Paediatric Non-Alcoholic Fatty Liver Disease (NAFLD)

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Outline of Talk

- Epidemiology
- Natural history
- Risk factors
- Pathogenesis
- Diagnosis
- Therapeutic options
Non-Alcoholic Fatty Liver Disease

- Emerging as a leading cause of chronic liver disease in children
  - 3-10% in general paediatric population
  - 80% in obese children
- Mirrors worldwide annual increment in obesity
- National Health Nutritional Examination Survey
  - 17% all children in Western countries are obese
Non-Alcoholic Fatty Liver Disease

Definition: Hepatic fat infiltration >5% hepatocytes assessed by liver biopsy

In the absence of:

• Viral, autoimmune, metabolic or drug-induced liver disease
• Excessive alcohol intake

Spectrum of disease

• Steatosis
• Steatohepatitis
• Fibrosis / Cirrhosis

Paediatric NAFLD histopathology has distinct characteristics compared to adults

- 2 different phenotypes – adult and paediatric
- Different pathogenesis

Associated with similar metabolic impairments as in adults

- Insulin resistance
- Hypertension
- Central abdominal obesity

→ Increased risk of Type 2 Diabetes Mellitus, metabolic syndrome, cardiovascular disease
Non-Alcoholic Fatty Liver Disease: Histopathology

2 histological patterns of NASH in children

Type 1: Adult type

• Less common and tends to occur in girls

• Classical histological findings
  - steatosis
  - ballooning → increased risk of disease progression to NASH
  - inflammation
  - fibrosis

• Steatosis in zone 3

• Lobular inflammation, ballooning and peri-sinusoidal fibrosis

World J Gastroenterol 2010;16(42):5286
Non-Alcoholic Fatty Liver Disease: Histopathology

Type 2: Paediatric type

- More common and tends to occur in boys
- Zone 1 (periportal) steatosis or panacinar steatosis
- Portal inflammation
- Ballooning uncommon
- Portal fibrosis

Paediatric NAFLD Histological score

- Steatosis (0-3)
- Ballooning (0-2)
- Lobular inflammation (0-3)
- Portal inflammation (0-2)
Determine the Severity of NAFLD:
Presence of NASH

Type 1 NASH (Adult)

Type 2 NASH (Paediatric)

Inverse Relation of Classic NASH and Type 2 NAFLD With Age

Non-Alcoholic Fatty Liver Disease: Epidemiology

Data from North America, Europe, Asia, South America and Australia

- Paediatric NAFLD prevalence: 3-10%
  - influenced by population characteristics especially lifestyle habits and diagnostic methods

- Liver biopsy gold standard
  - not feasible to detect disease prevalence

- Screening tests all have diagnostic limitations
  - BMI
  - ALT
  - Ultrasound

Non-Alcoholic Fatty Liver Disease: Epidemiology

NHANES III (USA population-based field report)

- Prevalence of ALT >30 IU/l in adolescents
  - 7.4% Whites
  - 11.5% Mexican-American
  - 6% Blacks
- ↑ALT: 12.4% males vs 3.5% females

Similar data from South Korea and Japan

Prevalence of NAFLD in adolescents at least 2.6 - 3.2%
- Probable underestimate as surrogate markers used for diagnosis

Prevalence of Steatohepatitis based on ALT levels in Children

- Comparison of children with suspected NAFLD and normal or mildly elevated ALT (n = 91) vs children with elevated ALT (n = 392)
- Plasma ALT may underestimate liver injury in NAFLD

Non-Alcoholic Fatty Liver Disease: Natural History

- Natural history and prognosis in children uncertain
  - very little long-term published data

- Documented progression to cirrhosis and hepatocellular carcinoma
  - need for liver transplantation

- Diagnosed as early as 2 years and cirrhosis as early as 8 years

- Genetic and environmental factors play a role
  - development and progression of disease

