The effect of Nystatin on urinary indican level in Dysbiosis

Abstract

Introduction

Changes in the composition of gut microflora are associated with an increase in chronic diseases. Urinary indican is one of the most common and easily assessable markers of intestinal dysbiosis. Candida albicans is the most common type of fungus in the gut and also one of the intestinal dysbiosis causes. The use of Nystatin to decrease Candida albicans is safe, less expensive and side effects.

Method

48 persons, 25-50 years old who were suspected to be intestinal dysbiosis required to answer the dysbiosis questionnaire until 30 subjects were selected by inclusion criteria. Nystatin was prescribed for all subjects twice a day continuously until repeated indican test at the end of the second and fourth week respectively.

Results

The results showed that was 96.67 percent of the negative result improvement in both second and fourth week, respectively. The negative result of the second and third tests was compared with the first test, both p-value < .001 respectively, but the second test compared with the third test showed it was not statistically significant.

Conclusion

Nystatin is likely to be beneficial for decreasing the urinary indican level. Candida albicans removal may relate to the improvement of the gut ecosystem.

Keywords : Nystatin, urinary indican, dysbiosis

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1. Introduction

Thailand has little available informative sources of intestinal dysbiosis. Most likely, dysbiosis is not prioritized as a common medical checked-up system, so the effects of dysbiosis are still unintentionally negligible. For supporting and improving an important medical evidence, we decided to study the effect of nystatin to urinary indican level in dysbiosis.

The digestive tract is an important human health system, there is normal flora in each part. [1] Microorganisms in the intestinal tract are living normally together as a microbiota. There are more than 99 % bacteria.[2] Urinary indican is one of the most common and easily assessable markers of intestinal dysbiosis.[3] There are thousands of different bacteria in the intestinal microbiota.[4]

Growing evidence shows that dysbiosis of the gut microbiota is associated with the pathogenesis of both intestinal and extra-intestinal disorders. Intestinal disorders include inflammatory bowel disease, irritable bowel syndrome (IBS), and coeliac disease, while extra-intestinal disorders include allergy, asthma, metabolic syndrome, cardiovascular disease, and obesity [5], systemic inflammation has been associated with autoimmune diseases, such as type 1 diabetes (T1D).[6,7] The common causes are unhealthy lifestyle affecting firstly [8] and also antibiotics, psychological and physical stress, and dietary factors etc. which contribute to intestinal dysbiosis [9], so clinicians would do well dealing with the causes of this condition.

Candida spp. is found in about 70 percent of all fungal colonies in the gut. [10] Candida albicans, the most abundant found in the fungal population in intestinal microbiota [11], the most common cause of fungal infections [12,13] leading to a range of life-threatening invasive to non-life-threatening mucocutaneous diseases. The yeast overgrowth is caused by the presence of the excessive number of Candida albicans. It can form a biofilm which destroys tissues and induce the immune system. Its colonization in the GI tract may impair mucosal barrier defense against gram-negative bacteria. The clinical role of gut antifungal prophylaxis in protecting against gut-derived gram-negative sepsis is speculative.[14] Yeast infections of the intestinal mucosa are uncertain clinical significance and their possible connection to irritable bowel syndrome. Mucosal yeast infections are treated with topically active polyene antimycotic drugs.[2] It is also the cause of many important

symptoms such as chronic fatigue syndrome, insomnia, allergy or rash by unknown cause. Non-immunocompromised subjects may cause unexplained GI symptoms.[15]

