Running title: Synbiotics & Urinary Oxalate, Maddahi *et al.*

Effects of Synbiotic Supplementation on Blood and Urinary Concentrations of Factors Related to Kidney Stone Formation in Overweight or Obese Patients with Hyperoxaluria: A Randomized Controlled Trial

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Keywords: synbiotics; calcium oxalate stones; hyperoxaluria; overweight; obesity

Abstract

Purpose: This study investigated the impact of synbiotics on blood and urinary concentrations of factors related to kidney stone formation in overweight or obese patients with hyperoxaluria.

Materials and methods: A randomized double-blind clinical trial was conducted involving forty-four patients assigned to either synbiotic or placebo groups. Participants received their respective capsules twice daily for 12 weeks. Blood and 24-hour urine samples were collected at baseline and week 12 for biochemical analyses.

Results: Urinary oxalate significantly decreased in the synbiotic group compared with both baseline (P=.001) and placebo (P=.001). Other biochemical markers showed no significant differences, while urine volume increased in both groups without between-group variance.

Conclusion: Synbiotic supplementation significantly reduced urinary oxalate but did not affect other blood or urinary parameters associated with kidney stone formation.

Introduction

Kidney stone disease (nephrolithiasis) affects approximately 12% of the population during their lifetime.[1–3] The predominant type, calcium oxalate stone, is strongly associated with hyperoxaluria. Overweight and obesity increase risk.[4,5] Despite improved management strategies, nephrolithiasis incidence continues to rise globally.[6]

Recent research links intestinal dysbiosis to elevated urinary oxalate.[7–10] Probiotic use may influence oxalate metabolism, but prior findings are inconsistent.[11–18] Given existing limitations, this study aimed to evaluate the effects of synbiotic supplementation on urinary and serum factors of stone formation in overweight or obese hyperoxaluric patients.

Materials and Methods

Trial design and ethical aspects

This double-blind, placebo-controlled parallel trial was conducted from December 2024 to March 2025, adhering to the Declaration of Helsinki. Ethical approvals were obtained (IR.SSU.SPH.REC.1403.148; IR.SBMU.RETECH.REC.1404.096). Trial registration: IRCT20120913010826N35.

Participants

Sample size was determined based on differences in urinary oxalate excretion (power 80%, α=0.05). Forty-four patients meeting inclusion criteria (diagnosed calcium oxalate stones, hyperoxaluria, BMI≥25 kg/m², age 18–65 years, normal serum creatinine) were recruited from two Iranian hospitals.

Randomization and intervention

Block randomization (block size 4) was applied. Synbiotic capsules contained twelve probiotic strains (each 1×10^9 CFU) and 21 mg fructo-oligosaccharides; placebo capsules contained identical excipients excluding probiotics. Both were produced by Zist Takhmir Pharmaceutical Company, Tehran. Investigators and participants remained blinded to group allocation throughout the study.

Measurements

Primary outcomes: urinary oxalate, citrate, urate, magnesium, calcium, phosphorus, sodium, potassium, pH, and volume. Plasma and urine samples were collected at baseline and week 12. Biochemical assays were carried out using enzymatic colorimetric and immunoassay techniques, with CV <6% for all parameters.

Anthropometric and dietary assessment

Body weight was recorded at baseline and week 12. Dietary intake was measured using three-day dietary recall and analyzed with Nutritionist IV software.

Compliance assessment

Capsule counts and biweekly telephone monitoring were used to assess compliance (>90% required).

Statistical analysis

Data were analyzed using SPSS v23. Independent and paired *t*-tests compared quantitative data, and repeated measures ANOVA assessed dietary variables. Categorical variables were analyzed using chi-square tests. $P \leq .05$ was considered statistically significant.

Results

Of 44 enrolled participants, two were excluded from the placebo group (one for poor compliance, one for antibiotic use). No adverse events occurred. Compliance averaged above 90%.

No significant baseline differences existed between groups (Table 1). Dietary intake did not change significantly throughout the trial (Table 2). Urinary oxalate significantly decreased in the synbiotic group (P=.001) compared to baseline and placebo, while other urinary indices remained stable (Table 3). Serum biochemical markers did not vary significantly (Table 4).

