micro-organisms. The exposure of stains to high humidity is observed to result in growth of fungi. *Kashyap* et *al* (1) have reported the presence of micro-organisms on stains exposed to 50 % or above relative humidity. The contami-

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nated B group blood stains are generally observed B antigen negative or false positive for H antigen (2). The change in nature of B antigen may be attributed to the presence of fungal contamination. There are various reports on secretions of enzymatic substances by fungi pathogens (3,4) . However, there is no report available, on the type of fungi infesting blood stains except that of *Kobayashi* et *al* (5) and the nature of enzymatic substances in their exudate. To delineate the cause of change in B antigen in fungal infested stains, in the present investigation, first, fungi infesting blood stains are identified and secondly substances secreted by them on stains are characterized.

MATERIALS AND METHODS

Chemicals and Reagents

All the chemicals used in the present investigation were of BDH analytical grade and substrates for enzymatic study, were purchased from Sigma Co., USA. Human blood of different groups (ten samples each) were collected from Central Blood Bank, Hyderabad. Anti-sera of different groups were purchased from Ethnor Ltd., Bombay.

Preparation of Stains

Ten stains of each blood group were made by pouring 5 mL blood of known group on sterile cotton cloth of 10x10 cm, size. The stained cloths were kept in petri dishes at 50% or above humidity and free passage of environmental air throughout the period of the study (15 days) was maintained (6,7).

Preparation of Culture

For identifying the fungi in contaminated blood stains, cultures and sub-cultures were grown on PSA (8) medium. For identification of enzymes secreted from the fungi Zapak's medium (9) was used.

Identification of Mycelia

Identification of mycelia was carried out in the laboratory by the standard identification methods (10,11).

Extraction of Enzymes

Isolated fungi were developed in cZapak's liquid culture medium with suitable substrates and mycelium and culture filtrates were used as a source of extracellular enzymes.

Identification of Enzymes

Proteases were identified by using case in as substrate (12) and other carbohydrates related enzymes i.e., β-glucosidase, amylase, invertase, cellulase, glycosidase, galacturonidase, were identified by using salicin, starch, sucrose, cellulose, p-nitrophenyl β-D-glucosid and pectin, respectively as substrates (13) .

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Detection of Blood group Antigens

Blood group antigens in stains were identified by absorption elution method (14).

RESULTS AND DISCUSSION

Table I and Figures 1&2 show various fungi infesting blood stains and their rate of growth on stains of different groups. *Aspergillus niger, Aspergillus nidulus, Aspergillus flavus* and *Penicillium notatum* are observed growing on all blood stains of all blood groups. However, the growth of different species are observed to have different degree of growth on different blood group stains. *Aspergillus niger* has highest growth rate and *Penicillium notatum* has the minimum. The other two species i.e., *Aspergillus nidulus* and *Aspergillus flavus* show moderate growth on all studied blood stains.

Table II depicts enzymes secreted by different fungi. The enzymes, protease, β-glucosidase, amylase, invertase, cellulase, glycosidase and galacturonidase are secreted by all the four species of fungi. However, *Penicillium notatum,* in addition to these enzymes, also secretes penicillin, an antibiotic in its exudate. The quantity and nature of different enzymes differ from species to species.

Table I. Fungi contaminating blood stains and their growth.

Sample number	*Group of blood stain*	*Growth*			
		A. niger	*A. nidulus*	*A. flavus*	*P. notatum*
1	A	+++	+++	++	+
2	B	+++	++	++	+
3	O	+++	++	++	+
4	AB	+++	++	++	+

Maximum : +++
Moderate : ++
Minimum : +

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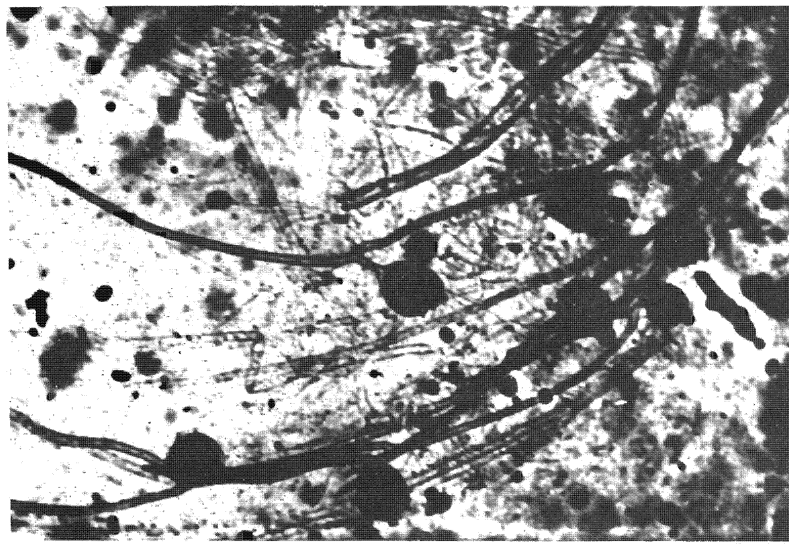

