### **Original papers**

# Consumption of sweetened beverages as a risk factor of colonization of oral cavity by fungi – eating habits of university students<sup>1</sup>

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ABSTRACT. Foods rich in sugar are an excellent substrate for the microorganisms that inhabit the initial sections of the gastrointestinal tract, and one of the most commonly available sources of sugar is the sweetened drink. Students represent an interesting sub-population; the large number of classes and associated stress levels promote fixing of unhealthy behaviors, e.g. tendency to consume a lot of sweetened drinks, for example cola-type or energetic drinks. Aim of this study was to determine the relationship between the amount of sugar consumed in beverages and the prevalence of fungi in the oral cavity. The investigated material consisted of oral washings. Participants completed original questionnaire regarding beverages consumed. The relationship between the consumption of sweetened beverages and risk of the presence of fungi in the oral cavity was determined. Fungi were isolated from 68.1% of examined subjects. Seven species of the genus Candida were observed. Higher prevalence of fungi was seen in the oral cavity of subjects who declared consumption of beverages containing sugar. 37.8% of respondents were found to consume with beverages doses of sugar exceeding the recommended daily requirement. Significantly greater prevalence of oral cavity fungi was noted in those exceeding the recommended GDA (76.3%), compared to of those who were not (68.7%). There were positive correlations between occurrence of fungi and consumption of sweetened carbonated drinks or adding sugar to coffee and tea. The addition of sugar to coffee/tea and sugar consumption above the recommended daily amount significantly increases the risk of colonization of the oral cavity by fungi. Students, due to invalid nutritional habits especially excessive consumption of beverages containing large amounts of sugar, belong to a group with a predisposition to the occurrence of fungi in the oral cavity.

Key words: Candida, fungi prevalence, sweetened beverages, sugar GDA, risk of oral cavity colonization by fungi

#### Introduction

Contemporary human lifestyle is conducive to perpetuating "anti-healthy" behaviour. As more time is spent on work and commuting, leaving less for relatives, interests and relaxation, such timesaving products as processed foods are becoming increasingly popular among consumers. Similarly, the fast-growing media and digital industries provide an ever-increasing variety of television programs, computer games and social networking websites which unfortunately further contribute to a sedentary lifestyle. Improper eating habits influence health. One example is the increased incidence of obesity and cardiovascular disease in people who consume highly processed foods, "fast foods", with high fat and sugar content [1–4]. The quality and quantity of food consumed influences the prevalence of such metabolic diseases as diabetes, as well as certain diseases of the gastrointestinal tract and the mouth, and of course, dental caries. The tendency to consume sweet foods has been found to increase with age. Stefańska et al. [1] report 11% and 7% greater consumption of sweets among 13–15 year old girls and boys, respectively, compared to 10–12

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year olds, and more frequent consumption of carbonated soft drinks than water and juices. Foods rich in sugar are an excellent substrate for the microorganisms that inhabit the initial sections of the gastrointestinal tract, and one of the most commonly available sources of sugar is the sweetened drink. According to the manufacturers, 250 ml of product can contain from a few grams of sugar, in the case of some juices, to more than 30 g, in carbonated sodas. Even so-called flavoured waters are in fact sweetened drinks with 15g of sugar per 250 ml (3 cubes to 1 cup).

Excess sugar can contribute to a disruption of the natural balance of microbiota inhabiting the various sections of the gastrointestinal tract, as well as an excessive proliferation of potentially pathogenic fungi [5,6]. The prevalence of fungi in humans continues to increase. While it ranges from 2 to 70% in the healthy population, it may be as high as 96% in people with weakened immune systems [7]. The ontocenosis most frequently exposed to colonization by yeasts and yeast-like fungi is the oral cavity, from which the most frequently isolated species is Candida albicans (25-75%). Other Candida species, such as C. glabrata, C. tropicalis, C. guilliermondii, C. krusei, C. dubliniensis, as well as Kluyveromyces marxianus, have been isolated less frequently, however, their prevalence has increased over the past decade [6,8-12]. Studies show that in the human body sexually reproducing stages, which have a higher enzymatic activity and thus greater the virulence of the host tissues, are becoming increasingly popular [11–13].

Students represent an interesting sub-population. Romanowska-Tołłoczko [14], and other studies, observes that the large number of classes and associated stress levels promote fixing of unhealthy behaviors. Most students do not respect the minimum time allowed for sleep and rest and do not take part in any physical activity. Many admit to smoking and drinking alcohol several times a week and eating fast food. Intensive learning, lack of sleep and whole nights parties favors the homeostasis disorders, which can lead to fungi colonization. University students are also group of people, who tend to consume a lot of sweetened drinks, for example cola-type or energetic drinks.

