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3. KONFERENCIE
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Aplikácia OMICS nástrojov v štúdiu vzniku chorôb a ich prevencie

Chemický ústav SAV, v. v. i., Dúbravská cesta 9, Bratislava

30. november 2022

Recenzent

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Editor

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ISBN 978 – 80 – 971665 – 4 - 0

Antibody-mediated immune responses against rare facultative pathogen *Candida utilis* in atopic patients with vulvovaginal candidiasis. Glucomannan as a new serologic biomarker

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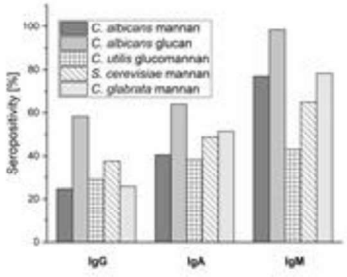

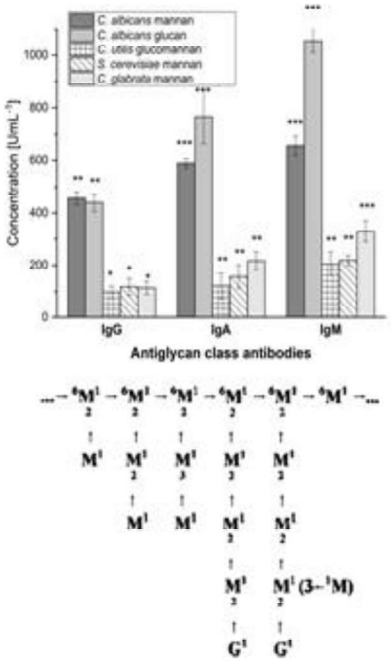

Introduction

Vulvovaginal candidiasis is one of the most commonly reported female genital tract infections, affecting approximately 70–75% of childbearing age women at least once during their lifetime. The fungal cell wall represents the important host-invader interface. Cell-wall polysaccharides represent biological response modifiers and the pathogen-associated molecular patterns and virulence factors. The most dominant cell-wall antigenic structures of *Candida* species as ß-glucan, α- and ß-mannans, glucomannan and other immunogenic polysaccharides are of particular relevancy for specific *in vitro* diagnosis and long-term follow-up of the *Candida* infection. The vaginal mycobiome represents the part of human mycobiome and covers approx. 0.1% of all fungal constituents of total human microbiome. Approximately 75% of women suffer at least one episode of vulvovaginal candidiasis in their life and approximately 40-50% experienced a recurrence. In general, the factors associated with vaginal colonisation and developing of vulvovaginal candidiasis are multifactorial i.e. changes in the physiological mucosal flora, dysbiosis,

pregnancy, active sexual life, systemic or intravaginal antibiotics therapy, oral contraceptive usage, estrogen therapy, hormone replacement therapy, diabetes mellitus type 2, cystic fibrosis, HIV, systemic immunosuppression, deteriorated local cell immunity, secondary and primary immunodeficiency, decrease of mannose binding lectin, allergy/atopy. (Sobel,2007; Denning et al.,2018; Hrubiško et al.,2003; *Faria-Gonçalves,* 2020; *Gonçalves,* 2016; Krüger et al., 2019; Hall and Noverr, 2017, Lines et al.2020, *Ardizzoni et al.,*) Recently, the studies of genetic predisposition to vulvovaginal candidiasis revealed polymorphism in the SIGLEC15 gene that was associated with RVVC (Jaeger et al.,2019). The relationship between polymorphism in the NLRP3 gene, higher production of IL-1α , low IL-1Ra levels and persistent hyperinflammatory state in RVVC patients has been studied (Rosati et al. 2020). Next, the association between Dectin-1 deficiency (Ferwerda et al.,2009), mannose-binding lectin codon 54 gene polymorphism (Wojitani et al., 2012; Nedovic et al., 2014), and interleukin-1 receptor antagonist gene polymorphism (Wojitani et al., 2012) and RVVC has been mentioned. The relationship between atopy/allergy and RVVC has been suggested by Bernstein (Bernstein et al., 2015) and Dondores (Dondores et al., 2018), based on the hypothesis that vulvovaginal candidiasis is an allergic reaction to the ability of Candida-specific IgE and prostaglandin E2 to inhibit vaginal cell-mediated immune response (Bernstein et al., 2015).

In this study we assessed the immunobiological activity of facultative pathogen *Candida utilis* cell glucomannan and its effectivity as *in vitro* serological marker for antibody testing. The novel serologic assay has been developed and optimized for *C. utilis* serodiagnosis. The comparison assays were performed to establish relationship between antibodies against *C. utilis*, *C. albicans* and *S. cerevisiae* main cell-wall antigens in patient sera.

Material and methods

The serological assays were performed in a patient cohort comprising 35 female participants (31.3 ± 5.2 years) with atopy and a history of recurrent vaginal mycosis (Department of Clinical Immunology and Allergy, Oncology Institute of St. Elisabeth, Bratislava, Slovakia).

