

In vitro antifungal effect of human milk

Emin Mete, M.D.,* Nüket Bavbek, M.D.,# Sabriye Dayı, M.D.,§ Mehtap Erkmen, M.D.,# and Fatih Andıran, M.D.§ (Turkey)

ABSTRACT

A lower incidence of infection occurs among breast-fed babies because of the presence of antibacterial, antiviral, and antiparasitic effects, but little is known about the antifungal effects to fungi other than *Candida albicans*. This study was undertaken to assess the antifungal effect of human milk to the fungi in the environmental air, which also may be allergenic. Milk samples were obtained from lactating mothers of healthy term infants between the 3rd and 8th days of lactation. Ninety-six Sabouraud agar petri dishes were separated into three groups, closed, and incubated in the same location after 15 minutes uncovered. The first group (group 1, $n = 48$ dishes) was used to detect the fungal flora of the environmental air. The second group (group 2, $n = 24$ dishes) was rubbed with a thin layer of human milk by a sterile pipette. The last group (group 3, $n = 24$ dishes) was rubbed with 0.9% NaCl solution. After 7 days of incubation, the colony-forming fungal growths of all dishes were evaluated by a microbiologist who did not know the groups of the dishes. The number of fungal colonies grown in human milk-rubbed dishes in group 2 was less than both of the other groups (group 1 and 3; $p < 0.001$ and $p < 0.001$, respectively). These results indicated that human milk may have antifungal effects to fungi present in the environmental air as tested by Sabouraud agar petri dishes. To prevent infections and allergic diseases, human milk must be considered the ideal food for newborns.

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In addition to well-known antimicrobial substances, a growing body of evidence has occurred in the last 10 years regarding the presence and function of immunomodulatory and anti-inflammatory factors present in human milk.¹ These substances in human milk modulate and mature the immune system of premature or mature newborns and protect them against infections. The incidence of gastrointestinal and respiratory infections in infants receiving human milk is lower than in those not breast-fed, because of the presence of the specific antibody and antiadhesion molecules in human milk.² Although it is well documented that breast milk has *in vivo* and *in vitro* antibacterial, antiviral, and antiparasitic effects,^{3–10} only a few studies are present concerning the antifungal effects. The phagocytosis and killing of *Candida albicans* by human milk macrophages has been reported.^{11–13} On the other hand, there is no study about the effects of human milk to the fungal flora of daily natural environmental air. Therefore, the purpose of this study was to determine the antifungal effect of human milk to the fungi in the air, which in some conditions may be allergenic.

MATERIALS AND METHODS

Milk samples were obtained from lactating mothers of healthy term infants between the 3rd and 8th days of lactation after colostrum, with a breast pump, and the milk was placed into sterile containers. All samples were stored at 4°C and processed within 2 hours of collection. Ninety-six Sabouraud agar petri dishes were used. All petri dish lids were closed and incubated in the Ankara Emek district, in the same location, on an unrainy day, after 15 minutes uncovered. Then, the dishes were separated into three groups. The first group (group 1, $n = 48$ dishes) was used to detect the fungal flora of the environmental air. The second group (group 2, $n = 24$ dishes) was rubbed with a thin layer of human milk by a sterile pipette. The last group (group 3, $n = 24$ dishes) was rubbed with 0.9% NaCl solution to determine whether the depletion of fungal growth in human milk-rubbed dishes was only caused by the prevention of contact of air to the agar or the antifungal effect of the human milk. After 7 days of incubation, the colony-forming fungal growths of all dishes were evaluated, counted, and recorded by a microbiologist who did not know the groups of the dish numbers.¹⁰ The statistical evaluation between the three groups was done with chi-square test and values of $p < 0.05$ were considered statistically significant.

RESULTS

The fungal growth in the groups is shown in Table 1. There was no statistical significance between the number of fungal colonies grown in normal groups and in

From the Departments of *Pediatrics, #Internal Medicine, and §Pediatric Surgery, Fatih University Medical School, Ankara, Turkey
Address correspondence and reprint requests to Emin Mete, M.D., Department of Pediatrics, Division of Allergy, Fatih University Medical Faculty, Hoşdere C. No :145, Y. Ayrancı, 06540 Ankara, Turkey
E-mail address: metee@hotmail.com
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Table 1 Colony-forming fungal growth in the groups

