**ORIGINAL STUDIES**

**ORIGINALNI NAUČNI RADOVI**

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**MORPHOLOGIC AND MORPHOMETRIC ANALYSIS OF ALTERNATIONS IN THE ORAL CAVITY CAUSED BY CANDIDA ALBICANS – EXPERIMENTAL WORK**

**MORFOLOŠKA I MORFOMETRIJSKA ANALIZA PROMENA U USNOJ DUPLJI KOJE JE IZAZVALA CANDIDA ALBICANS – EKSPERIMENTALNI RAD**

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**Summary**

**Introduction.** Candidiasis has become a human disease of increasing importance in the last decades. The aim of the study is to establish pathomorphological alterations caused by the blastospores of the *Candida albicans* as well as morphometric alterations.

**Material and Methods.** The experiment was carried out on 2.5-month-old rats, weighting 110–130 g. The study sample was divided into the animals infected by a submucous inoculation in the periodontal region and the controls. The gingival specimens were taken, preparations were done and stained by the hematoxylin-eosin and Periodic acid Schiff methods.

**Results.** The following alterations were found out by the stereological analysis: an average volume of nuclei of the gingival epithelial cells was 111.82 µm³ (SD=25.34) on the first day. A statistically significant increase in the volume of nuclei in the experimental group began to occur from the fourth day (202.97 µm³; SD=31.16, p<0.05) and the highest value of the nuclei volume was found out on the eight day of the experiment (316.83 µm³; SD=40.15).

**Conclusion.** Blastospores of *Candida albicans* are pathogenic for the gingival tissue where they cause degenerative necrotic alterations of the granulomatous character and after the fourth day from the inoculation, the development of the pseudohyphae was observed. The obtained values of stereologic measurements show the acute increase in the volume of nuclei.

**Key words:** Mouth; Candida albicans; Candidiasis; Rats; Gingiva; Spores, Fungal

**Sažetak**

Poslednjih godina kandidoza predstavlja oboljenje od posebnog značaja. Cilj ovog istraživanja bio je da se utverde i ispitaju patomorfološke promene uzrokovane blastosporom kandide (*Candida albicans*) kao i morfometrijske promene. **Materijal i metode.** Eksperimentalna studija je urađena na pacovima starosti 2,5 meseća, težine 110–130 g. Životinje su inficirane submukoznom inokulacijom. Takođe, formirana je kontrolna grupa. Uzimani su isečci sa gingive, napravljeni preparati su bojeni sa hematoxylin-eozin i Periodic acid Schiff metodom. **Rezultati.** Stereološkom analizom utvrđene su sledeće promene: u prvom danu srednja vrednost zapremine nukleusa je 111,82 µm³ (SD = 25,34). Zapaženo je statistički značajno povećanje zapremine jedra od četvrtog dana (202,97 µm³; SD = 31,16, p < 0,05), a najveća vrednost zapremine jedra iznosila je osmog dana eksperimenta (316,83 µm³; SD = 40,15). **Zaključak.** Blastospore kandide (*Candida albicans*) su patogene za tkivo gingive, gde uzrokuju degenerativne nekrotične promene i nakon četvrtog dana od inokulacije, javljaju se pseudohife. Dobijene vrednosti stereometrijske analize pokazale su postojanje akutnog uvećanja zapremine jedra.

**Ključne reči:** Usna šupljina; Candida albicans; Kandidijaza; Pacovi; Gingiva; Spore gljivica

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In memory of my father Prof. dr. Georgi Penev (1933-2012), Medical faculty University of Niš, Institute for Pathological Anatomy

