The Role of Probiotic in Reducing Hepatic Inflammation Among NAFLD Patients: an Evidence-based Case Report

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ABSTRAK

Tujuan: *mengetahui efektifitas peran probiotik dalam menurunkan peradangan pada pasien perlemakan hati non-alkoholik (PHNA).* ***Metode:*** *dilakukan pencarian literatur terstruktur untuk membuktikan apakah pemberian probiotik dapat mengurangi inflamasi hati pada PHNA.* ***Hasil:*** *enam artikel ditemukan dan ditelaah secara kritis. Semua artikel ini menyimpulkan bahwa probiotik dapat menurunkan peradangan hati, yang terlihat dalam penurunan enzim transaminase. Selain itu, probiotik juga menurunkan kandungan lemak dalam parenkim hati. Namun, apakah probiotik bermanfaat dalam menurunkan progresivitas PHNA menjadi sirosis dan menurunkan mortalitas masih perlu dipelajari oleh penelitian lanjutan.* ***Kesimpulan:*** *probiotik memiliki manfaat dalam penanganan PHNA. Probiotik dapat digunakan sebagai salah satu terapi pendamping pada kasus perlemakan hati non-alkoholik.*

Kata kunci: *perlemakan hati non alkoholik, probiotik, inflamasi.*

ABSTRACT

Aim: *to know the effectiveness of probiotic in reducing hepatic inflammation among non-alcoholic fatty liver disease (NAFLD) patients.* ***Methods:*** *we performed literature searching regarding the potential role of probiotic in reducing hepatic inflammation among NAFLD patients.* ***Results:*** *six articles were finally critically appraised. All six studies had good validity and importance. These studies unanimously reported that probiotic is useful in reducing hepatic inflammation, and liver fat content. However, further evidence is needed to show whether or not probiotic is beneficial reducing cirrhosis progression and liver-related mortality.* ***Conclusion:*** *probiotic owns robust potential to treat NAFLD. Probiotic reduce hepatic inflammation, as shown by the reduction of liver aminotransferase, and inflammatory markers. Based on this evidence based report, probiotic is a promising adjunct therapy for NAFLD.*

Keywords: *non-alcoholic fatty liver disease (NAFLD), probiotic, inflammation.*

INTRODUCTION

Non Alcoholic Fatty Liver Disease (NAFLD) is a disease characterized by lipid accumulation in liver parenchym without any history of significant alcohol consumption. It was first introduced by Ludwig et al in 1980 and has been increasingly recognized as a common liver disease afterwards.[1,2] The histologic finding of NAFLD encompass a wide spectrum of disorder from simple steatosis, steatohepatitis and liver cirrhosis. In the last two decades, NAFLD has been recognized as the most common liver disease in western countries. NAFLD affects 10% to 24% of the general population and the number increases up to 74% in obese population.[3,4] Indonesia is one of the high prevalence countries of NAFLD. Lesmana CRA et al.[5] reported fatty liver was found in 51% of patients coming for medical check up.

To be defined as Non-alcoholic steatohepatitis (NASH), the liver should meet other criterias in addition to the accumulation of fat. The histological diagnosis of NASH requires the finding of hepatocyte injury (manifest by swollen or 'ballooned' cells), an inflammatory infiltrate (predominantly neutrophils) with or without fibrosis and it typically distributed perivenular/ pericellular. As many as 50% of NASH patients develop liver fibrosis, 15% would develop cirrhosis, and 3% may progress to terminal liver failure requiring liver transplantation.[6,7]

Certain metabolic risk factors have been strongly linked to NAFLD. The association between obesity, diabetes mellitus (insulin resistance) and dislipidemia with NAFLD has been well documented. As the amount of obesity and diabetes cases increase, so do their complications. Therefore the prevalence of NAFLD is expected to increase.[6] Besides insulin resistance and oxidative stress, intestinal flora has an important role in the pathogenesis of NAFLD. Although the exact mechanism remains unknown, small intestinal bacterial overgrowth (SIBO) is postulated to induce inflammation through the production of cytokine.[8]

There is currently no effective therapy for NAFLD. However as insulin resistance and oxidative stress are the two underlying culprits of NAFLD, therapy targeted on these particular step of pathogenesis is proved to be effective and promising. One novel therapy that has gained so much attention lately is probiotic. Probiotic works through its effect in manipulating commensal intestinal flora. Whether or not probiotic can give beneficial effects for NAFLD patients needs to be further investigated.

