The association between serum fructosamine and random spot urine fructose levels with the severity of non-alcoholic fatty liver disease – an analytical cross-sectional study

H Kamuzinzi,1 MB ChB, Dip HIV Man (SA); M Kgomo,1 FCP (SA), PhD; P Rheeder,2 FCP (SA), PhD; N Dada,1 MB ChB, PGDipGUS; P Bester,3 MB ChB, PGDipGUS

1 Department of Internal Medicine, School of Medicine, Faculty of Health Sciences, University of Pretoria, South Africa
2 Department of Gastroenterology, School of Medicine, Faculty of Health Sciences, University of Pretoria, South Africa
3 Department of Radiology, School of Medicine, Faculty of Health Sciences, University of Pretoria, South Africa

Corresponding author: M Kgomo (mpho.kgomo@up.ac.za)

Background. Non-alcoholic fatty liver disease (NAFLD) in South Africa and Africa at large is considered a hidden threat. Our local population is burdened with increased metabolic risk factors for NAFLD. Our setting requires a reasonable approach to screen for and aid the diagnosis of NAFLD.

Objectives. To investigate serum fructosamine and random spot urine fructose levels as biomarkers for the screening, diagnosis and monitoring of NAFLD. The primary objective of this study was to compare serum fructosamine and random spot urine fructose levels between groups with different levels of NAFLD severity as measured by ultrasound. A secondary objective was to determine the association, if any, between serum transaminases, the aspartate aminotransferase (AST) to platelet ratio index (APRI) score, serum fructosamine and urine fructose in different groups with steatosis.

Methods. Using a cross-sectional study design, 65 patients with three different levels of NAFLD, as detected by imaging, were enrolled. The primary exposures measured were serum fructosamine with random spot urine fructose, and secondary exposures were the serum transaminases (AST and alanine aminotransferase (ALT)) and the APRI score. Patients identified at the departments of gastroenterology, general internal medicine and diagnostic radiology were invited to participate.

Results. There were 38, 17 and 10 patients with mild, moderate and severe steatosis, respectively. There was no significant difference between the groups regarding serum fructosamine, measured as median (interquartile range): mild 257 (241 - 286) µmol/L, moderate 239 (230 - 280) µmol/L and severe 260 (221 - 341) µmol/L, p=0.5; or random spot urine fructose: mild 0.86 (0.51 - 1.30) mmol/L, moderate 0.84 (0.51 - 2.62) mmol/L and severe 0.71 (0.58 - 1.09) mmol/L, p = 0.8. ALT (U/L) differed between groups: mild 19 (12 - 27), moderate 27 (22 - 33), severe 27 (21 - 56), p=0.03, but not AST (U/L) (p=0.7) nor APRI (p=0.9). Urine fructose and ALT were correlated in the moderate to severe steatosis group (R=0.490, p<0.05), but not in the mild steatosis group. Serum fructosamine was associated with age in the mild steatosis group but not the moderate-severe steatosis group (R=0.42, p<0.01).

Conclusion. Serum fructosamine and random spot urine fructose did not vary with the severity of NAFLD, indicating that they would not be useful biomarkers in this condition.


Liver disease not caused by alcohol, viruses or inborn errors of metabolism, but rather by metabolic risk factors associated with diet and obesity, is a global concern. Non-alcoholic fatty liver disease (NAFLD) in South Africa (SA) and Africa at large is considered a hidden threat. Our local population is burdened with increased metabolic risk factors for NAFLD. There is unfortunately a paucity of data in our local setting on NAFLD to reliably define our local knowledge base of SLD. The revised terminology allows the inclusion of atypical phenotype patients with SLD. A key consideration in the
Delphi consensus is the preservation of NAFLD data regarding its natural history, pathogenesis, biomarkers and clinical trials. It is with this background that NAFLD was used in this study as the term of reference for SLD with or without cardiometabolic risk factors.

It has been shown worldwide that the increasing rates of obesity and type 2 diabetes mellitus are a cause for concern – especially in relation to NAFLD. A recent global systematic review and meta-analysis demonstrated that the current global prevalence of NAFLD among adults is 29.8%. In Africa, the prevalence of NAFLD has been estimated to affect 13.5% of the adult population. SA has a paucity of data regarding NAFLD. A study performed in the Western Cape Province found a NAFLD prevalence in 47% of the population confirmed on histology. The demographic differs significantly from the rest of the SA population, and this finding may not be completely extrapolated to the rest of SA.

