OLIGOSACCHARIDE ANALYSIS – THE SCIENCE, INTERPRETATION AND PITFALLS

MARGARET THORNLEY
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BIRMINGHAM
WHY SCREEN FOR OLIGOSACCHARIDES?

A number of inherited genetic disorders show abnormal amounts of oligosaccharides in urine. Testing urine is a non-invasive procedure especially useful with young babies. Screening urine for oligosaccharides can initially identify some oligosaccharidurias. Some symptoms of oligosaccharidurias may also apply to other diseases and it is important to differentiate these.
COMMON OLIGOSACCHARIDOSES

GM1 Gangliosidosis
α-mannosidosis
β-mannosidosis
α-fucosidosis
Neuraminidase deficiency (sialidosis)
Sialicaciduria (ISSD, Salla disease)
Galactosialidosis
Aspartylglucosaminuria
THE SCIENCE

Medical / clinical
• Clinical symptoms
• Clinical diagnosis

Biochemical
• Screening
• Biochemical diagnosis
<table>
<thead>
<tr>
<th></th>
<th>Face</th>
<th>Dyst Multi</th>
<th>Neur</th>
<th>Organo megaly</th>
<th>Eyes</th>
<th>Histology</th>
<th>Others</th>
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</thead>
<tbody>
<tr>
<td>α-mann</td>
<td>Coarse</td>
<td>+++</td>
<td>MR</td>
<td>+++</td>
<td>Cats Cloud</td>
<td>Vacuoles</td>
<td>Hearing loss</td>
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<tr>
<td>β-mann</td>
<td>Dysmorphic</td>
<td>+/-</td>
<td>MR</td>
<td>-</td>
<td>Normal</td>
<td>Normal</td>
<td>Angio-keratoma</td>
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<tr>
<td>Fucosidosis</td>
<td>Coarse</td>
<td>++</td>
<td>MR</td>
<td>++</td>
<td>+</td>
<td>Vacuoles</td>
<td>Angio-keratoma</td>
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<tr>
<td>Sialidosis</td>
<td>Coarse</td>
<td>+++</td>
<td>MR</td>
<td>+/-</td>
<td>Cherry red spot</td>
<td>Vacuoles</td>
<td>Hydrops</td>
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<tr>
<td>GM1 gang</td>
<td>Dysmorphic</td>
<td>+</td>
<td>MR</td>
<td>++</td>
<td>Cherry red spot</td>
<td>Vacuoles</td>
<td>Skeletal</td>
</tr>
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SCIENCE - DEGRADATION OF COMPLEX OLIGOSACCHARIDES

Fig. 140-4 Probable steps in degradation of complex oligosaccharide structure.
SCIENCE - BIOCHEMICAL

Screening
- Thin layer chromatography
- HPLC
- MS / MS

Diagnosis
- Enzyme Analysis (except for ISSD)
- Mutation Analysis
SCREENING – THIN LAYER CHROMATOGRAPHY

TLC – separation of compounds utilising their different solubility properties

Thin layer of silica gel 60 on a plastic support

Stand in solvent to give ascending chromatography

Visualise by spraying with chemicals and heating to enable a colour reaction
TLC for oligosaccharides
- Solvent 1: Butanol / acetic acid / water
  2:1:1 run twice

TLC for sialic acid (N-acetyl neuraminic acid)
- Solvent 1: Butanol / acetic acid / water
  2:1:1 run once followed by
- Solvent 2: Propan-1-ol / nitromethane / water 5:4:3 run twice
TLC continued

Staining thin layers

• Oligosaccharides – orcinol stain
• Sialic acid – resorcinol stain (after spraying with stain place thin layer plate between glass to maintain the acid atmosphere during heating)
Bands running between the origin and level with the band from lactose (a disaccharide) are the oligosaccharides becoming sequentially larger as they near the origin.
Interpretation

Some identifiable patterns

$\alpha$-mannosidosis – strong trisaccharide band with other bands stepwise downwards

$\beta$-mannosidosis – strong disaccharide band with other bands stepwise downwards

GM1 gangliosidosis – strong band close to origin plus other band above

Sialidosis (neuraminidase deficiency) – many bands of bound sialic acid

Sialic storage (ISSD) – heavy band of free sialic acid

Galactosialidosis – mixture of sialidosis and GM1
TLC patterns

α-mann  α-mann  β-mann  Standards
post-BMT

[Image of TLC patterns with labeled bands]
TLC patterns

ISSD

Neur
TLC patterns

GM1
GalSial
α-man
Pitfalls

Patterns from young babies are often deceptive
Jaundiced babies show heavy patterns
Other causes, eg liver damage, produce oligosaccharides
Not all patients suffering from an oligosaccharidosis show oligosaccharides in the urine – $\alpha$-fucosidosis very difficult to spot
Needs experience to interpret patterns
Age makes a difference
Disease severity makes a difference
SOMETHING TO REMEMBER

THIS IS ONLY A SCREENING METHOD, NOT A DIAGNOSTIC METHOD

IF CLINICAL SYMPTOMS CLEARLY INDICATE A POSSIBLE OLIGOSACCHARIDOSIS, FOLLOW UP WITH ENZYME ANALYSIS EVEN IF THE SCREEN APPEARS NEGATIVE