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Oral candidiasis and inflammatory response: A potential synergic contribution to the onset of Type-2 Diabetes Mellitus

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CASE STUDY

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ABSTRACT

It is known that fungal pathogens activate cellular immune responses involving several inflammatory molecules. On the other hands, it is well known that diabetic patients show a chronic inflammatory state. In this study, we explore the hypothesis that yeast colonization of the oral cavity directly influences the tendency of an individual to develop type 2 Diabetes. According to this hypothesis, oral yeast colonization may influence the pathway in which the immune response handles subsequent responses to glucose intake. The first part of this work presents data concerning oral *Candida* carriage in subjects with pre-diabetes, the second part explores the potential role of oral *Candida* infection, related to inflammatory response in promoting insulin resistance.

Key Words
Candida, pre-diabetes, cytokines, immune response

Implications for Practice:

1. What is known about this subject?
Candida infection can activate cellular immune responses involving several inflammatory molecules; furthermore, diabetic patients show typically a chronic inflammatory condition.

2. What new information is offered in this case study?
We explored the potential role of oral *Candida* infection, related to inflammatory response in promoting insulin resistance.

3. What are the implications for research, policy, or practice?
It is probable that the colonization with Candida contribute to insulin resistance. Clinicians must consider the control of Candida infection in diabetic patients.

Background

Candida albicans is considered the most prevalent human fungal pathogen.[1] It is a yeast commensal living in the bowel flora, but it can be commonly discovered in the mouth, throat, and genitourinary tract. *C. albicans* and *C. glabrata* are the two most common *Candida* species found in the oral cavity of individuals.

The pre-diabetes represents a condition with dysglycemic states intermediate between normal glucose regulation and diabetes. Up to 70 per cent of pre-diabetic patients may acquire the disease over their lifetime.[2]

Pre-diabetes is also considered as an impaired glucose tolerance (IGT), an impaired fasting glucose (IFG), or a kind of insulin resistance. Some people show both IGT and IFG: in IGT, glucose levels are little higher than normal right after eating; in IFG, glucose levels are little high several hours after eating.[3]

In a recent study, oral *Candida* was isolated from 100 per cent of pre-diabetic patients, and the infection was found to be independent of glycemic status in such patients.[4]

Furthermore, local pathological condition of *Candida* oral infection may be affected by neutrophils accumulation and by increased levels of some cytokines already after 48 h

after *C. albicans* first colonization, without any clinically observable sign.[5]

On the other hands, in the literature has been reported that patients with prediabetes, have increased concentrations of subclinical inflammation which is mostly driven by postload glucose concentrations.[6]

Furthermore, there are increasing evidences about the role of chronic inflammation in type-2 diabetes progression.[7]
We, thus, hypothesized that the subclinical inflammation affecting the prediabetic patients, worsened by the presence of *Candida* infection, could lead such patients towards the onset of Type-2 Diabetes Mellitus.

Objective
With the aim to confirm our null hypothesis, we investigated *Candida* colonization that occurred in the oral cavity of pre-diabetic subjects without clinical signs of oral candidiasis.

Method

Forty-two subjects with clinical signs of pre-diabetes were included in this study. The control group consisted of healthy subjects. The inclusion criteria for the study group were: adult subjects aged 20-55y.o., no clinical signs of active oral candidiasis, no antibiotic or anti-inflammatory therapy in the last 3 month before the study, no immunosuppressant therapy, no partial or total dentures present in the oral cavity, no systemic diseases with the sole exception of the pre-diabetic state. A complete anamnesis and an accurate oral examination were performed to both groups. Subjects in both groups were non-smokers.