CLINICAL QUESTION

A 47-year old man came to the emergency room with a complaint of general weakness since one week before hospital admission. He also felt very thirsty and urinate a lot for the last one week. He felt nauseous and vomited a few times before hospital admission. He did not have any history of diabetes and hypertension. He denied any alcohol consumption.

In the emergency room, blood glucose meassurement was 640 mg/dl with keton level of 4.1. Blood gas analysis showed consistent result of metabolic acidosis, and the diagnosis of diabetic ketoacidosis (DKA) was made. The patients was then resucitate according to DKA protocol. Another laboratory examinations were as follow: Hb: 11.1 g/dl, leukocyte 11,000/µl, platelet count: 151,000/µl, and HbA1c: 12.3. The liver aminotransferase level was sligthly increased, aspartate aminotransferase: 57 U/L, alanin aminotransferase: 96 U/L. Hepatitis virus serology was negative. Bedside ultrasonography was performed with the result of bright liver, consistent with fatty liver disease, therefore the diagnosis of nonalcoholic fatty liver disease (NAFLD) was made.

After the acute period of ketoacidosis was succesfully managed, the patient was then transfered to the inpatient ward for further management. Apart from the treatment for his diabetes, we would like to give this patient an evidence based treatment for NAFLD. Apart from lifestyle modification, probiotic is an emerging therapeutic approach for NAFLD. To further confirmed probiotic efficacy in treating NAFLD, we performed an evidence based searching to justify the administration of probiotic among NAFLD patients.

Is probiotic administration effective to reduce hepatic inflammation among patients with NAFLD?

- P (Population):Patients with NAFLD
- I (Intervention): The administration of probiotic
- C (Comparison): Placebo or lifestyle modification
- O (Outcome): Liver transaminase improvement, cirrhosis progression, mortality.

METHODS

The search was conducted on PubMed,® Google scholar®, Proquest® on 9th November 2015, and 2nd December 2015 respectively, using the keywords 'NAFLD', 'probiotic', 'transaminase', 'mortality' along with its synonyms and related terms (**Table 1**). Search strategy, the inclusion and exclusion criteria are shown in **Figure 1**.

Table 1. Search strategy used in PubMed, Google scholar and Proquest (Conducted on November 9th 2015, and December 2nd 2015)

Database	Search terms	Results
Pubmed (9th November 2015)	((((("probiotics"[MeSH Terms] OR "probiotics"[All Fields] OR "probiotic"[All Fields]) AND ("non-alcoholic fatty liver disease"[MeSH Terms] OR ("non-alcoholic"[All Fields] AND "fatty"[All Fields] AND "liver"[All Fields] AND "disease"[All Fields]) OR "non-alcoholic fatty liver disease"[All Fields] OR "nafld"[All Fields])) OR nash[All Fields]) AND ("mortality"[Subheading] OR "mortality"[All Fields] OR "mortality"[MeSH Terms])) OR ("transaminases"[MeSH Terms] OR "transaminases"[All Fields] OR "aminotransferase"[All Fields]) AND (Clinical Trial[ptyp] AND "loattrfull text"[sb] AND "2010/11/16"[PDat] : "2015/11/14"[PDat])	1475
Google scholar (9th November 2015)	NAFLD NASH Probiotic transaminase mortality cirrhosis	242
Proquest (2nd December 2015)	pub(probiotic) AND pub(fatty liver or nash) AND pub(transaminase) OR ab(mortality)	952

