Urine kidney injury markers do not increase following gastric bypass: a multi-center cross-sectional study

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Introduction: *To determine if markers of kidney injury correlate with urinary oxalate excretion. If so, such biomarkers might be early predictors of oxalate nephropathy. Gastric bypass surgery for obesity is known to be associated with postoperative hyperoxaluria, which can lead to urolithiasis and kidney damage.*

Materials and methods: *Patients were recruited from four large academic centers > 6 months following completion of gastric bypass surgery. Patients provided a spot urine sample for analysis of three markers of kidney injury: 8-iso-Prostaglandin F2α, N-acetyl-β-D-Glucosaminidase, and Neutrophil gelatinase-associated lipocalin. Patients also provided 24 hour urine samples for stone risk analysis.*

Results: *A total of 46 study patients provided samples, the average age was 48.4 ± 11.3. There were 40 women and 6 men. There was no difference in the level of any of the three inflammatory markers between the study group and the reference range generated from healthy non-hyperoxaluric subjects. Neither oxalate excretion nor supersaturation of calcium oxalate correlated with any of the injury markers. There was no difference noted between those with hyperoxaluria (n = 17) and those with normoxaluria (n = 29) with respect to any of the injury markers.*

Conclusions: *Though hyperoxaluria was common after bypass surgery, markers of kidney injury were not elevated after surgery. No correlation was found between urine oxalate excretion and any of the injury markers.*

Key Words: obesity, gastric bypass, hyperoxaluria, biomarkers

Introduction

Using data from the National Health and Nutrition Examination Survey (NHANES) study, the CDC has estimated that approximately 35% of adults in the United States are obese.[1] Prevention and treatment of obesity with lifestyle modification such as changes in diet and increased exercise, unfortunately, remain ineffective. The most effective treatment for patients with obesity is surgical therapy. The adoption of bariatric surgery as a treatment for obesity has increased as evidence has accumulated that bariatric surgery improves glycemic control,[2] hypertension, obstructive sleep apnea, and hyperlipidemia.[3] The number of bariatric surgery procedures performed

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has increased drastically in the United States from 103,000 patients in 2003 to approximately 220,000 patients in 2008.[4] Current bariatric procedures include gastric banding, sleeve gastrectomy, biliopancreatic diversion with duodenal switch and roux-en-Y gastric bypass (RYGB). Nutritional deficiencies are common after gastric bypass procedures and are most often related to the malabsorptive physiology created as a consequence of gastric bypass. Nelson and colleagues were among the first to note the risk of postoperative hyperoxaluria, nephrolithiasis, oxalate nephropathy, and renal failure among patients undergoing RYGB.[5] Duffey et al demonstrated that patients undergoing contemporary RYGB demonstrated increased oxalate excretion and supersaturation of calcium oxalate at 3 months postoperatively.[6] Furthermore, these increases in oxalate excretion and supersaturation of calcium oxalate continued to worsen at 2 years post-RYGB.[7]

Hyperoxaluria is a significant risk factor for the development of calcium oxalate nephrolithiasis. In addition, hyperoxaluria may increase the risk of renal damage and loss of renal function in the absence of nephrolithiasis. Simple urine tests are currently available that can recognize renal inflammation and early renal damage. Enteric hyperoxaluria is commonly treated with calcium supplementation to reduce intestinal absorption of oxalate.[8] With early detection of renal damage in hyperoxaluric bariatric surgery patients, it may be possible to intervene to prevent loss of renal function. Without treatment, patients undergoing contemporary bariatric surgery could develop prolonged untreated hyperoxaluria that may lead to renal damage and functional renal impairment. We hypothesized that markers of renal damage would be correlated with the severity of hyperoxaluria in patients with malabsorptive bariatric surgery, and could serve as an early predictor of the subsequent development of overt renal disease. Bariatric surgery patients present a unique opportunity to study the relationship of hyperoxaluria with renal damage, since the onset of enteric hyperoxaluria can be reasonably estimated and patients can be evaluated early in the course of the hyperoxaluria exposure.

Materials and methods

Study patients were recruited from the University of Minnesota, Methodist Hospital Indianapolis, Wake Forest University and Johns Hopkins University. Each site obtained local IRB approval. All patients had malabsorptive bariatric surgery (either RYGB or bilio-pancreatic diversion) at least 6 months prior to performing the urine collections. Patients with a prior history of kidney stones were excluded from the study.