The study was approved by the Ethical Committee of "Universitatea de Medicina si Farmacie Tîrgu Mureș". All the subjects included in this study signed an informed consent.
After mouth rinses (1 minute) with saline water, samples of scraped mucosa were obtained from the *dorsum* of the tongue and from the buccal mucosa of each subject involved in this study. The samples were smeared and fixed on glass microscope slides. Each slide was stained with periodic-acid-Schiff (PAS) reagent: the following step was the microscopy examination of each sample, to assess the Candida infection in the subject analyzed. All microscopic evaluations were performed in a blind manner by one examiner.

After our clinical assessment and the harvesting of intraoral samples, the subjects investigated were finally addressed to a specific visit by diabetologist, in order to get a complete examination and an oral glucose tolerance test (OGTT).

Results

The mean age of the study group was 41±8y.o. and the control group reported a mean age of 43±6y.o.; gender distribution was approximately equal, with a slight predominance of female subjects in both study groups. The mean body-mass index (BMI) in control group was 22.04±2.21, while in the study group 85 per cent of subjects were overweight, with a mean BMI value of 27.75±3.09.

In the study group, the results of OGTT test, at 2 hours after the test, showed blood glucose levels of 140-178mg/dl, confirming the suspected diagnosis of pre-diabetes.

The detectable Candida carriage in control group was noticed only in 4.76 per cent of the cases; surprisingly, the 73.78 per cent of patients in the study group showed a detectable Candida carriage. Oral examination revealed no clinical infection in control group (Figure 1a and 1b), whereas the presence of yeast forms and proliferation of pseudo-hyphae, that indicate a sub-clinical infection, was detected in the study group (Figures 2a and 2b).

Discussion

The hypothesis of the study was that subclinical inflammation affecting the prediabetic patients, worsened by the presence of Candida infection, and might induce a hyperglycemic state contributing to diabetic state progression. Our results showed a strongly higher proliferation of pseudo-hyphae and yeast forms in the oral cavity of pre-diabetic subjects, compared with controls.

Several studies have shown oral *Candida* proliferation and colonization in subjects with insulin resistance/diabetes.[8] In addition, *Candida* growth and salivary glucose were shown to have a clear correlation in diabetic patients.[9] Subjects with pre-diabetes have an impaired glucose tolerance, which may lead to temporary acidosis, favoring oral candidal proliferation.

It is also known that *Candida* needs carbohydrates for its growth: in fact, one of the most effective therapeutic approaches against the fungal infection is to modify the daily diet, firstly reducing the carbohydrates. Thus, we may postulate that from this point of view, there is a two-way relationship between *Candida* and glucose tolerance. In one way, impaired glucose tolerance lead to Candida colonization, through several paths already described, and on the other way, the presence of *Candida* craves for glucose intake, which may lead to disturbances in glucose metabolism.

Epithelial cells are usually the first line of defense against *Candida* pathogens. Until recently, it was thought that the main role of epithelial cells was limited to providing an anchorage point for colonization and a food source for *Candida*. However, recent studies have changed the view of the importance of epithelial cells in host-fungal interactions. Specifically, it is well known that a fundamental role of epithelial cells is to target responses to *C. albicans* hyphae and to discriminate between commensal/colonizing and invasive/pathogenic *C. albicans*.[10-13]

Epithelial cells produce a large variety of molecules, including cytokines and chemokines, but the precise combination depends upon the *Candida* strain or species and the epithelial cell type involved [8-14]. For example, infection of oral epithelial cells with *C. albicans* results in the induction of several cytokines including IL-1α, IL-1β, IL-6. For *C. albicans*, cytokine induction appears to be associated with hyphae formation, and it is known that the species or strains that do not produce hyphae in culture conditions are unable to produce strong effector responses.[14-17] The secretion of epithelial pro-inflammatory cytokines and chemokines in response to *C. albicans* will result in the recruitment and activation of a variety of immune cells including neutrophils.[18]

It is known that immunity against fungal infection depends on activation of cellular immune response, namely T lymphocytes. These T lymphocytes are an important part of the host defense against fungi, known from the fact that patients with AIDS are highly susceptible to *C. albicans* and other fungal infections, due to the lack $CD4^+$ T cells.[19]

Several authors discussed about the concept of different T-helper (Th) cells, according to the types of cytokines secreted by each of them. They called “Th1 cells” those cells that produce interferon-γ (IFNγ), and “Th2 cells” those who produce interleukin (IL)-4. Thus, Th1 cells are essential for host defense against intracellular pathogens, whereas Th2 cells fight against parasites and allergies.