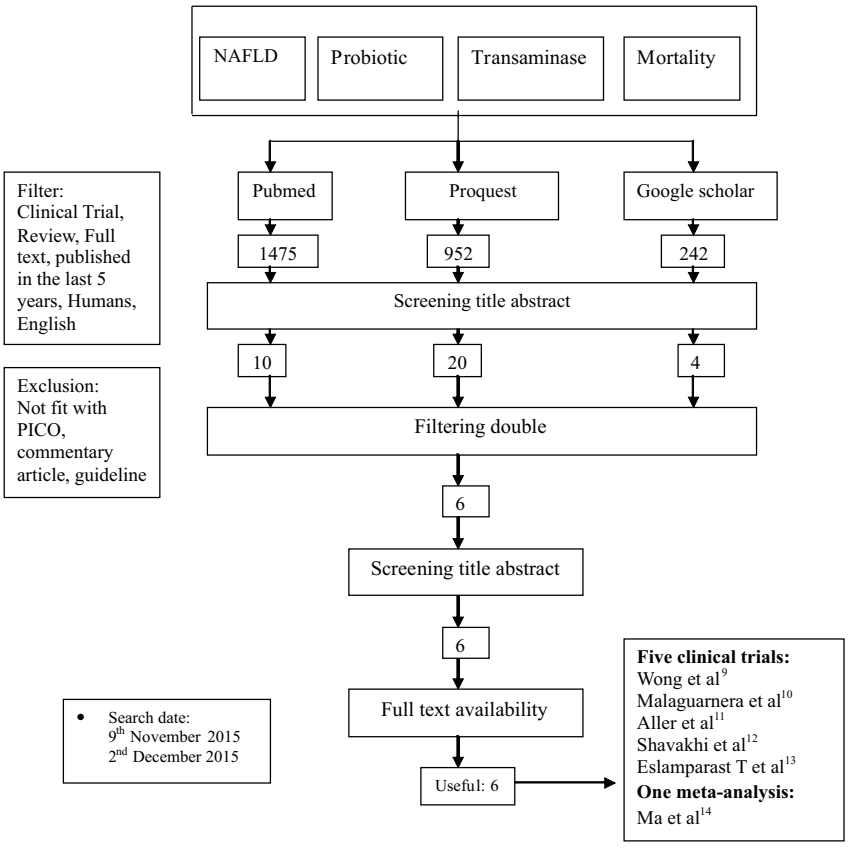

Figure 1. Flowchart of search strategy

RESULTS

Table 2. Critical appraisal of the useful randomized controlled trials based on criterias by centre of evidence medicine University of Oxford

Articles	Validity					Applicability				Levels of evidence*
	Study design	Randomization	Similarity treatment and control	Blinding	Comparable treatment	Intention to treat	Patient	Intervention	Outcome	
Wong et al[10]	+	+	+	-	+	+	+	+	+	1B
Malaguarnera et al[11]	+	+	+	+	+	+	+	+	+	1B
Aller et al[12]	+	+	+	+	+	+	+	+	+	1B
Shavakhi et al[13]	+	+	+	+	+	+	+	+	+	1B
Eslamparast T et al[14]	+	+	+	+	+	+	+	+	+	1B

+ stated clearly in the article; - not being done; ? not stated clearly.
*Levels of evidence based on The Oxford Centre of Evidence-based Medicine

Table 3. Critical appraisal of the meta-analysis

	Appraisal questions	Ma et al[14]
Validity	What question did the systematic review address?	+
	Is it unlikely that relevant studies were missed?	+
	Were the inclusion criterias appropriate?	+
	Were the included studies valid?	+
	Were the results similar from study to study?	+
Relevance	Patient	+
	Intervention	+
	Comparison	+
	Outcome	+
Importance	The result of this study is summarized in table 5	

+ stated clearly in the article; - not being done; ? not stated clearly;
* Levels of evidence based on The Oxford Centre of Evidence-based Medicine

DISCUSSION

Nonalcoholic Fatty Liver Disease (NAFLD) is a common liver disease worldwide. This phenomenon is partly influenced by environtment factors superimposed on genetic factors. Component of the metabolic syndrome, which includes obesity, insulin resistance and hypertension are some important risk factors of NAFLD. The multiple hit hypothesis has been the leading theory of the pathogenesis of NAFLD. The first hit of insulin resistance would make liver cells vulnerable to second hit in the form of oxidative stress. These hits would lead to liver fibrosis and cell death.[2]

Recent evidence showed the relation between intestinal bacterial overgrowth with NAFLD progression. Intestinal bacterial overgrowth increase hepatic oxidative stress by increasing endogenous ethanol production and releasing bacterial lipopolysaccharide (LPS). Both ethanol and bacterial LPS could stimulate inflammatory cytokine production, one of which is TNF-α by hepatocytes and kupffer cells. As intestinal bacteria plays an important role in the progression of NAFLD, one obvious way to control the development of NAFLD is by

