Correlation of Aerobic Exercise and High Nitrate Diet with Population of *Eschericia Coli* in the Digestive Tract of Liver Cirrhosis Individuals

Retti Nurlaili, Syifa Mustika***

*Faculty of Medicine, Universitas Brawijaya/Dr. Saiful Anwar Hospital, Malang
**Division of Gastroentero-hepatology, Department of Internal Medicine, Faculty of Medicine, Universitas Brawijaya/ Dr. Saiful Anwar Hospital, Malang

Corresponding author:
Syifa Mustika. Division of Gastroentero-hepatology, Department of Internal Medicine, Dr. Saiful Anwar General Hospital. Jl Jaksa Agung Suprapto No. 2 Malang Indonesia. Phone: +62-341-369384; Facsimile: +62-341-369384. E-mail: drtika_78@yahoo.com.

ABSTRACT

Background: *In liver cirrhosis, the population of E coli is increased. conditions such as reduced intestinal). Escherichia coli with 2 enzyme nitrate reductase (NRF and Nir) reduce nitrate to nitrite and subsequently converted to ammonia (99%) and nitric oxide (1%) in anaerobic condition. Regular aerobic exercise 2-3 times/ week for 30 minutes resulted in increased 2,3-DPG which reduces the activity of E. coli to reduce nitrate to nitrite and ammonia, which only works on the anaerobic state. High Nitrate Diets lead to increased nitrate reducing bacteria such as E. coli resulting in the reduction of nitrate excess produce nitrite and ammonia in large quantities. Probiotic Lactobacillus spp. can suppress the growth of bacterial endotoxins and pathogens such as E. coli and other Enterobacteriaceae. This study aimed to determine the correlation of aerobic exercise and a high nitrate diet in gastrointestinal populations of Escherichia coli gastrointestinal tract in patient with liver cirrhosis.*

Method: *This was a descriptive-experimental study in liver cirrhosis patients Child Pugh A/B in outpatient clinic Saiful Anwar Hospital in August 2015. Respondents were asked to fill out a questionnaire with information about the demographic data, the nitrate diet, aerobic exercise, other medical data and sanitation, and stool samples were taken for faecal culture. Eta Correlation statistical test was used to determine the correlation of aerobic exercise and a high nitrate diet high in population of E. coli. The significant difference are indicated by* $p < 0.005$.

Results: *A total of 36 patients diagnosed with liver cirrhosis Child Pugh A/B, 14 (39%) underwent aerobic exercise 3x /week, as many as 25 (70%) consume a high nitrate diet. There was a strong relationship between aerobic exercise and high nitrate diet with population of E. coli (*$\eta = 0.725$; $p < 0.05$).

Conclusion: *There was a strong relationship between aerobic exercise and high nitrate diet with a population of E. coli in liver cirrhosis. Giving probiotics in patients with liver cirrhosis suppressed the population of E. coli.*

Keywords: *Aerobic exercise, high nitrate diet, Escherichia coli, liver cirrhosis*

ABSTRAK

Latar belakang: *Populasi E. coli meningkat pada sirosis hati. Escherichia coli memiliki 2 enzim nitrat reductase (Nrf dan Nir) mereduksi nitrat menjadi nitrit dan amonia pada kondisi anaerob. Latihan aerobik 2-3x/ minggu selama 30 menit meningkatkan 2,3-DPG, mengurangi aktivitas E. coli mereduksi nitrat menjadi nitrit dan amonia. Diet tinggi nitrat meningkatan populasi E. col untuk mereduksi nitrat menjadi nitrit dan amonia dalam jumlah besar. Probiotik Lactobacillus spp. menekan populasi E. coli. Tujuan penelitian ini mengetahui hubungan latihan aerobik dan diet tinggi nitrat terhadap populasi E. coli pada sirosis hati.*

Metode: *Penelitian deskriptif-eksperimental pasien sirosis hati Child Pugh A/B di poliklinik rawat jalan Rumah Sakit Umum Daerah (RSUD) Dr Saiful Anwar pada Agustus 2015. Responden mengisi kuesioner data demografi, diet nitrat, latihan aerobik, selanjutnya diambil sampel feses untuk deperiksakan kultur feses. Korelasi Eta mengetahui hubungan latihan aerobik dan diet tinggi nitrat terhadap populasi E. coli. Perbedaan yang signifikan ditunjukkan dengan* $p < 0.05$.