Each patient provided a spot urine collection which was frozen at the site at the time of a clinic visit and then shipped to Litholink on dry ice for analysis. Samples were stored at -80°C prior to analysis. A 24 hour urine was collected within 2 weeks of the spot urine collection to estimate the patient's urine oxalate excretion and stone risk. The 24 hour urine collections were collected at room temperature and shipped by overnight courier to Litholink for routine clinical analysis. Chemistries measured in the 24 hour sample included oxalate, calcium, citrate, magnesium, sodium, potassium, chloride, phosphorus, sulfate, ammonium, pH, uric acid and creatinine. Urine supersaturations for calcium oxalate (SS CaOx) were calculated using the iterative program EQUIL 2.

The frozen spot urine was analyzed for concentration of three injury markers: 8-iso-Prostaglandin F2α (8-iso-PGF2α), N-acetyl-β-D-Glucosaminidase (NAG), and Neutrophil gelatinase-associated lipocalin (NGAL). 8-iso-PGF2α was determined using a competitive ELISA kit from Cell Biolabs, Inc. (San Diego, CA, USA), NAG was determined using a colorimetric assay kit from BioQuant Kits, Inc. (San Diego, CA, USA), and NGAL was determined using a rapid ELISA kit from BioPorto diagnostics (Gentofte, Denmark).

A group of 10 healthy subjects without hyperoxaluria (defined as urine oxalate to creatinine ratio < 40 mg/gram on a spot urine) were recruited to determine reference ranges for the injury markers. These subjects included 5 men and 5 women with an average age of 30.7 ± 14.4.

Statistical analysis was performed using JMP (SAS, Cary, NC, USA). Student t-test was used for well distributed matched pairs and the Wilcoxon Rank Sums 2-sample test was used for nonparametric analysis.

Results

A total of 46 patients completed 24 hour urine collection and spot urine collection for injury markers between January 1, 2009 and December 31, 2009. Twenty patients had one 24 hour collection and one spot urine for markers. Twenty-six patients had two collections for the 24 hour urine collection, the spot urine for injury markers, or both. For patients with two collections, the average of the two collections was used. Of the 46 study patients M:F ratio was 6:40, the average age was 48.4 ± 11.3 years and body mass index (BMI) was 35.41 ± 10.40.

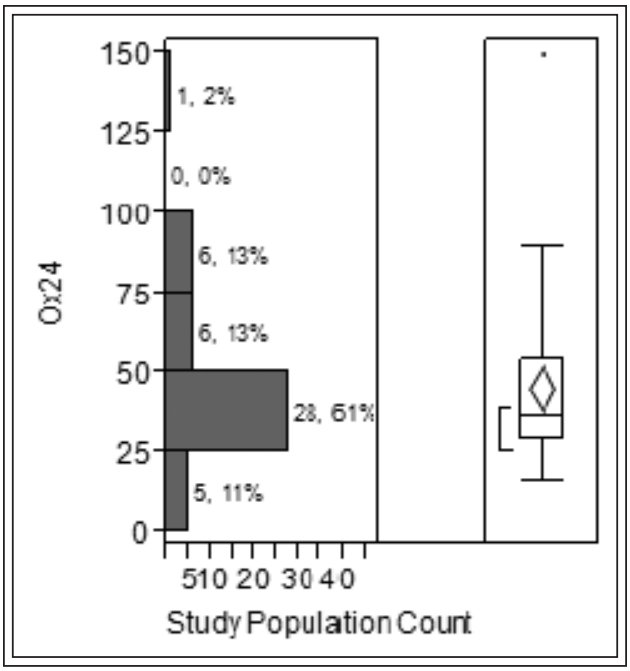

Figure 1a. Distribution of Ox24 in the study population.

Our primary hypothesis was that markers of injury would positively correlate with urine oxalate excretion (Ox24). For the study population, Ox24 had a median and inter-quartile range of 35.8 [29.1-54.3]. Seventeen patients had hyperoxaluria, defined as a urine oxalate excretion greater than 40 mg/day, Figure 1a. However, we found no correlation between any of the three injury markers and Ox24 in the study population: NGAL and Ox24 ($r^2 = 0.003$); 8-Iso-A and Ox24 ($r^2 = 0.003$); NAG and Ox24 ($r^2 = 0.0001$).

There was no significant difference between the study population and the normal healthy subjects with regards to any of the three injury markers. The median level and inter-quartile range for 8-iso-PGF2α was 2686.2 (pg/mL) [1370.1-3357.7] in the study group and 2586.8 [1203.33-3031.68] in the reference group (p = 0.6908). The median level and inter-quartile range for NAG was 3.29 (IU/L) [2.24-6.30] in the study group and 3.33 [2.17-4.15] in the reference group (p = 0.7241). The median level and inter-quartile range for NGAL was 20.21 (ng/mL) [11.41-37.48] in the study group and 23.1 [8.18-34.93] in the reference group (p = 0.7561), Table 1.