Yeast strains *C. glabrata* CCY 26-20-1, *C. albicans* CCY 29-3-32, *S. cerevisiae* CCY 21-4-13 and *C. utilis* CCY 29-38-18 (all from Culture Collection of Yeasts, Institute of Chemistry, *Center for Glycomics*, Slovak *C. glabrata* CCY 26-20-1, *C. albicans* CCY 29-3-32, *S. cerevisiae* CCY 21-4-13 cellular mannans were prepared and analysed as previously published (Peat*, et al.*, 1961), *C. utilis* CCY 29-38-18 glucomannan has been isolated and characterised by Kogan et al. (Kogan et al.,1993). *C. albicans* glucan was also obtained from Dr. Kogan.

ELISA assay and determination of specific anti-C. utilis CCY 29-38-18 glucomannan, anti-C. albicans CCY 29-3-32 mannan and glucan, anti-C. glabrata CCY 26-20-1 mannan and anti-S. cerevisiae CCY 21-4-13 mannan IgG, IgM and IgA antibodies were modified based on diagnostic kits Biogema a.s. Kosice.

Results and discussion

The present study reports the evaluation of unconventional opportunistic pathogen *C. utilis* cell wall glucomannan as serodiagnostic antigen and inducer of antigen specific antibody isotypes in

the cohort of atopic female subjects with recurrent *Candida vulvovaginitis*. Statistically significant sera values of specific anti-glycan IgM and IgA class antibodies were revealed. The results are suggestive for efficient serological application of *C.utilis* glucomannan as in vitro disease marker and prospectively for follow-up of the specific long-term antimycotic therapy. Generally, the highest values within the concentration range have been determined for anti-glycan specific isotypes IgM and IgA, followed by specific IgG (Table **1**). Specific IgM anti-*C. albicans* mannan and β-glucan and anti-*C. glabrata* mannan exerted high degree of statistical significance ($P < 0.001$), while sera values of specific IgM anti-*S. cerevisiae* mannan and anti-*C. utilis* glucomannan were lower, although statistically significant ($P < 0.01$). Next most profound reactive isotype has been IgA anti-*C. albicans* mannan and β-glucan ($P < 0.001$), IgA anti-*C. glabrata* while anti-*S. cerevisiae* mannan and *C. utilis* glucomannan were produced to a lesser extent ($P < 0.01$). The specific IgG anti-*C. albicans* mannan and β-glucan sera levels have been lower in comparison with IgM and IgA isotypes ($P < 0.01$). The determination of specific IgG anti-*C. glabrata* and anti-*S. cerevisiae* mannan and *C. utilis* glucomannan demonstrated the lowest levels of anti-glycan antibodies ($P < 0.05$). Evaluation of distribution of positive results revealed the majority of positive results over normal reference interval with IgM isotype antibodies against *C. albicans* β-glucan and mannan, *C. glabrata* mannan, to lesser degree in the case of *S. cerevisiae* mannan and *C. utilis* glucomannan (Figure **1 and** 2).

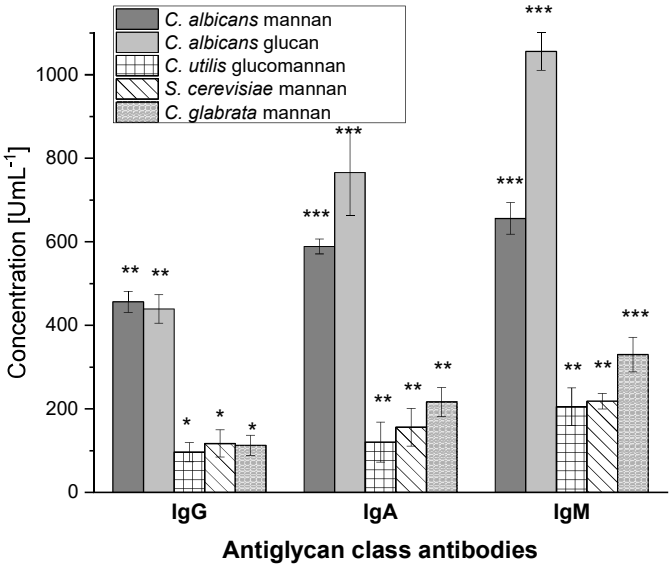

Fig.1. Serological profile of antiglycans IgG, IgA and IgM class antibodies. All data are presented as Mean ± 3SD. Serological tests were assayed in duplicate. Statistical significance of differences *(one-way ANOVA, post-hoc Bonferroni´s test)* is expressed as: *** $P < 0.001$, ** $0.001 < P < 0.01$, *$0.01 < P < 0.05$.