Hasil : *Total 36 pasien terdiagnosa sirosis hati Child Pugh A/B, sebanyak 14 (39%) menjalani latihan aerobic 3x/minggu, sebanyak 25 (70%) mengkonsumsi diet tinggi nitrat. Terdapat hubungan kuat antara latihan aerobic dan diet tinggi nitrat terhadap populasi E. coli pada saluran cerna (*$\eta = 0.725; p < 0.05$*).*

Simpulan: *Terdapat hubungan yang kuat antara diet tinggi nitrat dan latihan aerobik terhadap populasi E. coli saluran cerna pada sirosis hati. Probiotik menekan populasi E. coli pada sirosis hati.*

Kata kunci: *Latihan aerobik, diet tinggi nitrat, Escherichia coli, sirosis hati.*

INTRODUCTION

Digestive tract microbiota is an important component for body defense. A very complex group of microbes lives in human bodies, which is later known as microbiota. It is estimated that there are 10^{14} cells of microbiota in human body; the majority of which are found in the digestive tract.[1] Liver cirrhosis is a final pathologic phase of chronic liver disease. It has been known that intestinal microbiota plays an important role in the development of liver cirrhosis complications, including bacterial infection, the degree of hyperdynamic blood circulation, and hepatic encephalopathy.[2]

Intestinal microbiota is an ecosystem symbiosis which creates homeostatic balance inside the human body. Nevertheless, this balance can also be disrupted by a pathologic condition affecting intestinal physiology. In cirrhosis, several conditions take place such as decreasing of intestinal motility, decreasing of gastric acidity and pancreato-bilier secretion, and portal hypertension enteropathy (*colopathy*); all contribute to the disruption of intestinal microbe community which further increase pathogenic bacteria (*Enterobacteriaceae, E. coli*) and decrease *Fusobacterium spp* and *Clostridiales*.[3] *Escherichia coli* has 3 types of nitrate reductase enzymes which are active in anaerobic condition. This enzyme utilizes nitrate as an electron acceptor to produce nitrite that is toxic to cells. Nonetheless, there are 2 types of nitrate reductase enzymes in *E. coli* (Nrf and Nir) which will convert nitrite to ammonia (99%) and Nitrite Oxide (1%) to decrease the toxic effect of nitrite. However, excessive ammonia level may also cause problems, such as hepatic encephalopathy. *E. coli* can work in low haemoglobin condition such as in patients with chronic disease (liver cirrhosis) as these bacteria can produce heme and vitamin K. They are different from *Lactobacillus* which requires the supply of heme and vitamin K from outside to work optimally.[4]

It has been reported that routine aerobic exercises 2-3x/week for 30 minutes result in elevation of 2,3-DPG. 2,3-DPG is found in a large amount in red blood cells. This compound is a high-charged anions which is bound to ß deoxihaemoglobin chain. 2,3-DPG is a factor which influence haemoglobin-oxygen level. Higher binding of haemoglobin-oxygen will decrease *E. coli* activity to reduce nitrate to nitrite and ammonia which only occur in anaerobic condition.[5] Human obtaines nitrate intake from various ways; most of them is obtained exogenetic through the consumption of vegetables (such as spinach, cauliflower, broccoli, and tubers), meat, and water. World Health Organization (WHO) has established Acceptable Daily Intake (ADI) for nitrate ion. ADI for nitrate ion ($NO3^-$) is 0-3.7 mg/kg body weight. If consumed more than this limit, it will stimulate the elevation of nitrate reducing bacteria such as *E. coli* which leads to excess reduction of nitrate to produce nitrite and ammonia in large amount and are harmful to health.[6]

Probiotic can give beneficial effect to patients with liver cirrhosis. Probiotic *Lactobacillus spp.* can suppress the production of proinflammatory cytokines, such as TNF alpha, and CRP which accelerate liver injury; suppress endotoxin and growth of pathogenic bacteria, such as *E. coli* and other *Enterobacteriaceae*. Probiotic can also decrease the indicence of portal hypertension due to Nitrite Oxide production by *Lactobacillus sp*.[7,8,9] Although it has been widely accepted that liver cirrhosis causes characteristic changes in intestinal microbiota, yet only a few studies have been performed. Characteristic changes of intestinal microbiota can be identified by using faecal culture method. For the last few decases, faecal culture method independently

has been implemented in studies of human intestine, although sometimes the diversity of bacteria made culture difficult to perform. The is a need to design a study to know the association between aerobic exercise and high nitrate diet to intestinal microbiota population, particularly *E. coli*, in patients with liver cirrhosis, and the possibility of probiotic administration to decrease morbidity and mortality, in regard to the incidence of hepatic encephalopathy. The objective of this study is to identify the association of aerobic exercise and high nitrate diet to the population of *Eschericia coli* in the digestive tract of patients with liver cirrhosis.

