OVERLAPPING CLINICAL FEATURES BETWEEN NAFLD AND METABOLIC SYNDROME IN CHILDREN

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is a cluster of pathological liver conditions of emerging importance in overweight and obese children. NAFLD is associated with central obesity, insulin resistance, and dyslipidaemia, which are considered to be the main features of metabolic syndrome (MetS). Prevention of the adverse outcomes of NAFLD, as well as the risk of MetS, depends on the identification of genetic background and environmental factors that modulate susceptibility to these diseases. However, several lines of evidence highlight the strong correlation and co-currency of these two chronic diseases, both in children and in adults. In the present review, we provide an overview of the current clinical proofs on the link between NAFLD and MetS in children, with particular focus on all the possible overlapping features that connect them at paediatric age.

<u>Keywords</u>: NAFLD, MetS, adipocytokines, gene polymorphisms, obesity.

INTRODUCTION

The global prevalence of overweight and obesity has considerably increased in several industrialised countries over the past 20 years. In fact, based on the body mass index (BMI) definition, approximately one-third of the world's population is considered overweight (BMI 25-29.9 kg/m^2) or obese (BMI ≥30 kg/m^2). The International Obesity Taskforce (IOTF) estimates that around 1 billion adults and 150 million school-aged children are overweight, while around 475 million adults and 50 million children are classified as obese.[1] The 'obesity epidemic' is particularly relevant in some geographic areas (US, Europe, Australia) where more than 30% of children are obese. It is currently estimated that the continuation of this increasing trend will lead to an incidence of around 60% overweight/obesity in the worldwide population, with several associated early and long-term effects, including metabolic syndrome (MetS).[2] However, it is the presence of abdominal or 'central' obesity (CO), coupled or uncoupled to insulin resistance (IR), that constitutes a critical key risk factor for MetS in children.

Although there are some divergent opinions about the definition of paediatric MetS, it is widely accepted that it is characterised by a cluster of crucial metabolic components including CO, dyslipidaemia (high levels of triglycerides [TGs] and low-density lipoprotein cholesterol, and low levels of high-density lipoprotein cholesterol), hypertension, and IR.[3] In addition to the co-occurrence of these traits, the presence of a fatty liver, configuring to non-alcoholic fatty liver disease (NAFLD), identified by ultrasound, has been recently linked to MetS.[4-6] In fact, NAFLD is becoming one of the most important complications of childhood obesity, affecting approximately 3% of normal children and up to 80% of obese individuals, particularly in industrialised countries.[7]

The recent clinical implications of the long-term effects of MetS and NAFLD on liver cancer and cardiovascular disease development have highlighted the relevance of a full characterisation

of specific signs of these diseases that may appear early in life. Understanding the real role of contact-points between NAFLD and MetS development and progression may help with early identification of patients at risk and of those with some pathological traits; this could then help in the design of short and long-term personalised management programmes for child populations. The present review discusses the current evidence of the link between NAFLD and MetS in children, and addresses the overlapping features that make NAFLD and MetS two sides of the same coin.

NAFLD IN CHILDREN

NAFLD in children includes different patterns of liver diseases assessed by liver biopsy. The intra-hepatic accumulation of fat is defined alone as simple steatosis or non-alcoholic fatty liver (NAFL), whereas if it co-exists with various degrees of necrotic inflammation (lobular and portal inflammation) and ballooning degeneration, it is defined as non-alcoholic steatohepatitis (NASH).[8] In children, like in adult settings, this severe form of NAFLD may be coupled with the presence of long-standing mild-to-severe liver fibrosis.[9] Unfortunately, as published data with long-term follow-up are scarce, the natural history and prognosis of paediatric NAFLD are still uncertain. In susceptible individuals, NAFLD can evolve to cirrhosis and hepatocellular carcinoma, with the consequent need for liver transplantation even though this phenomenon is rare in children.[10] In fact, only a minority percentage of children, suffering from hepatic steatosis, progress to NASH and cirrhosis.[11]

Conventionally, the presence of steatosis in >5% of hepatocytes in the pathological section is considered to be the necessary criterion for NAFL diagnosis. However, adults and children display a different pattern of histological NAFL and NASH damage, making the paediatric form a distinct disease that requires a personalised in-depth evaluation and analysis. It is now widely accepted that the major predisposing risk factors to paediatric NAFLD, as well as for the adult form, are represented by obesity, visceral adiposity, IR, and other disorders, including glucose and lipid homeostasis deregulation, that define MetS. Therefore, to date, NAFLD is firmly considered as the hepatic manifestation of MetS, and several clinical and pathogenetic overlapping features have been found between these two diseases in child populations.

