Use of Probiotics in Patients with Nonalcoholic Fatty Liver Disease and Reduction of Liver Aminotransferases

Sandy Pham
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Abstract

Background

Nonalcoholic fatty liver disease (NAFLD) is one of the most common liver diseases characterized by increases in serum aminotransferases and accumulation of fat in the liver. NAFLD can lead to diabetes, cardiovascular complications, end stage liver disease, liver cirrhosis, and hepatocellular carcinoma. At the moment there is no drug treatment for the management of NAFLD. Other treatment options should be explored for this disease that can progress to liver cirrhosis and hepatocellular carcinoma. Individual studies and systematic analysis have shown that the consumption of probiotics can decrease liver aminotransferase levels in adults with NAFLD.

Methods

An extensive literature search was performed using MEDLINE-Ovid, Web of Science, and CINAHL using the terms “probiotics,” and “nonalcoholic fatty liver disease.” The studies were then limited to randomized control trials and restricted to human studies in the English language and performed within the last 5 years. Inclusion criteria included studies on adults diagnosed with NAFLD via ultrasonography or liver tissue biopsy. Studies were assessed for quality using GRADE criteria.

Results

Two studies were found which met the inclusion and exclusion criteria. Both studies were randomized control studies performed in Spain, Aller et al, and the second study performed in Iran, Nabavi et al. Both studies showed that taking probiotics reduced liver aminotransferases levels in patients with NAFLD. The Aller et al study found that the intervention group was statistically different at baseline and at 3 months post probiotic therapy for ALT and AST. The Nabavi et al study found that at the end of the 2 weeks probiotic yogurt consumption results in a reduction of 4.67 and 5.42 in ALT and AST levels, respectively, compared to the conventional yogurt group.

Conclusion

The studies demonstrated a reduction in liver aminotransferases with treatment of probiotics in patients with nonalcoholic fatty liver disease.

Keywords Probiotics, nonalcoholic fatty liver disease, liver aminotransferases

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Biography

[redacted]
Abstract

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An extensive literature search was performed using MEDLINE-Ovid, Web of Science, and CINAHL using the terms “probiotics,” and “nonalcoholic fatty liver disease.” The studies were then limited to randomized control trials and restricted to human studies in the English language and performed within the last 5 years. Inclusion criteria included studies on adults diagnosed with NAFLD via ultrasonography or liver tissue biopsy. Studies were assessed for quality using GRADE criteria.

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Two studies were found which met the inclusion and exclusion criteria. Both studies were randomized control studies performed in Spain, Aller et al, and the second study performed in Iran, Nabavi et al. Both studies showed that taking probiotics reduced liver aminotransferases levels in patients with NAFLD. The Aller et al study found that the intervention group was statistically different at baseline and at 3 months post probiotic therapy for ALT and AST. The Nabavi et al study found that at the end of the 2 weeks probiotic yogurt consumption results in a reduction of 4.67 and 5.42 in ALT and AST levels, respectively, compared to the conventional yogurt group.

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The studies demonstrated a reduction in liver aminotransferases with treatment of probiotics in patients with nonalcoholic fatty liver disease.

Keywords Probiotics, nonalcoholic fatty liver disease, liver aminotransferases
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To My parents: Thank you for your ever endless support in my journey to becoming a Physician Assistant. Your positivity and belief in me has carried me through.
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Table 1. Quality Assessment of Reviewed Studies
Table 2: Changes in ALT and AST from Aller et al Study

List of Abbreviations

NAFLD.............................................................Nonalcoholic Fatty Liver Disease
ALT.................................................................Alanine aminotransferase
AST.................................................................Aspartate aminotransferase
Use of Probiotics in patients with Nonalcoholic Fatty Liver Disease and Reduction of Liver Aminotransferases