Nystatin is a polyene antifungal agents, safe and efficacious, the most commonly used to treat Candida albicans in the gastrointestinal tract[16,17]but it has a very little effect on other microbes in the body. Nystatin is not absorbed from the gastrointestinal tract when orally administered.[18] Therefore, the topical use of nystatin is considered the most common route of administration in dentistry, as systemic exposure is minimal. .[19] It is suitable for the treatment of yeast infections and not killing the beneficial bacteria in the intestinal tract. Polyene is less expensive and side effects than fluconazole, which can be absorbed by the azole derivative.[16] Nystatin is superior to placebo in reducing localized and systemic symptoms in individuals with presumed fungus hypersensitivity as selected by a 7-item questionnaire. This superiority is probably enhanced even further by a sugar-and yeast-free diet.[20] Topically active antimycotics (such as nystatin preparations) are available for the treatment of superficial infections of the orogastrointestinal tract. Modulation of intestinal microflora with probiotics can suppress Candida colonization.[2] Oral nystatin prophylaxis efficiently prevented Candida spp. colonization in ICU patients at low risk of developing invasive candidiasis.[21]

Urinary indican (indoxyl sulfate) is produced by the reaction of bacteria to the intestines, a urinary marker of dysbiosis.[22] Most are eliminated through the feces, the rest is absorbed, detoxified and secreted by urine. Using urinary indican as an initial assessment is very convenient and the cost is affordable.[3] Large shifts in bacterial populations induced by the artificial sweetener saccharin have also been demonstrated by changes in indican excretion.[23] There is no age adjustment for reference limits is necessary since excretion has been shown to be constant for young and elderly control subjects.[24] The test sensitivity may be enhanced by oral loading 5 gram of tryptophan.[25] Case Studies Records from patients who had a urine indican ordered were examined for provisional diagnosis. There was 83 percent (15 out of 18) of those tested were positive.[26] There are not many standardized inspection facilities, so most of the treatments and follow-up are based on evidence from the patient's signs and symptoms. D-Arabinitol, a metabolite of most pathogenic Candida species [22,27], a substance that can be detected in the urine from the chemistry

of candida yeast. D-Arabinitol levels in urine increased in patients with invasive candidiasis. In addition, the measurement of serum level is one of the diagnostic criteria for invasive candidiasis[28,29] and urinary levels of the sugar alcohol, D-arabinitol, used as a reliable biomarker for invasive candidiasis which has reliable scientific support. [27 ,29] We have focused on the decreasing way of intestinal Candida albicans as a concept in urinary indican monitoring and evaluation. For furthermore of advanced study and opportunities for dysbiosis patients to be treated and prevented unintended consequences that can lead to serious complications.

2. Material and Method

2.1 Ethics

The study was performed in accordance with the recommendations of local ethics committee from Dhurakit Pundit University (110/1-4 Prachachuen Road, Laksi, Bangkok 10210, Thailand) www.dpu.ac.th. All subjects provided written informed consent in accordance with the Declaration of Helsinki, The Belmont Report, CIOMS Guildline and International conference on harmonization in good clinical practice or ICH-GCP. The protocol was approved by (Process number 002/60EX)

2.2 Subject selection and allocation

The purpose of this study is to evaluate the effect of Nystatin on urinary indican as the pre-experimental study. 49 persons, 25-50 years old both male and female, who suspected to be intestinal dysbiosis were required to answer the dysbiosis questionnaire. Those who attained the required score, more than 80 and 120 points in males and females respectively, were classified as a "possible dysbiosis" continued to attend the urinary indican testing. There were 48 persons who passed the required scores and went onto take the urinary indican testing, the 9 remaining of 48 persons did not take urinary indican testing due to their individual limitations. So 39 persons who could take the urinary indican testing. So there were only 30 subjects included in the study and divided into 2 groups. Urinary indican testing reported as a colorimetric display of indican concentrations ranging from level 0,1,2,3 and 4 respectively (negative to maximum). We selected only those who had positive results in level 2,3 and 4 could be included in the study and level 0 and

1 could be negligible**.** 15 persons of level 3 and 4 positive urinary indican classified as “High group”, and 15 persons of level 2 positive urinary indican classified as “Low group”. 3 persons were level 1, and 6 persons were level 0. These were both classified as “Negative group”.

2.3 Clinical assessment

Medical condition history attention was focused on pharmacological therapy, underlying disease.