- Progression to endstage liver disease can occur over 10-20 years

Non-Alcoholic Fatty Liver Disease: Natural History

- **66 children with NAFLD (mean age 13.9 yrs) - 20 yr follow-up** - total of 409.6 person-yrs follow-up - Retrospective hospital-based cohort study

- Metabolic syndrome present in 19 (29%) at time of NAFLD diagnosis

- **55 (83%) presented with at least one feature of the metabolic syndrome** incl obesity, hypertension, dyslipidaemia, hyperglycaemia

- 13 liver biopsies (5 pts) over a mean of 41.4 mths - progression of fibrosis stage in 4 children

- **Follow-up: 2 died and 2 underwent Liver Tx for decompensated cirrhosis** - NAFLD recurred in allografts; ReTx for cirrhosis (1 pt)

- **Tx free survival significantly shorter in NAFLD cohort** compared to expected survival in general US population - same age and sex (log-rank test, p<0.00001), with standardised mortality ratio 13.6
Paediatric NASH: Multiple Risk Factors

Male sex/age
Race/ethnicity
Family history

Intrauterine environment
Early infant feeding

Gut microbiome

Sedentary lifestyle
Obesity

Gene SNPs
PNPLA3
TNF
IL-6, KLF-6, INSIG

Dietary
Higher saturated fatty acids/cholesterol
Lower PUFA, antioxidants, zinc, and fiber
More meat and soft drinks
Fructose

Non-Alcoholic Fatty Liver Disease : Risk Factors

Obesity main risk factor for paediatric NAFLD

• 80% prevalence in obese children (USA, Europe, Japan)

Population-specific based study in Europe

• 111 paediatric obesity centres (Germany, Austria, Switzerland)
• 16,390 overweight, obese and morbidly obese children
• NAFLD defined by AST and/or ALT >50 ULN
• NAFLD 11% study population
  - M : F = 14.4 : 7.4 %
  - Obese vs extremely obese 9.5% : 17%

Japan : 219 children (6-12yrs) : NAFLD

• 3% normal weight, 25% overweight and 76% obese children

Non-Alcoholic Fatty Liver Disease: Risk Factors

Metabolic syndrome (strong association)

- Insulin resistance
- Type 2 diabetes mellitus
- Hypertriglyceridaemia/hypercholesterolaemia (20-80% children with NAFLD)

NAFLD increases risk of cardiovascular disease in adulthood

- Increased carotid intimal medial thickness (marker of atherosclerosis) in children with NAFLD

  → greater risk of atherosclerosis and future CVS events

Insulin resistance more severe in NASH than in simple steatosis

Non-Alcoholic Fatty Liver Disease : Risk Factors

Age

- Can occur in young children
- More prevalent in adolescents
  - sex hormones and insulin resistance in puberty
  - lifestyle – fast foods and sedentary lifestyle

Gender

- Male : Females: 2:1
  - ? oestrogens are liver protective
  - ? androgens aggravate NASH
  - ? role of alcohol

References:
Non-Alcoholic Fatty Liver Disease: Risk Factors

**Ethnicity**

- More common in Hispanic than Caucasian and Afro-American children
- Ethnic differences
  - higher rates of insulin resistance
  - ↑ visceral adiposity at equivalent BMI
- Socio-economic factors
  - type of diet
  - exercise
- Afro-American children - more risk factors for NAFLD
  - Obesity
  - IR
  - Type 2 DM

Only minority of patients with NAFLD → NASH

- Complex interplay between environmental and genetic factors
- 2 cohort studies and 1 community based study in different ethnicities
  - suggested 35-40% NAFLD patients have genetic predisposition
    (adjusted for age, gender and BMI)
- 1\textsuperscript{st}, 2\textsuperscript{nd} and 3\textsuperscript{rd} generation relatives demonstrate abnormally high fat fractions (MRI) relative to BMI

- **Genes associated with energy balance**
  - adiponutrin/patatin-like phospholipase domain-containing 3 (PNPLA-3)
  - apolipoprotein C3 (APOC3)