The identification of T cells subtypes lead to a change in Th1-Th2 paradigm and helped understanding several abnormalities in the original model. In mid-1990, suppressor T cells were re-discovered as Treg cells and were shown to have a major role preventing autoimmunity, by controlling the immune response to self-antigens. The $CD4^+$ population that produces IL-17 was named Th17.[10-25]

The main function of IL-17 secreting T cells is to mediate inflammation, by stimulating production of inflammatory cytokines such as TNF-α, IL-6 and IL-1ß. These T cells also stimulate production of some inflammatory chemokines, leading to recruitment of macrophages and neutrophils.

Th17 cells were shown to have a protective role against infection, helping the clearance of pathogens by increasing neutrophils recruitment in infected sites and macrophages activation.[22-25] Though Th17 cells are named after IL-17, they produce a wide range of cytokines, such as TNF-α, IL-6, IL-21, IL-22, IL-17A and IL-17F, molecules that have distinct roles in host defense against infection. Moreover, T cells are a major source of IL-17, but they are not the only source for this cytokine. Neutrophils also produce Th-17 as a response to IL-15.[24]

It has been demonstrated that IL-17 promotes TNF-α and IL-1ß, as well as chemokine production, and thereby promotes inflammation and tissue damage. Since TNF-α and IL-1ß have well known roles in the pathogenesis of chronic inflammatory disorders, we can conclude that IL-17 might mediate its effect by increasing production of these pro-inflammatory cytokines.[24-26] Thus, Candida infection may induce or perpetuate an elevated systemic chronic inflammatory state, aggravating glycemic status of pre-diabetic subjects, contributing to the development of diabetes.

In our study, the mean BMI of pre-diabetic subject demonstrated that 85 per cent were overweight. This evidence suggests that oral candidiasis have systemic effects that extend beyond the local oral environment. It is likely that Candida infection leads to certain food addictions and unhealthy diet habits that favor metabolic disorders, increase in body mass index and development of diabetes mellitus.

It is known that systemic inflammation is significantly elevated in subjects with increased BMI. One of the major functions of adipose tissue is the endocrine function. Adipose tissue produces a wide range of hormones commonly called ‘‘adipokines.’’, who have important roles in insulin sensitivity, regulation of appetite, regulation of blood pressure and mediate the immune function.[27] When the body fat changes, the production and function of adipokines is altered. Increase BMI is correlated with increase number and dimension of adipocytes, cells with an intense metabolic activity who produce important quantities of TNF-α and IL-6. Actually, the adipose tissue produces about one-third of the total amount of circulating serum IL-6.[28] Although the exact physiological pathways are still unknown, obesity can increase insulin resistance by

elevated TNF-α and IL-6 production and decrease adiponectin production.[28-31] Infusion of TNF-α in healthy human induces insulin resistance in skeletal muscle and reduces glucose uptake and use. If pharmacologic agents are used to block TNF-α, a decrease of insulin serum levels and a better insulin sensitivity can be observed in some subjects but not in others.[32] Adiponectin antagonizes many of the effects of TNF-α and improves insulin sensitivity.[33] As body mass increases, adiponectin production decreases; thus, obesity results in elevated TNF-α levels and decreased adiponectin levels, both of which result in insulin resistance. IL-6 stimulates TNF-α production; thus, elevated levels of IL-6 produced by adipocytes in obese subjects lead to elevated TNF-α level, and may further exacerbate insulin resistance. Hepatic CRP may also increase insulin resistance, since increased production of TNF-α and IL-6 stimulates greater hepatic CRP production.[30-34] There are many mechanisms involved in regulation of insulin sensitivity and resistance, including adipokines and inflammatory mediators.[35]

