Serum biotinidase activity as a plausible biomarker for monitoring patients with hepatic glycogen storage diseases

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Abstract:
Biotinidase is an essential enzyme for recycling biotin. Patients with hepatic glycogen storage disorders (GSDs) tend to have higher biotinidase levels compared to controls. To evaluate the potential of biotinidase activity level as a biomarker for monitoring patients with hepatic GSDs, we measured biotinidase activity in subjects with GSD I (a, b) (n = 25) and GSD III (a, b) (n = 20). Retrospective chart review was done to assess correlation of biotinidase with relevant laboratory and imaging findings known to be associated with these GSD.

Our findings showed variation in biotinidase levels among subjects with GSD I, and III, where high biotinidase activity correlated with hypertriglyceridemia in both GSD I (r = 0.47, P = 0.036), and GSD III (r = 0.58, P = 0.014). GSD I cohort, who had urine organic acids measured (n=12), 5 patients with elevated 3-methylglutaconic acid (3-MGA) had significantly higher levels of biotinidase activity compared to patients without 3-MGA (P = 0.0063). In GSD III, biotinidase activity correlated negatively with age (r = -0.50, P = 0.03) and was relatively reduced in subjects with severe liver fibrosis (P = 0.002).

Longitudinal data (2-3 years) was collected on 6 patients. Overall, our findings suggested that biotinidase activity varied among subjects with GSD I, and III, potentially reflecting some underlying metabolic and pathologic liver changes. To confirm these findings, prospective, multi-center, longitudinal studies designed to assess the significance of monitoring biotinidase activity on larger cohorts with hepatic GSDs are warranted.

Introduction:
• Hepatic glycogen storage diseases (GSDs) are a group of inborn errors of glycogen metabolism and storage.
• Patients with GSD I have deficiency of glucose-6-phosphatase complex (GSD Ia) or its transport (GSD Ib). Patients present with severe fasting intolerance, hypoketotic hypoglycemia, hyperlipidemia and hepatomegaly due to defective glycogenolysis and gluconeogenesis.
• Patients with GSD III (a, b) have low debrancher activity leading to defective glycogenolysis with intact gluconeogenesis. Patients usually have ketosis, hyperlipidemia, and less severe fasting hypoglycaemia, they progress to develop chronic liver disease with signs of fibrosis and cirrhosis.
• In GSD, biochemical markers (lactate, uric acid, CK and triglycerides) fluctuate in blood and are influenced by diet, food intake, time of collection and tourniquet.
• Biotinidase is synthesized in the liver and secreted in blood.
• Biotinidase plays an important role in recycling biotin to conserve its co-factor activity for essential cellular metabolic pathway reactions involving biotin dependent carboxylases.
• Previous studies have reported the usefulness of biotinidase as a diagnostic biomarker in patients with hepatic GSDs.

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Subjects and Methods:

GSD I and III, and control subjects:

- 45 subjects with confirmed diagnoses of GSD I (n = 25, 13F, 12 M), or GSD III (n = 20, 12F, 8M) were enrolled in this study.
- Reference ranges for biotinidase activity was determined by analyzing residual samples from presumed healthy controls (n=62) from biochemical genetics laboratory (BGL). Leftover samples from confirmed Pompe disease patients (GSD II, n = 28), were used as laboratory controls (non-hepatic disorder without hypoglycemia).
- Individuals included in this study were consented in accordance with the requirements of the Institutional Review Board at Duke University Medical Center (IRB# PRO00013699, PRO00047556 and PRO00000034).

Biotinidase activity assay

- The plasma biotinidase activity was determined using the quantitative colorimetric assay that measures the release of para-aminobenzoate from biotinyl-para-aminobenzoate (Wolf et al 1983). The summary of biotinidase activities (see below) excludes patients that are post-transplant, and those that have renal dysfunction associated with nephropathy and insulin requiring type 2 diabetes mellitus.

Clinical laboratory and pathological findings

- Retrospective medical records review was performed on subjects enrolled in our study (GSD I and III).
- Analysis included; albumin and coagulation parameters (synthetic liver function), bilirubin (excretory liver function), and alanine transaminase (ALT) and aspartate transaminase (AST) (for hepatocellular function).
- Metabolic control markers for GSD I: lactate, triglycerides (TG), uric acid, and glucose levels; and for GSD III: glucose and TG were analyzed.
- Liver imaging results analyzed included; abdominal ultrasound (US), computerized tomography (CT) scan, and magnetic resonance imaging (MRI); as well as hepatic histopathological reports obtained during liver biopsy when available. For GSDIII, histopathological findings were stratified from stages 1 to 4 using the Batts Ludwig Scoring system (stage 1- portal fibrosis, stage 2- periportal fibrosis, stage 3- bridging fibrosis and stage 4- regenerative nodule formation, cirrhosis) and liver biopsy results were considered severe if reports showed stages 3 or 4 fibrosis (Halaby et al 2019).