Table 4. Summary of the RCTs' result

No	Author	Pts Number	Follow-up	Types of probiotic	Control	Result
1.	Wong et al[10]	20 NASH patients	6 months	Probiotic formula containing Lactobacillus plantarum, Lactobacillus delbruckii ssp. Bulgaricus, Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium bifidum	Lifestyle modification	The administration of probiotic reduce IHTG from 22.6 ± 8.2% to 14.9 ± 7.0% in the probiotic group (mean reduction 7.7%, 95% confidence interval 0.7% to 14.7%, P=0,034). Liver aminotransferase is also reduced significantly in the probiotic group. The use of probiotics was not associated with changes in body mass index, waist circumference, glucose and lipid levels.
2.	Malaguarnera et al[11]	66 NASH patients	24 weeks	Bifidobacterium longum with FOS	Placebo+ lifestyle modification	The administration of probiotic significantly reduce liver aminotransferase AST (109 ± 23.2 to 39.4 ± 28.2 p<0.05), LDL cholesterol (3.91±0.77 to 3.07±0.61, p<0.001), CRP (7.0 ± 3.4 to 4.1 ± 3.1, p≤0.05) and TNF-α (1.28±0.28 to 0.83 ± 0.36, p<0.001)
3.	Aller et al[12]	28 NAFLD patients	3 months	Lactobacillus bulgaricus, Streptococcus thermophiles	Placebo	Probiotic administration improved liver alanine aminotransferase level (67.7 ± 25.1 to 60.4 ± 30.4 UI/L, p < 0.05), aspartate aminotransferase activity (41.3 ± 15.5 to 35.6 ± 10.4 UI/L, p < 0.05) and gammaglutamine transferase levels (118.2 ± 63.1 to. 107.7 ± 60.8 UI/L, p<0.05)
4.	Shavakhi A et al[13]	64 NASH patients	6 months	Metformin 2x500mg/ day + 2 tablets of protexin	Metformin 2x500mg + 2 placebo tablets	The administration of metformin and probiotics provide greater reduction in terms of liver aminotransferase (ALT level at the end of treatment: 45.2 ±32.5 vs 112.5±68.7, p<0.001). There was significant improvement in liver radiological image as documented by USG in the probiotic group.
5.	Eslamparast T et al[14]	52 NAFLD patients	28 weeks	2 tablets of protexin	Placebo	There was significant reduction in terms of ALT level, γ-glutamyltransferase, TNF-α

IHTG: intrahepatic triglyceride content; FOS: fructo-oligosaccharides; ALT: alanine aminotransferase; AST: aspartate aminotransferase;LDL: Low Density Lipoprotein ; CRP: C-Reactive Protein; TNF-α: Tumor Necrosis Factor-α; USG: Ultrasonography

Protexin (made by Science and nature in balance Co, UK) contained: *Lactobacillus acidophilus* 1 × 10^8 CFU, *Lactobacillus casei* 5 × 10^8 CFU, *Lactobacillus rhamnosus* 7.5 × 10^7 CFU, *Lactobacillus bulgaricus* 1.5 × 10^8 CFU, *Bifidobacterium breve* 5 × 10^7 CFU, *Bifidobacterium longum* 2.5 × 10^7 CFU, *Streptococcus thermophilus* 5 × 10^7 CFU, fructooligosaccharides 350 mg.

Table 5. Result of meta-analysis

Author	Study included in the analysis	Total patients	Result
Ma et al[15]	Four studies	134	The use of probiotic is associated with reduction of liver aminotrasnferase (mean difference -23.71,95%CI: -33.46 to -13.95, P<0.00001) , total cholesterol (-0.28, 95%CI: -0.55 to -0.01, P = 0.04), TNF- (-0.32, 95%CI: -0.48 to -0.17, P<0.0001). Most of the result showed no heterogeneity

manipulation of the intestinal bacteria.[8]

One novel way to manipulate intestinal bacteria is by the administration of probiotic. Although the exact mechanism of probiotics in the treatment of NAFLD is incompletely understood, it is thought that probiotic interfere with NAFLD progression through its effect in eradicating pathogenic bacteria in the intestine. Probiotic also reduce ethanol production and reduce inflammation by altering cytokine signaling.[9]

After a thorough searching, six articles were finally critically appraised. All six studies, five studies were randomized controlled trials and one article was meta-analysis, have good validity, importance and applicability as shown in **Table 2 and 3**. These studies also matched our clinical question. Each of these studies provide different types of endpoint including liver aminotransferase, lipid profile, inflammatory marker, and liver radiological improvement.

Three clinical trials appraised in this report (Wong et al[10], Malaguarnera et al[11] and Aller et al[12]) were also included in the meta analysis. However, since these three studies provide interesting end points, we still included these studies in our appraisal. The importance of treatment effect can not be calculated in the form of relative risk due to the variable used in this trials are numerical data. Instead, we showed the mean difference in every trials, with their respective confidence interval.

All six studies reported significant reduction of liver enzymes (either ALT, AST or both) after the administration of probiotic. Beside the reduction of liver enzymes, inflammatory cytokines, one of which is TNF-α, were also reduced. TNF-α plays an important role in inducing hepatic inflammation, which is postulated to be the underlying process of NAFLD progression into cirrhosis.

