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EXOGENIC REINFECTION - A POSSIBLE CAUSE OF RECURRENT GENITAL CANDIDOSIS IN WOMEN

SUMMARY

Recurrent genital candidiasis (RGC), the infection that afflicts an extremely large number of women worldwide, is characterized by at least three vaginal fungal infection episodes per year, even in spite a conducted therapy with antimycotics. The aim of the paper was to determine in which percentage the exogenic re-infection can be a potential cause of RGC. The samples of vaginal swabs of 70 female RGC patients and 70 healthy women of control group were submitted for mycological analysis (direct microscopic examination, culture and antifungal susceptibility test). Mycological analysis also included the samples (swabs of urethra and glans penis) of 140 male partners of the examined women. By CandiFast test, the strains of yeasts of the genus *Candida* were differentiated and their antifungal susceptibility was examined *in vitro*. The isolated strains of *Candida spp.* obtained from the female genital tract material and their partners were compared and labeled as identical in case of the same biochemical activity and sensitivity to antimycotics. Identical resistotypes of *Candida albicans* were identified in 11 female RGC patients and their partners. Statistically significant difference ($p < 0.005$) was found compared to control group. By using a commercial antimycogram test, a high percentage of strains sensitive to amphotericin B, nystatine, 5-fluorocytosine, ketoconazole was recorded. The exogenic reinfection, as the possible cause of RGC, was found only in 15.7% of women with this chronic infection.

Key words: recurrent genital candidosis, exogenic reinfection, antifungal susceptibility

INTRODUCTION

Worldwide, about 8-19% of women suffer from recurrent genital candidosis (RGC), the disease characterized by three or more episodes of genital fungal infection in the course of one year (1-4). In these women, the most important and well-known risk factors, such as pregnancy, *Diabetes mellitus*, long-term use of antibiotics, cytostatics, hormonal therapy, idiopathic and acquired immunodeficient conditions have not been verified (1-3).

In the referent literature there have been

many studies concerned with this theoretical and practical problem, as well as various and controversial theories regarding the cause of RGC.

The first theory supports the thesis that the therapy with antimycotics eradicates *Candida spp.* from vaginal mucosa, and that repeated episode of genital candidiasis is the consequence of reinfection. *Candida spp.*, a colonizer and potential pathogen, can reach vaginal mucosa by sexual transmission, when the exogenic reinfection occurs (2, 5).

The aim of the paper was to examine the presence of the *Candida spp.* in the genital tract

material of female patients with RGC, as well as to ascertain a possible presence of the same species in their partners. Resistotypization of the same isolated species of *Candida spp.*, with the aim of confirming their being identical, should determine in which percentage the exogenous reinfection can be the potential cause of constant relapses of genital fungal infection in women.

MATERIAL AND METHODS

The investigation included 70 female patients in whom, regardless of the therapeutic treatment, the genital candidosis had been diagnosed (clinically and microbiologically) at least two times in the year before. This test group comprised female RGC subjects in the reproductive age, aged 23-45 years (mean age 31 years), in whom genital candidosis was not the consequence of pregnancy, diabetes, idiopathic or acquired immunodeficient conditions. This test group did not include women who had been using hormonal, antibiotic or cytostatics therapy for a long time. The patients of the test group (T group), at the moment of examination, were in the phase of manifested vaginal candidosis.

The control group included 70 women being in the reproductive age, aged 23-43 years (mean age 29 years). As for this group, there were two criteria which had to be met: that the women did not suffer from RGC, and that, at the moment of examination, these women did not have the symptoms of fungal vaginitis.

This survey also included 140 male partners of examined women from both groups.

The investigation was undertaken in the laboratories of the Center for Microbiology with Parasitology, Public Health Institute and Institute of Microbiology of the Faculty of Medicine in Nis.

The samples of vaginal swabs of 70 female RGC patients and 70 healthy women of control group were submitted for mycological analysis (direct microscopic examination, culture and antifungal susceptibility test). Mycological analysis also included the samples (swabs of urethra and glans penis) of 140 male partners of the examined women.

Fungi were identified by standard microbiological procedures, including the biochemical, by using the commercial CandiFast tests (Mycolasma, France); *C. albicans* was identified by formation of germinative tube and by chromogenic media (Cromogen albicans, Parquetecnologico de Madrid, Spain). By commercial CandiFast test, the sensitivity of *Candida spp.* to amphotericin B, nystatine, 5-fluorocytosine, ketoconazole, myconazole and fluconazole was examined. In cases of positive

finding of the same species of *Candida spp.* both in female subjects and their partners, the resistotypes of isolated strains were compared. These strains were labeled identical by meeting the following criteria: the same biochemical activity and the same sensitivity to antimycotics in the CandiFast test.

Statistical analysis

The data entry and the analysis were performed using the Epi Info (Ver.6.04) software and SPSS (Ver. 8.0 for Windows). The performed tests were the χ^2 test and the Fisher's exact test. A P-value of <0.05 was considered significant.