- **Genes involved in inflammation, oxidative stress and fibrogenesis**
  - SOD2

Associated with NAFLD and severity of liver injury

Genetic Variants Influence Developmental Susceptibility to Paediatric NAFLD and NASH

- Analysis of genetic background may identify susceptible children
- Discoveries of pathogenetic mechanisms may lead to therapeutic options

- SNPs – altered gene expression or protein function

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<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>SNP</th>
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<td>rs738409 C&gt;G</td>
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<tr>
<td>GCKR</td>
<td>Glucose reuptake, lipogenesis</td>
<td>rs738409 C&gt;T</td>
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<td>Antioxidant response</td>
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<td>Insulin signaling</td>
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<td>LPIN1</td>
<td>Lipogenesis, adipogenesis</td>
<td>rs13412852 C&gt;T</td>
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Non-Alcoholic Fatty Liver Disease: Pathogenesis

Complex interplay: Visceral adipose tissue, Gut, Liver and Immune system in genetically susceptible individuals

In obese patients with NAFLD, adipose tissue IR influences histologic and metabolic defects
Metabolic Consequences of NAFLD
Diagnosis and Management important

↓ Insulin clearance  ↑ Insulin resistance  ↑ Glucose production  ↑ Cytokines (systemic inflammation)

Hyperinsulinemia  Type 2 diabetes  Atherogenesis

↑ TG/ ↓ HDL  ↑ Apo-B

Heart disease: ↓ ATP generation  Lipotoxicity  Ischemia  Diastolic dysfunction

Myocardial dysfunction

Cardiovascular disease

Pediatric NAFLD Clinical Practice Guidelines

Biannual screening (ALT & AST) in children with:

• BMI ≥ 95th percentile

• BMI between 85th and 94th percentile for age & sex with other risk factors:
  - Metabolic syndrome
    ~ Insulin resistance: acanthosis nigricans
    ~ Type 2 diabetes: Fasting blood glucose, HBA1C, family history
    ~ Central obesity and other features of metabolic syndrome
  - Obstructive sleep apnea
  - Nocturnal BP on retinal microvasculature - retinopathy
    ~ positive relationship between hepatic fibrosis & degree of retinopathy

Acanthosis Nigricans: Best Predictor of Insulin Resistance in Paed NAFLD

• ALT 25 IU/L in boys and 22 IU/L in girls

NASH in Children With OSA Often Associated With Worse Liver Fibrosis

- Prospective study of obese children (N = 25) aged 10-18 yrs with liver biopsy–proven NAFLD

Limited Value of AST or ALT to Establish NASH Severity in Children: NAS

- Prospective study of 176 children with NAFLD and available liver biopsies

Limited Value of AST or ALT to Establish NASH Severity in Children: Fibrosis Stage

- Prospective study of 176 children with NAFLD and available liver biopsies

Non-Alcoholic Fatty Liver Disease: Diagnosis

- Exclusion of other liver diseases
- Exclusion of other causes of steatosis
- **Liver biopsy is gold standard, but invasive**
  - distinguish between NASH and simple steatosis
  - determine severity of disease
  - exclude AIH, Wilsons disease and metabolic diseases
  - before starting potentially hepatotoxic medication

- **Non-invasive tests**
  - anthropometric parameters: BMI, abdominal circumference
  - biochemistry (LFTs, fasting glucose/insulin/lipid profile), biomarkers
  - imaging/fibroscan/predictive models
    → together with ethnicity, gender, lifestyle and general health

Non-Alcoholic Fatty Liver Disease: Diagnosis

Autoantibodies

- ANF positive in 15.4%
- Anti-smooth muscle antibody positive in 10%
- Positive autoantibodies associated with higher fibrosis stages

Non-Alcoholic Fatty Liver Disease: Diagnosis

**Imaging**

**Ultrasound**
- Diagnostic sensitivity decreases when
  - hepatic steatosis <30%
  - BMI ≥40
- Cannot rule out steatohepatitis or fibrosis
- Overall sensitivity 60-94%, specificity 84-100%

