SUMMARY

FEATURES DETERMINING OF THE PATHOGENICITY OF GASTROINTESTINAL MICROFUNGI IN HEALTHY PERSONS AND PATIENTS WITH COLORECTAL CANCER

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The aim of the study was to assess the ecophysiological diversity of fungi colonizing different ontocenosis of healthy subjects and colorectal cancer patients, highlighting species frequently recorded in medical mycology and species rarely described but with clear ecological expansion tendencies in relation to the human ontosphere. The following research tasks were adopted to achieve the objective:

1. Mycological evaluation of materials collected by oncologists (macro- and micro-cultures, biochemical tests) - identification of fungi.
2. Verification of the correctness of fungal identification using MALDI TOF mass spectrometry.
3. Determination of enzyme activity of isolated fungi using Biomerieux API ZYM tests.
4. Determination of drug-susceptibility of fungal isolates using the agar gel diffusion method.
5. Attempt to determine the fungal growth rate in analyzed sections of the gastrointestinal tract using strains of *Candida albicans*, the species capable of forming "germ tubes".

Fungi isolated from the oral cavity, large intestine and anus of 30 healthy persons-control group (15 females, 15 males - 55 enrolled persons) and 51 patients with histopathologically diagnosed colorectal cancer (24 females, 27 males - 65 enrolled persons) constituted the research material. Swabs, intraoperative samples, endoscopic samples taken by doctors who have been collaborating with the Department of Microbiology and Mycology at the University of Warmia and Mazury for years were used in the study.

The biological materials were subjected to the standard diagnostics recommended in mycological laboratories (macro-cultures on Sabouraud medium, micro-cultures on Nickerson medium, biochemical tests, CHROMagar Candida chromogenic medium). To analyze certain physiological characteristics of the tested isolates, their pharmacological susceptibility to 8 anti-fungals was assessed by the agar gel diffusion method, using MastDiagnostics anti-fungal discs, and enzyme activity was assessed using the API ZYM tests from BioMerieux. All strains were subjected to MALDI-TOF (mass spectrometry) identification, performed at the Olsztyn Regional Epidemiological Station.

The results obtained indicate the presence of fungi in the gastrointestinal tract of both healthy and diseased individuals. In the control group, such individuals constituted 54,5% and in patients with colorectal cancer 78,5%. In the control group, 10 species belonging to four genera were identified: Candida, Saccharomyces, Rhodotorula and Trichosporon. The total number of isolates was 61. The most abundant genus was Candida (43 isolates = 70,4%), with a clear dominance of *C. albicans* - 22 isolates. Rarely recorded in medical mycology were *Rh. glutinis, Rh. minuta, S. cerevisiae, T. beigelii* and *T. inkin*. The number of isolates obtained in

different ontocenosis was comparable in men and women: highest in the oral cavity (14 and 16), slightly lower in the rectum (11 and 13), lowest in the colon (3 and 4), but still higher in men. In patients with colorectal cancer, 11 species attributed to 3 genera were identified: Candida, Rhodotorula, Trichosporon. The genus Candida was the most abundant, dominated by *C. albicans*. Only in females *C. tropicalis* was found, while in males *Rh. glutinis* and *T. inkin*- as many as 15 isolates. The number of species recorded in each ontocenosis is very similar, ranging from 8 to 9 in females and 10 species in males, as is the number of isolates: from 24 (females) to 26 (males) in the oral cavity, from 15 (females) to 29 (males) in the rectum and from 21 (females) to 24 (males) in the colon ontocenosis. These numbers are always slightly higher in men.

The results on enzymatic activity very clearly show that in cancer patients, the activity and spectrum of enzymes produced, of the same fungal species, in the same ontocenosis reach much higher values in cancer patients than in healthy subjects. In healthy subjects, enzymatic activity was at low to medium levels (up to 20 nano-moles of hydrolyzed substrate). In cancer patients, the enzymatic activity reached high and very high levels (20- 40 and > nano-moles of hydrolyzed substrate). This is especially true for fungi colonizing the tumor-affected large intestine.

The testing of the efficacy of the tested drugs did not bring very promising results. In general, very high and high drug resistance was found, and in numerous isolates the sensitivity was low or not recorded. An exception is amphotericin B. Only in its case no resistant or secondary resistant isolates were recorded, and in a few species, also weakly sensitive.

The growth of fungi obtained from healthy subjects was distinct and its individual phases easier to capture than in cancer patients. Although the fungi from the latter started to grow faster, their growth was irregular. In the case of the present study, the rate and extent of change were largely tumor-dependent. This was most evident in the results concerning the enzymatic activity of the fungi, which was significantly higher in diseased individuals. Moreover, the activity of some enzymes was higher in species with increasing expansiveness in the colon, altered by cancer. This is a disturbing fact, as enzymatic properties determine the degree of pathogenicity of fungi. In our study, high levels of enzymatic activity generally correlated with a decrease in drug susceptibility, and variation in this activity entailed variation in growth rate.

It should be emphasized that the presence of fungi in cancer patients may be a consequence of tumor development or the fungi, having previously conquered the ontosphere, were the biological carcinogenic factor.

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