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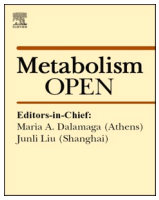

Editorial: Special issue: Non-alcoholic fatty liver disease: From molecular basis to therapeutic advances

Non-alcoholic fatty liver disease (NAFLD) has become one of the most common causes of chronic liver disease worldwide with a prevalence of 25% in the general population [1,2]. The prevalence of NAFLD is greater in patients suffering from various metabolic disorders such as dyslipidemia, hypertension, type 2 diabetes (T2DM) and obesity, as over 60% of people diagnosed with T2DM and over 90% of people with obesity develop NAFLD [3]. Taking into the account the increasing rate of obesity and diabetes prevalence from around 12.5% of adults in 2020 to 15.4% in 2030 and 20.9% by 2050, it is estimated that NAFLD will become the most common cause of chronic liver diseases worldwide [3].

Obesity is often interconnected with elevated risk of hypertension and dyslipidemia and CVDs complications [4]. A study by Crisostomo T. et al., published in this special issue, demonstrated that the cardiovascular, hepatic and renal alterations during early stages of obesity (after 10% increase in the body mass) in rats treated with high-lipid (HL) diet during their juvenile age, can be reversed after administration of 4 doses (80 mg/kg) of angiotensin (Ang) [3,4] (Val-Tyr) at 12h intervals. Elevated hepato-renal index along with increased systolic and diastolic hypertension after HL diet treatment was reversed in rats that received Ang-(3–4) through inhibition of Ang-(3–4)-sensitive Ang II→AT1R axis as well as RAAS-mediated alterations in renal Na + -transporting ATPases [5].

NAFLD can be described, at least in part, as a hepatic expression of metabolic syndromes. Given that, Zarghamravanbakhsh P. et al., gave a comprehensive review of the literatures on metabolic causes and consequences of NAFLD. Beside describing the impact of obesity, T2DM, genetics, and microbiome dysbiosis, on the initiation and progression of different stages of NAFLD, data regarding the cause of ''lean'' NAFLD has been widely explored by the writers. While not enough attention has been given to the "lean" NAFLD in both research and review articles, highlighting its frequency and the involvement of metabolic factors such as insulin resistance (IR) in the pathogenesis of lean NAFLD are an added value to this review article [6].

The hepatic IR observed in lean NAFLD have been associated with aberrant expression of Fibroblast growth factor 21 (FGF21), a hepatokine, and consequent activation of p38a. New findings indicate that FGF21 overexpression and "FGF21 resistance" along with activation of p38, in the liver can result in severe liver steatosis while loss of p38a protect against its development. It appears that increased hepatic p38 activation leads to induced hepatic FGF21 secretion and consequence liver derived FGF21- induced lipolysis in the adipose tissue. This mechanism has been proposed as a most likely explanation of lean phenotype observed in non-obese NAFLD as highlighted by an commentary article by Liu J. and Dalamaga M [7]. based on a study by Hao Ying and colleagues published in Diabetes [8].

Increased inflammation and oxidative and ER-stress observed in NAFLD patients has been associated with Zinc deficiency. The review by Barbara M. and Mindikoglu A.L, have summarized the mechanistic link between zinc deficiency and NAFLD progression and highlighted the potential role of zinc supplementation for NAFLD treatment. Additionally, they have collected data from both human and animal studies indicating association between NAFLD risk factors, specifically; diabetes mellitus, obesity, hypertension and dyslipidaemia and reduced blood zinc levels. Interestingly, zinc supplementation showed beneficial metabolic effects in people with the NAFLD risk factors [9].

The role of impaired autophagy in the progression and development of NAFLD has been widely studied, however, the mechanism remains un-elucidated [10]. During recent years scientist attention has been dragged towards the role of Sterol Regulator Elementing Binding Protein 1c (SREBP1c) on regulation of autophagy during various pathogenic conditions especially those developed in high glucose/high lipid environments [11,12]. An article by Sozen E. et al., published in this special issue revealed that autophagy was increased after SREBP1c silencing of oleic acid (OA) and non-OA treated AML12 hepatocytes. Interestingly, SREBP1c silencing resulted in increased peroxisome proliferator-activated receptor alpha (PPAR-α) mRNA levels and reduced size and number of lipid droplet (LD), indicating the crucial role of SREBP1c and PPAR-α in autophagy-induced LDs catabolism [12]. As recently autophagy-modulating drug draw massive attention for treatment of NAFLD, this study shed light on SREBP1c silencing as a potential therapeutic target.