Statistical Analysis

- Descriptive statistical analysis was performed and reported as (mean ± SD). The significance of two group comparisons was determined by a 2-tailed, unpaired t-test; ANOVA was used for multiple comparisons. Simple linear regression was used for correlation studies. P value of ≤ 0.05 was considered statistically significant.
- To evaluate the significance of measuring biotinidase activity, we used the Receiver Operating Characteristic-Area Under the Curve (ROC-AUC).
- Statistical analysis was performed using GraphPad Prism version 9.0.2 for Windows, USA.
Biotinidase activities correlated with triglyceride (TG) levels in GSD I

Biotinidase activity in individuals with GSD I (a, b) and the extent of liver involvement

Subjects | Age (years) | Biotinidase activity (U/L) | Liver involvement
--- | --- | --- | ---
GSD I (n=25) | (interval: 2-49 yrs) | | 
1 | 4 | 20.1 | Ultrasound: hepatomegaly, echogenic, steatosis
2 | 5 | 16.1 | Ultrasound: hepatomegaly
3 | 6 | 12.5 | Ultrasound: hepatomegaly, echogenic, steatosis
4 | 8 | 12.5 | Ultrasound: hepatomegaly, echogenic, liver
5 | 9 | 11.9 | Ultrasound: hepatomegaly, echogenic, steatosis
6 | 11 | 14.0 | Ultrasound: hepatomegaly, echogenic, steatosis
7 | 13 | 17.1 | Ultrasound: hepatomegaly, echogenic, steatosis
8 | 14 | 16.9 | MRI: hepatomegaly, steatosis, multiple adenomas
9 | 15 | 10.8 | Ultrasound: hepatomegaly
10 | 18 | 12.8 | MRI: hepatomegaly, fatty, multiple adenomas
11 | 18 | 16.7 | N/A
12 | 20 | 21.6 | Ultrasound: hepatomegaly, echogenic, steatosis
13 | 24 | 16.9 | MRI: hepatomegaly, multiple adenomas
14* | 24 | 18.1 | MRI: severe hepatomegaly, steatosis, multiple adenomas
15 | 30 | 15.9 | MRI: hepatomegaly, diffuse steatosis, multiple adenomas
16 | 35 | 6.8 | Post-liver transplant (no liver prior to transplant)
17 | 36 | 9.3 | Mild hepatomegaly, kidney stones
18 | 38 | 15.6 | MRI: hepatomegaly, steatosis, multiple adenomas
19* | 49 | 16.5 | MRI: hepatomegaly, steatosis, multiple adenomas,

GSD I (n=5) | (interval: 9-22.6 yrs) | | 
20 | 2 | 16.7 | Ultrasound: hepatomegaly, echogenic, coarse
21 | 7 | 17.5 | Ultrasound: hepatomegaly, echogenic, coarse
22* | 9 | 19.8 | Ultrasound: hepatomegaly, echogenic, coarse
23 | 11 | 15.9 | MRI: hepatomegaly, diffuse steatosis, multiple adenomas
24* | 29 | 10.6 | CT: hepatomegaly, portal hypertension, kidney failure
25 | 32 | 12.7 | MRI: hepatomegaly, steatosis, multiple adenomas

Metabolic parameters in individuals with GSD I with and without adenomas

| Age (years) | Biotinidase activity (U/L) | Lactic Acid (RI: <2.5 mmol) | Triglycerides (mmol/L) | Urea (mmol/L: (n=10 for females, n=15 for males)) | GFR (mL/min/1.73 m²)
--- | --- | --- | --- | --- | ---
GSD I participants without hepatocellular adenomas (n=15) | | | | | 
2 | 16.7 | 2.5 | 2.1 | 16.6 (12.7-22.6) | 7.4 (4.8-10.7) | 4.5 (2.5-6.4)
4 | 20.1 | 6.0 | 5.9 | 2.0 | N/A
5 | 16.1 | 4.1 | 2.4 | 5.4 | N/A
6 | 12.3 | 2.4 | 2.7 | 4.4 | N/A
7 | 17.5 | 3.8 | 2.1 | 7.2 | 4.3
8 | 12.5 | 2.4 | 3.2 | 5.8 | 4.4
9 | 13.9 | 3.8 | 3.6 | 6.1 | 5.9
10 | 19.8 | 7.8 | 4.0 | 10.4 | 4.4
11 | 14 | 4.5 | 5.1 | 6.1 | 5.0
12 | 17.5 | 7.8 | 2.1 | 7.7 | 5.3
14 | 16.9 | 9.7 | 9.1 | 9.4 | 2.4
15 | 13.8 | 5.5 | 4.5 | 8.3 | 4.4
16 | 16.7 | N/A | N/A | N/A | N/A
28 | 10.6 | 6.0 | 3.1 | 6.0 | 7.8
36 | 9.3 | 6.7 | 4.1 | 10.6 | 4.9
Mean ± SD (interval: 9-22.6 yrs) | | | | | 
GSD I participants with hepatocellular adenomas (n=25) | | | | | 
11 | 15.9 | 2.6 | 5.4 | 6.4 | 5.7
18 | 12.8 | 10.0 | 22.8 | N/A | N/A
20 | 21.8 | 6.3 | 6.2 | 7.3 | 3.4
24 | 16.9 | 7.6 | 11.6 | 7.3 | 5.3
28 | 18.1 | 7.4 | 2.9 | 8.0 | 3.9
32 | 15.9 | 5.8 | 6.1 | 7.4 | 4.4
32 | 12.7 | 2.8 | 5.9 | 7.1 | 8.4
38 | 15.6 | 8.4 | 4.0 | 10.7 | 2.5
Mean ± SD (interval: 9-22.6 yrs) | | | | | 
GFR (mL/min/1.73 m²) | | | | |
Biotinidase activity in individuals with GSD III (a, b) and the extent of liver involvement