The definitive pathogenesis of NAFLD is not well understood. The currently accepted hypothesis for NAFLD development is the ‘two-hit hypothesis’. This hypothesis places insulin resistance as the cornerstone of hepatic steatosis that contributes to the base on which NAFLD develops, also understood as the ‘first hit’. The ‘second hit’ is what results in the necroinflammatory inflammation needed to establish steatohepatitis. The ‘second hit’ comprises oxidative stress and subsequent lipid peroxidation, with the activation of pro-inflammatory cytokines and adipokines. Other confounding factors in NAFLD pathogenesis include genetic predisposition, antioxidant deficiencies and the actions of specific gastrointestinal hormones.

Dietary fructose is primarily metabolised by colonic enterocytes, but can also be directly delivered to the liver via the portal vein. In instances of excess fructose intake (>25 g per day – at which point the absorptive capacity of the enterocytes has been exceeded), there is resultant overflow of fructose and its metabolites to the liver. By the hydrolysing action of gut bacteria on the fructose, multiple organic acids such as butyrate, propionate and acetate are subsequently released into the hepatic parenchyma. These metabolites, along with the high load of fructose in the gut, trigger spontaneous hepatic lipogenesis with subsequent significant hepatic steatosis.

The radiological investigations used for NAFLD are ultrasound, computed tomography and magnetic resonance imaging (MRI). Ultrasound is the most available radiological investigation for the diagnosis of hepatic steatosis, but has a low sensitivity as it detects steatosis of ≥15%. MRI is the most sensitive and specific as it detects steatosis >5%. Ultrasound B-mode scoring is the most common technique used, among other scoring systems, to grade NAFLD.

NAFLD patients are typically asymptomatic. Patients with NAFLD are usually identified by associated medical conditions (diabetes mellitus, hypercholesterolaemia, hypertension and obesity), hepatomegaly and raised aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels. The AST to platelet ratio index (APRI) is a well-developed non-invasive indicator of NAFLD-associated liver fibrosis. Current scores and biomarkers are inadequate for diagnosis and monitoring of NAFLD. There is a need to investigate and validate novel biomarkers.

Objectives
Primary objective: to determine the association, if any, between serum fructosamine and random spot urine fructose levels with the degree of hepatic steatosis on ultrasound.

Secondary objective: to determine the association, if any, between serum fructosamine, random spot urine fructose levels, serum transaminases and the APRI score in patients with varying degrees of steatosis as assessed by ultrasound.

Methods
Study design, conduct and oversight
A cross-sectional study was approved by the University of Pretoria Faculty of Health Sciences MMed Committee and Research Ethics Committee (ref. no. 31/2023) as well as all relevant hospital authorities. All research participants provided written informed consent prior to enrolment into the study.

The study was conducted at Steve Biko Academic Hospital (SBAH), Pretoria, in Gauteng Province, SA. SBAH is a quaternary health institution rendering highly specialised healthcare services to patients in the immediate Tshwane region and Mpumalanga Province.

Enrolment criteria
Patients were eligible for inclusion in the study if they were diagnosed with NAFLD as evidenced by imaging or biopsy, and were ≥18 years of age. Patients were excluded if they were not able to give consent for procedures, had a history of significant alcohol use (men: ≥2 units/day; women: ≥1 unit/day), were taking medication that causes steatosis (i.e. amiodarone, didanosine, stavudine, valproic acid, carbamazepine, tamoxifen, methotrexate, glucocorticoids), were on chemotherapy, or had confirmed alternative diagnoses for hepatic steatosis (pregnancy-related hepatic steatosis, Wilson disease, thyroid dysfunction, alpha-1 antitrypsin deficiency, autoimmune hepatitis or any other established chronic liver disease).

Study procedures
The study was conducted from 1 June 2023 to 31 October 2023. Patients identified at the departments of gastroenterology, general internal medicine and diagnostic radiology were invited to participate. A systematic review of imaging was performed using the radiology department’s database, where steatosis was noted in their imaging reports. Additional patients were referred directly from the above-mentioned clinics or directly from the ultrasound department during the study duration. It was assumed that patients were referred without bias.

Eligible patients who had provided written informed consent were interviewed and had their medical records analysed for exclusion criteria. Information recorded for the study included their demographics and comorbidities. Blood sampling (random serum fructosamine, platelet count, aspartate aminotransferase and alanine transaminase levels), urine sampling (random spot urine fructose) and hepatic ultrasound were performed. The spot urine fructose level was determined using a BioChrom Libra-11 spectrophotometer set at 518 nm. Normal urinary fructose levels are <2 mmol/L. The serum fructosamine level was determined using a Roche/Hitachi Cobas C system with the quantitative determination based on the fructosamine’s ability to reduce nitroblue tetrazolium in an alkaline medium. The normal serum fructosamine level range is 200 – 285 umol/L.

