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The Potential Association of Human ABO Blood Group in *Candida albicans* Germination

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Abstract

Candidiasis is an infection caused by *Candida* spp., mostly *Candida albicans*. Infection with *Candida* spp. in immunocompetent individuals is often limited to the mouth, genital area or skin. However, patients who are immunocompromised due to infections or medications including corticosteroids or anticancer drugs are more susceptible to systemic candidiasis and candidemia. Upon *C. albicans* infections, the pathogenic yeasts enter the bloodstream and disseminate to the remote organs, leading to the formation of germ tube. Dimorphic adaptation is one of contributing virulent factors in *C. albicans*, which is responsible for disease pathogenesis and severity. However, little is known about whether differences among human ABO blood groups facilitate the development of *C. albicans* germ tube. Therefore, this study investigated the germ tube formation of *C. albicans* that grown in human serums from different ABO blood groups. Human pool serums varying ABO blood groups from healthy individuals were tested for *C. albicans* germ tube induction. After incubation for 2 h at 37 °C, the rate of germ tube formation at 15-min intervals was determined. Serum from human blood group A exhibited the highest rate of germ tube formation. Humans with blood group A exhibited the unique serum biochemical structure that possibly helps facilitate germ tube formation during *C. albicans* infection.

Keywords: Germ tube, *Candida* spp., *Candida albicans*, Candidiasis, Candidemia, ABO blood group, Yeast

Introduction

Pathogenic fungi have increasingly been associated with high rates of morbidity and mortality. Nowadays, there is an increase in reports of standard antifungal resistance [1] particularly in yeasts such as *Candida* spp. [2] and *Cryptococcus neoformans* [3], as well as in molds such as *Aspergillus* spp., *Scedosporium* spp. and *Mucor* [4,5]. The main-contributing factor to the increase in antifungal drugs resistance is the indiscriminate and uncontrolled of antimicrobial drugs. Furthermore, misidentification of pathogenic fungi has resulted in improper and ineffective treatments. Previous studies have also identified several patient risk factors that contribute for specific fungal pathogenesis [2,3]. The other risk factors are patients with underlying diseases, cancer patients receiving chemotherapy and patients receiving immuno-suppressive drugs and state of the host immune system. A number of studies have been conducted on *Candida* spp., which are commonly used as a model for fungal research due to their high infection rates across all age groups worldwide [6]. In patients infected with *Candida* spp. on the skin mucous membranes and bloodstream, the infections rate was found to be higher in individual with blood group type O compared to other blood group [7,8]. In mold infection, a previous study reported a higher prevalence of dermatophytes infection, among patients with blood group A [9,10]. The study was consistent with *Candida* spp. when considering the pathogenesis and the severity of infections in both yeast and strains. It was found that fungal germination was a fundamental and correlated factor, which led to aggression and severity in the host [11,12]. However, the relationship between germination and fungal aggression within different blood groups remains poorly investigated. Therefore, the present study aimed to determine the association between blood groups of Thais and the germination and invasion of pathogenic fungi, which are prevalent in Thai people. This research study focused on changes in fungal germination in relation to the blood groups. The finding of this research will provide a valuable

foundation for further studies on genomic correlation between Thais and fungal infection as well as for clinical research, which will lead to further development an effective fungal treatment and surveillance.

Materials and methods

ABO blood group testing

Thirty-two blood samples were collected from volunteers' vein blood vessel and placed in 4 mL clot blood tube. The blood sample (clot blood tube) was centrifuged at 3,000 rpm for 15 min after that serum was collected for further experiments. Blood glucose was measured with a POCT glucose meter to assess the risk of primary diabetes mellitus [13]. The blood sugar should be below 126 mg/dL. In case of non-fasting (random blood sugar), the sugar value should not exceed 200 mg/dL. ABO blood grouping was performed using the slide method [14]. Briefly, a drop of blood sample was placed onto the slide and reacted with liquid anti-A, anti-B or anti-A, B monoclonals (The Thai Red Cross Society). The volunteers' blood groups were identified based on the agglutination patterns. Blood pH was measured after centrifuging the blood samples at 3,000 rpm for 15 min using litmus paper (MERCK). The isolated serum samples were stored at −80 °C for subsequent analysis.