Overlap between NAFLD and MetS

Because of the many different definitions used to diagnose MetS, its prevalence in children ranges between 0-60%. However, despite this epidemiologically wide range, the definition by the International Diabetes Federation (IDF) highlights that obesity is an essential criterion, IR is a prerequisite, and dyslipidaemia is the most frequent metabolic derangement.[12] Furthermore, a simplification of the IDF definition highlights that waist circumference, considered as percentiles rather than absolute values, should represent the main component of MetS in children and adolescents.[13] Therefore, to date, paediatric MetS is differently defined by three age-groups: 6-10 years, 10-16 years, and ≥16 years (considered as adults).[14]

Of note, CO, IR, and dyslipidaemia are considered to be the most prevalent risk factors associated with NAFLD development, providing strong proof of a cross-correlation between MetS and liver damage occurring in liver disease. In paediatric NAFLD the connections with CO (defined by an apple shape), IR, and dyslipidaemia are described in several clinical studies. Despite the multifactoriality of both diseases, their strong association may be explained by a common genetic susceptibility and an analogous pattern of low-grade inflammatory circulating adipocytokines (Figure 1).

Clinical Evidence of Paediatric MetS and NAFLD Connection

One of the first lines of evidence that associates NAFLD with MetS in children is provided by a retrospective review including 43 American children with biopsy-proven NAFLD, which demonstrated that approximately 95% of patients were obese and 95% were insulin-resistant as assessed by BMI and homeostasis model assessment of IR (HOMA-IR).[15] A few years later, Manco et al.[16] performed a cross-sectional study on 197 Caucasian children with NAFLD, highlighting that 92% and 84% of these patients presented a BMI >85th percentile and waist circumference ≥90th percentile, respectively. Furthermore, these authors also demonstrated that CO measured by waist circumference was strongly associated in this cohort of children. This significant association was confirmed by a case-control study on 300 overweight/obese children (150 with biopsy-proven NAFLD and 150 without).[17] This study reported that children with MetS traits had five-times the odds of having NAFLD compared to age-matched obese children without MetS.
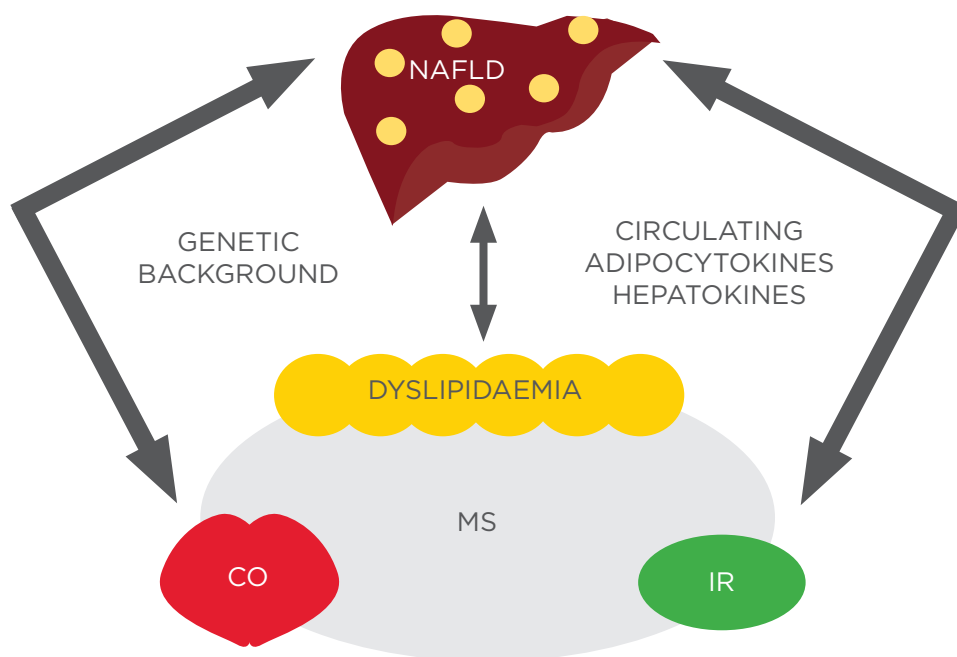
Figure 1: Schematic representation of NAFLD/MetS nexus.
NAFLD: non-alcoholic fatty liver disease; CO: central obesity; MS/MetS: metabolic syndrome; IR: insulin resistance.

