NAFLD & NASH

Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of disorders ranging from fatty infiltration of liver cells, or hepatic steatosis, to steatohepatitis, fibrosis and eventually cirrhosis. Ultimately cirrhosis itself can lead to hepatocellular carcinoma. The histological, imaging and laboratory features of NAFLD are indistinguishable from alcohol-induced steatosis and subsequent steatohepatitis and, therefore, the diagnosis is made in the absence of a history of significant alcohol intake.

In the majority of cases, NAFLD develops in association with features of metabolic syndrome and insulin resistance (IR). Within the spectrum of metabolic syndrome one usually finds a cluster of clinical features, namely IR, glucose intolerance or full-blown diabetes, obesity, hypertension and dyslipidaemia all of which result in a significantly increased associated cardiovascular risk.

Epidemiology

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide, both in adults and children. Due to its strong association with other metabolic diseases such as obesity and diabetes, the western world is seeing an increase in both incidence and prevalence of NAFLD and NASH. The statistics vary depending on the geographical region and diagnostic parameters but research suggests that up to 24% of the general population of developed countries is affected by NAFLD. The British Society of Gastroenterology suggests that this figure is closer to 33% for the UK population whilst a Korean study suggested a prevalence of 51%. When associated with other diseases the prevalence of NAFLD increases dramatically particularly in patients with obesity (60–95%), type 2 diabetes mellitus (28–55%) and hyperlipidaemia (20–92%). NAFLD affects 2.6% of children and this figure increases to between 22.5% - 52.8% in the obese child population. Progression to NASH is a clinically significant event and epidemiological research suggests that its prevalence is 1.2% – 4.8%. Importantly, studies from tertiary care centres, community-based and population-based databases suggest that liver disease is the third leading cause of death among people with NAFLD.
Pathogenesis

The pathogenesis of the disease is extremely complex, not fully understood and beyond the scope of this feature, however it is important to understand some of the basic mechanisms underlying its development. Early explanations suggested a “2-hit hypothesis” with initial hepatic triglyceride (fat) accumulation, or steatosis, increasing the liver’s susceptibility to further injury mediated by “second hits” from pro-inflammatory cytokines, oxidative stress and cellular dysfunction. This leads to hepatic inflammation, or steatohepatitis, and in turn, fibrosis\textsuperscript{11,12}. However, more recently, free fatty acids (FFA) have been attributed with a direct toxic effect which works in tandem with and, in fact, triggers other hepatotoxic pathways and prevents hepatic cellular regeneration, a “third hit”\textsuperscript{13}.

Another contributory mechanism by which inflammatory pathways can be activated is the production of pro-inflammatory products by intestinal bacteria. There is a clear association between intestinal bacterial overgrowth and the development of NAFLD and NASH with some of the specific mechanisms being identified\textsuperscript{14}. An association between small intestinal bacterial overgrowth (SIBO) and severity of steatosis has been identified, adding further evidence to the hypothesis that the gut microflora plays a key role in the development of the disease\textsuperscript{15,16}. It is thought that the gut microbiota contributes to the development of NASH through the increased production of ethanol and lipopolysaccharides which stimulate inflammatory cytokine production through TNF-\textalpha and NF-\kappaB mediated mechanisms\textsuperscript{14}. In addition, luminal bacteria metabolise dietary choline (which is required for hepatic lipid export) thereby promoting steatosis. These pathways, in turn, increase intestinal permeability thus enhancing hepatic exposure to other gut derived endotoxins that are directly or indirectly hepatotoxic\textsuperscript{17}.