Apart from the well-recognized underlying mechanism of NAFLD pathogenesis, alterations in mRNA splicing may count as significant causal factors for development of several metabolic-related components such as NAFLD [13]. In fact, alternative RNA splicing may generate many different proteins which influence the function and expression of more than 70% of human genes. In a commentary article by Dalamaga M. and Liu J. the key points of a recent and interesting study by Li Y. et al. published in Cell Metabolism [14] have been summarized. A crucial role of death-associated protein kinase-related apoptosis-inducing kinase-2 (DRAK2), identified as a nuclear apoptosis promoter belonging to the death-associated protein kinase (DAPK) family, in mRNA alternative splicing has been revealed by comprehensive phosphoproteome and transcriptome analyses [15]. DARK2 knockdown in the liver or hepatocyte-specific DRAK2 deficiency in mice exert beneficial effects on HFD-induced hepatic steatosis and HF/high-cholesterol in addition to high fructose (HFF) diet-induced hepatic steatohepatitis. Furthermore, by performing transcriptomic exonic analysis, they

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realized that DRAK2-SRSF6 (Serine And Arginine Rich Splicing Factor 6) signalling pathway regulates the alternative splicing of mitochondrial function-related genes involved in NAFLD progression indicating the potential of DRAK2-SRSF6 as a therapeutic target for treatment of NAFLD [14,15].

Hepato-protective effect of natural origin compounds are promising therapeutic tools in managing NAFLD. As such Anethum graveolens (dill), is a herbal-derived cholesterol lowering product [16]. In order to evaluate the beneficial effect of dill on liver cholesterol 7 alpha-hydroxylase and liver fat accumulation in rats, Abbasi E. et al., examined the effect of daily treatment with dill tablet or dill extract (300 mg/kg) on HC-HF induced NAFLD rat models. Thirty days of treatment with both dill tablet and extract significantly decreased serum cholesterol, TG and LDL-C levels and increased HDL-C levels, improved liver histology and exert an anti-oxidant levels through reduction of malondialdehyde (MDA) levels. This study indicates that dill treatment significantly reduced liver fat and hepatic oxidative stress, at least in part, through increased mRNA and protein levels of cholesterol 7 alpha-hydroxylase (CYP7A1) enzyme. Additionally, dill treatment reduced foam cells formation, necrosis and other morphological changes observed in the liver of HC-HF treated rats. Of note, overexpression of CYP7A1 led to reduction of hepatic oxidative stress, inflammation, cholesterol levels and fibrosis in NAFLD animal models [16].

Silymarin (milk thistle) is also a powerful anti-oxidant agent of natural origin known to counteract the pathophysiological alterations caused by elevation of hepatic oxidative stress. Based on existing data, Silymarin is able to exert hepato-protective effects on intact liver cells or cells which have not been yet irreversibly damaged, highlighting the importance of early initiation of Silymarin therapy in patients with fatty liver diseases [17,18]. On an attempt to evaluate the effect of Silymarin on amelioration of NAFLD's severity among obese people before bariatric surgery as well as during bariatric surgery in the upper abdomen (for reduction of fat content and liver size), Mirhashemi S.H. et al., assigned fifty-two morbidly-obese patient candidates for bariatric surgery (mean age: 38.90 $\pm$ 10.28 years; n = 41 women and 11 men) with NAFLD to receive Silymarin supplementation (140 mg four times daily for a total of 560 mg) for period of 8 weeks. A significant reduction in both AST, ALT levels as well as AST/ALT ratio, BMI and number of patients with high sonographic grading ($p<0.05$) was found after Silymarin treatment without any adverse effects. Nevertheless, no significant effect was observed on fibroscan staging, Fib-4 and NAFLD scores. The findings of this study revealed that Silymarin supplementation for 8 weeks prior laparoscopic bariatric surgery can improve the technical challenges observed in liver retraction by calorie restriction as well as reducing BMI, providing adequate access to the gastro-esophageal junction [18].

Apart from evaluating the efficiency of natural compound in the alleviation of NAFLD, employment of new advanced technologies such as nanoparticle therapy for the treatment of NAFLD has attracted increasing attentions [2]. An article by Abbasi E. et al., indicated that treatment of both carbon tetrachloride (CCl4) and high-fat diet (HFD) rats with cerium oxide nanoparticles (CeO2 NPs) for 4 weeks (0.1 mg/kg, i.v. twice a week) results in normalizing the ALT, AST, ALP levels as well as alleviation of oxidative stress demonstrated by increased levels of hepatic intestinal total antioxidant capacity (TAC) and glutathione (GSH). Moreover, CeO2 NPs treatment reduced the elevated levels of malondialdehyde (MDA) and total oxidant status (TOS) in the liver, and intestine of the NAFLD and CCl4 group compared with control rats. Finally, the histomorphometry analysis revealed reduced inflammation, fibrosis and necrosis in the intestine and liver of NAFLD and CCl4–treated rats treated with NPs as compared to the control group, indicating their ability for being considered as a potential therapy for the treatment of liver disease [19].