Inclusion Criteria

1. The results of 25-50 years old male and female subjects who passed the dysbiosis questionnaire scoring and the urinary indican testing are shown in level 2 3 and 4 of Indican Color Chart respectively.

2. The exclusions were congenital dieseases, underlying diseases and gastrointestinal diseases which needed to be treated with continuous medications, volunteers without the following conditions: digestive and absorption disorders, hypochlorhydria, cancer, concurrent intestinal fungal infection, bowel obstruction, irritable bowel syndrome (IBD), irritable bowel syndrome (IBS), diverticulitis, diverticulosis.

3. No treatment by antibiotics, NSAIDs, other anti-inflammatory drugs, immunosuppressants, steroids, antacids at least 1 month before the first urinary indican testing.

4. No probiotics at least 7 days before the testing and also during the trial.

5. No history of Nystatin allergic reaction.

6. No intake of tryptophan-contained supplements. (False positives may be found)

Exclusion Criteria

1. Subjects who had got adverse reactions from intake of nystatin.

2. Subjects had to be treated by admittance to a hospital, antibiotics, anti-inflammatory drugs, steroids, antacids, during the study period.

3.Subjects who did not cooperate, participate and continuously intake nystatin for more than 24 hours.

2.4 Indoxyl sulfate urinary determination

Urinary indican level evaluation is based on the amount of color intensity measured by a certified standardized test kit sourced from the certified Cellfix company limited in Bangkok Thailand. Fresh urine samples were collected in the morning after fasting overnight, 2 ml of urine were mixed with 2 ml of Obermeyer reactant and the mixture was allowed to stand for 5 minutes at room temperature. The product of this reaction is indigo, which is intensely blue. Then 2 ml of chloroform were added and mixed together for 10 minutes in order to separate the lower colored layer from the oily phase. Urinary indican testing reported as a colorimetric display of indican concentrations ranging from level 0,1,2,3 and 4 respectively (negative to maximum).

2.5 Nystatin

Nystatin, the product of T.O. Chemicals (1979) Co.,Ltd. Bangkok Thailand, is only one provider in Thailand. 500,000 international unit of oral suspension was prescribed for all subjects twice a day in morning and evening continuously until repeated urinary indican testing at the end of the second and fourth week respectively. During the study period, all subjects were tested a total of 3 times.

2.6 Statistics

Non-sensitive data of all the subjects were entered into a password-protected database. Indican levels are the ordinal scale variable. The statistic analysis performed using a nonparametric test as appropriate. Percent of improvement were reports of comparison between before and after trials from the total number of samples (n) due to the urinary indican level is an ordinal scale.

-The Cochran Q test statistics was performed to analyze the difference of the ratio of negative results among 3 times of urinary indican tests.

-McNemar test was performed to analyze the difference of negative urinary indican results in each comparison of the first testing (prior to intake of nystatin) to the second and third testing respectively and also the second testing compared to the third testing.

-Chi-square test Fisher Exact Test was performed to compare the ratio of negative results between the high and low group.

-The statistical analysis of data was carried out using SPSS software.

-The value of $p < 0.05$ was set as the limit of statistical significance.

3. Results

A study of the effects of urinary indican levels before and after taking Nystatin. Percentage of improvement is calculated from the total number of samples (n) due to the urinary indican level as an ordinal scale.

Table 1 The results of all three urinary indican tests of all 30 subjects in Low and High groups.

Results of all tests	Low group n=15 (%)	High group n=15 (%)	Total n=30 (%)
The first test			
Negative	-	-	-
Positive	15 (50)	15 (50)	30 (100)
The second test			
Negative	15 (50)	14 (46.67)	29 (96.67)
Positive	-	1 (3.33)	1 (3.33)
The third test			
Negative	15 (50)	14 (46.67)	29 (96.67)
Positive	-	1 (3.33)	1 (3.33)

Data are reported as percent of improvement

The first test is “prior Nystatin trial”, The second test is “after 2 weeks of Nystatin trial”, The third test is “after 4 weeks of Nystatin trial”.