The following ultrasound B-mode scoring system was used:
• Score 1: mild – slight but diffuse increased liver echogenicity, with the diaphragm and portal vein wall normally visualised.
• Score 2: moderate – moderate increase in liver echogenicity, with the diaphragm and portal vein wall appearance impaired.
• Score 3: severe – significant liver echogenicity, with minimal to no visualisation of the diaphragm, portal vein wall and posterior section of the right lobe of the liver.

Statistical analysis
For an effect size between 0.5 and 1 (moderate to large), 30 participants in two groups would be sufficient to compare means at 80% power. Data collected were recorded in Table format using Excel (Microsoft, USA). Data analysis was performed using R 4.3.1.
Severity was classified as follows in increasing order to distinguish three groups for comparison: mild, moderate and severe steatosis. Groups were also collapsed into two, with mild and moderate-severe groups.

Data analyses consisted of descriptive data analyses as well as group comparisons using the Kruskal-Wallis test, ANOVA and the Welch two-sample t-test, dependent on the distribution of the data. For assessment with multiple variables, logistic regression was used. For the secondary analysis regarding association, non-parametric correlation was used.

Results
A total of 322 patients were possible study candidates. Of these, 65 patients were enrolled in the study (Fig. 1). One patient had no fructosamine recorded, and another patient did not have urine fructose recorded.

Table 1 compares the three groups of steatosis. Differences between groups were noted in ALT levels. Due to the small numbers in groups, participants were pooled into two groups: mild v. moderate-severe. There was no statistically significant association with serum fructosamine, urine fructose or their respective logarithmic derivatives (Table 2).

Figs 2 and 3, using box plots, depict the absence of a statistically significant relationship between both random spot urine fructose and serum fructosamine across the varying degrees of hepatic steatosis. Urine fructose had a wider spread in the moderate-severe steatosis group, but this was not statistically significant.

Multivariate adjusted analysis with logistic regression shows no association between serum fructosamine or urine fructose and steatosis category. We used a relatively simple model due to the small sample size.

In Table 3 this model shows that white patients had 6-fold greater odds of being in the moderate-severe steatosis category. Serum fructosamine had no association with steatosis category (odds ratio 1.00, p=0.97).

In Table 4, white patients had the greatest risk, with urine fructose not significantly increasing the odds for a higher steatosis category (odd ratio 1.11, p=0.56).

The secondary objective examining associations across different steatosis categories is shown graphically in Fig. 4.

Discussion
Key results
There were 38, 17 and 10 patients with mild, moderate and severe steatosis, respectively.

Regarding the primary objective, there was no significant difference between the severity groups on ultrasound regarding serum fructosamine (p=0.42) and random spot urine fructose (p=0.97) levels.

Regarding the secondary objective, only ALT (U/L) differed between the severity groups on ultrasound, with a p-value of 0.027, the highest being in the moderate-severe steatosis group.

There was a statistically significant association between ALT and spot urine fructose levels in patients with moderate-severe steatosis (p<0.05).

There was a statistically significant association between serum fructosamine and increasing age in the mild steatosis group (p<0.01).

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Patients identified as potential study participants with a history and imaging suggestive of hepatic steatosis (n=322)

Patients excluded at recruitment stage (n=257)
Incomplete medical records;
history of significant alcohol use: not taking steatosis-inducing medication;
confirmed alternative diagnoses for hepatic steatosis or any other established chronic liver disease

Complete profiles (n=65)

Patients recruited into study (n=63)

Incomplete profiles (n=2)
Patient 50 (fructosamine lab error)
Patient 57 (AST, ALT, urine fructose lab errors)