So far, liver biopsy's is a gold standard for diagnosis of liver fibrosis severity. Evaluation of the degree of hepatic fibrosis is the most important factor in determining the risks associated with NASH progression. However, given the high prevalence of disease, pursuing each patient for the liver biopsy is not a pragmatic clinical approach. Apart from that, high resources necessary for biopsies along with pain, infection, bleeding, pneumothorax in patients can be count as limitations of this procedure [20,21]. These limitations highlight the need for development of simple, reproducible non-invasive methods for determination of the degree of fibrosis in NAFLD patients. A comprehensive review by Bernstein D. and Kovalic A.J. summarized the existing data on non-invasive methods used for NAFLD diagnosis. They have separated the data from existing literatures into 1) non-invasive serum biomarkers based on conventional testing, 2) non-invasive serum biomarkers targeting collagen turnover and extracellular matrix remodelling, 3) non-invasive imaging modalities and 4) combination, sequential, and algorithmic testing. Additionally, this article provides the readers with a quick overview of data through various tables illustrating necessary information regarding the sensitivity, specificity and threshold of methods used in each study [21]. Due to the lack of feasibility to utilize liver biopsy regularly, easily accessible laboratory non-invasive tests such as NFS and FIB-4 currently serve as the non-invasive diagnostic tool for fibrosis assessment, however, no single test to identify steatosis, to early diagnose NASH, or to predict the disease progression is available. Therefore, there is an emerging need for development of accurate and adequate non-invasive assessment of hepatic fibrosis thus discovering the suitable non-invasive panels and strategies based on genetic, epigenetic, and transcriptomic modalities should be the aim for future clinical studies.

There is a bidirectional relationship between NAFLD and T2DM. More specifically, diabetes promotes NAFLD progression and increases the risk of NASH, cirrhosis and HCC development. On the other hand, patients suffering from NAFLD show an increased risk of developing T2DM [22]. A review article by Alam S. et al., highlighted the importance of monitoring liver function in patients with T2DM by measuring hepatic enzymes levels. In this study which was conducted on 386 T2DM patients from North India the levels of ALT, total bilirubin and ALP in T2DM were significantly higher compared to healthy control. Interestingly, 62.53% of T2DM subjects showed abnormal hepatic enzymates activities, emphasizing the importance of prompt diagnosis and management of abnormal liver parameters in DM for prevention of liver-related morbidity and mortality in the diabetic population [23].

Apart from the direct risk factors for the development of NAFLD such as obesity and T2DM, obstructive sleep apnoea (OSA) is a known independent risk factor. It has been shown that hypoxia observed during OSA regulates the expression of genes involved in triacylglycerol (TAG) and lipid metabolism in mouse through heterodimeric transcription factor known as hypoxia-inducible factors (HIFs). HIFs control many cellular processes such as energy metabolism and inflammation, both involved in the pathogenesis of NAFLD, through regulation of the expression of genes involved in the pathogenesis of NAFLD [24]. An article by Hazlehurst J.M. et al., shed light into the effect of acute model of intermittent hypoxia in human hepatoma cell lines, male Wistar rats and in healthy male volunteers. This study revealed that increased hepatic *de novo lipogenesis* (DNL) in humans and rodent models is associated with the exposure to intermittent hypoxia (AIH) in a HIF-dependent manner. Exposure of cells with AIH (1% O2, 24 h) had an effect on hepatic DNL but not on the β-oxidation indicated by increased expression of regulatory element-binding protein 1 (SREBP1), fatty acid synthase (FASN) with no effect on adipose tissue lipolysis factors [25]. As the exact mechanism underlying development of NAFLD in each individual is still unclear, this study revealed a possible role of HIFs on this processes and its potential for treatment of NAFLD in patients suffering from metabolic disorders related-OSA.

The main goal of this special Issue "NAFLD: from molecular basis to therapeutic advances'', of the ''Metabolism Open'', was to gather and highlight selection of original basic, clinical and translational research articles investigating the epidemiology and risk factors, pathophysiological mechanisms and diagnostic techniques (biomarkers) of

Nonalcoholic Fatty Liver Disease/Metabolic Associated Fatty Liver syndromes and examining potential novel molecular and cellular targets used for prevention and treatment of NAFLD. We hope that the contribution of the world experts in this field in this special issue has provided valuable resources to both clinicians and basic scientists.

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