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Original Scientific Article

METABOLIC PARAMETERS IN TYPE 2 DIABETIC PATIENTS WITH POSITIVE CANDIDA CULTURES

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Abstract. *The gut microbiota plays an important role in host metabolism, immunity, digestibility and even behaviour. Candida spec. is common resident of the gastrointestinal tract and integral part of the microbiota. The aim of the study was to evaluate if positive Candida cultures in the stool influence metabolic parameters in type 2DM patients. 46 patients with type 2 DM and oral antidiabetic treatment, were divided into a study group (S=18 patients with positive Candida sp. cultures in stool) and the control group (C= remaining 28 patients). Besides medical history and clinical examination, all patients were tested for coproculture, fasting glycaemia (FPG), HbA1C, total cholesterol (CL) triglycerides (TG), high- density lipoproteins (HDL) and low- density lipoproteins (LDL). Study group patients had a significantly higher BMI (31.41 ± 5.29 vs. 25.18 ± 3.58; p<0.001); HbA1C (9.8 % ± 1.74 vs 6.9% ± 1.89; p<0.05) as well as FPG (10.87 ± 1.35 vs 7.47 ± 1.03; p<0.01), compared to the control group. Even though the study group patients had higher TG, CL, LDL and HDL, compared to the control group, there was no statistical significance verified. Uncontrolled glucoregulation is one of the host condition which favours candida colonization and subsequent infection. This may be related to the decrease in commensal bacteria, probably as the result of yeast-bacterial competition. On the other hand, we have to keep in mindthat a significantly increased number of Candida colonies can affect the rate of digestion and absorption of carbohydrates and consequently increase the level of glycaemia in patients with diabetes*.

Key words: *Candida, microbiota, diabetes, metabolic disorder, hyperglycaemia*

Introduction

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycaemia, caused by reduced insulin secretion, reduced biological action of insulin, or both [1]. It is estimated that over 500 million people worldwide suffer from diabetes, among whom approximately 90%, has type 2 diabetes mellitus T2DM [2]. Epidemic proportion of diabetes, progressive course of the disease and link to the numerous micro- and macrovascular complications, are burden for health, economics and society around the world. That is why it is of great importance to determine all factors influencing the occurrence of DM as well as modifying its clinical course. One of such potential factors is bacterial and fungal microbiota of the gastrointestinal tract. Numerous studies, in the last decade, have revealed that the gut microbiota plays an important role in the host metabolism, immunity, digestibility and even behaviour [3]. It is pointed out that bacterial and fungal intestinal microbiota play a potential role in the development and progression of diabetes mellitus [4]. Since the processes of digestion and absorption can modify the occurrence of autoagression, one of the apostrophized causes of DM, gut microbiota influence on the speed of decomposition of complex food components, may potentially influence pathogenesis of DM [5]. Altered microbiota composition can change the speed of digestion and absorption of carbohydrates and substantially lead to the postprandial hyperglycaemia changing the clinical course of DM [6]. Experimental studies on the animal models, demonstrated that gut microbiota may

Candida spec. are considered normal inhabitant of the gastrointestinal tract and integral part of the microbiota. These opportunistic microorganisms commonly colonize cutaneous and mucosal surfaces. The gastrointestinal tract represents the ultimate human reservoir for most *Candida* species [8]. Number of 10 to 10^3 fungal colony forming units (CFU)/g faeces are considered as normal range. However, when mucosal surface is damaged or host immunity is compromised, common commensal *Candida sp.* can be the cause of life-threatening invasive infection. Patients with DM have higher predisposition to fungal infections, including *Candida sp.*, due to several mechanisms, such as: higher salivary glucose levels [9], reduced salivary flow [10], microvascular degeneration, but mostly due to diabetes mellitus immunosuppressive effect.

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Even though, *Candida sp.* are one of the most prevalent fungus in gut microbiota, literature data about frequency in DM patients and interplay between host and *Candida* are limited.

Objectives

This retrospective study is conducted with the following aims:

1. To estimate the frequency of *Candida sp*. in patients with type 2 diabetes mellitus.
2. To evaluate if *Candida sp.* presence influences metabolic parameters in type 2DM patients.

Patients and Methods

The study included 46 patients with type 2 DM, 20 women and 26 men. Based on the positive or negative *Candida sp.* in stool, patients were divided into following groups: study group (+C = 18 patients with positive *Candida* sp. cultures in stool; 39.13%) and control group (-C = remaining 28 patients; 60.87%). The number of CFU/g faeces above 10^3 was considered as positive. Patient's characteristics, age, gender, diabetes duration and therapy (antidiabetic and hypolypemic) are provided in Table 1. All the subjects were white Caucasians. The antidiabetic therapy consisted of oral hypoglycaemic agents, metformin and sulfonylurea (SU) preparations (glimepiride or gliclazide). Besides medical history and clinical examination (including BMI) stool and blood samples were collected from all patients participating in the study. Stool samples were used for *Candida sp.* testing and blood samples for determining the following parameters: fasting plasma glucose levels (FPG), glycated haemoglobin (HbA1c) and lipid profile (total cholesterol (CL), triglycerides (TG), high density lipoproteins (HDL-C) and low density lipoproteins (LDL-C)).