Fig. 1. Patient enrolment. (AST = aspartate aminotransferase; ALT = alanine aminotransferase.)
Table 1. Comparison of the 3 groups of steatosis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mild, n=38</th>
<th>Moderate, n=17</th>
<th>Severe, n=10</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)*</td>
<td>51 (16)</td>
<td>55 (10)</td>
<td>50 (10)</td>
<td>0.5</td>
</tr>
<tr>
<td>Gender, n (%)†</td>
<td></td>
<td></td>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td>Male</td>
<td>16 (42)</td>
<td>6 (35)</td>
<td>7 (70)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>22 (58)</td>
<td>11 (65)</td>
<td>3 (30)</td>
<td></td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td>0.046</td>
</tr>
<tr>
<td>Black</td>
<td>17 (45)</td>
<td>2 (12)</td>
<td>1 (10)</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>18 (47)</td>
<td>14 (82)</td>
<td>9 (90)</td>
<td></td>
</tr>
<tr>
<td>Coloured</td>
<td>1 (2.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Indian</td>
<td>2 (5.3)</td>
<td>1 (5.9)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Diabetes‡</td>
<td></td>
<td></td>
<td></td>
<td>0.4</td>
</tr>
<tr>
<td>No</td>
<td>27 (71)</td>
<td>13 (76)</td>
<td>5 (50)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>11 (29)</td>
<td>4 (24)</td>
<td>5 (50)</td>
<td></td>
</tr>
<tr>
<td>AST‡</td>
<td>22 (17 - 29)</td>
<td>25 (20 - 31)</td>
<td>25 (19 - 29)</td>
<td>0.7</td>
</tr>
<tr>
<td>Platelets‡</td>
<td>259 (222 - 338)</td>
<td>285 (248 - 331)</td>
<td>272 (195 - 330)</td>
<td>0.7</td>
</tr>
<tr>
<td>APRI‡</td>
<td>0.22 (0.15 - 0.29)</td>
<td>0.24 (0.1 - 0.30)</td>
<td>0.22 (0.15 - 0.31)</td>
<td>&gt;0.9</td>
</tr>
<tr>
<td>ALT‡</td>
<td>19 (12 - 27)</td>
<td>27 (22 - 33)</td>
<td>27 (21 - 56)</td>
<td>0.027</td>
</tr>
</tbody>
</table>

*One-way ANOVA.
†Fisher's exact test.
‡Kruskal-Wallis rank sum test.
AST = aspartate aminotransferase; APRI = aspartate transaminase to platelet ratio index; ALT = alanine aminotransferase.

Table 2. Comparisons of steatosis severity: serum fructosamine and urine fructose

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mild, n=38</th>
<th>Moderate-severe, n=27</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum fructosamine (µmol/L), median, IQR*</td>
<td>257 (241 - 286)</td>
<td>245 (227 - 290)</td>
<td>0.4</td>
</tr>
<tr>
<td>Urine fructose (mmol/L), median, IQR*</td>
<td>0.86 (0.51 - 1.30)</td>
<td>0.75 (0.54 - 1.35)</td>
<td>&gt;0.9</td>
</tr>
<tr>
<td>Log (serum fructosamine), mean (SD)†</td>
<td>5.57 (0.16)</td>
<td>5.56 (0.21)</td>
<td>0.9</td>
</tr>
<tr>
<td>Log (urine fructose), mean (SD)†</td>
<td>-0.25 (0.83)</td>
<td>-0.10 (1.12)</td>
<td>0.6</td>
</tr>
</tbody>
</table>

*Wilcoxon rank sum test.
†Welch two-sample t-test.
IQR = interquartile range; SD = standard deviation.

Fig. 2. Serum fructosamine (µmol/L) v. steatosis severity.
White patients had a higher chance of being in the moderate-severe group of steatosis than the mild steatosis group ($p<0.005$).

**Interpretation and implications**

Serum fructosamine and random spot urine fructose, as per the findings, do not have direct statistically significant correlations with the severity of hepatic steatosis on B-mode scoring on ultrasound assessment. This would not qualify them as reliable serum biomarkers for NAFLD diagnosis and monitoring.

The serological marker for hepatic dysfunction that correlated with sonographic hepatic steatosis was serum ALT. This relationship is already established in the literature and may be based on the pathophysiological principle of hepatocellular injury being at the core of steatosis and steatohepatitis.$^{[9]}$

The association between serum ALT and spot urine fructose levels in patients with moderate-severe steatosis is partially supported by evidence in the literature,$^{[18]}$ where steatosis is associated with increased fructose intake levels when analysed with serum ALT.

A meta-analysis provided a good indication that sugar-sweetened beverages provide excess energy at high doses, leading to small, but important, increases in ALT along with hepatic steatosis. Unfortunately, there is uncertainty regarding the effect of other sources of fructose-containing sugars such as fruit, fruit juice, dried fruit, sweetened dairy alternatives, sweets and desserts.$^{[18]}$

**Table 3. Logistic regression results for serum fructosamine**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Odds ratio</th>
<th>Confidence interval</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.17</td>
<td>0.01 - 4.18</td>
<td>0.284</td>
</tr>
<tr>
<td>Serum fructosamine</td>
<td>1.00</td>
<td>0.99 - 1.01</td>
<td>0.968</td>
</tr>
<tr>
<td>Dyslipidaemia</td>
<td>2.15</td>
<td>0.48 - 10.69</td>
<td>0.323</td>
</tr>
<tr>
<td>White v. other</td>
<td>6.25</td>
<td>1.89 - 25.14</td>
<td>0.005</td>
</tr>
</tbody>
</table>