RESULTS

In 65 female RGC patients, the cause of genital fungal infection was *C. albicans*, while in 5 cases, the cause was *C. tropicalis*. Only in 11 male partners of female RGC patients, the colonization/infection of the glans penis with *C. albicans* was recorded. Statistically significant difference ($p<0,005$) was determined among the groups (in none of the male partners of control group subjects, the positive finding of *Candida spp.* in the genital tract material was found) (Table 1).

Table 1 *Candida spp.* in material of genital tract of investigated women and their partners

	Test group		Control group	
	Women	Men	Women	Men
<i>Candida spp.</i>	70	11*	0	0
<i>C. albicans</i>	65	11*	0	0
<i>C. tropicalis</i>	5	0	0	0

* Statistically significant difference ($p<0.005$)

In eleven couples, the presence of *C. albicans* was registered, and by comparing the resistotypes in all the couples, the identical resistotypes of this species were determined.

By examination of sensitivity of isolated species of *Candida spp.* to antimycotics present in the commercial CandiFast test, a high percentage of strains of *C. albicans* sensitive to amphotericin B (100%), nystatine (96.92%), 5-fluorocytosine (87.69%) and ketoconazole (76.92%) was recorded. Considerably lower percentage of *C. albicans* strains sensitive to the effects of econazole (23.07%), myconazole (35.38%) and fluconazole (32.31%) was registered (Table 2). Strains of *C. tropicalis* (only five isolates) showed good sensitivity to amphotericin B (100%), nystatine (100%) and 5-fluorocytosine (100%). However, this species proved to be more resistant to azole derivatives (myconazole 60%; ketoconazole 60%, econazole 60%;

fluconazole 40%, (Table 2).

Table 2. Antifungal susceptibility of *Candida spp.* isolates

	<i>C. albicans</i> /76 isolates	<i>C. tropicalis</i> /5 isolates
Antifungals	% of susceptibility isolates	% of susceptibility isolates
Amphotericin B (4 µg/ml)	100	100
Nystatin (200 IU/ml)	96.9	100
5-Fluorocytosine (35 µg/ml)	87.7	100
Econazole (16 µg/ml)	23.0	60
Ketoconazole (16 µg/ml)	76.9	60
Myconazole (16 µg/ml)	35.4	60
Fluconazole (16 µg/ml)	32.3	40

DISCUSSION

Different data about the possible cause of a chronic episode of vaginal candidosis can be found in the reference literature. Even after numerous analyses, the pathogenesis of primary RGC remains enigmatic to world experts in the fields of microbiology, immunology, epidemiology, gynecology, and persistence of these infections in women appears to be an unsolved problem in the everyday practice. Scientific attainments and interests in proving the cause of recurrent genital fungal infection in women have taken two directions. Generally, the two theories affected the major courses of investigation. One theory supports the thesis that the therapy with antimycotics eradicates the fungi of the *Candida spp.* from vaginal mucosa, and that the repeated episode of genital candidiasis is the consequence of reinfection (6, 7). *Candida spp.*, the colonizer and potential pathogen, reaches the vaginal mucosa from the digestive tract of a woman (endogenous reinfection), or by sexual transmission (exogenous reinfection) (6, 7).

The other hypothesis is based on the opposite thesis. According to this theory, the frequent episodes of RGC are the consequence of a failure to eradicate *Candida spp.* from the female genital tract (2). Antimycotics probably do not conduct eradication, in the case of which, probably, a certain number of causes remains on vaginal mucosa. After the therapy, a considerably decreased number of microorganisms of *Candida spp.* does not give the symptoms of infection, and clinically, the patient is

facing the phase of remission. In the case of the environmental condition change on vaginal mucosa, which is beneficial for fungi to replicate, a number of causes of genital candidosis augments, the infection begins, and, clinically, the patient is afflicted with a new episode of RGC. According to this theory, RGC is the consequence of relapses rather than reinfection (2).

In a large number of studies, RGC can be explained by the change of microorganisms (resistance to antimycotics, augmentation of pathogenicity, antigenic and phenotypic variations of *Candida spp.* (2, 3); by the change of non-specific mucosa resistance (the change of microbial flora as a natural barrier on vaginal mucosa, the influence of reproductive hormones on the change of environmental conditions, on virulence of causes and attenuation of immune defense mechanisms on vaginal mucosa (2, 8-10); and, in addition, by immune non-reactivity or immune dysregulation (systemic *Candida* specific immunosuppression, establishing of IgE-mediated hyperreactivity reaction) (11-13).

Up to date, there have been controversial data, so that, according to consensus of opinion, all the enumerated causes of vaginal candidiasis relapses can be only the risk factors.

The first theory about reinfection points to sexual transmission as the cause of RGC. This conclusion has been supported by the fact that *Candida spp.* is diagnosed four times more frequently in men, the partners of women with genital candidiasis, than in others (2, 4, 5, 7, 14). Besides, the strains of fungi found in male partners of female RGC patients are identical to the strains of *Candida spp.* isolated from the material of their wives in the phase of active fungal infection. By the use of DNA tests for determining the genome of *C. albicans* strains, the cause of RGC, and with the aim to compare them with the strains of the same species of fungi detected in the partners' material, one group of authors have proved that the strains of fungi are the isolates from the material of the female genital tract and their partners, and that they are identical or highly identical (7, 15).