BACKGROUND

The prevalence of nonalcoholic fatty liver disease (NAFLD) is rising and becoming the most common form of chronic liver disease in adults and children.\(^1\) NAFLD is a broader term used to describe hepatic changes seen in patients with steatosis, nonalcoholic steatohepatitis, more progressive liver diseases like cirrhosis and end stage liver disease, all of which can lead to the development of hepatocellular carcinoma.\(^1\) The pathology behind NAFLD is an active area of research. There are several predicted factors that contribute to NAFLD such as lifestyle, diet, exercise, genetic predisposition, and hormonal dysregulation.\(^2\) Research has shown that intestinal microflora contributes to NAFLD by way of translocation of endotoxins and ethanol produced by the intestinal lumen to the mesenteric circulation. The endotoxins then activate Kupffer cells located in the liver and thus stimulate the production of TNF alpha and IL-6.\(^1\) One study\(^3\) states that the prevalence is an estimated 2.8% to 46% throughout the world and varies depending on the study population and the diagnostic tool that is used to diagnose NAFLD (ie: blood serum liver enzymes, ultrasound, or liver biopsy).\(^3\) The development of NAFLD has been linked to an increase risk of diabetes, cardiovascular problems, cirrhosis and even hepatocellular carcinoma. One study\(^4\) states that individuals with NALFD have a higher mortality rate than healthy people.\(^4\)

Although the prevalence of NAFLD is becoming more and more common, there is no approved treatment for this disease. There have been many studies\(^5\) that look into the role of nutrients in the development, progression, and treatment of NAFLD. In adults
and children, obesity is the main cause; therefore, data reviewed confirms that weight loss and lifestyle changes remain the first choice treatment for adults and children with NAFLD.\textsuperscript{5} Other treatment modalities include insulin sensitizers, antioxidants, and hepatoprotective agents such as ursodeoxycholic acid, and the use of probiotics.\textsuperscript{4,1} The use of probiotics in the setting of NAFLD is a new area of research that warrants more studies to confirm its potential benefit.

Probiotics are live microorganisms which when consumed confer a healthy benefit to the host.\textsuperscript{6} Probiotics have numerous reported effects such as maintaining intestinal microflora, reducing cholesterol levels, promoting a healthy immune system, lowering risk of cancers, and helping with diabetes. It is shown that changes in our intestinal microbiome composition could increase endotoxemia in response to a high-fat diet, which in turn would trigger the development of obesity and diabetes.\textsuperscript{7} In summary, given that obesity is a common risk factor in people with NAFLD, the use of probiotics could potentially be a treatment with promising benefits either as an alternative therapy or as an adjunct therapy in combination with other therapeutic modalities. Probiotics come in many forms including pills, gummy form, mix drinks, and can be found in multiple yogurts. They can be found at any store and are cost friendly. There are no studies that show risks associated with taking probiotics. Taking into consideration the facts above, one would find it a reasonable alternative or adjunct therapy in reducing the effects of NAFLD.

NAFLD is characterized by elevated serum aminotransferases levels and fat accumulation in the liver. Alanine aminotransferase (ALT) and aspartate...
aminotransferase (AST) are liver markers measured with a blood test and can be useful in telling us about overall liver function and are indicators of hepatocellular injury. Although there are other ways of measuring liver disease, the 2 mentioned above are the focus of this review. The aim of this review is to focus on the role of probiotics in reducing liver aminotransferases in patients with NAFLD.

**METHODS**

An exhaustive literature search was performed using MEDLINE-Ovid, Web of Science, and CINAHL using the terms “probiotics,” and “nonalcoholic fatty liver disease.” With Web of Science, the search also included the search term “liver aminotransferases.” The search was limited to randomized control trials (RCT) and restricted to human studies in the English language. The search was also restricted to studies published within the last 5 years. In addition to using the above search databases, articles pulled from certain references were used for relevant information. The studies were then appraised and assessed for quality using GRADE.

**RESULTS**

The initial search using MEDLINE-Ovid yielded 5 articles. Using PICO for inclusion and exclusion criteria, the 5 articles were narrowed down to 2 randomized control studies (see Table 1). Web of science yielded these same 2 articles. A search with the key terms on CINAHL yielded 8 results, all of which did not meet eligibility criteria or did not consist of randomized control studies.
**Study Description**—This randomized control study,\textsuperscript{10} published in 2011 in the European Review for Medical and Pharmacological Sciences, examined a cohort of 30 patients diagnosed with NALFD. The main purpose of this study was to examine the effects of taking probiotic tablets daily on liver aminotransferases and gammaglutamine transferase levels. The diagnosis of NAFLD was made via percutaneous liver biopsy. The exclusion criteria included patients with hepatitis B or C, cytomegalovirus, Epstein Barr infections, non organ-specific autoantibodies, alcohol consumption, diabetes mellitus, impaired glucose tolerance, medications (ie, statins and blood pressure), and hereditary defects (iron and copper diseases).\textsuperscript{10} The study was a double blind randomized clinical trial that included 2 groups. Group I (intervention group) was given 1 tablet a day of 500 million of *Lactobacillus bulgaricus* and *Streptococcus thermophile*. Group II was the control group that was given a placebo tablet of 120 mg of starch. Each participant was given 90 tablets making it a 3-month treatment window. Food intake was recorded at baseline and after the 3 months. The participants recorded their food intake for 3 days and incorporated the use of food scales to enhance portion size accuracy.\textsuperscript{10}