Discussion

Hyperoxaluria plays a pivotal role in calcium oxalate stone recurrence.[19–21] Synbiotic administration lowered urinary oxalate by 18 mg/day after 12 weeks, aligning with earlier studies showing oxalate reduction following probiotic use.[11–13] Other studies reported no effect, possibly due to differing strains or durations.[14–18]

Proposed mechanisms include degradation of intestinal oxalate by *Lactobacillus* and *Bifidobacterium* species and enhancement of oxalate transporter SLC26A6 expression via short-chain fatty acids.[22–25]

In this study, urine volume increased likely due to hydration recommendations, rather than synbiotic effect. Serum PTH and 25-hydroxycholecalciferol were unaltered, consistent with some previous trials.[28,29]

Limitations include relatively small sample size, short intervention duration, and lack of microbiota profiling. Future studies should extend duration and include microbial analysis.

Conclusions

Synbiotic supplementation significantly reduced urinary oxalate in overweight or obese adults with hyperoxaluria but had no impact on other biochemical indices associated with nephrolithiasis.

Summary

Synbiotic use reduced urinary oxalate in overweight or obese hyperoxaluric patients, indicating potential benefit in reducing kidney stone risk.

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Conflict of Interest

The authors declare no conflicts of interest.

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Table 1- Baseline characteristics of patients with a history of calcium oxalate kidney stones in the synbiotic and placebo groups

Variables [a,b]	**Synbiotic (n=22)**	**Placebo (n=20)**	***P*-value**
Age, year; mean ± SD (range)	47.0± 7.8 (35-64)	46.7 ± 10.0 (30-63)	0.914
Sex, n (%)			
Men	16 (73)	17 (85)	0.460
Women	6 (27)	3 (15)	
Smokers, n (%)	9 (41)	8 (40)	1.000
Alcohol consumption, n (%)	5 (23)	4 (20)	1.000
History of kidney stone surgery, n (%)	19 (86)	17 (85)	1.000
Weight, kg; mean ± SD			
Baseline	87.0±12.0	89.5±10.5	0.459
Week 12	85.0±11.0	89.0±9.5	0.298

[a] Continuous variables were compared by independent samples t-test

[b] Categorical variables were compared by chi-square test

Table 2- Dietary factors of patients with a history of calcium oxalate kidney stones in the synbiotic and placebo groups [a]

Factors [b,c,d]	Baseline	Week 6	Week 12	Within-group *P*-value
Energy (Kcal/day)				
Synbiotic	2056±273	2002±190	2009±154	.632
Placebo	2074±316	1982±157	2020±162	.397
Between-group *P*-value	.857	.725	.830	
Protein (g/day)				
Synbiotic	64±16	57±10	57.5±13	.137
Placebo	63±14	57±9	61±11	.407
Between-group *P*-value	.839	.857	.416	
Carbohydrate (g/day)				
Synbiotic	246±58	241±40	254±31	.664
Placebo	260±77	244±31	248±33	.573
Between-group *P*-value	.538	.803	.554	
Fat (g/day)				
Synbiotic	91±23	93±16	89±12	.759
Placebo	87±25	89±12	90.5±12	.814
Between-group *P*-value	.629	.379	.635	
Fiber (g/day)				
Synbiotic	16±3	15±3	14±3	.181
Placebo	17±6	14±3	14±3	.066
Between-group *P*-value	.524	.789	.727	
Calcium (mg/day)				
Synbiotic	591±237	503±156	473±154	.128
Placebo	500±116	469±121	464±169	.719
Between-group *P*-value	.147	.459	.866	
Phosphorus (mg/day)				
Synbiotic	901±88	891±142	931±167	.101
Placebo	901±69	863±116	947±177	.135
Between-group *P*-value	.981	.511	.786	
Magnesium (mg/day)				
Synbiotic	232±50	223.5±46	225±46	.527
Placebo	237±29	218±33	239±49	.213
Between-group *P*-value	.753	.653	.379	
Potassium (mg/day)				
Synbiotic	1814±248	1836±330	1925±330	.220
Placebo	1929±197	1773±285	1957.5±426	.113
Between-group *P*-value	.131	.534	.793	

[a] N=22 for the synbiotic group and N=20 for the placebo group.
[b] All values are presented as mean ± SD
[c] Within-group comparisons were performed by analysis of variance for repeated measurements
[d] Between-group comparisons were performed by independent samples t-test