Fungi	*Environmental Fungal Flora (Group 1; n = 48)		Environmental Fungal Flora with Human Milk (Group 2; n = 24)		Environmental Fungal Flora with 0.9% NaCl (Group 3; n = 24)	
	Number of Colonies	Ratio of Colony to Total (%)	No. of Colonies	Ratio of Colony to Total (%)	Number of Colonies	Ratio of Colony to Total (%)
<i>Rhizopus</i>	120	33.8	31	31.9	58	32.9
<i>Cladosporium</i>	85	23.9	23	23.7	40	22.7
<i>Penicillium</i>	49	13.8	10	10.3	20	11.3
<i>Alternaria</i>	29	8.1	9	9.7	15	8.5
<i>Mucor</i>	23	6.4	8	8.2	14	7.95
<i>Aspergillus</i>	20	5.6	6	6.1	11	6.25
<i>Hormodendrum</i>	12	3.3	4	4.1	7	3.9
<i>Laccaria</i>	6	1.6	3	3.09	4	2.27
<i>Monilia</i>	6	1.6	1	1.03	3	1.7
<i>Stemphilum</i>	3	0.8	1	1.03	2	1.1
<i>Myxomycele</i>	2	0.5	1	1.03	2	1.1
Total	355	100	97	100	176	100
Mean number of colonies $\pm$ SD	7.56 $\pm$ 3.6		3.9 $\pm$ 2.2		7.32 $\pm$ 3.1	

the 0.9% NaCl group. The number of fungal colonies grown in human milk-rubbed dishes in group 2 was less than both of the other groups (groups 1 and 3), which was statistically significant ($p < 0.001$ and $p < 0.001$, respectively). *Cladosporium*, *Alternaria*, *Aspergillus*, *Penicillium*, *Stemphilum*, *Mucor*, *Stachybotriys*, *Calvatica*, *Ureidinisporium*, *Hormodendrium*, and *Rhizopus* sp. fungi were grown and fungus species were similar in all of the dishes. In the human milk group, the number of fungal colonies was significantly lower, but the percentages of fungal species were similar.

DISCUSSION

A lower incidence of infection occurs among breast-fed babies than among artificially fed babies. Much of the antibacterial effect of milk has been ascribed to the powerful bacteriostatic function of fluid-phase components, including buffer, lactoferrin, IgA antibody, and lysozyme. There also are polymorphonuclear leukocytes, macrophages, and lymphocytes present in human milk.^{2,5-8,14}

Although it is well documented that breast milk has *in vivo* and *in vitro* antibacterial, antiviral, and anti-parasite effects,³⁻¹⁰ only a few number of studies are present regarding the antifungal effects. Cummings *et al.* have reported that phagocytosis and killing of *Candida albicans* by milk macrophages were identical to that by blood monocytes.¹¹ The *in vitro* phagocytosis and killing of *Staphylococcus aureus*, *Escherichia coli*, and

C. albicans by human milk macrophages and neutrophils after opsonization in aqueous phase have been shown by Robinson *et al.*¹³

The significantly less fungal growth in human milk-rubbed agars than in simple Sabouraud agars in our study may suggest an *in vitro* antifungal effect of human milk, which could be an important factor in protection from mycotic infections in breast-fed infants. In one study, this antifungal effect in milk cells has been reported to be lower to *C. albicans* than to serum or blood cells, which may be a result of inadequate complement activation because killing is more complement-dependent than opsonization.^{13,15}

Fungi threaten human life not only by infections, but also by causing allergic reactions as aeroallergens.¹⁶⁻¹⁹ Before causing allergic reactions, mucosal blockage of fungi on entering the human body can prevent the onset of allergic bronchitis, asthma, and skin diseases such as urticaria. Providing the production of IgG-type blocking antibodies against the aeroallergens such as fungi is one of the mechanisms of vaccination in asthmatic patients.²⁰ Because of its antifungal effect,¹⁶ human milk may be proposed to protect infants from allergic diseases caused by fungi *via* some mechanism of induction of cytokines and secretory IgA. Having established the *in vitro* antifungal effect of human milk against normal saline, other substances such as cow's milk and infant formula also would be examined for this antifungal effect.

In conclusion, human milk shows antifungal effects to not only *C. albicans* but also to other fungi present in environmental air as tested by Sabouraud agar petri dishes. To prevent infections and allergic diseases, human milk must be considered the ideal food for newborns. Additional studies are needed to determine the blocking antibodies and antifungal factors and their mechanisms present in human milk.

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