The aim of this study was to determine if consumption of large doses of sugar with beverages can be a risk factor for colonization of oral cavity by yeast and yeast-like fungi.

#### **Materials and Methods**

Study participants were volunteers and represent a student's population with typical behaviors. The study included 119 students, 83 female and 36 male, of the faculties of Medicine, Dentistry, Public Health and Laboratory Medicine, Medical University of Lodz. All participants completed our own original questionnaire regarding the quality and quantity of beverages consumed. All respondents declared good health and proper dental care, over 90% had never been treated due to fungal infection. The investigated material consisted of oral washings collected with liquid Sabouraud medium. Fungal growth was evaluated microscopically, positive cultures were then passaged on Sabouraud solid medium in order to obtain pure isolates. The obtained fungi were identified using API 20C AUX tests (Biomerieux). The relationship between the consumption of sweetened beverages and risk of the presence of fungi in the oral cavity was then determined.

The declared amount of sugars consumed by the respondents in different types of beverages was analyzed and compared with the adult guideline daily amount (GDA) of glucose: 90g for women and 110g for men. Based on the information about the types and quantities of beverages consumed and information from product labels, it was possible to determine how many grams of sugar per day respondents consumed in beverages.

Student's t-test was used to identify relationships within the data obtained in the survey and from the test results. All statistical analyses were performed using STATISTICA 10.0 software (Statsoft Polska). Results were found to be significant at p<0.05.

#### **Results**

In total, fungi were isolated from 68.1% (±4.95) of examined subjects. A significantly higher prevalence was noted in men (75%±4.95) than women (65.1%±4.95) (p=0.045). Seven species were observed, all of the *Candida* genus. *Candida albicans* was the most common (79%), while the other species occurred significantly less frequently: *C. tropicalis* (6.2%), *C. guilliermondii* (4.9%), *C. famata* (3.7%), *C. lusitaniae* and *C. humicola* – 2.5% both and *C. lipolityca* (1.2% of cases) (Fig. 1).

Fungi were most frequently isolated from students of Dentistry or Public Health (75%±3.18 both), slightly less were found in those from the Faculty of

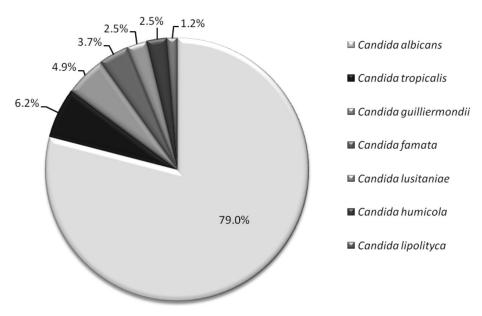


Fig. 1. Yeast and yeast-like fungi species isolated from oral cavity of students examined

Laboratory Medicine ( $67.6\pm3.18\%$ ), and the least were found in students of Medicine ( $61.9\pm3.18\%$ ). However, the differences between these groups were not statistically significant (p>0.05).

With respect to the age of the examined students, a higher prevalence of fungi was observed in groups of students aged 24–26 ( $85.7\pm8.3\%$ ) and 27–30 years old (100%). A lower incidence of fungi was noted in students in the first three years of the course, aged 18–20 ( $63.9\%\pm12.9$ ) or aged 21–23 ( $69.4\%\pm8.28$ ). However, these observed differences were, again, not statistically significant (p>0.05).

A higher prevalence of yeasts and yeast-like fungi can be seen in the oral cavity of the subjects who declared consumption of beverages containing sugar (Fig. 2). The proportion of carriers was 74.6% (±6.4%) of those drinking sweetened carbonated beverages, and 65.5% ( $\pm 2.1\%$ ) of those who do not: the difference not being statistically significant (p>0.05). Fungi were found to be prevalent within groups of students who prefer clear soft drinks (83.3%±6.4), energy drinks (76.3%±6.4), cola drinks (75.4%±6.4), and orange drinks (only 53.8%±6.4), however, the differences were not statistically significant (p>0.05). There was a positive correlation between consumption of carbonated beverages, and the presence of fungi in the oral cavity, the correlation coefficient 0.5395. Taking into account the type of beverage positive correlation was seen with clear soft drinks (correlation coefficient 0.7085) and tonic (correlation coefficient 0.9878). The prevalence of fungi was 71.8% (±4.5%) of students consuming

