ABSTRACT: Not long after birth, yeast, predominantly *Candida albicans*, colonizes the epithelium of oral cavity and the whole gastrointestinal tract. *C. albicans* lives in yeast, a non-harming form, as a commensal member of the microbial flora, but may turn into pathogen infective form under certain conditions that encourage its overgrowth. In this phase, it may damage the intestinal wall and enter the bloodstream, causing invasive candidiasis with high mortality rate. It is essential to recognize candidaemia and start the lifesaving therapy on time. Recognizing the risk factors which allow candida to overgrow is the most important step in preventing candida’s overgrowth and chronic candidiasis, the previous status of invasive candidiasis. If this recognition is missed, and the overgrowth advances, a question remains how to discover and treat it and in which phase it should be done. A stool culture requires time and proves the presence of live yeast cells only. If the live yeast cells are not present in the stool, the result of the culture will be negative. In this paper, the author presents her experience of stool analysis under dark-field microscope, as a rapid, easy to carry out method for detecting the presence of live or dead yeast cells and yeast overgrowth.

KEYWORDS: yeast infection, candida infection, candida overgrowth, chronic candidiasis, stool analysis, dark-field microscope

INTRODUCTION

The first publication of *Candida* overgrowth in the intestine after antibiotics administration was written by Irene Neuhauser in 1954. In 1972, Iwata Kazuo published his observation of drunken people who never drank alcohol, but had high levels of blood alcohol produced by overgrown candida in their gut. He called this condition “drunkenness without drinking alcohol.” Later, Orian C. Truss published his book “The Missing Diagnosis” in 1983, William G. Crook published his first book “The Yeast Connection” in 1986 and John P Trowbridge’s book “The Yeast Syndrome” was also published in 1986. At that time, their observations and findings were not accepted as scientific, but later on, * Corresponding author. E-mail: mariakleinlaszlo@gmail.com
more and more researchers have proved that these first works were right. Still, there is no consensus among doctors. Some believe, and the others do not, that yeast can overgrow in gut and cause disturbances in the functions of the whole body, and that candida syndrome or chronic candidiasis really exists.

The yeast, predominantly candida species, in small amount is normally the member of the intestinal microbial flora of a healthy person. C. albicans is the most common yeast species isolated from human stool. It colonizes the surfaces of the oral cavity and the whole gastrointestinal tract early after birth (Forbes et al. 2001) and remains there in yeast form without any sign of its presence until the intestinal flora maintains its balance and the immune system functions properly. It is commensal, unicellular yeast, but has dimorphic feature – ability to turn from round or oval unicellular budding yeast form to pseudo- or hyphae (filament) and mycelium forming multicellular fungal form. It is also an opportunistic pathogen. It means that under certain circumstances, when the friendly bacteria are reduced, or the cell mediated immune defence is depressed (following antibiotics, steroids, or immunosuppressive therapy, in immunocompromised persons, in diabetic person, in seriously ill patients treated in intensive care unit, and in the case of long term unhealthy nutrition, rich in sugar and refined carbo-hydrates) the benign yeast form begins to overgrow, spreads on the epithelium surface of the gut, turns into fungal form, damages the epithelium, and at the end, may cross through the intestinal mucosa and enter into the bloodstream, causing candidaemia and systemic candidiasis – fungal infection of any organ (Jawetz, Melnick and Adelber, 2001; Miranda, 2009). During the past decade, the incidence of mycotic infections has been increasing such as the mortality of systemic, invasive mycosis.

Before entering the bloodstream, candida overgrows on the mucosal surface of the gastrointestinal tract. The colonies are attached to the mucosa of the intestine wall with adhesin. This attachment is relatively stable and essentially irreversible (Kennedy, 1988). I surmise that the existence of this attachment may be the explanation of why in some patients at high risk with candida overgrowth, the colonies of the yeast/fungus are not present or just few of them is found in the stool, and consequently, the microscopic analysis and the culture are negative. However, after the administration of purgative, live yeast/fungus detaches from the surface and appears in the stool and becomes visible under dark-field microscope, as the dead yeast/fungus is visible after the administration of fungicide.