Statistical Analysis

1) The result from the Cochran Q test: At least one time of testing that was significantly different of the proportion of negative urinary indican results at p-value < 0.05.

2) The result from the Mc Nemar test: The prior trial of negative urinary indican result was compared with the second and third testing, p-value = .000 respectively, these showed that the proportion of the negative results were statistically significant at p-value < 0.05 but on the second

testing compared with the third testing showed there was p-value = 1.00.

3) The result from the Chi-square test Fisher Exact Test, the proportion of negative results between high and low group compared, the result of the second and third testing were not statistically significant, p-value = 1.00

The study showed that at least two weeks of nystatin intake twice a day until after the second and fourth week could significantly decrease urinary indican level in dysbiosis. However, the effect in the fourth week compared to the second week was not statistically significant. The negative urinary indican level results between low and high group compared even in the second and fourth week also showed there were not statistically significant.

4. Conclusion

It is likely to be beneficial on a cost-effective scale when we add Nystatin as one of dysbiosis treatment as the concept of Candida albicans removal and decreasing the level of urinary indican in dysbiosis. Urinary indican testing is a safe, comfortable, and inexpensive procedure for dysbiosis screening, thus it may be one of the first choice indicators for dysbiosis. Additional and combined treatments are always recommended for individual and effective management of dysbiosis.

5. Discussion

Urinary indican (indoxyl sulfate), is produced by the reaction of bacteria to the intestines, a urinary marker of dysbiosis[22], performed as an initial assessment, very convenient and the cost is affordable.[3] Factors that may affect urinary indican levels such as digestive and absorption disorders, (e.g. oral loading of tryptophan [25][30], Antibiotics [31], cancer of gastrointestinal tract, bowel obstruction, small intestinal obstruction, diverticula, fistulae, surgical blind loop, previous ileo-caecal resections and/or motility disorders [32], irritable bowel disease [33]) If we exclude all mentioned factors that can induce positive urinary indican but the testing still showed positive, we may consider the intestinal yeast overgrowth to be one of the remaining possible cause leading to the positive result. The effect of the urinary indican levels may be linked to many serious complications such as the progression of chronic renal failure [34], glomerular necrosis in uremic patients, a potent co-carcinogen

for the urinary bladder in animal models [35], sporadic colorectal carcinoma (CRC). [36]

The concept of decreasing the number of pathogenic microorganisms, the most common cause of fungal infections is Candida albicans [12][13], the most abundant found in the fungal population in intestinal microbiota. [11] Nystatin, is the most commonly used to treat Candida albicans in the gastrointestinal tract, may lead to improvement in the gut ecosystem, is safe and efficacious [16][17], is not absorbed from the gastrointestinal tract when orally administered.[18]

However, this is a preliminary, pre-experimental study, and how to confirm whether Candida albicans has been decreased may be additionally investigated. A standardized laboratory examination is necessary to solve the overgrowth of intestinal Candida albicans such as urine D-arabinitol, urine organic marker of invasive candidiasis. [22][27][29] None of the previous studies have addressed the effect of Nystatin on urinary indican level and alteration in gut microbiota associated with dysbiosis.

Further studies on fecal microbiota are warranted to investigate the potential role of gut dysbiosis [3] and the most accessible source of the GI microbiota. [37] SIgA is the most abundant antibody molecule on mucosal surfaces of humans and most other mammals [38][39][40], while the gold standard for diagnosing small intestinal bacterial overgrowth is still microbial investigation of jejunal aspirates. [27][41] Therapy for any type of dysbiosis should be integrated to solve all causes, symptoms and complications, and individuals.

6. Conflict of Interest Statement

- The authors declare that the study was no conflict of interest and not supported by any other foundation.

7. Acknowledgement

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