

The Impact of Probiotic Therapy on the Levels of Butyrate Acid in Inflammatory Bowel Disease

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Abstract : Inflammatory bowel disease (IBD) is a term applied to a group of bowel disorders in which inflammation is a major feature, it is widely accepted that these diseases have long been associated with imbalances in mucosal levels of micro-flora-produced short-chain fatty acids such as butyrate. 10 Patients received a 2 –week probiotic treatment and 10 control patients received a placebo for the same period. For the pre- and post-intervention assessments, stool samples were analysed for their level of butyrate acid with gas chromatography. This study showed that the mean concentration of butyrate acids in pre- and post- probiotic therapy was 6.49 ± 2.64 versus 21.07 ± 8.01 $\mu\text{mol/g}$. Compared to the group who received the placebo, the group who received the probiotic therapy showed a significant increase in the level of butyrate acid ($P < 0.001$). Future research should be done to study the effects of the therapy with larger samples over longer periods.

1 INTRODUCTION

Ulcerative colitis (UC) and *Crohn's disease* (CD) are 2 of the primary types of Inflammatory Bowel Disease (IBD) (Friedman S and Blumberg RS, 2010; Ng SC *et al*, 2017). There is a high incidence of both UC and CD in Europe, England and North America. Worldwide, the incidence of UC is between 10 to 20 per 100,000 population per annum with a prevalence of 50 to 100 per 100,000 population. There are many factors which can cause IBD: however, at present, changes in the mikroflora in the intestines is believed to be the primary factor in the pathogenesis of IBD; such changes make it possible that the supply of a beneficial micro-organism through changes/addition to/in the diet could reduce the incidence of inflammation (Olendzki BC *et al*, 2014).

A Probiotic is a living micro-organism which if given in a sufficient quantity will bring health benefits to the sufferer (WHO, 2001). Lactobacillus and bifidobacteria are probiotic anaerobic microbes which produce lactic acid as a final metabolite from fermentation of carbohydrates. The primary products from the anaerobic microbial fermentation which are found in the large intestine and which affect the physiological condition of the large intestine are short chain fatty acids (SCFA), namely acetate,

propionate and butyrate acids (Mattu B and Chauhan A, 2013). A most important part of the imbalances that develops in the composition of the micro-biota is the significant decrease in the digestive tract of microbiota coming from the butyrate acid.

The decrease in the butyrate reflects the increase in oxidation of the SCFA by colorectal mucosa (Kelly D *et al*, 2005, Chen W *et al* ,2012). The purpose of this study is to measure the levels of butyrate acid in IBD patients, both before and after they are given a probiotic treatment.

2 PATIENTS AND METHODS

The samples for this research were faeces from IBD patients who were confirmed by colonoscopy and who met the criteria for inclusion. The sample was gathered by asking successive patients with IBD whether they would agree to participate in the study. Patients who met the criteria for inclusion were asked to agree in writing to participate in the research by signing an informed consent agreement after they had been briefed on the purpose and the nature of this research. The sample patients were split into two groups, an intervention or treatment or experimental group (IG.) and a control Group (CG):

and the pre-treatment research samples (ie., the faeces from the patients) were collected from both groups to measure the level of butyrate acid in them. Next the IG were given a probiotik treatment while the CG were given a placebo for 2 (two) weeks: After that the post-treatment faeces were collected from the two groups for analysis of the butyrate content, to see the effect of the treatment.

Criteria for Inclusion : 1). The patients is aged between 18 and 65, either male or female ; 2) The patient has Indonesian heritage as proven by her/his KTP and, through interview, can provide a family tree of three generations, namely the patient, her/his parents and the four grandparents ; 3) The patient is suffering from IBD as proven by a colonoscopy examination and (s)he agrees to consume a probiotik or a placebo for 2 (two) weeks and (s)he agrees to provide her/his faeces for examination of the butirate content; 4)The patient agrees to be interviewed, is co-operative and can communicate well, (s)he agrees to provide specimens to be examined as material for the study and for the results to be published; such agreement to be confirmed by signing an informed consent agreement.

Criteria for Exclusion : 1). The IBD patient does not meet the requirements for examination of faeces and colonoscopy (for example (s)he is suffering from a toxic megacolon, fulminan colitis, perforated colon and/or other very serious condition ; 2) The patient has previously had antibiotic therapy, corticosteroids, amino salisilate acid and/or has used imunomodulators (Azatrioprin, 6-merkaptopurin, siklosporin and/or metotreksat) and/or therapy specifically for other intestinal inflammation (inflicsimab, tocolizumab, filgrastim) in the past three (3) months.

This study was an experimental study designed to use case control with a two (2) group pre-test & post-test design (before and after), the first group or the intervention group (IG) are IBD patients who are given the experimental treatment and the second or control group (CG) are IBD patients who are given a placebo. This design enables comparison between the two groups and measurement of the differences which have occurred as a result of the treatment. The purpose of this study was to measure the concentration of butirate acid in the faeces of IBD patients before and after being given a probiotik for three weeks and of a control group of IBD patients after being given a placebo for the same period and to compare the difference in butyrate acid content between the two groups before and after the treatment.