METHOD

This study was a descriptive-experimental study with questionnaire approach to identify health problem, particularly liver cirrhosis Child Pugh A/B which was found in outpatient settings to patients in Dr. Saiful Anwar Hospital Malang in August 2015, which was aimed to identify the influence of aerobic exercise and high nitrate diet to the population of *E. coli* in liver cirrhosis patients. The study population was all individuals who fulfilled inclusion and exclusion criteria and was within the scope of Dr. Saiful Anwar Hospital Malang.

Sample in this study included 36 respondents from patients in outpatient clinic in Dr. Saiful Anwar Hospital Malang with liver cirrhosis Child Pugh A/B in August 2015. Before being included in the study, respondents were initially requested to fill out a questionnaire on demographical data, nitrate diet data, aerobic exercise data, and other medical data and environmental sanitation data. Through the questionnaire results, respondents who suffered from severe illness/sepsis, have been diagnosed or is currently diagnosed with cancer, or under antibiotic treatments in the previous 1 week, were not incuded in this study. The location of this study was in Dr. Saiful Anwar Hospital Malang and Microbiology Laboraty of Faculty of Medicine Brawijaya University Malang; the reason for selecting this location was because this hospital was easily accessible and has a considerable number of population.

Operational definitions from variables in this study, (1) Aerobic exercise is physical exercise such as jogging, swimming, cycling performed for 30 minutes with the frequency of 2-3x/week; (2) High nitrate diet is diet which contains nitrate more than 0-3.7 mg/kg body weight; (3) *E.coli* population was presented in percentage compared to the entire population of bacteria in faecal culture.

Study instruments used in this study were questionnaire which was adapted from NHANES-FOOD QUESTIONNAIRE and has been modified and WHO-Global Physical Activity Questionnaire, which has been translated to Indonesian language (bahasa Indonesia). In this study, data was collected for one month, consisting of phase I-III. This study will be performed in 3 phases, which are: (1) Phase I: in this phase, researcher will collect study sample obtained from liver cirrhosis Child Pugh A/B patients from outpatient ward of Dr. Saiful Anwar Hospital Malang. The data collection method being used was primary (direct oberservation by observing and distributing questionnaires). Filling out of this questionnaire was performed by interview technique conducted by healthcare workers; (2) Phase II: in this phase, researcher will collect faecal sample from respondents with liver cirrhosis Child Pugh A/B. All faecal sample was placed in a sterile box and was stored in anaerobic room with the temperature of 4^0C. Sample was freezed for 4 hours under the temperature of -20^0C and 200 mg aliquot was taken for storage and analysis in the next phase; (3) Phase III: In this phase, researcher performed microbiota analysis using conventional method; faecal sample was serially diluted with 1ml phosphate buffered saline (PBS, pH 7,2) and was further placed on the selective media agar plate, and incubate the plate in aerobic condition (for 24 hours) or anaerobic condition (for 48 hours) in the temperature of 37◦C. First bacterial identification was performed by gram staining and morphology of the coloni; further identification was performed with API and VITEK system. Finally, quantification (enumeration) of *E. coli* bacteria in the selective media, was gathered in the form of percentages.

Inclusion criteria included: (1) Giving consent to provide information on demographical data, diet data, aerobic exercise data, and other medical data, and environmental sanitation data; (2) Giving consent to provide faecal sample; (3) Currently diagnosed with liver cirrhosis Child Pugh A/B. Exclusion criteria include: **(**1) Past or current history of cancer; (2) Was suffering from severe infection/sepsis; (3) Was suffering from chronic disease other than liver cirrhosis (diabetes mellitus, hypertension, dyslipidemia, heart disease); (4) Was consuming antibiotic in the previous 1 week.

Data was collected and analysed using SPSS. Normality of the data was assessed by using nonparametric kolmogorov-smirnov test. Further, homogeneity from varian was performed using Levene

test. Eta correlation statistical test was used to know the association between aerobic exercise and high nitreate diet towards *E. coli*. Significant difference was shown by $p < 0.05$. Association strength was determined by eta coefficient value: poor (0-0.3), moderate (> 0.3-0.6), strong (> 0.6-0.8), very strong (> 0.8-1).

Implementation of activities was planned for 2 (two) months which include 1 month of data collection, 0.5 month in phase II, and 0.5 month of phase III. Period of study activities was planned from August 2015 to October 2015.