Alanine amino transferases, aspartate aminotransferase, bilirubin, and gammaglutamine transferase were determined by enzymatic colorimetric assay.\textsuperscript{9} Other parameters were measured: weight, blood pressure, glucose, total cholesterol, triglycerides, and IL6 and TNF alpha. These were measured at baseline and after the 3-month period.
**Study Results**—Out of the 30 patients that started the study, 28 participants finished out the study. They excluded the 2 participants because they consumed less than 90% of the tablets they were given. The results after 3 months showed that group I had an improvement (a reduction) of ALT, AST, and gammaglutamine transferase levels after 3 months of probiotic therapy compared to baseline. This is in comparison to the control group that had increased levels of ALT, AST, and gammaglutamine transferase (See Table II). The results were analyzed with a confidence interval of 95% and a significant p-value of <0.05. When comparing intergroup statistics, it showed that the intervention group was statistically different at baseline and at 3-months post probiotic therapy for ALT and AST. When comparing the placebo group vs. the intervention group there is a difference of 11 points when comparing differences in ALT. Overall, the study concluded that a tablet containing 500 million of *Lactobacillus bulgarius* and *Streptococcus thermophiles* in patients with NAFLD was associated with ALT and AST improvement.¹ The limitations to this study include a small cohort, short treatment window, and the use of surrogate outcomes. The large standard deviations suggest that future studies should include a larger cohort of participants or following the participants for a longer duration of time. The study used surrogate outcomes (ALT and AST) to indirectly measure liver disease. Using these biomarkers to measure liver disease is not a direct way to measure whether or not liver function is improving.

**Nabavi et al**

**Study Description**—This randomized control study,² published in 2014 in the American Dairy Science Association, examined a cohort of 72 patients with NAFLD,
aged 23-63 years, with body mass index ranging from 25-40 kg/m$^2$. Recruitment of the participants were patients referred over from a gastroenterologist who had just diagnosed these participants as new cases of NAFLD. The diagnosis was made via ultrasonography rather than liver biopsy. The exclusion criteria included patients with kidney disease, another type of liver disease, hepatitis B or C, inflammatory intestinal disease, thyroid disorders, immunodeficiency disease, Wilson disease, hemochromatosis, lactose intolerance, tobacco or alcohol use, pregnant or breast feeding, receiving cholesterol-lowering medication, estrogen, progesterone, or diuretics, and consumption of probiotic yogurt or other probiotic products within the last 2 months. The study was a double blind randomized clinical trial that included 2 groups. There were no differences in gender and age distribution between the 2 groups. Group I had a mean age of 49.4 and group II had a mean age of 44.3. One group was given probiotic enriched yogurt and the other group was given conventional yogurt. ALTs and ASTs were measured using Bio System kits and an auto analyzer set. Fasting blood glucose, triglycerides, HDL, and LDL were also measured. The length of the study was 2 weeks with no participants dropping out.

**Study Results**—All the participants of the study ended without compliance issues. The study found that at the end of the 2 weeks, probiotic yogurt consumption resulted in a reduction of 4.67 and 5.42 in ALT and AST levels, respectively, compared to the conventional yogurt group. The probiotic group had a decrease in the number of abnormal cases of ALT after intervention (13 to 10) whereas the conventional group had no change. The results of this study trends towards a positive result similar to that of the Aller et al study.
DISCUSSION

The prevalence of NAFLD is significant and with the prevalence increasing worldwide, pharmacological and non-pharmacological treatments are being sought out. The use of probiotics in liver disease, specifically NAFLD, has little data that supports it. Results emerging from the above studies,\(^8,10\) demonstrate that an acute course of daily probiotics induced a reduction in liver aminotransferases in adults diagnosed with NAFLD. Data from the Aller et al\(^10\) study are in keeping with the results of the Nabavi et al\(^8\) study. Within the studies,\(^8,10\) there was no adverse reactions when taking the probiotics.