C. albicans culture and inoculum preparation

For control strains, *C. albicans* ATCC 90028 was employed and grown on Sabouraud dextrose agar (SDA: Himedia) at 35 °C for 24 h. The *C. albicans* inoculum was prepared by suspending 5 colonies with 3 mL sterile NaCl (0.85 % v/v). The cell density was adjusted with spectrophotometric method at a wavelength of 530 nm to achieve a turbidity equivalent 0.5 McFarland standard [15]. The cell suspension was adjusted with sterile NaCl (0.85 % v/v) to a concentration 1 ~ 5×10^6 colony forming units (CFU/mL). For the treatment, 300 µL the *C. albicans* cell suspension was mixed with 300 µL of the serum sample and incubated at 37 °C for 1 h. Then, 1 drop of the serum supernatant (~ 20 µL) was used to examine the germ tube formation under a light microscope (400× magnification). The experiments were conducted at 15-min intervals. The length of germ tube was measured at 5 min intervals for 2 h using Dino-Eye Microscope Eye-Piece camera with DinoCapture 2.0 program (AnMo Electronics Corporation, Taiwan).

Growth rate calculation

The measured length of germ tube was obtained from Dinocapture 2.0 program. The growth rate was calculated using the formula [16] described below:

$$\text{Growth rate} = {}^{/1}\left(\frac{Present}{Past}\right)^{n}\text{-}1$$

where, Present = Length value in minute 120; Past = Length value in minute 0; n = Number of storage periods.

Data was showed as mean (Mean) ± standard deviation (S.D.).

Statistical analysis

Statistical processing of data was carried out with the computer program GraphPad Prism 7. $p < 0.05$ was considered to indicate a statistically significant difference.

Ethics

Thirty-two volunteers were recruited in this study. The healthy volunteers were 18 years old or older, had no history of diabetes, has discontinued the use of the steroid drugs at least 3 weeks prior to the study, and had no history of antifungal use (both orally and topically) at least 2 weeks prior to the study. The volunteers willingly participated in the project and provided signed informed consent. This study was approved by the Burapha University-Institutional Review Board for Protection of Human Subject in Research (BUU-IRB), approved protocol number HS 020/2563. Additionally, this study was approved by the Burapha University-Institutional Biosafety Committee (BUU-IBC), with an approved protocol number IBC 016/2563.

Results and discussion

This study was conducted to assess the relationship between ABO blood groups and germination of *C. albicans* germ tube. Thirty-two volunteers aged between 18 and 25 years participated in the study, a higher representation of females. There were 8 volunteers per blood group (A, B, AB and O). The blood group of each individual was determined using the direct heamagglutionation method and samples were further catagorized based on their blood groups. However, the individual serum was measured blood glucose levels by POCT glucose meter. There were no significant differences in average venous blood glucose levels onto blood groups (100.88 in A, 99.00 in B, 89.63 in AB and 101.38 mg/dL in O types) (**Table 1**). Therefore, the average venous blood sugar levels of each blood groups could not effect on *C. albicans* germ tube formation.

Table 1 Venous blood glucose levels from 32 volunteers into 4 ABO blood groups.

Blood Group A	Blood glucose (mg/dL)	Blood Group B	Blood glucose (mg/dL)	Blood Group AB	Blood glucose (mg/dL)	Blood Group O	Blood glucose (mg/dL)
A01	94	B01	104	AB01	77	O01	94
A02	97	B02	90	AB02	100	O02	115
A03	94	B03	89	AB03	89	O03	95
A04	108	B04	123	AB04	84	O04	103
A05	104	B05	105	AB05	101	O05	123
A06	124	B06	93	AB06	90	O06	84
A07	94	B07	98	AB07	91	O07	103
A08	92	B08	90	AB08	85	O08	94
Average 100.88 mg/dL		Average 99.00 mg/dL		Average 89.63 mg/dL		Average 101.38 mg/dL	

The measured length of germ tube was obtained using Dinocapture 2.0 program. The germination of a germ tube was determined at 0, 15, 30, 45, 60, 75, 90, 105 and 120 min. The length of the germ tube for each blood group was determinded in triplicates (**Table 2** and **Figure 1**). The growth of the germ tube was calculated using the formula described in the methods section. The average germ tube growth rates of *C. albicans* per 2 h in blood group A, B, AB and O were 0.838, 0.533, 0.445 and 0.612 μm, respectively. Blood group A exhibited higher germ tube growth than blood group B, AB and O (**Figure 2**).

Table 2 Average germ tube growth (lenhth: μm) of *C. albicans* in blood group A, B, AB and O.