In our investigation, we also proved that in 15.7% of female RGC patients, the reason of constant relapses of genital fungal infection can be the exogenous reinfection. By comparing the isolated strains of female RGC patients and their partners, we have proved the identical resistotypes of *C. albicans* in 11 couples. Statistically significant difference was found when compared to the control group, in which not a single one male partner of healthy women had a positive finding of *Candida spp.* in the examined material.

The results of this study are in accord with the findings of other studies investigating the same

theme, as in some other analyses the positive finding of *Candida spp.* was found only in 20% of partners of female RGC patients (2).

Such low percentage (15.7–20%) of partners of female RGC patients with confirmed fungal infection/colonization of the genital tract raises the question about practical problem solving, whether all the partners of female RGC patients should be treated *a priori* or after the microbiological examination.

Analyzing the effect of simultaneous treatment of couples by application of antimycotics, one group of authors (5, 16) have proved that only simultaneous treatment of partners of women with RGC by using antimycotics, who also have positive findings of *Candida spp.* in genital tract material, lowers the percentage of women with repeated episode of genital candidiasis. However, even if the couples are treated, without microbiological analysis of the samples taken from men, there is no significant difference among female patients with RGC after the appearance of a new episode of fungal infection, no matter whether the partners are simultaneously treated or not (16).

Academically observed, microbiological examination of partners of female patients with RGC would be recommendable, as well as prescription of simultaneous therapy only in the case of positive finding of *Candida spp.* in the partners' genital tract material. The practical problem solving relies on the principle that has been applied in practice for a long time, and which requires the simultaneous treatment of partners of the women suffering from genital candidosis, no matter whether it occurs in the

sporadic or recurrent form. As both principles are incomplete, so far, a general attitude has been adopted that the results of some future investigations in this field will offer new prospects and solutions of this practical problem.

By using the commercial antimycogram test, we have determined significantly high percentage of *Candida spp.* strains resistant to econazole, myconazole and fluconazole. In the reference literature, there is a small number of papers dealing with the antifungal susceptibility of *Candida spp.* as the cause of RGC *in vitro*. The existence of contradictory findings about antimycotic efficacy *in vivo* and *in vitro*, a different range of antimycotics in commercial tests, as well as non-standardized methods of examination of fungi sensitivity to antimycotics *in vitro* are the reasons why there is little basis to compare these findings (16-22).

Based on statistically significant difference in the finding of *Candida spp.* in the material of male partners of female RGC patients, when compared to control group, we can say that in 15,7 % of cases the cause of RGC can be the exogenic reinfection. It has been ascertained that *C. albicans* stands for the commonest cause of RGC in women, and therewith the commonest cause of genital fungal colonization/infection in the examined partners of women with this infection. In our investigation, we have concluded that the most efficient antimycotics *in vitro* in the treatment of RGC subjects and *Candida* infection/colonization of the partners' genital tract are the following: amphotericin B, nystatine, 5-fluorocytosine and ketoconazole.

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EGZOGENA REINFEKCIJA - MOGUĆI UZROK REKURENTNOSTI GENITALNE KANDIDOZE ŽENA

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SAŽETAK

Rekurentnu genitalnu kandidozu (RGK), infekciju od koje boluje izuzetno veliki broj žena, karakterišu minimum tri gljivične vaginalne infekcije u toku jedne godine, uprkos sprovedenoj terapiji antimikoticima. Cilj rada bio je utvrditi u kom procentu egzogena reinfekcija može biti potencijalni uzrok RGK. Uzorci vaginalnog sekreta 70 žena sa RGK i 70 zdravih žena kontrolne grupe, ispitivani su standardnim mikološkim pregledom (mikroskopiranje direktnih nativnih i bojanih preparata, kultivisanje i ispitivanje osjetljivosti na antimikotike). Mikološka analiza obuhvatila je i ispitivanje prisustva gljiva roda *Candida* u materijalu genitalnog trakta (uretralni sekret, bris glansa penisa) partnera ispitivanih žena. Primenom CandiFast testa (Mycoplasma International-France) diferencirani su izolovani sojevi gljiva roda *Candida* i ispitana je njihova osjetljivost na antimikotike u uslovima *in vitro*. Izolovani sojevi gljiva roda *Candida* iz materijala genitalnog trakta žena i njihovih partnera upoređivani su i proglašavani identičnim u slučaju iste biohemijske aktivnosti i osjetljivosti na antimikotike. Identični rezistotipovi vrste *Candida albicans* utvrđeni su kod 11 žena sa RGK i njihovih partnera. Utvrđena je statistički značajna razlika ($p < 0,005$) u odnosu na kontrolnu grupu. Korišćenim komercijalnim antimikogram testom utvrđen je visok procenat osjetljivih sojeva prema amfotericinu B, nistatinu, 5-fluorocitozinu i ketokonazolu. Egzogena reinfekcija kao mogući uzrok recidiviranju RGK utvrđena je samo u 15,7% žena sa ovom hroničnom infekcijom.

Ključne reči: rekurentna genitalna kandidoza, egzogena reinfekcija, osjetljivost na antimikotike