**CT Scan**
- More specific
- Limitations of radiation exposure

**MRI**
- Greatest accuracy for determining fat content, but costly
# Noninvasive Liver Fibrosis Tests in Pediatric NAFLD

<table>
<thead>
<tr>
<th>Marker</th>
<th>Interpretation</th>
<th>Cost</th>
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</table>
| Transient elastography  | - TE value 5-7 kPa: F1  
                          | - TE value 7-9 kPa: F1-2  
                          | - TE value > 9 kPa: F3-4  | +++ |
| MR elastography         | - Liver stiffness value of 2.71 kPa gives sensitivity of 88% and specificity of 85% for $\geq$ F2 fibrosis | +++  |

302 children, mean age 12.3 ± 3.1 years, mean BMI percentile of 94.3 ± 6.9, and NASH present in 67%
PNPM AUROC : 0.737

242 children, mean age $12.4 \pm 3.1$ years and 15% had advanced fibrosis

AUROC 0.74

Performance of PNFS vs APRI, FIB-4 Index, NAFLD Fibrosis Score

- Single cohort of 242 children in Italy

Biomarkers: CK-18 for Prediction of NASH

Cytokeratin-18 fragments: Apoptosis by-product

CK-18 Levels in Children With vs Without NASH

Performance of CK-18 Level for Diagnosis of NASH

Non-Alcoholic Fatty Liver Disease: Prevention and Treatment

- **Limited knowledge of molecular pathogenesis**
  - cross-talk between gut, immune system and liver in genetically susceptible individuals

- **Multi-targeted approach**
  - diet and lifestyle modification
  - therapeutics: Insulin sensitisers, hepatoprotective agents, antioxidants

- **Decrease incidence of known risk factors**
  - individual and public health level

- **Good pregnancy care**
  - prevention of low birth weight infants
  - encourage breast feeding
Lifestyle Recommendations: Paediatric NAFLD

First step: Address obesity

- Weight loss of 10% for those at adult height
  - Weight maintenance otherwise

- Aerobic exercise (play) 60 min/day for 6 days/week at moderate intensity

- Less than 2 hours screen time/day

- Low refined sugar, low trans-fat, low GI fruits and vegetables

- Home-cooked meals with family engagement

Non-Alcoholic Fatty Liver Disease: Treatment

**Lifestyle modification ± anti-oxidant therapy**

- 53 children (5.7-18.8yrs) with biopsy proven NAFLD randomised to
  - lifestyle modification plus anti-oxidant therapy (a-tocopherol 600 IU/day and Vit C 500 mg/day vs lifestyle modification plus placebo for 24 mnths

- Both groups showed improvement in steatosis, lobular inflammation, ballooning and the NAS

- Improvement in ALT, TG, Chol, glucose, insulin and insulin sensitivity

- No additional benefit from anti-oxidant therapy

Hepatology 2008;48:119
Non-Alcoholic Fatty Liver Disease: Treatment

Pharmacotherapy: targeted IR or oxidative stress

TONIC study: Vitamin E

- Multicentre USA study – 8-17 year olds with NAFLD (N=173)
  - double-blind, placebo-controlled, randomized, multicenter phase II trial
  - compared efficacy of Vitamin E (800 IU/day) vs Metformin (500mg bd)
    vs placebo over 96 weeks (58 pts in each group)

- No difference in sustained ALT reduction in all 3 groups
  - 50% less than baseline

- Resolution of NASH
  - 58% (Vit E) vs 41% (Metformin) vs 28% (placebo)