RESULTS

Table 1. Characteristic of Study Respondents

Characteristic	Total
Age (years old)	
20-30	4
31-40	10
41-50	10
51-60	11
> 60	1
Sex	
Male	15
Female	21
Child Pugh Score	
Child Pugh A	16
Child Pugh B	20
Aerobic exercise	14
High nitrate diet	25
E. coli population :	
≥ 50%	23
< 50%	13

Table 2. Correlation coefficient and significance of the relation of aerobic exercise and high nitrate diet and *E coli* population

Dependent	Independent	Correlation coefficient	η^2	p
Percentage of *E coli*	Aerobic exercise	0.725	0.526	0.00
Percentage of *E coli*	High nitrate diet	0.854	0.730	0.00

Based on the analysis above, it was obtained that $p < 0.005$ which means that there was a significant association between aerobic exercise and *E. coli* population in the digestive tract. Eta coefficient value of 0.725, means that there was strong association between both. Eta squared value of 0.526 (52.6%) means that 52.6% of *E. coli* population in this study was influenced by aerobic exercise factor.

Based on the analysis of the results above, it was obtained that $p < 0.005$ which means there was a significant association between high nitrate diet with *E. coli* population in the digestive tract. Eta coefficient value of 0.854, means that there was a very strong association between the two variables. Eta squared value of 0.73 (73%) means that 73% of *E. coli* population in the study was influenced by high nitrate diet.

DISCUSSION

Aerobic exercise is intensive physical activity which increases heart rate and is performed in a long period, at least for 20 minutes. The types of exercise activities include jogging and swimming. Aerobic exercise can be defined as exercise below which lactic acid level in the blood increase rapidly. Aerobic metabolism is much more efficient compared to anaerobic, which produces 38 molecules of adenosine triphosphate (ATP); ATP is a component which moves muscle contraction per glucose molecule, compared to the anaerobic one which is only 2 molecules, because it produces less lactic acid. Aerobic exercise is relatively more enjoyable. Excess oxidation of fat ensure the energy supplies to the extent of exercise period.[5]

It has been reported that routine aerobic exercises 2-3x/week for 30 minutes stimulate the elevation of 2,3-DPG. 2,3-DPG is found in a large amount in red blood cells. This compound is a high-charged anions which is bound to ß deoxyhaemoglobin chain. 2,3-DPG is a factor which influences the haemoglobin-oxygen level. The higher the binding of haemoglobin-oxygen, the less *E. coli* activity will be in reducing nitrate to nitrite and ammonia which only occurs in anaerobic condition. It can be stated that routine aerobic exercise 30 minutes for 3x/week will decrease the incidence of hepatic encephalopathy in liver cirrhosis patients.[5]

Human body acquires nitrate intake through various ways; most of it is obtained by exogenetic means through consumption of vegetavles (such as spinach, lettuce, cabbage, celery, carrots), meat and water. World Health Organization (WHO) has established Acceptable Daily Intake (ADI) for nitrate ion. ADI for nitrate ion (NO3-) is 0-3.7 mg/kg body weight. If consumed more than this limit, it will stimulate the elevation of nitrate reducing bacteria such as E. coli; thus, excess nitrate reduction occurs which produce nitrite and ammonia in large amount and is harmful for health.[6]

Development of hepatic encephalopathy is based on the accumulation of various toxins in the blood circulation which pass through the blood brain barrier. Ammonia is a toxic molecule to the cell and is known to play important role in the development of hepatic encephalopathy because of its increased level in patients with liver cirrhosis. *E. coli* has 3 types of nitrate reductase enzymes which work in anaerobic condition. This enzyme uses nitrate as an electron

acceptor to produce nitrite which is toxic to cell. However, there are 2 other types of nitrate reductase enzymes in E coli (Nrf and Nir) which will convert nitrite to ammonia (99%) and Nitrite Oxide (1%) to reduce the toxic effect from nitrite. Yet, excessive levels of ammonia also causes problems such as hepatic encephalopathy. From the study results, it could be obtained that higher nitrate diet will stimulate the growth of *E. coli* bacteria population which increase the incidence of hepatic encephalopathyi.[6]

Lilly and Stillwell introduced the term "probiotic" in the year 1965 for substance which is produced by microbes which promotes the growth of other microbes. Probiotic is a living organism which is able to give beneficial effect for its host's health if consumed in an adequate amount by restoring intestinal microfloral balance as it is ingested to the digestive tract.[8]

The benefits of probiotic to the health can occur through 3 (three) mechanisms of function: [8] (1) Protective function, which is the ability to inhibit pathogens in the digestive tract. The development of probiotic colonization in the digestive tract will result in nutrition competition and adhesion location between probiotic and other bacteria, particularly pathogens; (2) Body immune system function, which is the enhancement of body immune system through probiotic ability to induce the development of IgA, activation of macrophage activation, modulation of profile cytokines, and induction of hyporesponsiveness towards antigen which originates from food; (3) Probiotic metabolite function, which is metabolite produced by probiotic, including the ability of probiotic to degrade lactose in fermented dairy products to be safely consumed by those who suffer from lactose intolerance.