Figure 1. *Aspergillus nidulus.*

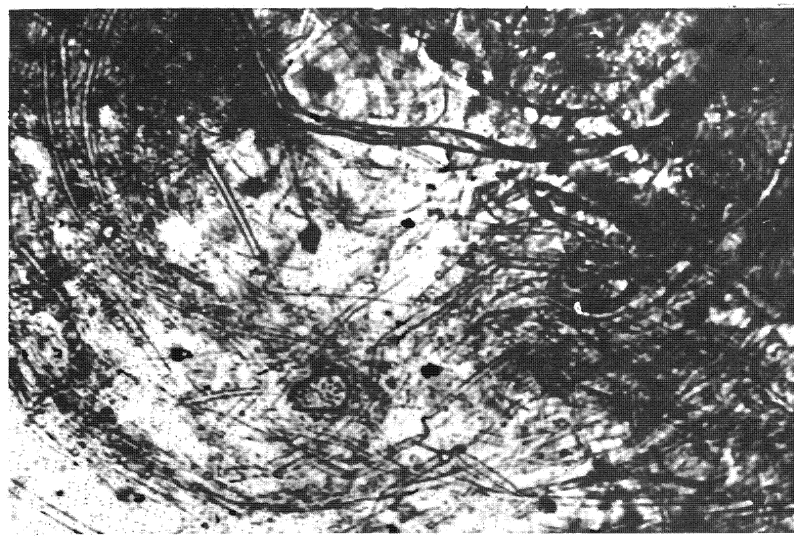

Figure 2. *Penicillium notatum.*

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Table II. Enzymes secreted by blood stains infesting fungi.

Sample number	*Fungi*	*Enzymes identified*
1	*Aspergillus niger*	Protease, ß-glucosidase, galacturonidase, cellulase, glycosidase, amylase, invertase
2	*Aspergillus nidulus*	Protease, ß-glucosidase, galacturonidase, amylase and invertase and cellulase
3	*Aspergillus flavus*	Protease, ß-glucosidase, amylase, invertase, galacturonidase, cellulase, glycosidase
4	*Penicillium notatum*	Protease, amylase, cellulase, ß-glucosidase, galacturonidase, glycosidase, penicillin

Table III. Influence of fungal contamination on blood group antigens.

Sample number	*Stains of blood group*	*Number of stains examined*	*Antigen identified in stain*	
			Number of stains	Antigen
1	A	10	8	A
			2	None
2	B	10	4	B
			6	H
3	AB	10	6	AB
			4	A
4	H	10	10	H

The influence of fungal contaminations on different blood group antigens is shown in Table III. The results show that A group antigen is not found altered in any of the 10 fungal infested stains except in 2 cases, no antigen could be detected by the employed method. Out of 10 contaminated stains of B group, in 6 cases, H antigen was detected instead of B. Similarly in 4 stains of 10 AB stains, only A antigen could be detected. H antigen in blood stains was not observed influenced by contaminating fungi growing on blood stains or their exudate.

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When the fungi isolated from contaminated blood stains were independently allowed to grow on different blood group stains, it is found that the rate of growth of different fungi species is different and also varies with the nature of stains. Perhaps, the difference in growth rate is due to the varied nature of constituents present in different blood stains which serve as substrate for growth of fungi. When ABH antigens were examined in contaminated blood stains, it is found that majority of B positive stains could not be detected and instead of B, H antigens positive reaction were observed. This change in nature of antigen reaction could be attributed to the enzyme/s present in exudate of contaminating fungi.

A detailed systematic study about the enzyme responsible for altering/deteriorating for antigenicity of B antigen will be presented separately.

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