JAMA 2011;305:1659
# Alternative Pediatric NAFLD Therapeutics

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<thead>
<tr>
<th>Therapy</th>
<th>Possible Target</th>
<th>Proposed Mechanism</th>
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<tbody>
<tr>
<td>Probiotics/antibiotics</td>
<td>Gut microbiome</td>
<td>Change in TLR, endotoxin nutrient metabolism</td>
</tr>
<tr>
<td>Pioglitazone</td>
<td>PPAR-gamma NHR</td>
<td>Hepatic macrophages</td>
</tr>
<tr>
<td>Fish oil/omega-3 fatty acids</td>
<td>Anti-inflammatory</td>
<td>Eicosanoid metabolism</td>
</tr>
<tr>
<td>Elafibranor</td>
<td>PPAR-alpha/delta receptor</td>
<td>Pleiotropic nuclear hormone transactivator</td>
</tr>
<tr>
<td>Hyperimmune colostrum</td>
<td>Endotoxins in gut</td>
<td>Hepatic inflammation reduction</td>
</tr>
<tr>
<td>Metformin</td>
<td>AMP-kinase</td>
<td>Reduced insulin resistance</td>
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Non-Alcoholic Fatty Liver Disease: Conclusions

- Emerging leading cause of chronic liver disease in children
- Paediatric NAFLD prevalence: 3 - 10%
- Important to exclude other causes of steatosis esp metabolic disorders in young patients
- **Mainstay of treatment is lifestyle modification → sustained weight loss**
  - diet and aerobic exercise
  - address co-morbid conditions: Diabetes Mellitus, hyperlipidaemia, hypertension
- Therapeutics: ? Role of Vitamin E in patients with documented NASH
- Untreated NAFLD
  - can progress to cirrhosis and increased risk of HCC
  - increases risk of cardiovascular disease in adulthood
Health Disparities: sub-Saharan Africa

Burden of liver disease in sub-Saharan Africa is substantial.

Challenges:
• Lack of data to accurately establish disease prevalence
• Lack of access to health facilities - diagnostic and interventional
• Access and cost of medications

Apply similar programmes to HIV/AIDS to combat liver disease in SSA - PEPFAR - Global Fund to Fight AIDS, TB and Malaria. → brought medication at affordable prices to SSA
Probiotics

- Specific nutrients increase intestinal permeability to bacterial endotoxins
  - activating immune-mediated inflammatory response of liver resident cells (Kupffer cells, hepatocytes, lymphocytes, stellate cells)
  - → profibrogenic phenotype

- Animal models - restoring gut microflora
  - → protect liver from steatosis and prevents cardiovascular disease

- Animal model - effect of probiotics on intestinal microbiota
  - modulate expression of nuclear receptors
  - correcting IR in liver and adipose tissue
  - protect against steatohepatitis

Ongoing clinical trials

CyNCh: Cysteamine DR for Treatment of Paediatric NAFLD

- Randomized, double-blind, placebo-controlled, multicenter phase IIb trial
- Cystine-depleting aminothiol
- 70% boys, mean age 13.7 yrs

Children aged 8-17 yrs given standard care diet and exercise followed by 120-day screening period: 2 visits; within 90 days of liver biopsy (N = 169)

MRI

5 on-treatment visits: Wk 4, 12, 24, 36, 52

Cysteamine DR
9-12 mg/kg, twice daily* (n = 88)

Placebo
(n = 81)

Wk 52: Liver biopsy and MRI; D/C study drug

Posttreatment follow-up at Wk 76

* Total dose: 300 mg if pt weighed ≤ 65 kg, 375 mg if pt weighed 65-80 kg, 450 mg if pt weighed > 80 kg.

Primary Endpoint: Improvement in NAS ≥ 2 Points Without Worsening of Fibrosis

- End of treatment (Wk 52) biopsies in 81% on drug, 93% on placebo ($P = .03$)

- ITT analyses also performed for 4 histologic features including fibrosis, steatosis, ballooning and lobular inflammation
  - Not significant with statistical correction

Secondary Outcomes at Wk 52

- No change in serum lipids, cholesterol, insulin sensitivity
- No difference in adverse events

Improvement in Histology by Weight at Enrollment

**Responder:** patients with improvement in NAFLD activity score ≥ 2 points without worsening of fibrosis