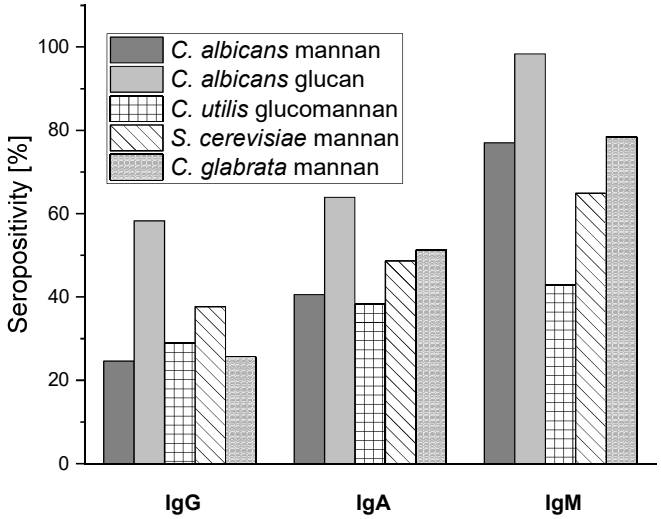

Fig.2. Distribution of positive results of glycan-specific IgG, IgM and IgA class antibodies in study population. *The cut-off values for diagnostic tests were calculated according to blood donors´* IgG/IgM/IgA anti-glycan antibodies´ values *(average±3SD).*

Tab.1. Concentration range (min and max values *(*UmL^{-1}*))* of specific anti-*C. albicans, C. glabrata , S. cerevisiae, and C. utilis anti-glycan IgG, IgM and IgA antibodies in patient cohort.*

	C. albicans mannan		*C. albicans β-glucan*		*C. utilis glucomannan*		*S. cerevisiae mannan*		*C. glabrata mannan*	
IgG	48.08	930.2	64.3	720.3	35.2	210.3	41.8	288.2	31.7	418.2
IgA	53.54	1211.5	38.2	1851	25.8	268.1	34.7	332	32.6	559.3
IgM	47.63	3652.3	40.7	3177.6	38.6	480.2	45.2	383	45.3	603.2

Antibody mediated antifungal immunity is engaged in protection against fungal pathogens and consequently against fungi-mediated local or systemic inflammation. As mentioned before, the main etiological agent of fungal vulvovaginal mycoses *C. albicans* accounts for 80-95% of all episodes of vulvovaginal candidiasis. The increase of non-albicans spp. (Mushi et al., 2019), and participation of the

other yeasts *as S. cerevisiae* has been demonstrated (Sobel et al.,1993, Holland et al., 2003). Generally, the specific antibodies against critical fungal cell virulence factors might play important protective or therapeutic role. Several mechanisms of effective antibody protection as direct neutralization of fungi and fungal cell antigens, growth inhibition capacity, inhibition of exopolysaccharide cell release and biofilm formation were reviewed (Elluru et al., 2015). Antibodies are engaged in process of opsonophagocytosis, activation of complement cascade and participate in antibody-dependent cytotoxicity. Moreover, antifungal antibodies have an important role in immunomodulation and in preventing inflammation-mediated tissue damage (Casadevall and Pirofski, 2003 and 2012; Lionakis et al., 2017).

The serologic analysis of anti-glycan antibodies in a cohort of atopic females with episodes of mycotic colpitis revealed, that the highest concentrations of anti-glycan antibodies are of IgM isotype, followed by IgA class antibodies (Figure 1, Table 1). Highest values resulted principally for *C. albicans* β-glucan, followed by mannan, this order could be presumably associated with re-arrangement of β-glucan masking in the cell wall of *C. albicans* following morphoforms switch from yeast to filamentous growth associated with infection and β-glucan exposure (Goodridge et al., 2009). As concerned seropositivity (Figure 2) the majority of positive results over normal reference interval is observed with IgM isotype antibodies against *C. albicans* β-glucan and mannan, followed by *C. glabrata*, to lesser extent in the case of *S. cerevisiae* mannan and *C. utilis* glucomannan (Table 1). The seropositivity of IgA isotype antiglycan antibodies reflected IgM trend. Detection of serum specific IgA antibodies to *C. albicans* in parallel with specific IgG in women with recurrent genital candidiasis has been evaluated (Tasic et al., 2003). The anti-*Candida* β-glucan and mannan and *Saccharomyces* mannan specific IgM prevalence in different patient cohorts with vulvovaginal mycosis was previously reported (Paulovicova et al. 2015; Paulovicova et al., 2017; Paulovicova et al., 2019). The increased specific IgM anti-mannan isotype antibodies over IgG-anti mannan isotype antibodies were recorded in candidemia patients (Meng et al., 2020).

Acknowledgments

This publication was created with the support of the Operational Program Integrated Infrastructure for the project „Study of structural changes of complex glycoconjugates in the process of inherited metabolic and civilization diseases“, ITMS: 313021Y920, co-financed by the European Regional Development Fund.

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