3 RESULTS

The age and gender characteristics and the butirate acid content before and after treatment, of the 20 IBD patients from the Dr Zainoel Abidin Provincial General Hospital in Banda Aceh who were the subjects of this research, are set out in Table 1, which follows:

Table 1; Gender, Age & Butyrate Acid Content of IBD patients in IG and CG

Variable	Intervention group (% or SD)	Control group (% or SD)
Sex		
Male	8 (80%)	2 (20%)
Female	2 (20%)	8 (80%)
Age group (years)		
≤ 30	3 (30%)	4 (40%)
31 – 40	2 (20%)	3 (30%)
41 – 50	3 (30%)	2 (20%)
≥ 50	2 (20%)	1 (10%)
Butyrate acid level(umol/g)		
Pre-treatment	6.49 ± 2.64	19.66 ± 12.99
Post-treatment	21.07 ± 8.01	13.84 ± 12.76

Note: IG, IBD patients, got probiotic treatment; CG, IBD patients got placebo treatment

To summarise the results: The level of butyrate acid in the IG increased between the pre-treatment and the post-treatment from 6 to 21 umol/g with an increase in SD from 3 to 8 while for the CG the change in level between the pre-test and the post-test was actually a decrease from 20 to 14 umol/g while the SD stayed at 13.

Table 2. Comparison butyrate acid’s level between two group

Group	Paired Sample	Mean Differences	t-Test	p-value
Interventi on group	Pre test- post test	-14,58	-6,748	< 0,001 *
Control group	Pre test- post test	5,82	,521	0,163

From the results in Table 2, it can be seen that for the Intervention Group the average difference in the level of butyrate acid from the pre-test to the

post-test was -14,58. The negative value indicates that the level of butyrate acid in the post-test was higher than that in the pre-test. Furthermore, the value of t-calculated from the paired sample t-test for the level of butyrate acid for the Intervention Group was -6,748 while the p-value was much smaller at 0.001. Accordingly, it can be said that for the Intervention Group the average level of butyrate acid increased significantly between the pre-test and the post-test. In other words, the treatment given significantly increased the average level of butyrate acid in the intestines of the treated patients. Meanwhile the level of butyrate acid in the Control Group actually decreased by 5.82 between the tests and the value of t-calculated from the paired sample t-test for the level of butyrate acid for the Control Group was 1.521 while the p-value was smaller at 0.163. Thus, in conclusion, the placebo did not improve the level of butyrate acid in the CG patients.

4 DISCUSSION

Butyrate acid is known to promote the benefits of healthy intestines; it effectively stimulates the proliferation of intestinal mucosal cells. The lowering of the level of butyrate acid is believed to contribute to getting IBD (Prideaux L *et al*, 2012). The purpose of this study was to measure the levels of butyrate acid in IBD patients before and after getting treatment with probiotics.

The data from this study showed that the minimum levels of butyrate acid in the pre-tests from the IG and the CG were 3,70 $\mu\text{mol/g}$ and 3,50 $\mu\text{mol/g}$ respectively, while the maximums were 10 $\mu\text{mol/g}$ and 38,60 $\mu\text{mol/g}$ respectively and the average levels were 6,49 $\mu\text{mol/g}$ and 19,66 $\mu\text{mol/g}$ respectively.

Then for the Treatment Intervention Group (IG) the post-test minimum, maximum and average levels were 13,90 $\mu\text{mol/g}$, 36,80 $\mu\text{mol/g}$ and 21,07 $\mu\text{mol/g}$ while for the CG the average level actually decreased to 13.84 $\mu\text{mol/g}$.

Butyrate acid has important functions to protect gastrointestinal health because it functions as a primary source of energy for colonocytes, increases the integrity of the epithel barrier and disrupts inflammation (Ringel Y *et al*, 2012). In their research, Faujan *et al*, 2010. compared the levels of butyrate acid in faeces from a normal population with those from IBD patients and found that the butyrate acid levels from the IBD patients were significantly lower. Results from this study are in line with those from Li *et al*, 2017. who also studied the relationship

between probiotik treatment and the level of butyrate acid in faeces. From 81 patients studied there was a significant increase in the level of butyrate acid in the faeces after taking *B. Bifidum* and there was also clinical improvement in the IBD patients verified by colonoscopy.

The results from this study are similar with those from a study by Takaishi *et al* who found that that the concentration of butyrate and propionate acids in the faeces of IBD patients was significantly lower than that in a normal population (Takaishi H *et al*, 2008). Vernia *et al*, 1988. reported that there was a similar decrease of butyrate acid in UC patients. The butyrate molecule is important for the remission of colitis. Reduction in butyrate acid reflects an increase in oxidation of SCFA by colorectal mucosa (Ishikawa H *et al*, 2011). The consumption of probiotics can reduce inflammation and improve health due to the production of SCFA in the large intestine and can reduce the production of the hydrogen peroxide radical. As well as that, the benefits of probiotics for oxidative stress biomarkers is possible because of the intestinal production of butyrate acid. The results of this research are in agreement with those from the study by Geinaert *et al* in 2015 in Belgium which reported the increase in butyrate acid content (of faeces) after 13 days treatment with probiotics compared to a control group. According to Geirnaert, the use of probiotics and similar natural anti-microbial antagonists have potential to be alternative therapies because they have a better pharmaceutical effect remembering that the use of artificial drugs is increasing greatly at present (with potential for drug-resistant bacteria to develop) (Soleimani A *et al*, 2016).

The results from this study were also in agreement with those found by Tursi *et al*, 2010. in Italy, where 47.7% of UC patients that used probiotics went into remission compared with only 32.4% of those who took a placebo, although statistically not a very large increase, this was possibly because the period of the study was too short. A study by Sood *et al*, 2009. of 147 patients in India found that taking probiotics had a significant benefit whereby 41.9% went into remission compared with only 15.7% from the control group. Tursi *et al*, 2010. used VSL#3 which contains lactobacillus, bifidobacterium and streptococcus thermophilus, with UC patients who reported significant remission of their illnesses after using it for 8 weeks.

5 CONCLUSIONS

There was a significant increase in the levels of butyrate acid in the faeces of IBD patients who got a probiotik treatment compared to the control group of IBD patients who only got a placebo but did not get the probiotic treatment.

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