Probiotic consumption is usually applied in the manufacturing of processed food products, such as yoghurt, cheese, refreshment drinks, ice cream, probiotic drink, sweets, and frozen yoghurt. The minimal number of probiotic strains in the food products is 106 CFU/g while the number of probiotic strains which needs to be consumed daily is approximately 108 CFU/g, with the aim to balance the reduction in the number of probiotic bacteria inside the digestive tract.[27] Probiotic may be beneficial for liver cirrhosis patients. Probiotic *Lactobacillus sp* can suppress the production of inflammatory cytokines, including TNF alpha, and CRP which may accelerate the development of liver injury; suppress endotoxin produced by pathogenic bacteria such as *E. coli* and other *Enterobacteriaceae*. Probiotics may also decrease the incidence of portal hypertension due to Nitrite Oxide production by *Lactobacillus sp*.[7,8]

In liver cirrhosis, the hepatic encephalopathy that occurs could be life-threatening. This is associated with the rise of ammonia in the body of liver cirrhosis patients. *Lactobacillus spp* will be decreased in chronic liver condition such as liver cirrhosis, a condition in which anaerobic metabolism increased. This elevated anaerobic metabolism allows the population of *E. coli* to increase and inhibit *Lactobacillus* population. *Lactobacillus* differs from *E coli* in its capacity of reducing nitrate to nitrite, ammonia and NO, in which *Lactobacilus spp* will convert nitrite to NO (70%) and ammonia (30%); thus the risk of hyperamonia decreases and increase the acidity of digestive tract to inhibit the growth of pathogenic bacteria. Through administration of probiotics, it is expected that *Lactobacillus spp* population increases and *E. coli* population decreases; hence, the production of ammonia will be reduced with the end result of decreased incidence of hepatic encephalopathy.[4]

CONCLUSION

This study showed strong association between aerobic exercise with *E. coli* population in liver cirrhosis. There was also very strong relationship between high nitrate diet with *E. coli* population in liver cirrhosis. Administration of probiotic in liver cirrhosis patients is needed to suppress the population of *E. coli* and other pathogenic bacteria.

REFERENCES

1. Clemente JC, Ursell, LK, Parfrey LW, Knight R. The impact of the gut microbiota on human health: an integrative view. Cell 2012;148:1258-70.
2. Emmanuel AT, Jaime B, Andrew KL. 2014. Liver Chirrosis [serial online] [cited 2016 August 19]. Available from: http://dx.doi.org/10.1016/ S0140-6736(14)60121-5
3. Valerio G, Vincenza DG, Valerio I. Microbiota and the gut-liver axis : Bacterial translocation, inflammation and infection in chirrosis. World J Gastroenterol 2014;20:16795-810.
4. Mauro T, Alan NS. 2015. Nitrate Reduction to Nitrite, Nitric Oxida and Ammonia by Gut Bacteria under Physilogical Conditions. Molecular Medicine Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland, United States of America.
5. Isprayoga Ibnu. 2015. Efektivitas Latihan Aerobik Pagi dan Malam Hari terhadap Kadar Hemoglobin dan Kadar Leukosit. Universitas Negeri Semarang.
6. Novasari Fuzy. 2011. Karateriksasi dan Analisis Kandungan Nitrat Tanaman Pakis Sayur di Kecamatan Dermaga Bogor. Institut Pertanian Bogor.

7. Robert SL, Andrew SA and Jan GF. 2014. Is There A Role for Probiotics in Liver Disease? The Scientific World Journal Volume 2014;874768:xx-xx.
8. Rahayu, E. S. 2008. Probiotic for Digestive Health. Food Review-Referensi industri dan teknologi pangan Indonesia. [serial online] [cited 2016 August 19]. Available from: http://www.food review.biz/login/preview.php?view&id=55932.
9. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature 2012;486:207–14.
10. Senok, A. C. 2009. Probiotics in the Arabian Gulf Region. Food & Nutrition Researc. [serial online] [cited 2016 August 12]. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC 2651754/pdf/FNR-53-1842.