**Introduction**

*Candida species* fungi are commonly present in healthy individuals, and *Candida albicans* is the most prevalent species [1, 2]. The leading cause of candidiasis, *Candida albicans*, is a dimorphic fungus that resides as a commensal of the oral mucosa and the gastrointestinal tract mucosa. Changes in the oral ecosystem or in the immunological system of the host can lead to candidiasis development [2–4]. Candidiasis has become a human disease of increas-
ing importance in the last decades due to the increasing number of patients with immunological involvement associated with the infection by the human immunodeficiency virus (HIV) and the use of immunosuppressive agents after organ transplantation or antineoplastic therapy [1]. In immunocompromised hosts, however, saprophytic colonization often leads to opportunistic mucosal or life-threatening deep organ infection. Invasion of the human gastrointestinal mucosa by Candida albicans and its passage across the bowel wall into the bloodstream is an important portal of entry for this opportunistic pathogen in the neutrophic host, leading to systemic or disseminated candidiasis [5]. In addition, hematogenous candidiasis is a frequent complication in treatment of patients with acute leukemia [6]. Many researchers developed several experimental models in rats in order to understand the mechanisms related to the pathogenesis of oral candidiasis. The oral cavity of these animals is easily colonized by Candida and develops similar lesion in relation to those observed among human beings [1, 3]. Many predisposing factors for oral candidiasis have been studied in experimental models, such as: broad-spectrum antibiotics therapy [7, 8], the use of acrylic prosthesis [9], diabetes mellitus [10], topical use of corticosteroids [11], xerostomia [12, 13] and immunosuppressive therapy [4, 14]. An important cofactor associated with the pathogenesis of oral candidiasis appears to be the virulence of the infecting organism [15, 16]. The specific features of the fungus that contribute to the development of oral candidiasis include its ability to adhere to and colonize the oral mucosa [17], its ability to form cylindrical appendages termed germ tubes [18], and its cell surface hydrophobicity [19]. In addition, phenotypic and genotypic switching [20, 21], extracellular aspartyl proteinase secretion [22, 23], and phospholipase production [24] appear to play a subsidiary role in the pathogenicity.

Animal models represent powerful tools in elucidating the molecular and cellular pathogenesis of candidiasis (previously reviewed in [1, 3]). The principal advantage in studying animals instead of human beings is that the animal and its environment can be controlled [4], allowing a precise cause-and-effect longitudinal analysis of host-pathogen interactions. In addition, these models obviate the procurement of tissue samples from human patients, which can often be problematic. The usefulness of animal models of candidiasis includes not only the study of pathogenesis but also the in vivo assessment of novel antifungals, immunomodulators and potential Candida vaccines [25, 26]. The aim of this study was to establish pathomorphological alterations caused by the blastospores of Candida albicans as well as morphometric alterations in the rats.

Material and Methods

The experiment was carried out on 2.5-month-old rats, weighting 110–130 g. A group of ten rats was infected with blastospores of Candida albicans in the dosage of 400.000 in 0.5 ml of physiological solution for an animal (determined in the Spenser chamber). The animals were infected by a submucous inoculation in the periodontal region. The control group consisted of three animals which were kept under the same conditions as those for the experimental group. The rats were sacrificed after 24 hours, after the second, fourth, sixth and eighth days from the moment of infection. The cuts of gingival tissue were taken, preparations were done and stained by the hematoxylin-eosin (HE) and Periodic acid Schiff (PAS) methods.

Stereologic analysis of the average volume of the nuclei of the gingival epithelial cells was also carried out according to the Gunderssen "nucleator principle" (1988).

The sinus-dependent test system was used to measure the intercept length according to the formula \( V_v = (l \times \pi / 3) \). The x100 objective was used.

The Student t-test was used for the statistical analysis of the obtained results.

Results

The alteration took place only at the gingival level in the gingival tissue in the acute phase up to the fourth day from the infection (Figure 1).

Inflammatory-edematous alterations were ascertained by the presence of the budding blastospores and pseudomicellar fibers in the gingival tissue in the acute phase and from the fourth day onwards granulomatous alterations occurred with abscess formation in addition to numerous predominantly eosinophile elements and pseudohyphae. Giant cells of the Langhans type and the foreign body type were present in complexes. The alterations appeared due to the effects of blastospores and candidine, their metabolic product (figures 2 and 3).

Figure 1. Gingival tissue with edema and blastospores. HE, X 400.

Slika 1. Tkivo gingive sa edemom i blastosporama HE, X 400
The development of excess fibrous connective tissue, that is fibrosis and sclerosis, occurred in the chronic phase (Figure 4).