Germ tube growth (μm)				
Time (min)	Blood group			
	A	B	AB	O
0	7.30	11.14	11.27	10.83
5	8.45	11.76	11.43	11.92
10	9.87	12.56	11.94	12.54
15	10.37	13.09	12.25	13.43
20	10.73	13.75	12.70	14.15
25	11.70	14.59	13.07	14.71
30	12.24	15.20	13.82	15.39
35	12.98	15.61	14.23	16.26
40	13.45	16.30	14.78	16.79
45	14.45	17.06	15.31	17.82

Time (min)	Germ tube growth (µm)			
	Blood group			
	A	B	AB	O
50	15.02	17.74	15.85	18.81
55	15.71	18.13	16.40	19.45
60	16.41	18.81	16.98	20.46
65	16.95	19.81	17.56	20.87
70	17.22	20.61	17.72	21.88
75	17.68	20.92	18.09	22.55
80	18.40	21.67	18.75	23.40
85	18.93	22.13	19.30	23.78
90	19.82	22.65	19.60	24.10
95	20.21	23.37	20.01	24.35
100	21.09	23.82	20.52	24.77
105	21.63	24.21	21.05	25.19
110	22.34	24.80	21.44	25.73
115	23.25	25.41	21.97	25.71
120	23.81	25.72	22.89	26.28

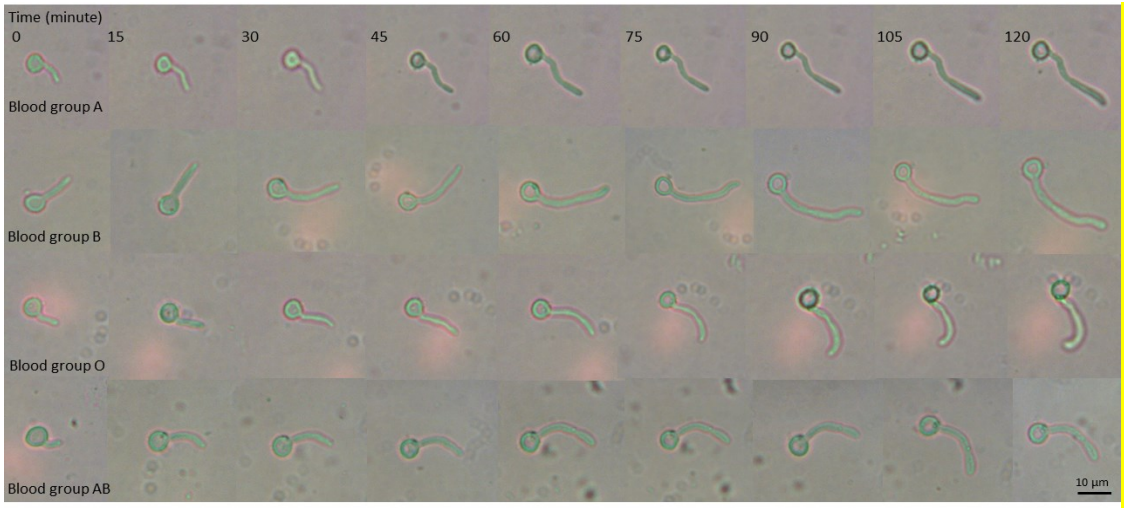

Figure 1 Germ tube growth of *C. albicans* in blood group A, B, AB and O.