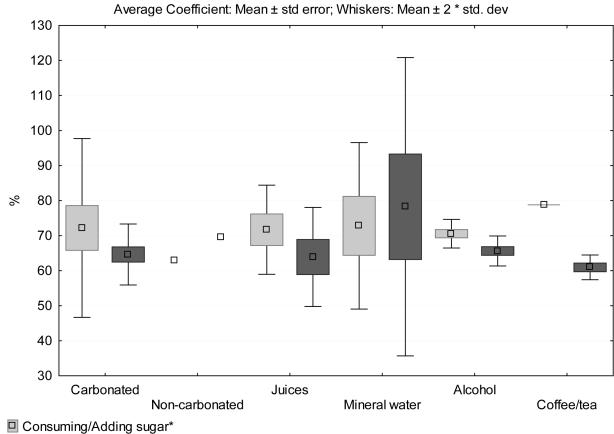
fruit juices, and  $64.1\% (\pm 5\%)$  in those who do not. A higher proportion of carriers was observed among those preferring squeezed juices than those who drank purée:  $76.2\% (\pm 4.5\%)$  and  $67.2\% (\pm 4.5\%)$ , respectively (Table 1).

Fungi were found significantly more frequently (p=0.04) in the oral cavity of students consuming alcoholic beverages  $(70.6\% \pm 2.05)$ , than people declaring abstinence (65.7%±2.1). There was a positive correlation between the presence of fungi and the consumption of spirits (correlation coefficient 0.7267) and negative for consumption of medium-grade alcohol (correlation coefficient -0.7901). A significantly higher incidence of fungi in the oral cavity was also found in those adding sugar to hot beverages such as coffee or tea (78.8% of cases) compared to those who do not  $(60.6\% \pm 1.7)$ : the difference being statistically significant at p=0.004. A positive correlation was observed between the presence of fungi and adding sugar to the coffee (correlation coefficient 0.9691), similar relationship is not observed in the case of tea.

A surprisingly large prevalence of fungi in the oral cavity (81.2%) was observed in the oral cavity of the respondents consuming carbonated mineral water, while the prevalence in those who prefer non-carbonated water was much less (64.4%). Interestingly, 93.3% of students who do not consume non-carbonated water were found to have fungi present in the oral cavity (Table 1). Negative correlation between the presence of fungi and drinking non-carbonated mineral water (correlation coefficient -0.8616) has been reported.

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Table	Table 1. Prevalence of fungi in the oral cavity with respect to the type of beverages consumed	n the oral cavity with	n respect to th	ie type of bever	ages consumed					
				Students consur	Students consuming/adding sugar*	çar*	Stud	ents not consur	Students not consuming/not adding sugar*	sugar*
	Beverages type	es type	Nimber	Maan 02	Prevalenc	Prevalence of fungi	Munhar	Maan 02	Prevalenc	Prevalence of fungi
			TAULIDE		Amount %	Mean %	INUITION		Amount %	Mean %
		cola type	65		49 75.4		54		32 59.2	
-	Sweetened	orange	13	33.5(±11.8)	7 53.8	25(±9)	106	85.5(±11.8)	74 69.8	56(±9.2)
-	carbonated beverages	clear	18	28.1(±9.9)	17 83.3	74.6(±6.4)	101	71.8(±9.9)	66 65.3	$(5.5(\pm 2.1))$
		energy drinks	38		29 76.3		81		52 64.2	
5	Sweetened non-carbonated beverages	nated beverages	27	$27(\pm 0)$ $22.7(\pm 0)$	17 62.9	$17(\pm 0)$ 62.9(±0)	92	$92(\pm 0)$ 77.3( $\pm 0$ )	64 69.6	$64(\pm 0)$ $69.6(\pm 0)$
۲		purcé	61	62(±1)	41 67.2	44.5(±3.5)	28	57(±1)	40 68.9	36.5(±3.5)
)	coone.	squizeed	63	52.1(±0.8)	48 76.2	71.8(±4.5)	56	52.1(±0.8)	33 58.9	64.1(±5)
4	Minarol wotar	carbonated	32	68(±36)	26 81.2	46.5(±20.5)	87	51(±36)	55 63.2	34.5(±20.5)
-		non-carbonated	104	57.1(±30.2)	67 64.4	68.4(±8.4)	15	42.8(±30.2)	14 93.3	67.6(±8.4)
		low-grade	99		46 69.7		53		35 66.1	
2	Alcohol	medium-grade	59	$55.7(\pm 7.1)$ $46.8(\pm 5.9)$	43 72.9	$39.3(\pm 5.2)$ $70.6(\pm 1.2)$	60	$63.3(\pm 7.1)$ $53.2(\pm 5.9)$	38 63.3	$41.7(\pm 5.2) \\ 65.8(\pm 1.2)$
		spirits	42		29 69.1		77		52 67.5	
9	Coffee/ tee	coffee*	52	52(±0)	41 78.8	41(±0)	37	52(±15)	23 62.2	31.5(±8.5)
		tee*	52	43(±0)	41 78.8	78.8(±0)	67	43.7(±12.6)	40 59.7	60.6(±1.2)