The control sample (Figure 5) showed intact epithelial lamina with subepithelial muscle tissue. The following alterations were determined by the stereological analysis:

- an average volume of nuclei of the gingival epithelial cells on the first day was 111.82 µm³, (SD=25.34).
- a statistically significant increase in the volume of nuclei in the experimental group began to occur from the fourth day (202.97 µm³; SD=31.16, p<0.05);
- the highest value of the nuclei volume was found out on the eight day of the experiment (316.83 µm³; SD=40.15).

Discussion

The animals that inoculated by *Candida albicans* in the oral cavity developed clinical and microscopy lesions of candidiasis in the tongue dorsum even without presenting predisposing factors, such as the administration of antibiotics, immunosuppression, carbohydrate-rich diet or xerostomia. These data confirm that the experimental candidiasis can be induced by a simple inoculation of a pathogenic strain of *Candida albicans* [28]. The recognition that *Candida* is an important pathogen, particularly in the immunocompromised host, has resulted in a vast body of *in vitro* investigations evaluating its virulent attributes in an attempt to elucidate the pathogenesis of the disease. The progress made in understanding some of these features, such as the mechanisms that result in adherence to host tissues [29], cell surface hydrophobicity [30], switching phenomena of the yeast [22, 31], secretion of aspartyl proteinases [24], and phospholipase production [25], is very impressive. Nonetheless, *in vivo* studies either in humans or in animals are essential to elucidate and fully comprehend the mechanisms leading to candidal infection. The host oral defenses against *Candida* essentially fall into two categories: nonspecific immune
mechanisms (e.g., integrity of the mucosae, commensal bacteria, polymorphonuclear leukocytes, microphages, and salivary factors) and specific immune mechanisms (e.g., serum antibodies, secretory antibodies, and cell-mediated immunity) [32]. The stratified squamous epithelium of the oral mucosa forms a continuous surface that protects the underlying tissues and functions as an impervious, mechanical barrier. The protection so provided is dependent on the degree of keratinization and the continuous desquamation or shedding of epithelial cells. Indeed, the latter mechanism is considered to play a pivotal role in maintaining a healthy oral mucosa and in limiting candidal colonization and infection. The interaction between Candida species and the commensal microflora is perhaps the next critical mechanism modulating oral candidal colonization [33]. The commensal flora regulates yeast numbers by inhibiting the adherence of yeasts to oral surfaces by competing for sites of adherence as well as for the available nutrients. A number of studies have also shown, both in vivo in gnotobiotic mice and in vitro, that candidal colonization of epithelia could be suppressed by streptococci, which are the predominant resident commensals of oral mucosal surfaces [33–35]. Consequently, the process of infection can be viewed as a competition between the ability of fungal cells to multiply and the host antimicrobial response. Obviously, for an infected host to survive and recover, it is crucial to impede the ability of pathogens to multiply [36]. Although different species of Candida, such as Candida glabrata, Candida tropicalis, Candida krusei and Candida dubliniensis, are at present recognized as increasing opportunistic pathogens specially in HIV infected individuals and acquired immunodeficiency syndrome (AIDS) patients, Candida albicans still remains the most common yeast isolated in humans [37]. In some ways, it is surprising that Candida albicans is uniquely associated with animals and human, as it has no specific nutrient requirements that would prevent it from surviving in the outside environment [38]. The use of mouse models appears appropriate since progression of both systemic and oral candidiasis closely resembles that observed in humans [39].

Conclusion

Based on the results obtained from the experimental testing of effects of blastospores of Candida albicans the following conclusions can be drawn:

1. Blastospores of Candida albicans given in dosage of 400,000 in 0.5 ml of the physiological solution are pathogenic for the gingival tissue where they cause degenerative necrotic alterations of the granulomatous character. We suppose that candidine, as a metabolic product of Candida albicans, play a great role in the pathohistological alterations of the gingiva.

2. Development of the pseudohyphae was found after the fourth day from the inoculation.

3. The obtained values of stereologic measurement in the acute increase of the nuclei volume is probably a consequence of the nearby focus of infection or toxic effects of Candida albicans.

References