Due to the escalation of NAFLD and MetS in children over the last few decades, we have witnessed an increasing amount of attention on this hot-topic from worldwide clinicians and national health care systems.

A cross-sectional study conducted on 1,107 Iranian children and adolescents (6-18 years) demonstrated that overweight or abdominal obesity was the most sensitive predictor of paediatric NAFLD assessed by surrogate markers (i.e. alanine aminotransferase, ALT) and ultrasound.[18] More recently, 254 children enrolled in the Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN) were included in a retrospective study that confirmed not only a prevalence of MetS in NAFLD patients with respect to general population, but also a significant association of CO and IR with the histological severity of liver damage (i.e. fibrosis).[19] Accordingly, a UK cross-sectional descriptive study reported that 34 out of 216 obese children presented increased levels of ALT and other traits of MetS, including elevated BMI and alterations of glucose metabolism.[20] Furthermore, Fu et al.[21] demonstrated that, among 861 Chinese obese children, 68.18% had NAFLD and 25.67% had MetS, and an overlap between features of the two diseases was found in 84.61% of subjects. Therefore, NAFLD should be considered as an early mediator that mirrors MetS status, which could be screened by liver ultrasound.

Finally, a recent study by Silveira et al.[22] has well documented that intra-abdominal fat positively correlates with NAFLD and MetS in children. They showed that intra-abdominal fat was positively correlated with NAFLD (p=0.005), MetS (p=0.013), dyslipidaemia (p=0.001), and HOMA-IR (p=0.007) in 180 subjects aged between 6 and 16 years. Further evidence of the NAFLD-MetS connection highlights that other MetS-related features, including type 2 diabetes, dyslipidaemia, and albuminuria could be at the cross-road between metabolic homeostasis imbalance and liver damage in children.[23-25]

Genetic Polymorphisms in Paediatric NAFLD and Susceptibility to MetS

The escalation of paediatric NAFLD and MetS worldwide prevalence is partly due to over-nutrition and a sedentary lifestyle, which characterises urban adolescents and children.[26] However, the aetiology of MetS and its contribution to NAFLD is complex and is closely related both to lifestyle and genetic predisposing factors.[27] Several potential single nucleotide polymorphisms (SNPs) in genes have been studied in children with NAFLD. These SNPs include: the polymorphism of a gene coding

for the Kruppel-like factor 6 (KLF6), which is associated with fibrosis; the polymorphism of a gene coding for insulin receptor substrate-1 (IRS-1), which is associated with fibrosis; the polymorphism of a gene coding for adiponutrin/ patatin-like phospholipase domain-containing 3 (PNPLA3), which is associated with the severity of different histopathological features of NASH (i.e. steatosis, ballooning, inflammation, and fibrosis); the polymorphism of a gene coding for manganese-dependent superoxide dismutase (SOD2), which is associated with liver fibrosis; and the polymorphisms on LPIN1 gene (coding for Lipin-1), which displays an inverse association with disease severity.[28-32] Recent studies demonstrated that the presence of one or more of these SNPs may predispose children to the more severe forms of NAFLD (e.g. NASH and fibrosis), and interestingly, homozygosity for the 148M PNPLA3 allele is associated with a lower response to therapy with docosahexaenoic acid.[33,34]

It is now widely accepted that the components of MetS are also strongly inherited.[35] In fact, data from numerous studies provided challenging evidence suggesting that gene-environment interactions (i.e. the modulation by a genetic polymorphism of a dietary component effect on a specific phenotype) could interact in a way that increases susceptibility to MetS.[36] During the last 5 years numerous genome-wide association studies identified in children many SNPs associated with a large number of conditions related to obesity and traits of MetS per se.[37-39] Although all of these SNPs potentially affect the metabolic function of encoded proteins that may also predispose to more severe NAFLD, until now, there has been no evidence of their association with hepatic damage in children.