Table 1 Characteristics of the studied patient groups

	Candida positive group (+C) (n = 18)	Candida negative group (-C) (n = 28)
Male : Female (n)	10:8	14:14
Age (yrs.)	68.21 ± 10.4	66.97 ± 9.7
Duration of diabetes (yrs.)	9.89 ± 5.04	10.13 ± 6.21
Diabetes treatment	Metformin + SU (18)	Metformin + SU (28)
Statin therapy*	4 (33.33%)	20 (58.82%)

The presented data are means ± SD.
*significant differences within the two compared groups of patients; ($p < 0.01$) – Mantel- Haenzel Chi-squared test

The main exclusion criteria were antibiotic therapy, administration of probiotics and corticosteroids 30 days before stool sampling.

Statistical data processing and determination of significance between the study and control group were performed in the SPSS program.

Results

The average age of all patients was 67.83 ± 9.72 years. The youngest patient was 30 years old, and the oldest was 77. No statistical significance was found relating to the average age in the study (+C) and control group (-C) (68.21 ± 10.4 vs. 66.97 ± 9.7 years).

In the +C group, most patients (12; 66.67%) had some lipid status disorder (mixed hyperlipidaemia, isolated hypercholesterolemia, or isolated hypertriglyceridemia). The majority of patients had mixed hyperlipidaemia (6; 33.33%), followed by isolated hypercholesterolemia (4; 22.22%), and only 2 patients had isolated hypertriglyceridemia (2; 11.11%).

In the -C group, the number of patients with a lipid status disorder was similar (18; 64.28%). Mixed hyperlipidaemia was present in 28.57% (8 patients), isolated hypercholesterolemia in 21.42% (6 patients), and isolated hypertriglyceridemia in 14.29% (4 patients). The distribution of hyperlipidaemia types is shown in Table 2.

Table 2 Hyperlipidaemia types distribution in the studied patients groups

Lipide profil type (n; %)	Candida positive group (+C) (n = 18)	Candida negative group (-C) (n = 28)
Mixed hyperlipidaemia	6; 33.33%	8; 28.57%
Isolated hypercholesterolemia	4; 22.22%	6; 21.42%
Isolated hypertriglyceridemia	2; 11.11%	4; 14.29%
Normal lipid profile	6; 33.33%	10; 35.71%

Higher values of TG (2.03 ± 1.13 vs. 1.80 ± 0.58), CL (5.23 ± 1.23 vs. 4.73 ± 0.92), LDL-C (3.27 ± 0.75 vs. 2.86 ± 0.83) and HDL-C (1.63 ± 0.25 vs. 1.08 ± 0.44) were verified in +C compared to the -C, but without statistical significance (Table 3).

Table 3 Total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol in the studied patients groups.

Parameter	Candida positive group (+C) (n = 18)	Candida negative group (-C) (n = 28)
CL (mmol/l)	5.23 ± 1.23	4.73 ± 0.92
TG (mmol/l)	2.03 ±1.13	1.80 ± 0.58
HDL-C (mmol/l)	1.63 ± 0.25	1.08 ± 0.44)
LDL-C (mmol/l)	3.27 ± 0.75	2.86 ± 0.83

A significantly smaller number of patients in the +C group (4) had statin therapy, compared to the -C group (20) of patients (33.33% vs. 58.82%, $p<0.01$) with high correlation degree between statins and a negative *Candida sp.* findings.

Study group patients had a higher BMI (31.41 ± 5.29 vs. 25.18 ± 3.58; $p<0.001$), HbA1C (9.8 % ± 1.74 vs 6.9% ± 1.89; $p<0.05$) as well as FPG (10.87 ± 1.35 vs. 7.47 ± 1.03; $p<0.01$) compared to the control group (Table 4).

Table 4 Laboratory and antropometric parameters (HbA1C, FPG and BMI) in studied patients

Parameter	Candida positive group (+C) (n = 18)	Candida negative group (-C) (n = 28)
HbA1C[#] (%)	8.02 ± 1.74	7.9 ± 1.89
FPG[§] (mmol/l)	7.87 ± 1.35	7.47 ± 1.03
BMI[*] (kg/m2)	31.41 ± 5.29	25.18 ± 3.58

The presented data are means ± SD.

[#]significant differences within the two compared groups of patients; ($p < 0.05$)

[§]significant differences within the two compared groups of patients; ($p < 0.01$)

*significant differences within the two compared groups of patients; ($p < 0.001$)

Discussion

Human microbiota represents the complete set of microorganisms in the human body, while the term microbiome defines total genetic material of the microbiota. Such a powerful "microcosm" carries a huge genetic information, which is 150 times larger than the human genome. Precisely for this reason, the concept of microorganisms being either pathogenic or commensal is slowly being abandoned. An increasing number of studies confirm that it is a matter of complex interactions between microbiota and humans, which have a very significant impact on metabolism, the immune system, and even on the host's behaviour [3]. The knowledge that microbiota can modulate hormonal secretion was the foundation for a new field known as microbiological endocrinology.