Not consuming/Not adding sugar\*

Fig. 2. prevalence of fungi in oral cavity with respect to type of drink preferred

With regard to the daily amount of sugar consumed in beverages, 37.8% (±14.9) of the respondents in total were found to consume doses exceeding the recommended daily requirement: 22.9% of the female respondents compared to 52.8% of the male respondents (Fig. 3). A significantly greater prevalence of oral cavity fungi was noted in those exceeding the recommended GDA  $(76.3\%\pm2.6)$ , compared to of those who were not (68.7%±7.8) (p=0.02). Among women, 78.9% of those consuming more than the daily requirement of sugar were found to have fungi present, compared to only 60.9% of those who declared a lower intake, however, for men, 73.7% who exceeded the GDA value were found to have fungi present in the oral cavity, compared to 52.8% who reported consuming a lower amount of sugar. The differences were not statistically significant (p>0.05).

#### Discussion

As the incidence of fungi in the oral cavity is associated, *inter alia*, with a correct homeostasis and functioning fungal-human relationship, mucosal colonization by fungi is asymptomatic in patients without systemic diseases or other risk factors. Fungi are least frequently found in the oral cavity in children (20.6–31.7%) but this prevalence grows with age, becoming more common in adults aged 18–48 years (57.7%), and most common in the elderly (63.1%) and old people between 71 and 92 years of age (74.2%) [7,15–17]. In the above mentioned studies, *C. albicans* was found to be the dominant species (49% of children and 53.3% in adults), but *C. glabrata* and *C. krusei* (2–10%) were also observed, albeit to a lesser degree. Other species of the genera *Candida*, *Saccharomyces* and *Trichosporon* were identified in individual cases [7,15,17].

Students comprise a specific group who, because of a lifestyle comprising *inter alia* irregular meals, low physical activity, too little sleep and an excessive consumption of stimulants, may have a weakened immune system and disorders of homeostasis [14]. Negroni et al. [18] isolated fungi of the genus *Candida* from 21.4% of students of Dentistry, 68.7% of which were *Candida albicans*. In students of Biology and Veterinary Medicine Biedunkiewicz [19] found the prevalence of fungi to be 46.5–48%, with the most frequently listed

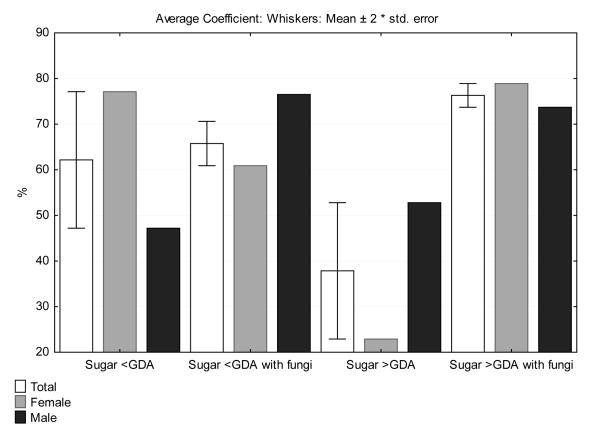


Fig. 3. Prevalence of fungi in oral cavity with respect to guideline daily amount (GDA) of sugar consumed

species being *C. tropicalis* (41.5%) and *C. albicans* (38.5%). Bonassoli and Svidzinski [20] report the presence of fungi in 68% of nursing students, 59.6% of which was *C. albicans*. These authors warn of the possibility of the transmission of fungi in a hospital environment, as well as a potential threat to the medical staff (doctors, nurses and students) and the patients under their care. A similar percentage of carriers of fungi was found in the present study. The increased prevalence of fungi among medical science students may be the result of more frequent contact with patients belonging to risk groups of fungal infection or with biological materials derived from patients.

In patients demonstrating favorable factors, such as the weakening of the immune system or cancer, the prevalence of fungi is higher. Fungi were detected in 60.6% of patients with chronic periodontitis, and 68.8% of patients with aggressive periodontitis [21]. A higher prevalence of fungi is also seen in diseases of the oral mucosa: 81.6% for the inflammation of the tongue, 87.5% for oral atrophic inflammation, 91.7% for prosthetic inflammation and up 96.4% for leukoplakia; the most frequently isolated species is *Candida albicans* (74.4%) [22]. In addition, 70% of patients with damage to the salivary glands were found to be carriers of fungi in the oral cavity, of whom *C. albicans* was identified in up to 89% of cases, *C. parapsilosis* 33%, and *C. tropicalis* 13%. In many cases, two or three species were identified simultaneously [23]. The presence of fungi has been noted in 60% of those infected with HIV, and in 80% of patients in whom AIDS develops [24].