As an inflammatory condition, candidiasis may also play a role in this process. Elevated circulating levels of several pro-inflammatory cytokines have been found in individuals with candidiasis, but also in obese subjects. Since overweight subjects have an increased risk for Candida colonization, we may postulate that when these two conditions meet, there is an elevated general inflammatory state that may alter insulin resistance, potentially leading to type 2 diabetes development.

An aspect of interest is that many clinical conditions could simulate other ones, as the infection by Staphylococcus aureus occurring in patients with immunological deficit, or in patients with mucosal trauma.[30-35] Also the stress is able to induce alteration of glucose levels in the blood, thus, it should be evaluated some professional categories, some specific pathologies and the role of the prevention of such oxidative conditions.[35-40]

Pre-diabetic patients who also have candidiasis may have a high systemic inflammatory condition due to elevated serum levels of IL-6, TNF-a, and CRP, which can worsen insulin resistance and thereby aggravate glycemic control. This could explain why candidiasis increases the risk of poor glycemic control in patients with type 2 diabetes. Thus, the treatment of fungal infection may decrease serum levels of the inflammatory mediators that cause insulin resistance, thereby positively affecting glycemic control.

The clinical relevance of our study is that oral Candida carriage can predict the evolution of glycemic state in subjects with pre-diabetes.

Despite other studies has been carried out on similar topics, leading to the conclusion that experimental colonization of *Candida glabrata* is not associated with an inflammatory immunopathogenic response or biofilm formation, many of these researches are focalize on vaginal infections and they have taken into consideration patients already affected by diabetic condition.[41]

As a limitation of the study, we can mention the reduced number of patients and the fact that we did not perform immunological analysis for these subjects, the present study being a preliminary one. Immunological analyses will complete the current results in a follow-up study. Further studies are necessary to sustain our hypothesis. Another topic that remains to be explored is how antifungal treatment can improve general inflammatory state, reducing the risk for developing type 2 Diabetes (Table 1).

Conclusion

Candidiasis and diabetes mellitus are highly prevalent chronic diseases and they are closely associated. Some associated conditions such as increased body mass index and insulin resistance may be of great importance in this relationship. Insulin resistance increases the risk of oral Candida colonization; less unclear is the impact of oral candidiasis on glycemic control and the mechanisms involved. It is probable that the colonization with Candida initiates or contributes to insulin resistance in a similar way to obesity, worsening glycemic control. Further researches are needed to elucidate this two-way relationship between oral candida colonization and the development of type 2 diabetes.

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PEER REVIEW

Not ommissioned. Externally peer reviewed.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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PATIENT CONSENT

The authors, *Monea A, Santacroce L, Marrelli M, Man A*, declare that:

1. They have obtained written, informed consent for the publication of the details relating to the patient(s) in this report.
2. All possible steps have been taken to safeguard the identity of the patient(s).
3. This submission is compliant with the requirements of local research ethics committees.

Figure 1: Cytological examination from a subject in control group. No smear, PAS stain, a) 10x magnification, b) 20x magnification