When overgrown, yeast/fungus may cause local symptoms of the intestine and distal symptoms of malfunction of any organ, as the whole body is affected. In literature this is known as candida syndrome or chronic candidiasis. The longer period they have been overgrowing, the larger surface they occupy, the symptoms become more complex and by time more severe. Therapy will take longer time, months or even years until the stool is cleared from yeast/fungus. If the complex therapy is not long enough, and/or was stopped prematurely, the remained yeast/fungus continues to overgrow and the symptoms reoccur. As of recently, discovered pathogenesis and symptoms can be learned in the paper (Klein, 2009) and the book (László, 2014) written by the author.
Overgrowing candida irritates the mucosa of the intestine causing inflammation, mucus production, pain, rise in temperature, diarrhoea or constipation. Fermenting the sugar, yeast releases carbon dioxide, which leads to bloating and flatulence without smell, and alcohol damaging the liver or even causing drunkenness. If the covered surface of the mucosa is larger, the food digestion and nutrients absorbance are poor, and some nutrients deficiency occur with their typical symptoms. The damage of the intestine’s wall may cause “leaky gut”. This allows extraneous substances to enter the bloodstream developing food intolerance and allergy. The histamine blood level is higher causing itching anywhere, rash, overproduction of stomach acid, even asthma. Yeast needs sugar to overgrow, thus sugar, sweets and bread craving is very typical and leads to overweight. Starvation leads to the drop of the blood sugar (hypoglycaemia) with its typical symptoms. Many metabolic products of the yeast are absorbed into the bloodstream causing disturbances all over the body with a lot of different symptoms like chronic fatigue, irritability, attention deficit, headache, loss of memory and concentration, palpitation, cold arms and legs, recurrent infections, hormonal imbalance, fluid retention, and many others.

In most of the patients suffering mostly from chronic health problems, the cause/causes remain unrecognized, as in many cases of gastrointestinal disturbances the cause remains unknown, because the possibility of yeast overgrowth is not considered. Most doctors still refuse to admit that yeast can cause such numerous symptoms or illnesses due to their intestinal overgrowth.

To prove the connection between the candida overgrowth in gut and symptoms is not easy, because presently available diagnostic methods are imperfect. Candida antibodies (IgM, IgG) are present in blood of all people with normal humoral immunity as candida lives in everyone. Early skin prick test to candida is also positive in case of normal cellular immune response. In case of weakened immune system, the blood level of the antibodies is very low or immeasurable, and the early skin prick test to Candida is negative. People with weakened immune system presumably have certain overgrowth of candida. The same problem is with the stool analysis – microscopic and culture. Positive results can be seldom obtained from healthy people. The question is the quantity of yeast in their stool, normally there should be only a few single yeast cells. Rarely, in people with yeast/fungus overgrowth the stool may be yeast free, or just a few single cells can be seen, as the yeast/fungus colonies are strongly attached to the intestine mucosa. However, after the administration of purgative or fungicide, live or dead colonies of yeast/fungus, become visible in the stool by naked eye, as well as by dark-field microscope.

Dark-field illumination is a technique used to observe unstained samples, causing them to appear brightly lit against a dark, almost black background. In bright-field illumination, the object is lit from below the stage, resulting in a larger, contrasted image that can be studied. A dark-field microscope blocks this central light with a condenser so that only oblique rays hit the object. These rays from the side of the field make the samples bright, usually white against the dark background as the aperture of the condenser is larger than the aperture of the lens.
AIMS

By using the dark-field microscope, the author aims to identify the presence and estimate the quantity of yeast in the stool of the patients suffering from chronic health problems, with increasing number of complaints over a long period of time, but with unidentified causes. The study is prospective, started ten years ago, and is still on-going. Until now the author has examined over 10,000 stool samples and made several thousand photographs. In this paper, the author presents her results of dark-field microscopic stool analysis of the mentioned people. Almost all patients have negative records (findings) of different examination carried out in the past (laboratory, endoscopy, US, etc.). None of them had microscopic stool analysis and almost none of them had candida stool culture. Just few of them had records of candida positive culture of the stool, but no doctors paid attention to the result.

MATERIAL AND METHODS

The study is carried out in the author’s medical office. The samples are taken from the fresh stool delivered on the day of the analysis.