The Aller et al\(^10\) study was a pilot trail designed to evaluate the effects of acute treatment with a mixture containing 500 million of \textit{Lactobacillus bulgaricus} and \textit{Streptococcus thermophilus} per day in patients with NAFLD. The study concludes that in conditions in which intestinal microflora may be a cofactor of NAFLD, modification of gut flora with the use of probiotics may be a potential treatment.

Another double blind RCT\(^11\) done on children showed that obese children with NAFLD (average age of 10.7) treated with \textit{Lactobacillus rhamnosus} for 8 weeks showed a significant reduction in ALT amongst other parameters. The research and studies done around the use of probiotics and its ability to reduce liver aminotransferases warrants its consideration as a therapeutic modality in slowing down the progression and development of NAFLD as well as treating the disease.

There is a lack of randomized control studies regarding this topic. With more studies that contain a larger sample size along with a longer duration of treatment, a better understanding of the effects of using probiotics for NAFLD will be uncovered.
Both studies\textsuperscript{8,10} used blood serum ALT and AST levels to measure the effectiveness of probiotics. Given that these parameters indirectly measure liver disease, it is hard to examine if it is actually helping the progression and treatment of the disease. In future studies, measuring fatty deposits as a direct biomarker for NAFLD would give a better idea on its benefits in the progression of NAFLD. Within the studies, had the panel followed the participants for a longer duration of time to see if they needed a liver transplant, this would be a more direct way to measure the effectiveness of probiotics.

**CONCLUSION**

There is evidence presented in the Aller et al\textsuperscript{10} and Nabavi et al\textsuperscript{8} studies that consuming probiotics in any form correlates with improvement of liver aminotransferases in patients with NAFLD. Both studies did not mention any harm or adverse reactions in using probiotics. Despite the lack of RCTs that contain a larger sample size, the studies reviewed within this article as well as research done on this topic show enough of a positive trend that medical providers should suggest this as a treatment option if patients wish to try it. If patients want to try probiotics, providers should let them know that taking probiotics have not been shown to have any adverse reactions and may or may not help with improving liver aminotransferase levels. However, with any new treatment modality, the more studies that are done, the better the validation of the effectiveness of the treatment. Indeed, the effects on liver enzymes and on some other parameters related to NAFLD such as BMI, glucose levels, and lipid levels, the low cost, and no adverse effects, it would appear that probiotics as an alternative option or adjunct therapy would be a well-reasoned recommendation.
References


### Table 1: Quality Assessment of Reviewed Articles

<table>
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<tr>
<th>Study</th>
<th>Design</th>
<th>Limitations</th>
<th>Indirectness</th>
<th>Inconsistency</th>
<th>Imprecision</th>
<th>Publication bias</th>
<th>Upgrade Criteria</th>
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<td>Aller et al&lt;sup&gt;a&lt;/sup&gt;</td>
<td>RCT</td>
<td>Not Serious</td>
<td>Serious&lt;sup&gt;↑&lt;/sup&gt;</td>
<td>Not Serious</td>
<td>Serious&lt;sup&gt;↑&lt;/sup&gt;</td>
<td>Unlikely</td>
<td>N/a</td>
<td>Low</td>
</tr>
<tr>
<td>Nabiavi et al&lt;sup&gt;a&lt;/sup&gt;</td>
<td>RCT</td>
<td>Not Serious</td>
<td>Serious&lt;sup&gt;↑&lt;/sup&gt;</td>
<td>Not Serious</td>
<td>Serious&lt;sup&gt;↑&lt;/sup&gt;</td>
<td>Unlikely</td>
<td>N/a</td>
<td>Low</td>
</tr>
</tbody>
</table>

<sup>a</sup>Use of surrogate outcomes (AST, ALT, and GGT)
<sup>↑</sup>Small sample size

### Table II: Changes in ALT and AST from Aller et al Study<sup>a</sup>

<table>
<thead>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
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<tr>
<td>ALT</td>
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<tr>
<td>AST</td>
<td>31.7±13.1</td>
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<tr>
<td>Gammaglutamine transferase</td>
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