Few concerted efforts have been made to investigate SNPs that may recognise subjects with a simultaneous high risk for MetS and NAFLD in children. A recent study, conducted on 250 NAFLD and 200 healthy Chinese children aged between 6 and 16 years, demonstrated that the rs1800849 variant of uncoupling protein 3 (UCP3) gene is associated either with MetS traits (elevated BMI and waist circumference) or increased risk of NAFLD.[40] Furthermore, Nobili et al.[41] recently demonstrated that the severity of obstructive sleep apnoea (OSAS) was associated with the presence of NASH and with the severity of histological necroinflammation and fibrosis, independently of

CO, IR, and MetS. However, as OSAS is associated with increased risk of MetS and higher plasma levels of fatty acid binding protein 4 (FABP4), the presence of selective SNPs in the gene encoding for this protein could explain the OSAS common trait in children with MetS and NAFLD.[42] In order to prevent the adverse outcomes of NAFLD, as well as the risk of MetS, the identification of genetic susceptibility profiles for these diseases and their severe patterns (e.g. hepatic fibrosis and cardiovascular disease) will be crucial in designing and testing multi-panels of SNPs as non-invasive markers.

Low-Grade Inflammatory Circulating Adipocytokines

The link between NAFLD and IR in children is now a widely recognised fact, even though the causal/effect relationship between them is still a matter of debate.[6,22,25,43] However, accumulating evidence has demonstrated that NAFLD and IR are strongly associated with low-grade inflammation characterised by the release of circulating adipocytokines.[44]

Adipocytokines such as tumour necrosis factor-α (TNF-α), interleukin 6 (IL-6), adiponectin, leptin, and resistin, which are synthesised and secreted by adipose tissue to regulate energy balance, glucose homeostasis, and insulin sensitivity, seem to be critical mediators of the pattern of low-grade inflammation that often characterise subjects with IR and NAFLD.[45] Therefore, it is not surprising that circulating levels of adipocytokines have also been considered as overlapping features in children. In fact, Nobili et al.[46] demonstrated that values of fasting serum leptin increased concomitantly to steatosis, inflammation, ballooning, and fibrosis worsening, suggesting that hyperleptinaemic status observed early in NAFLD children could be a precondition for promoting IR, overweight, and obesity. Interestingly, 3 years later Lebensztejn et al.[47] found that adiponectin and resistin negatively correlated with grade of liver steatosis at ultrasound, suggesting a protective anti-inflammatory role of these two circulating molecules. However, the same authors demonstrated that only hypoadiponectinaemia was significantly contemporaneously connected with a reduced NAFLD and IR. As adiponectin and leptin control the expression and secretion of TNF-α and IL-6, it is not surprising that these adipocytokines may also be associated with IR and NAFLD.[43]

Although the real role of these two adipocytokines in paediatric NAFLD is still under investigation, a recent study demonstrated that a meal high in saturated fat induced postprandial dyslipaemia, hyperinsulinaemia, and altered lipoprotein expression and low-grade inflammatory profile in obese children with and without NAFLD.[48] Finally, the potential role of adipocytokines as biomarkers for both paediatric NAFLD and IR has been confirmed by three more recent studies, even though discrepancies among the specificity and sensibility of the single mediators as tags for the severity of disease have emerged.[49-52] In addition to the most studied adipocytokines, the circulating levels of retinol-binding protein 4 (RBP4), which is associated with IR pathogenesis, also present an inverse correlation with the degree of liver damage in children with NAFLD.[53] On the contrary, very recently Boyraz et al.[54] demonstrated that RBP4 levels positively correlated with ALT and NAFLD at ultrasound in 63 obese children.