First, the stool specimens are observed by naked eye and then examined with dark-field microscope.

Observation with naked eye:
1. The shape of the stool can be normal, sausage-like, or small globules stick together with or without mucus, covered with mucous (Figure 4), lose or even diarrhoea.
2. On the surface of the stool can be seen white, yellow or brown mucus or patches of different dimension (Figure 2, Figure 3), or the surface is normal, but deep scratching discovers the previously mentioned formations (Figure 1).

Dark-field microscope analysis is carried out with Zeiss Axioscop 40 microscope, with 10x and 40x Zeiss Achromat lenses.

The native specimens are taken from two different parts of the stool, or more if the previous two are yeast free; from the mucus or the patches if present, and placed on the slide (Figure 5).

Frequency of analysis:
In the case of positive findings at the first analysis, the next one will be carried out after two weeks of antifungal treatment. In the cases of negative findings, the patients also receive antifungal treatment and recall for first control examination after two weeks as well. To the author’s experience, two weeks of therapy is sufficient for killing some quantity of colonies which then detach from the epithelium and become visible in the stool by naked eye and by microscope.
RESULTS

The microscopic images of the stool may sometimes be yeast/fungal free (Figure 25), due to their stable adhesions to the wall of the intestine, or show different shape, dimensions and quantity of yeast/fungus as follows:

1. A few big single round live yeast cells (Figure 6)
2. A few big round live yeast cells, small colony of small round yeast cells and single hypha (Figure 7)
3. More small round, budding yeast cells (Figure 8)
4. More big round and oval live yeast cells without forming colonies (Figure 9)
5. A lot of small round live yeast cells without forming colonies (Figure 10)
6. A lot of small round live yeast cells with forming colonies (Figure 11)
7. A few big round live yeast cells and small round cells colony (Figure 12)
8. Big round live yeast cells colony with gas (bubbles) production (Figure 13), (in these cases patients complain of bloating and flatulence gas without smell)
9. Round live yeast cells, few or a lot of live conidia (Figure 14, 15, 16)
10. Live mycelia (Fig. 17) and live yeast colony inside the mycelia (Figure 18)

After two weeks of antifungal treatment the findings may be as follows:

1. Big single round dead yeast cells (Fig. 19) and cracking big oval cells (Figure 20)
2. Big round yeast cells colony with live and dead cells (Figure 21)
3. Big round dead yeast colony with gas production (Figure 22) (these patients complains of very unpleasant bloating and flatulence of stink gas)
4. Dead conidia (Figure 23)
5. Dead mycelia (Figure 24)
6. Yeast free stool (Figure 25)

Figure 1. White spots visible only after scratching
Figure 2. A lot of white spots on and inside the stool

Figure 3. White mucus in the stool
Figure 4. Small pieces of stool covered with white mucus

Figure 5. White spots of different dimension
Figure 6. A few big live single round yeast cells

Figure 7. Single big round live yeast cell, single hyphae, small yeast colony
Figure 8. More single small round, one big oval live cell and budding

Figure 9. More big single live oval and round yeast cells
Figure 10. A lot of small single live yeast cells without forming colonies

Figure 11. A lot of single round live yeast cells, a colony
Figure 12. A few big single live yeast cells, a small yeast colony

Figure 13. Big live yeast colony producing bubbles
Figure 14. Live yeast cells and a lot of live conidia

Figure 15. A few single live yeast cells and a lot of live conidia
Figure 16. A single live yeast cell and conidia (enlarged image)

Figure 17. Live mycelia
Figure 18. Live yeast colony inside the live mycelia

Figure 19. Single dead yeast cells
Figure 20. Small round live yeast cells and big cracking oval cells

Figure 21. Big yeast colony: live (white) and dead (black) cells
Figure 22. Dead yeast colony, gas production