TABLE 2. **Reference range**

	Reference range
8-iso-PGF2α (pg/mL)	523.8-10,304.3
NAG (IU/L)	1.34-10.32
NAGL (ng/mL)	0-76.6

A reference range was determined for each marked based on the healthy subjects

Based on the samples from the healthy subjects, a reference range and an upper limit of normal was determined for each injury marker, Table 2. The study population was analyzed to compare the Ox24 between those above and below the threshold. No patients had 8-iso-PGF2α level above the reference range. Three patients had NAG levels above the threshold with no difference in Ox24 compared to those with NAG within the reference range (54.3 ± 23.1 versus 44.1 ± 25.6, p = 0.5119). Seven patients had NGAL above this level with no difference in Ox24 (41.1 ± 12.3 versus 45.4 ± 27.2, p = 0.6910).

The study population was divided into those 17 patients with hyperoxaluria (> 40 mg/day) and 29 with normal oxaluria. These groups were not different in respect to M:F (3:14 versus 3:26; p = 0.4778), age (47.0 ± 12.31 versus 49.24 ± 10.35; p = 0.5220), or BMI (38.42 ± 14.61 versus 35.08 ± 8.54; p = 0.3527). There was no difference between either group with respect to any of the injury markers, Table 3.

In the study population there was no correlation between any of the three injury markers and SSCaOx:

TABLE 1. **Comparison of kidney injury markers**

	Study group	**Reference group**	**Wilcox 2-sample**
8-iso-PGF2α (pg/mL)	2686.2 [1370.1-3357.7]	2586.8 [1203.33-3031.68]	p = 0.6908
NAG (IU/L)	3.29 [2.24-6.30]	3.33 [2.17-4.15]	p = 0.7241
NAGL (ng/mL)	20.21 [11.41-37.48]	23.1 [8.18-34.93]	p = 0.7561

There was no significant difference in any of the three injury markers between the study group and the normal healthy subjects

TABLE 3. **Comparison of hyperoxaluric and non-hyperoxaluric groups**

	Hyperoxaluric group n = 17	**Non-hyperoxaluric group n = 29**	**Wilcox 2-sample**
8-iso-PGF2α (pg/mL)	2554.5 [1329.5-3321.0]	2698.8 [1370.1-3402.9]	p = 0.8016
NAG (IU/L)	3.16 [2.20-7.45]	3.88 [2.16-6.33]	p = 0.9637
NAGL (ng/mL)	23.8 [12.43-65.9]	18.4 [10.45-32.75]	p = 0.3742

There was no significant difference found between either group with respect to any of the injury markers

NGAL and SSCaOx ($r^2 = 0.0187$); 8-Iso-A and SSCaOx ($r^2 = 0.1784$); NAG and SSCaOx ($r^2 = 0.1502$). There was no correlation between any of the three injury markers and BMI: NGAL $r^2 = 0.0927$; 8-Iso-A $r^2 = 0.1166$; NAG $r^2 = 0.0701$. There was also no correlation between Ox24 and BMI ($r^2 = 0.0231$) or Ox/Cr and BMI ($r^2 = 0.0855$). There was no correlation between age and any of the injury markers in the entire population. In the study population, there was no correlation between age and Ox24 ($r^2 = 0.0017$). 27 patients had SSCaOx level less than 6 (normal). The median and interquartile range was 5.369 [3.416-8.357], Figure 1b.

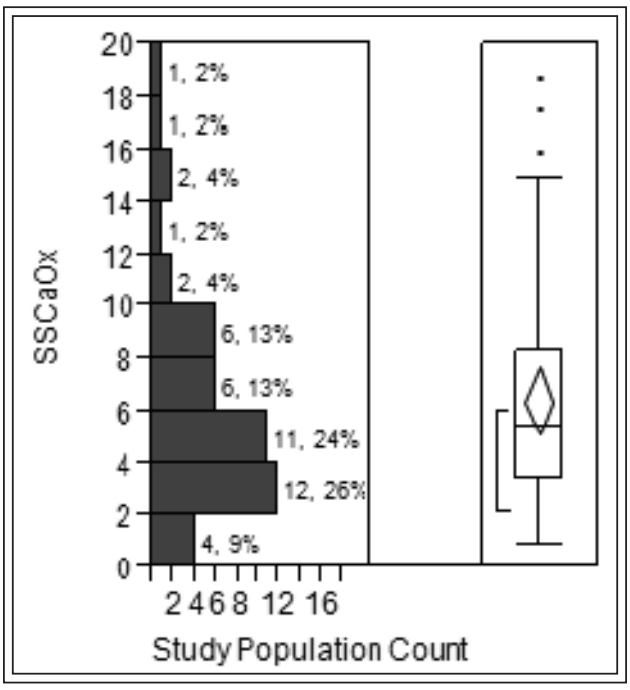

Figure 1b. Distribution of SSCaOx in the study population.