Candida belongs to a group of opportunistic microorganisms that are normal inhabitants of the skin and mucous membranes. In faeces, the number of *Candida sp.* colonies should not exceed $10\text{-}10^3$. However, when the anatomical barrier is violated, candidiasis occurs, which can significantly threaten the life of the host [11]. *Candida albicans* infection is the most widespread invasive mycosis with a mortality rate of up to 40% [12, 13].

Our study results showed that 26% of included patients had positive *Candida sp.* in the stool, which is a slightly higher percentage, compared to the data from the literature [11]. Dietary habits in the region from which the study patients are, consist of many products important for *Candida sp.* growth. Food such as bread (containing yeast), white flours, smoked or cured pork meat (containing mould), wine and beer (alcohol which is fermented using yeast), sugar itself and dried fruits (which have been jarred) are well-known to make a suitable environment for *Candida sp.* overgrowth. It has been described that *Candida sp.* is more frequent in the faeces of patients with type 1 and type 2 DM with poor glycemic control as opposed to healthy subjects [4]. In our study, all patients were type 2 DM and significantly higher HbA1C and FPG in the *Candida positive* group pointed out that poor glycaemic control favours development of the candidiasis among DM patients. There are several factors largely influencing the balance between host and yeasts, favoring the transition of *Candida* sp. from commensal to pathogen and causing infection. One of the main reasons is direct effect of elevated blood glucose levels, creating specific conditions for intensive fungal colonization [4]. *Candida sp.* uses glucose as a source of energy, necessary to synthetize biofilms and the polysaccharide matrix as protection from the environment [14, 15]. Our study results also showed that Candida positive group of patients had significantly higher BMI. The compositional changes of intestinal fungi have been found in patients with obesity [16]. Also, different methods confirmed correlation between increased *Candida sp.* and obesity [17]. However, causal relationship between intestinal fungi and obesity has only been confirmed in the animal experimental model. *Sun et al.* in their experimental study showed that the treatment with amphotericin B and fluconazole significantly repressed the progress of obesity in mice [18]. Further investigations in this field will certenly give the answers about physiological functions and underlined mechanism of intestinal fungi during the development of obesity.

The patients of the Candida positive group, had higher levels of CL, TG, LDL-C and HDL-C compared to the Candida negative control group, but without statistical significance. There are opposite literature data regarding the correlation between *Candida* and lipid status. Gosiewski et al. in their research, verified a negative correlation between *Candida* in the large intestines and serum lipid levels in T2DM subjects, while Netea et al. in the experimental study, pointed out that hyperlipoproteinaemia enhances susceptibility to acute disseminated *Candida albicans* infection [4, 19]. We have to emphasize that in our study patients, significantly higher number of patients in Candida negative group were using statin therapy (58.82% vs. 33.33%). There is a growing evidence that statin therapy has ability to reduce the incidence of positive culture of *Candida spec.* By inhibiting 3-hydroxy-3-methyl-glutaryl coenzyme A reductase, statins lead to the reduction of mevalonate, thereby reducing cholesterol levels in humans and possibly ergosterol in fungi. Considering that ergosterol is an essential lipid component of the cell membrane of fungi, they will be more vulnerable especially to the action of antifungal drugs [11, 20]. Although our study included a relatively small number of patients, the results are consistent with the literature data, showing that statin therapy in patients with diabetes is important not only in reducing the concentration of CL and TG, but also in providing some protection against the intestinal *Candida* overgrowth.

The classic, well-known role of intestinal microbiota is in the digestion of carbohydrates and their fermentation to fatty acids. Hyperglycaemia and poor glycaemic control in DM, favour *Candida sp.* overgrowth. On the other hand, increased number of *Candida* colonies can affect the speed of digestion and absorption of carbohydrates leading to the higher postprandial glycaemia levels in DM patients [6]. Microbial endocrinology, reveals that the intestinal microbiota affects neuropeptides such as

ghrelin, insulin and leptin, crucial controllers of appetite and metabolism. Altered microbiota could be one of the underlying pathophysiological mechanism of obesity [7, 21].

Our study limitations are the sample size of the study groups, inclusion individuals with relatively long DM duration and single-point examination. Thus, the obtained results require confirmation on a greater number of patients.

Conclusion

Candida fungi appear to be more prevalent in the T2DM patients with poor glycemic control, higher FPG and higher BMI. Statin therapy is strongly correlated with negative *Candida* findings. Although microbiological endocrinology is a relatively new field, there is growing evidence of the intestinal microbiota importance and its influence on metabolism, appetite, immunity, reproduction and behaviour. Further research should elucidate these complex interactions between the host's endocrine system and gut flora. The development of preventive and therapeutic measures such as probiotics, statins and certain dietary products would certainly be of great help in better understanding of diabetes.

List of abrreviations

DM - Diabetes mellitus
T2DM - type 2 diabetes mellitus
T1DM - type 1 diabetes mellitus
BMI - Body mass index
FPG - fasting plasma glucose
HbA1c - glycated haemoglobin
CL - total cholesterol
TG - triglycerides
HDL-C - high density lipoprotein cholesterol
LDL-C - low density lipoprotein cholesterol
CFU - colony forming units

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