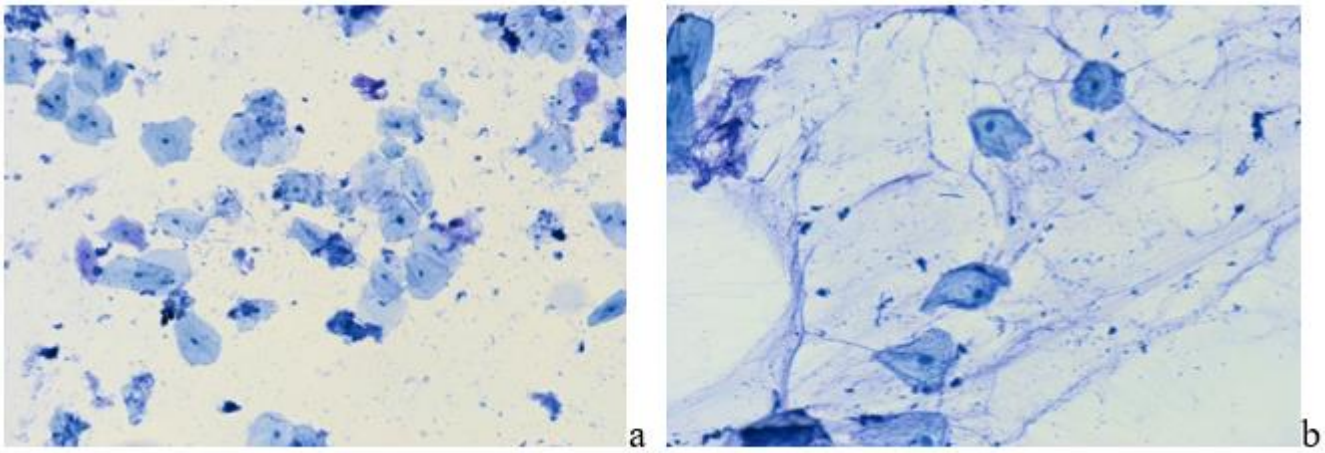

Figure 2: Cytological examination from a pre-diabetic subject. Scattered yeast forms, proliferation of pseudo-hyphae. PAS stain, a) 10x magnification, b) 20x magnification (Arrows indicate the pseudo-hyphae)

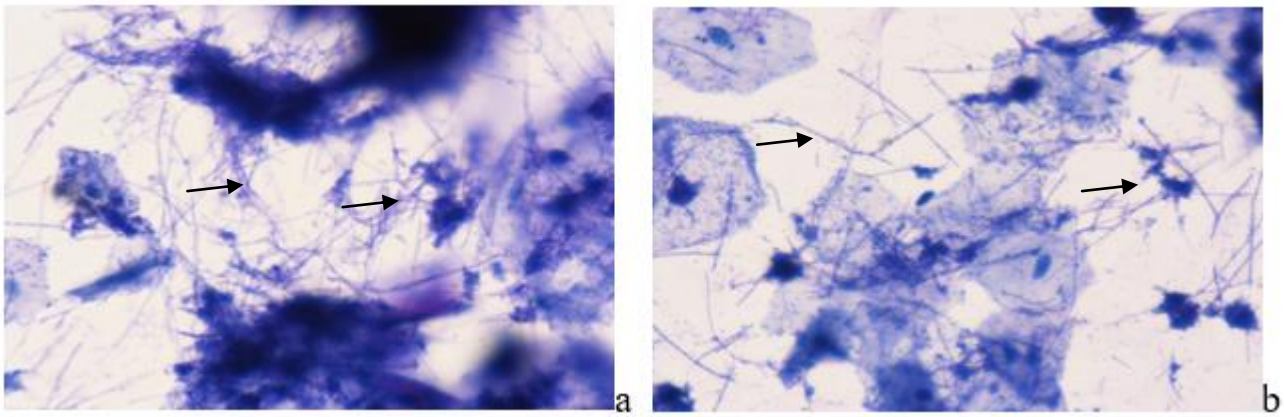

Table 1: Analysis of subjects enrolled in the study group and control group with a sub-analysis about the differences related to B.M.I. (Body Mass Index) in each group

Subjects Group	Mean age (y.o.)	Gender (M/F)	Normal B.M.I.	Overweight B.M.I.	Obese B.M.I.	Candida Carriage
Study	41±8	22/20	8%	85%	7%	73.78%
Control	43±6	25/17	100%	0%	0%	4.76%