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age (years) (interval: 2-58 yrs)</th>
<th>Biotinidase activity (U/L)</th>
<th>Liver involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSD IIIa (n=14)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>20.0</td>
<td>Ultrasound: hepatomegaly, steatosis</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>12.5</td>
<td>N/A</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>21.1</td>
<td>Ultrasound: hepatomegaly, steatosis</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>12.6</td>
<td>Ultrasound: hepatomegaly</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>8.5</td>
<td>Ultrasound: coarse liver, cirrhosis, steatosis</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>9.1</td>
<td>Ultrasound: hepatomegaly, bridging fibrosis on biopsy</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>17.7</td>
<td>Ultrasound: hepatomegaly, mild echogenicity</td>
</tr>
<tr>
<td>8</td>
<td>13</td>
<td>13.8</td>
<td>Ultrasound: hepatomegaly, increased echogenicity</td>
</tr>
<tr>
<td>9</td>
<td>16</td>
<td>15.8</td>
<td>MRE: hepatomegaly, steatosis, no focal findings</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Stage 3 (bridging fibrosis) on liver biopsy, coarse echogenicity on ultrasound with diffuse steatosis, fatty liver as type 2 diabetes mellitus (insulin requiring)</td>
</tr>
<tr>
<td>10</td>
<td>16</td>
<td>19.2</td>
<td>MRE: hepatomegaly</td>
</tr>
<tr>
<td>11</td>
<td>35</td>
<td>14.7</td>
<td>Stage 3 (bridging fibrosis), on liver biopsy, evidence of steatosis, coarse echogenicity on ultrasound</td>
</tr>
<tr>
<td>12</td>
<td>36</td>
<td>15.0</td>
<td>Stage 4 (regenerative module formation, cirrhosis) on liver biopsy</td>
</tr>
<tr>
<td>13*</td>
<td>38</td>
<td>10.6</td>
<td>Stage 4 (regenerative module formation, cirrhosis) on liver biopsy</td>
</tr>
<tr>
<td>14</td>
<td>57</td>
<td>10.0</td>
<td>Stage 4 MRI: cirrhosis, fibrosis, dysplastic module</td>
</tr>
</tbody>
</table>

GSD IIIb (n=6)

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age (years)</th>
<th>Biotinidase activity (U/L)</th>
<th>Liver involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>10</td>
<td>14.8</td>
<td>Ultrasound: liver normal</td>
</tr>
<tr>
<td>16</td>
<td>11</td>
<td>12.0</td>
<td>CT abdomen: hepatomegaly</td>
</tr>
<tr>
<td>17</td>
<td>17</td>
<td>11.5</td>
<td>CT abdomen: hepatomegaly</td>
</tr>
<tr>
<td>18</td>
<td>41</td>
<td>12.8</td>
<td>Ultrasound: hepatomegaly</td>
</tr>
<tr>
<td>19*</td>
<td>47</td>
<td>5.7</td>
<td>Stage 4 MRI: cirrhosis, fibrosis, dysplastic module</td>
</tr>
<tr>
<td>20</td>
<td>58</td>
<td>9.3</td>
<td>Stage 4 MRI: cirrhosis, fibrosis, steatosis</td>
</tr>
</tbody>
</table>

Conclusions:

- Biotinidase activity levels varied in patients with GSD I and III.
- Biotinidase activities correlated with triglyceride (TG) levels in GSD I and III, suggesting increased compensatory needs of the liver to restore biotin levels for altered metabolic regulatory pathways involving gluconeogenesis, lipid regulation, and amino acid metabolism.
- Pathologic changes associated with advanced parenchymal liver disease and cirrhosis may limit liver’s capacity to synthesize biotinidase, which may explain reduced levels of biotinidase activity in patients with GSD III with advancing liver disease and age.
- Our finding suggests that GSD related metabolic (disturbed gluconeogenesis, and fatty acid synthesis) and pathological processes (advancing fibrosis and cirrhosis) may influence biotinidase activity levels thus making it a potential biomarker for long-term disease monitoring, with altered metabolic status, when interpreted alongside other clinical findings.
- Advanced liver disease associated with GSD I and III may result in unreliable levels of biotinidase.
- Prospective multi-centered studies designed to understand biotinidase role in disease monitoring and outcome are needed.

El-Garbawy et al., submitted