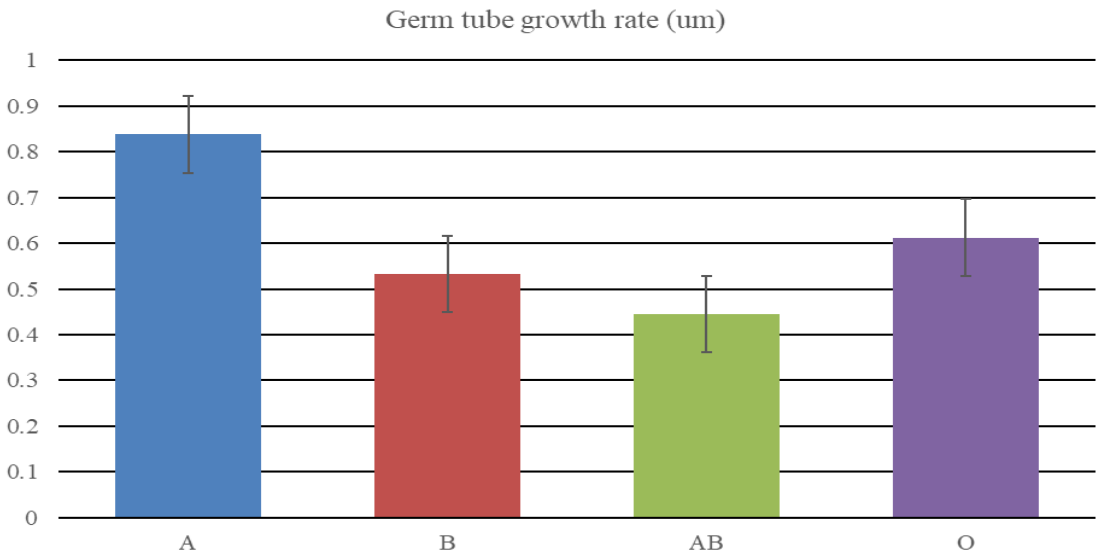

Figure 2 Germ tube growth rate of *C. albicans* in blood group A, B, AB and O.

An association between ABO blood group and susceptibility to certain infections, including candidiasis, has been shown in several studies. However, conflicting results have been reported by other investigators and the relationship between ABO blood group antigens and oral candidiasis is still inconclusive [7,8]. Therefore, the objective of this study was to assess the germ tube formation of *C. albicans* and its relationship with ABO blood groups. *C. albicans* is a human opportunist pathogen that can grow as yeast, pseudo hyphae or true hyphae *in vitro* and *in vivo*, depending on environmental conditions such as temperature, time and pH [11,12]. The transition of *C. albicans* from yeast to germ tubes is a critical factor in its virulence. In the pathogenesis of Candidiasis of *Candida* spp. by helping the infection to invade the host and increase the number and infection by various organs. When *C. albicans* invades the bloodstream under suitable body temperature conditions, it can initiate the formation of a germ tube [17]. Previous studies by Jain *et al.* [8] have investigated the association of blood group and proliferation of oral candidiasis and found a dependent increase in the number of *C. albicans* in the oral cavity individuals with blood group O. Blood group antigens may act as one of the predisposing factors that can either contribute to or prevent disease processes [18,19]. In this experiment, the pool serum was used from a total of 32 subjects, divided into 4 ABO blood groups and repeated 3 times. In addition, the environmental factors including curing temperature, curing time and serum acidity-base (pH) were equally controlled in all blood groups. Including the sampling procedure, we measured the glucose levels of all the volunteers. The results showed that the mean blood glucose levels of all subjects, A, B, AB and O blood groups were valuable. The results showed that blood group A had higher germ tube growth rate than other blood group. In studies by Imanishi *et al.* [20], it was found that protein is an essential component and influences germ tube formation by different germ tube growth rates. Iron is the main factor in fungal growth, which were multiple channels that can carry iron from the host and influence the formation of hyphae by the different growth effects of hyphae in the blood group [21-23]. Furthermore, the germination in *Aspergillus fumigatus* and *Rhizopus* spp. in Blood group A was higher rate than other group (data not shown). This result that individuals with blood group A may have a higher risk a developing *C. albicans* infection compare to individual with other blood groups. Further research is the differences in growth rates of germ tubes in each blood group were not statistically significant. Further studies are needed to prove the role of blood groups in germ tube proliferation. In the future, serum factors that cause the germ tube growth rates of fungi in each blood group were to be studied differently. In preliminary investigation, we found that blood group A exhibited a higher rate of germ tube formation than other blood groups; type B, type AB and type O. Thus, people with blood group A may have higher risk of *C. albicans* infection and more severity than other blood groups.

Conclusions

This study investigated the germ tube formation of *C. albicans* in 32 human serums from different ABO blood groups (blood group A, B, AB and O), 8 people per blood group. The environmental factors including curing temperature, curing time and serum acidity-base (pH) were equally controlled in all blood groups. The results showed that blood group A had higher germ tube growth rate than other blood groups; B, AB and O. The average germ tube growth rates of *C. albicans* per 2 h in blood group A, B, AB and O were 0.838, 0.533, 0.445 and 0.612 μm, respectively. Therefore, the finding of this study indicated that people with blood group A may have higher risk of *C. albicans* infection and more severity than other blood groups.

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