Discussion

Hyperoxaluria is a well-documented complication of mal-absorptive bariatric surgery with reports of subsequent calcium oxalate stones and oxalate nephropathy.[5] Given the increasing utilization of bariatric surgery for the treatment of morbid obesity, the need for early detection of renal damage in hyperoxaluric patients has heightened, with the hope of decreasing rates of subsequent nephropathy.

We used three different markers of kidney injury in order to maximize the chance of finding a useful marker for oxalate induced disease in the bariatric population. 8-iso-PGF2α (also known as Isoprostaglandin F2a type-III) is a compound produced by in vivo free radial peroxidation of arachidonic acid which has been shown to be a useful marker of oxidative stress[9] and has been studied in ESRD patients and kidney transplant patients.[10,11] NGAL is a protein which is released from renal tubular cells during acute kidney injury.[12] The release of NGAL into the blood and urine has been shown to precede any detection in change in GFR[13] and has been used as an established biomarker of AKI.[14,15] Elevated urinary NGAL has been shown to be a marker of decreased renal function in several renal diseases such as primary FSGS,[16] membranous nephropathy,[17] and has correlated with the degree of proteinuria in IgA nephropathy[18] and membranous nephropathy.[16,17] NAG is a lysosomal enzyme which is located predominately in the proximal tubule of the nephron[19] and is a well-established urinary marker of proximal tubule cellular injury.[20] NAG was found to be elevated in children with urolithiasis and nephrocalcinosis, however was not elevated in children with only hyperoxaluria.[21] Other studies have shown a positive relationship between urinary

NAG/Cr ratio and urinary oxalate excretion.[22,23] Elevated NAG has also been shown in rodent models of hyperoxaluria.[24]

Hyperoxaluria is the most common significant urine abnormality in patients following bariatric surgery.[25] Duffey et al reported findings of a prospective cohort study which followed patients who underwent gastric bypass surgery for 2 years.[7] They found a significant increase in urinary oxalate excretion after 3 months that continued to rise until 1 year post op. This level remained stable at the 2 year time point in a significant hyperoxaluric range (> 45.0 mg/day) with an average of 63 ± 29 mg/day. This level of hyperoxaluria was significantly higher than the population in our study (42.66 ± 19.62). The time point for our collections was > 6 months following surgery. It is possible that inflammatory changes in response to hyperoxaluria may not become evident until later in follow up with increased duration and intensity of exposure. The relative early timing of our collections post operatively is one possible explanation that no increase of inflammatory markers was seen in our study. Prior studies have demonstrated a risk of oxalate nephropathy from enteric hyperoxaluria, including subjects with RYGB and biliopancreatic diversion. Whether an associated factor, such as acute volume depletion, is required for the development of oxalate nephropathy is not clear.

In contrast, the level of hyperoxaluria seen in our study does align with postoperative values previously reported in the literature. A recent review article by Canales et al pooled data related to bariatric surgery and kidney stone risk.[26] They identified six articles which were prospective cohort studies and another six which were retrospective studies. In all 12 of these studies the patients undergoing bariatric surgery were non-stone formers, similar to our study population. In the prospective group, the pooled patients totaled 277 and the average of urinary oxalate increased from a mean of 28 mg/d to 44 mg/d. The retrospective group was comprised of 177 patients with a mean postop urinary oxalate of 54 mg/d. However, an earlier study of postoperative bariatric patients without kidney stones had found 26% of subjects had a urine oxalate greater than 100 mg/d on at least one of two urine collections.[27] In the current study only one subject (2.2%) had this severe level of hyperoxaluria. Of note, even this patient did not have an elevated of urine injury markers.

Our study has some limitations. The fraction of patients of patients with severe hyperoxaluria was smaller than a prior study of a similar population using the same laboratory, limiting our ability to detect oxalate related injury. Patients undergoing bariatric surgery have other possible medical risk factors for the development of renal disease such as hypertension, diabetes, while our control group consisted of individuals without significant comorbidities. Despite this we did not demonstrate differences in urine kidney injury markers between patients and controls. While patients and controls were excluded if they had history of kidney stones, it is possible that there were stones which were clinically undetected. Whether a different set of urine injury markers might have proved more sensitive of detecting oxalate related kidney injury is not known.

In summary, urinary kidney injury markers do not appear to be an effective means of screening for post-bariatric renal dysfunction secondary to hyperoxaluria.

Conclusion

Hyperoxaluria was found in 37% of the study population; however, there was no difference seen in the level of any of the injury markers. Further research is needed to determine if there are injury markers which could provide clinically meaningful information in patients following gastric bypass surgery.

Disclosures

Dr. Asplin and Rocky Pramanik are employees of Litholink. All other authors report no conflicts of interest. □

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