**Table 4. Logistic regression results for urine fructose**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Odds ratio</th>
<th>Confidence interval</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.13</td>
<td>0.03 - 0.39</td>
<td>0.001</td>
</tr>
<tr>
<td>Urine fructose</td>
<td>1.11</td>
<td>0.79 - 1.68</td>
<td>0.562</td>
</tr>
<tr>
<td>Dyslipidaemia</td>
<td>2.31</td>
<td>0.55 - 11.13</td>
<td>0.266</td>
</tr>
<tr>
<td>White v. other</td>
<td>7.58</td>
<td>2.05 - 37.22</td>
<td>0.005</td>
</tr>
</tbody>
</table>

**Fig. 3. Random spot urine fructose (mmol/L) v. steatosis severity.**

![Graph showing random spot urine fructose (mmol/L) v. steatosis severity.](image)
Moreover, there is uncertainty regarding the pathophysiological implications of this finding, which need to be investigated further – this is a limitation of the meta-analysis where fructose levels were not compared between groups of 'high' and 'low' dietary fructose load. Clinical implications of this finding may need to be validated in a larger study where the outcome variables are serum ALT and random spot urine fructose. If proven, these markers may be used diagnostically in NAFLD.

The association between serum fructosamine and increasing age in the mild steatosis group is well established in the literature. The literature demonstrates increasing insulin resistance with increasing age: baseline serum fructosamine levels increase with age. A clinical implication of this finding may be to suggest lowering the threshold to investigate for NAFLD in older populations to identify them in the early disease stages.

Regarding white patients having higher chances of being in the moderate-severe steatosis group, this is influenced by the majority \(n=41\) of patients in the study being white. This is, however, supported by a meta-analysis of US populations. Clinically in the SA setting, this may not be wholly relied upon to evaluate the pre-test probability of NAFLD in a primary care setting.

Study limitations
The gold standard for the diagnosis of NAFLD is biopsy. Due to the high risk of a biopsy procedure, establishing the sensitivity and specificity of serum biomarkers against biopsy results would prove challenging. This study focused on clinically assessed steatosis not due to alcohol consumption. The degree to which the clinical assessment may truly predict histopathological NAFLD is undetermined.
A true reflection of insulin resistance and dysfructosaemia was not elucidated, as the patients had their samples taken without starving or after a specific fructose load challenge.

Potential bias may have influenced the study at the point of study participant recruitment. This is due to patients being recruited at the discretion and awareness of the diagnosis by the treating clinician, who contacted the principal investigator.

The study had limited representation of non-white groups. This may have been influenced by the demographic of the local population of the hospital. This limits the true generalisability of the findings to the greater SA population, and the finding of increased risk of white patients falling into the moderate-severe steatosis group.

Further research
A larger study (ideally involving multiple hospitals to diversify the patient populations) should be embarked upon to produce more data relating to insulin resistance and the hepto-inflammatory process related to hepatic steatosis in our local setting. There may be another element in the pathophysiology of NAFLD that, when corrected for total body fructose levels, may add clarity to, or refute the role completely for fructose-mediated hepatic steatosis. In the African setting, we may need to explore genetic predispositions and specific dietary elements outside the realm of carbohydrates to elucidate the true nature of this hidden threat.

Conclusion
Serum fructosamine and random spot urine fructose did not vary with the severity of NAFLD. This would not support the establishment of serum fructosamine and random spot urine fructose levels as biomarkers for the screening, diagnosis and monitoring of NAFLD in our local setting. Other findings were note: serum ALT and random spot urine fructose (using combined parametric correlation) are associated with moderate-severe steatosis, and serum fructosamine was associated with age in mild steatosis.

Declaration. This research was conducted and completed as part of fulfilment of the degree MMed (Internal Medicine) (Prep) at the University of Pretoria, Faculty of Health Sciences, Pretoria, South Africa to be conferred to HK.

Acknowledgements. The authors acknowledge all study participants and staff members in the Departments of Internal Medicine, Gastroenterology and Radiology of SBAH.

Author contributions. MK, PR and HK conceptualised the study. HK, ND and PB performed the study. ND and PB performed diagnostic ultrasonography. PR conducted statistical analysis and interpreted the results. HK wrote the first draft of the manuscript. MK and PR supervised the research study, and reviewed and edited the manuscript. All authors have read and agreed to the published version of the article.

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Conflicts of interest. None.

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