Wong et al reported that probiotic reduce intrahepatic triglyceride content, as meassured by proton magnetic resonance spectroscopy (p:0.034). Proton magnetic resonance spectroscopy is one of the most accurate non-invasive test for liver fat. Therefore this observation is a reliable measure of liver fat after probiotic administration.[10]

As insulin resistance play an important role in NAFLD patophysiology, metformin is often prescribed among NAFLD patients. Shavakhi A et al[13] reported that probiotic enhance the effect of metformin in terms of reduction of liver aminotransferase and improve liver radiological image as documented by USG.[14]

One meta-nalysis by Ma et al[15] further support the beneficial effect of probiotic for NAFLD treatment in terms of reduction of aminotransferase, improvement of cholesterol level and inflammatory markers (TNF-α). We also explore for heterogeneity in this meta-analysis and most of the result showed no heterogeneity.

One study performed by Loguercio et al is not included in this appraisal, due to the difference in patients characteristics. However, this study provide additional important information that the beneficial effect of probiotic (VSL#3) extend beyond NAFLD patients. This study also investigate the efficacy of probiotic administration among patients with alcoholic liver cirrhosis, and cirrhosis patients with hepatitis C. The administration of probiotic reduce lipid peroxidation, which is a key component of liver steatosis in patients with NAFLD and alcoholic liver cirrhosis. But limited effect was observed in patients with hepatitis C.[16]

TNF-α is a well-established marker for inflammation. Most of these studies showed consistent result that probiotic reduce the level of TNF-α among NAFLD patients. Whether or not the level of TNF-α is correlated with the degree of liver fibrosis is studied by Lesmana et al[17]. According to this study, increased TNF-α level was associated with a more severe fibrosis stage among NASH patients. So reduction of TNF-α is logically linked to reduction of inflammatory condition in the liver, and in the long run, would hopefully reduce the progression from NAFLD to liver cirrhosis.

Collectively, these unanimous result confirms previous animal studies on the potential role of probiotic as NAFLD treatment. There were multiple animal studies that reported beneficial effect of probiotic in improving lipid profile, and liver fat content.[9] Although all these studies reported similar finding that probiotic has

potential therapeutic role for NAFLD, cautious intepretation of this result is warranted. All these studies are relatively small, with a limited period of follow up. Another question need to be addressed in future studies is the type of probiotic can be used. There is currently no consensus regarding the strain and the amount of bacteria that would produce beneficial effect in the treatment of NAFLD.

Is the result of these studies can be applied to our case scenario? First, the characteritics of our patients match the characteristics of two studies performed by Malaguarnera M et al[11] and Wong et al[10]. Our patient had elevated level of liver aminotransferase, negative for hepatitis markers, and did not consume alcohol for the last six months. So, the result of these studies can be applied in our scenario.

Regardless of all these positive findings, all the endpoints in these studies are surrogate outcome, not hard endpoint (eg. mortality). Surrogate markers, like inflammatory markers or liver enzymes, are postulated to be related in the progression of NAFLD into cirrhosis. So, it is logical to assume that the reduction of these surrogate markers would translate into reduction of cirrhosis, or liver related-mortality. However, it may be premature to think that inflammatory parameters improvement is equivalent to hard end point benefits, without confirmatory data. Whether or not probiotic improve liver histology is also yet to be determined. Based on our literature searching, there has not been any published clinical trials that study the beneficial effect of probiotic in improving liver histology, as proven by liver biopsy.

Probiotic administration is a potential therapeutic approach, with the caveat that there is a lack of published robust clinical trials regarding its efficacy in reducing cirrhosis progression or liver related mortality. Future studies with a larger population and longer period of follow up would hopefully answer some lingering questions such as, whether or not probiotic provide hard endpoint benefit among NAFLD patients. Second, what is the best strain, the right dosage, and the duration of treatment required to produce significant beneficial effect.

An effective therapy for NAFLD is the current unmeet need. Since probiotic is affordable, widely available and safe, this novel therapy is a potential approach in the treatment of NAFLD. Based on existing evidence, probiotic can be used as an adjunct therapy for NAFLD.

CONCLUSION

Currently, there has not been any effective treatment for NAFLD. Probiotic has a robust potential in the treatment of NAFLD. Probiotic reduce hepatic inflammation, as shown by the reduction of liver aminotransferase, and inflammatory markers. Further evidence is needed to show whether or not probiotic is beneficial in terms of cirrhosis progression and liver-related mortality. Probiotic is widely available, safe and relatively affordable, therefor probiotic is a promising adjunct therapy for NAFLD.

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