Clinical Management

NAFLD and NASH are managed based on the progression and severity of the disease. Simple steatosis alone can be managed in the primary care setting with lifestyle modifications the mainstay of treatment\textsuperscript{18}. The main strategy is to manage obesity and other associated conditions within the spectrum of metabolic syndrome. This is then supplemented with treatment of specific liver related consequences. There are no targeted medications for NAFLD or NASH themselves although there are certain medications that have shown benefit in its management.
Lifestyle modification aimed at weight reduction and increased physical exercise is the first line treatment for all patients with NAFLD. That being said, the optimal diet for NAFLD patients has not yet been determined and, until further research is carried out, a calorie restricted diet (600Kcal less than a maintenance intake) should be recommended until target weight is achieved. A Mediterranean diet (high in monounsaturated fatty acids), as compared with a diet low in fat and high in carbohydrates, has been shown to reduce hepatic steatosis and improve insulin sensitivity in non-diabetic subjects with NAFLD. It has also been demonstrated that moderate intensity training, high intensity training and resistance exercise result in improvements in liver enzymes and reduction in liver fat, independent of weight loss. There is also a role for bariatric surgery in particularly obese patients in order to aid weight loss.

Pharmacotherapy

Individual components of the metabolic syndrome such as diabetes, hypertension and dyslipidaemia are managed as they would be without the presence of NAFLD with appropriate medication. Metformin has been investigated as an adjunct to lifestyle changes in the management of NASH with varying results. It has been shown to aid weight loss and reduces risk of diabetes related morbidity and mortality. Its role in those patients with NAFLD, but no components of diabetes, is controversial and is therefore not a definitive recommendation in those patients. Other insulin sensitisers are recommended as second line treatment and there is some evidence to suggest they can improve inflammation and steatosis in NASH.

In terms of liver directed pharmacotherapy, pioglitazone and vitamin E have been shown to have some benefit in patients with biopsy proven NASH. These benefits have been demonstrated in patients with or without associated diabetes. For example, a recent meta-analysis has demonstrated that pioglitazone treatment in NASH significantly improves steatosis, inflammation and to a lesser degree, fibrosis. While it appears to be an effective treatment there are concerns over its long term safety with the most serious reported risk being an increase in congestive cardiac failure, clearly an important consideration in patients already at risk of cardiovascular morbidity and mortality. The situation is similar with vitamin E whose efficacy in management of NASH has been demonstrated in a large trial showing improvements in steatohepatitis but there remains a concern over its long term safety with a potential increased risk of haemorrhagic stroke and prostate cancer.

Clinical Need

The brief overview of NAFLD and NASH above highlights the main points with regards to the clinical need for innovative management options. It is a growing problem with significant clinical implications affecting both adults and children. There is also no specific recommended pharmacotherapy for its treatment and the ones that are available have question marks over their efficacy and/or safety. There are other medications that have been explored but the evidence is minimal and so it is clear to see that new and safe therapies that can potentially help are invariably going to be welcomed by the clinical community.
Probiotics and NAFLD/NASH

Given the importance of the gut microbiota in the development of NAFLD and NASH it comes as no surprise that this is a growing area of research within the probiotic community. Their ability to alter the intestinal microbial population, restore gut barrier function, interact with the immune system and modulate inflammatory responses has attracted great interest. Several in vitro and animal models have been used to identify specific mechanisms by which they may exert their beneficial effects in NAFLD.

Mechanisms

The most obvious mechanism by which probiotics help in NAFLD is their ability to modulate the bowel flora. Given that SIBO is a significant trigger in the pathogenesis, reducing the overgrowth of pathogenic bacteria is a logical first step. The way in which probiotics are able to do this has been well documented and is discussed elsewhere in this booklet but includes competition for nutrients, direct production of antimicrobials, lowering luminal pH and preventing adhesion and translocation of pathogens, amongst other mechanisms.

Perhaps more interestingly, probiotics are able to modulate some of the pathways involved in the pathogenesis of the disease as discussed above. They are able to improve the barrier function of the gut and repair damaged intestinal linings through a number of complex pathways mediated by the enhancement of tight junction function, preventing local inflammation and preventing apoptosis of intestinal epithelial cells. As a result, the liver is exposed to fewer absorbed endotoxins as there are fewer pathogenic bacteria producing them and they are not absorbed as frequently due to the reduced intestinal permeability. In vitro and animal models have been used to demonstrate that probiotics also reduce inflammatory and oxidative damage to the liver through modulation of TNF-α and PPARα pathways. Other studies have shown that specific probiotics are able to reduce fat deposition, improve triglyceride content and reduce serum endotoxin levels in NAFLD rats.