It is plausible that the apparent divergences among the adipocytokines profile and NAFLD/IR association could be ascribable to a different ethnic-dependent pattern distribution of polymorphisms in genes encoding for these molecules. Further multicentre studies that evaluate profiles of circulating adipocytokines and exome-sequencing of the related SNPs to define their nexus with NAFLD and IR co-occurrence in children are needed.

Dual Role of the Hepatokines

In addition to an inflammatory response, the steatotic liver may also contribute to an altered pattern of release of other circulating factors known as hepatokines, directly affecting metabolism and contributing to MetS.[55] Among the hepatokines, two - including fetuin-A, fibroblast growth factor 21 (FGF21), and insulin-like growth factors (IGFs) I and II - could be important as potential non-invasive biomarkers and have been suggested as promising therapeutic targets for MetS and/or NAFLD in children. In fact, Reinehr et al.[56] demonstrated that circulating levels of fetuin-A, which inhibits tyrosine kinase activity of hepatocellular insulin receptor, were higher in NAFLD children and (in these subjects) were also related to MetS features, including IR. Furthermore, Reinehr et al.[57] detected higher values of circulating levels of FGF21 in obese children than in normal-weight children. Despite this, the study demonstrated no association

between FGF21 levels and NAFLD, while recently an inverse correlation of this hepatokine with hepatic damage in obese children with NAFLD was reported, suggesting its potential dual role in metabolic and hepatocellular damage.[57,58]

It has become apparent that IGFs may influence not only growth, but also protein, carbohydrate, and lipid metabolism, thus protecting individuals from several MetS features.[55] Interestingly, Cianfarani et al.[59] very recently linked the decreased levels of IGF I and II not only with IR but also with more severe degrees of steatosis, inflammation, and ballooning in paediatric patients with NAFLD. All these studies suggest that although the mechanisms that could explain a potential dual role of hepatokines in paediatric NAFLD and MetS remain fully elucidated, these circulating molecules could represent novel markers of liver and metabolic damage progression.

CONCLUSION

Clinicians need to be aware that to contain the evolution of MetS and NAFLD in children, it would be necessary to diagnose the disease as soon as possible, and particularly before the occurrence of related organ damage (i.e. liver fibrosis and cardiovascular disease). Furthermore, it has recently emerged that NAFLD co-occurs with MetS signs (including elevated BMI and increased triglyceride levels) after the first 5 years of liver transplantation MetS, which probably affects post-transplant survival of patients.[60] Finally, it is interesting that in a recent case-report a non-obese child with acute lymphoblastic leukaemia, preconditioned with total body irradiation before bone marrow transplantation, developed early hepatic steatosis, mild hypertriglyceridaemia, and IR, suggesting that a risk of MetS and NAFLD combination should also be monitored after cancer-related short and long-term treatments that alter MetS-related features.[61]

Childhood cancer survival is now excellent for certain malignancies in which total body irradiation treatment is a mainstay treatment. Therefore, the oncologists should consider a follow-up that includes evaluation of all possible *de novo* metabolic effects that could exacerbate MetS and NAFLD phenotypes. In this context it becomes very relevant to understand the pathogenic connections between MetS features and NAFLD development in the paediatric population,

either for establishing the primary determinant, and/or for extrapolating possible background predisposing conditions that, in the presence of other aetiological environmental factors (i.e. lifestyle), may promote severe liver damage and cardiovascular disease in adulthood.

Therefore, there is a need for new and sensitive early screening methods that are able to provide a large-scale of information about subjects at risk or who are presenting early signs of MetS and NAFLD. These methods could include: the analysis of polymorphism patterns on certain genes encoding for pathway regulatory molecules involved in IR and for circulating mediators associated with a low-grade inflammatory state; and/or the quantitative assessment of plasma circulating mediators that pathogenetically link NAFLD to MetS. In the near future, for patients whose SNPs and circulating profiles are known, it would be possible to draw-up a personalised prevention programme. In fact, the plethora of novel clinical/biological information extrapolated by this type of study could also have a strong impact on the evaluation of the therapeutic properties of drugs currently used and those being tested.

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