Figure 23. Dead conidia
Figure 24. Dead mycelia

Figure 25. Yeast free stool
DISCUSSION

In some cases of those who had yeast free stool at the first microscopic examination, after two weeks of antifungal treatment different shape and quantity of live and dead yeast/fungus appears, as it is in the first positive findings. In other cases with the first yeast negative stool, after two weeks of treatment the stool remains yeast negative. The follow-up of these patients should be discontinued, advising them a further search for the cause of their health problems and teaching them how to prevent candida overgrowth. There is no correlation in shape and quantity of live and dead yeast/fungus between the first, second, and all following examinations, but by time, if the treatment is efficient, the live yeast/fungus decreases in number and disappears at the end, while the quantity of dead ones increases at the beginning of the effective therapy and disappears at the end too. During the treatment, sometimes it is possible to see a few very big live and dead colonies, then smaller and yet smaller ones and again a few very big ones, until they all disappear. The next analyses are carried out from time to time during the treatment. Duration between the two analyses depends on the patient’s status, complaints, way of therapy and microscopic findings. The dark-field microscope stool analysis is very useful in monitoring the effectiveness of the therapy, and if necessary it can be modified in time. The aim of the treatment is to reduce, minimize, or even clear the patient’s symptoms and clear the stool from fungus and reduce the yeast to minimum. The length and the way of therapy is person-dependent, complex and may last from several months to more years. The longer is the yeast overgrowing, the bigger are the colonies, the larger is the territory they occupied and longer lasts the treatment. When the stool is yeast/fungus free (Figure 25), it doesn’t mean that the therapy is over. After several months, yeast/fungus can be visible again by microscope, because often the remained yeast begins to overgrow if the treatment was stopped prematurely and the circumstances which encourage the yeast to overgrowth are still present and, of course, the symptoms return. Despite the fact that some doctors do not admit candida overgrowth as a possible source of candidaemia and invasive candidiasis in people at high risk, others emphasize the benefit of prophylactic antifungal treatment (Ozturk, 2006; Damjanovic, 1993) even in people at low risk (Normand, 2005).

SUMMARY

Native stool analysis under dark-field microscope is an easy-to-carry-out examination, rapid, with no preparation, and may be carried out in general practice.

The freshly sampled stool from a person without any preparation is first observed with the naked eye and then, a small amount of it, taken from two or three different parts, should be placed on the slides, covered and observed under the dark-field microscope.
Rather soon, the doctor gets information whether the candida started to overgrow in patients at risk. Also, he/she is able to follow up the changes in the stool over time, to start with therapy on time and to monitor the effectiveness of therapy, aiming the prevention of candidaemia and invasive candida infection.

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АНАЛИЗА СТОЛИЦЕ МИКРОСКОПОМ С ТАМНИМ ПОЉЕМ – ЊЕГОВЕ МОГУЋНОСТИ У ОТКРИВАЊУ ПРЕКОМЕРНО РАЗМНОЖЕНИХ ГЉИВИЦА

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РЕЗИМЕ: Недуго након настанка, гљивице, углавном Candida albicans, насељавају слузокожу усне дупље и целог гастроинтестиналног тракта. Candida albicans живи у облику гљивица које нису штетне саме по себи, односно као саставни део микробне флоре, али може да постане патогена и инфективна под одређеним околностима које подстичу њено бујање. У тој фази, она може да оштети зид црепа и уђе у крвоток узрокујући инвазивну кандидијazu са високом стопом смртности. Од суштинског је значаја препознати обољевање од кандиде и започети на време терапију која може да сачува живот. Препознавање фактора ризика који омогућавају кандиди да буја је најважније у превенцији њеног раста и хроничне кандидијазе, што је претходни степен инвазивне кандидијазе. Ако ово препознавање изостане и бујање узнапредује, поставља се питање како стање открити и лечити и у којој фази то урадити. Узгајање културе из узорка столице захтева време, а осим тога доказ је само присуства живих гљивичних ћелија. Ако живе гљивичне ћелије нису присутне у столици, резултат културе ће бити негативан. У овом раду, ауторка представља своје искуство с анализом столице под микроскопом с тамним пољем, као брзом методом лаганом за извођење преко које се утврђује присуство животних или мртвих гљивичних ћелија и бујање гљивица.

КЉУЧНЕ РЕЧИ: гљивична инфекција, инфекција кандидом, бујање кандиде, хронична кандидијаза, анализа столице, микроскоп с тамним пољем