A significant factor favoring candidiasis which is associated with the course of cancer is the inhibition of immune system activity by chemo-and radiotherapy. A higher prevalence of fungi has been identified in patients with head and neck cancer treated with radiation: 68.2% being noted before treatment, rising to 80% in the third week of treatment, followed by a decrease to 57.1% in the last day of treatment and a final increase to 100% after three weeks of completion. In this case, C. albicans was found to be the dominant species, even though its frequency decreased from 60% in the first study to 40% in the last. Other species during subsequent tests were isolated much more frequently. After therapy, the participation of C. glabrata in the oral cavity mycobiota increased from 10% to 20%, C. guilliermondii from 0% to 20%, C. kefyr from 3.3% to 10%, and C. lusitaniae

from 0% to 10% [25]. A similar relationship was observed by Pytko-Polończyk et al. [26], who note the presence of fungi in 46.3% of patients before radiotherapy, followed by an increase to 68.3% after two weeks of therapy rising to 70.7% after 4 weeks, and then a fall to 43.9% after finishing treatment. During treatment, the prevalence of various fungi were found to fluctuate, for example, *C. albicans* rose from 68.4% prior to therapy to 78.6% during therapy before falling to 65.5% and then finally rising again to 77.8% after treatment. After treatment, *C. krusei* and *C. kefyr* were not observed in patients, while *C. pseudotropicalis* appeared to have replaced them.

The present study shows that students of medical faculties consume large amounts of sweetened beverages, especially sodas and low-level alcoholic beverages. It was noted that increased consumption of sweetened coffee/tea and alcohol significantly increases the prevalence of fungi. Similar observations have made by other authors [5,6,16,19].

According to the results of the present study, 28.1% of surveyed students consume sweetened sodas, although clear differences can be seen with regard to the consumption of different types of beverages. While orange drinks were consumed by only 10.9% of students, cola type by 54.6%. Few students consumed energy drinks, which is quite surprising in the context of studies conducted by Kopacz et al. [27], who reported the consumption of highly sweetened energy drinks by 58–62% of students.

The tendency to prefer products with a high content of sugar and fat grows with age. According to Stefańska et al. [1], the percentage of children consuming sweets between meals was about 6–11% higher among 13 to 15-year-olds than among younger children. Furthermore, approximately 50% of university students consume products such as chips, ice cream, donuts and cakes several times a month [4].

Improper eating habits can affect health. Body Mass Index has been observed to increase with age. In school-age children, overweight (BMI between 25–30) was found to occur with a frequency of 12–17%, and obesity (BMI $\geq$ 30) 10–27% [1,2]. In young people and students, these values rise to 16% for overweight and 12% for obese [28]. Of people aged 40 to 50, overweight was observed in 32% and obesity in 44%, while in those over 60, the respective values rose to 53% and 47% [3].

Collison et al. [2] and Park et al. [28] report a correlation between an increase in BMI and waist circumference and intake of sweetened carbonated beverages. Park et al. [28] also reveal that soda consumption is related to worse academic performance and poorer physical condition in young people. Both diet, access to computer games and time spent on social networking websites, reduce the amount of time dedicated to physical activity, which indirectly affects health. Studies conducted by Biedunkiewicz [19] suggest a correlation between the state of the immune system of students, and the risk of colonization of the human body by yeast and yeast-like fungi. The author also draws attention to the relationship between fungal colonization and alcohol abuse and smoking tobacco. Tobacco, usually smoked in the form of cigarettes, is a source of aromatic hydrocarbons, which may be a substrate for the fungi present in the oral cavity and can promote pathological changes in the mucous membranes [29,30].

The studies presented in this paper show that consumption of sweetened beverages is a predisposing factor for the colonization of the oral cavity by yeasts and yeast-like fungi. The results based on adult GDA values presented above show that a diet rich in sugar increases the risk of colonization of the oral cavity and may be a factor contributing to the development of mycosis of the gastrointestinal tract in patients. The research performed indicate a relationship between eating habits with the risk of oral colonization by fungi. It should be noted that the frequent and excessive intake of sweetened beverages and the addition of sugar to drinks may result in fungal colonization of upper sections of the gastrointestinal tract, thereby creating a reservoir of endogenous fungal infections in human organism.

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