Clinical Evidence

Given the volume of preclinical research demonstrating the mechanisms by which probiotics might improve NAFLD it is surprising to note that there are relatively few good quality clinical trials investigating their use in humans. That being said, the results from some of these trials are highly encouraging and certainly warrant further investigation.

A study published in 2011 by Malaguarnera and colleagues looked at the effects of a synbiotic alongside lifestyle modifications in patients with NASH. The synbiotic contained a *Bifidobacterium longum* strain with fructooligosaccharide (FOS) as the prebiotic and was given for a total period of 24 weeks with multiple biochemical and histological variables assessed. All 66 patients with a diagnosis of NASH were given the same lifestyle modification advice and then randomised into equal groups to receive the synbiotic or placebo. Lifestyle advice included detailed dietary and exercise schedules and was monitored by diéticians.

All 66 patients completed the study and improvements were seen in both groups. However, there were much greater improvements seen in the synbiotic group compared to placebo in a number of parameters:

- AST (P<0.05),
- LDL cholesterol (P<0.001),
- CRP (P<0.05),
- TNF-α (P<0.001),
- HOMA-IR (P<0.001),
- Serum endotoxin (P<0.001),
- Histological evidence of steatosis (P<0.05),
- NASH activity index (P<0.05)

The synbiotic group had greater improvements in measures of liver function, inflammation, insulin resistance and endotoxin levels as well as improvements in grading of disease.

Similar results were obtained by a group of researchers in 2013 looking at a multi-strain probiotic mixture with 7 strains of bacteria including 4 *Lactobacilli*, 2 *bifidobacteria* and a *Streptococcus*. A total of 64 patients with histologically confirmed NASH were recruited for the trial and all were given
lifestyle advice and metformin. They were randomised into equal groups to receive the probiotic mixture or a placebo for 28 weeks. Liver function was assessed using measurements of hepatic enzymes, and ultrasound was performed to grade the extent of the liver disease. This was done at baseline and after treatment with monitoring throughout the 6 month period. After the treatment period there were improvements in all parameters in both groups as would be expected. However, there was significantly better improvement in the probiotic group with the main results summarised below:

The above results show a clear improvement in liver function and these are corroborated by the dramatic improvements seen in the ultrasound grading of the liver. The probiotic group also showed a significantly greater improvement in BMI at the end of the study.

These results were further corroborated in 2014 by another group of researchers using the same mixture in patients with NAFLD. A total of 52 patients were enrolled in this study and received dietary and lifestyle advice. They were then randomised to receive the probiotic mixture or placebo. Both groups showed improvements in ALT, AST, γ-GT, hs-CRP, TNF-α and BMI when compared to baseline. However, the group receiving the probiotic mixture showed significantly greater improvements compared to placebo in all biochemical parameters (p<0.001 in all tests). There was no difference seen between the two groups in the change in BMI (p=0.13).
Conclusion

The effects of probiotics in NAFLD and NASH are yet to be conclusively evaluated but the preclinical and initial clinical data shows huge potential for their use as a safe and effective treatment option for these patients. In addition, other clinical trials have been performed with encouraging results in diabetes, cholesterol reduction and metabolic syndrome demonstrating the potential of probiotics to be used as an adjunct to treatment in metabolic disorders. As more is learned about the way in which probiotics are able to exert their beneficial effects more products will become part of the routine therapy for these complex disorders.

About the author:

Dr Mayur R Joshi MBBS, BSc, AICSM
Dr Joshi is a fully registered doctor and has spent most of his clinical work in colorectal surgery, before working for Probiotics International as a Medical Advisor. Dr Mayur Joshi is currently working as a Pharmaceutical Physician.

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