Table 3- Urinary concentrations of factors associated with kidney stone formation in the synbiotic and placebo groups [a]

Parameters [b,c,d]	**Baseline**	**Week 12**	**Changes** [e]	**Within-group *P*-value**
Oxalate (mg/24h)				
Synbiotic	51.5 ± 10.0	33.5 ± 8.0	-18.0 ± 12.0	**.001**
placebo	50.5 ± 7.0	47.5 ± 7.5	-3.0 ± 7.0	.094
Between-group *P*-value	.693	**.001**	**.001**	
Citrate (mg/24h)				
Synbiotic	583 ± 274	590 ± 157	7 ± 303	.920
Placebo	476 ± 151	583 ± 187	107 ± 266	.087
Between-group *P*-value	.122	.905	.261	
Urate (mg/24h)				
Synbiotic	552 ± 149	611 ± 138	58 ± 204	.193
Placebo	605 ± 154	569 ± 185	-35 ± 108.5	.161
Between-group *P*-value	.268	.414	.074	
Sodium (mEq/24h)				
Synbiotic	178±93	164±69	-14±74	.378
Placebo	176±67	148±62	-28±78	.122
Between-group *P*-value	.926	.424	.555	
Calcium (mg/24h)				
Synbiotic	167±93	146±69	-21±69	.158
Placebo	170±94	172±82	2±23	.747
Between-group *P*-value	.907	.260	.148	
Phosphorus (mg/24h)				
Synbiotic	573±173	674±207	101±255	.077
Placebo	534±197	613±134	79±219	.121
Between-group *P*-value	.496	.258	.766	
Magnesium (mg/24h)				
Synbiotic	83±26	94±38	11±40	.211
Placebo	90±26	80±25.5	-10±33	.178
Between-group *P*-value	.366	.177	.068	
Potassium (mEq/24h)				
Synbiotic	50.5±17	46.5±23	-4.0±28	.513
Placebo	50.0±27	46.0±30	-4.0±39	.658
Between-group *P*-value	.923	.946	.992	
Urine Volume (mL/24h)				
Synbiotic	1708±577	2248±357	540±667	**.001**
Placebo	1473±497	1815±652	342±648	**.029**
Between-group *P*-value	.167	.013	.335	
Urine pH				
Synbiotic	5.73±0.48	5.70±0.33	-0.03±0.29	.669
Placebo	5.82±0.42	5.82±0.39	0.00±0.18	1.000
Between-group *P*-value	.508	.291	.722	

[a] N=22 for the synbiotic group and N=20 for the placebo group.
[b] All values are presented as mean ± SD
[c] Within-group comparisons were performed by paired t-test
[d] Between-group comparisons were performed by independent samples t-test
[e] Changes reflect week 12 – baseline values.

Table 4- Serum concentrations of factors associated with kidney stone formation in the synbiotic and placebo groups [a]

Parameters [b,c,d]	**Baseline**	**Week 12**	**Changes** [e]	**Within-group *P*-value**
Uric Acid (mg/dL)				
Synbiotic	5.5 ± 1.2	5.3 ± 1.2	-0.2 ± 1.0	.403
Placebo	5.3 ± 1.7	5.8 ± 1.9	0.5 ± 1.84	.232
Between-group *P*-value	.641	.342	.147	
Sodium (mEq/L)				
Synbiotic	139.0±1.8	139.0±1.8	0.0±1.8	.637
Placebo	138.0±1.9	138.0±2.5	0.0±2.6	.289
Between-group *P*-value	.437	.227	.503	
Calcium (mg/dL)				
Synbiotic	9.4±0.5	9.6±0.5	0.2±0.6	.125
Placebo	9.4±0.4	9.4±0.4	0.0±0.3	.304
Between-group *P*-value	.730	.140	.065	
Phosphorus (mg/dL)				
Synbiotic	3.5±0.6	3.4±0.5	-0.1±0.7	.462
Placebo	3.4±0.5	3.6±0.6	0.2±0.6	.086
Between-group *P*-value	.708	.123	.085	
Magnesium (mg/dL)				
Synbiotic	2.1±0.2	2.1±0.2	0.0±0.2	.483
Placebo	2.1±0.2	2.1±0.1	0.0±0.3	.340
Between-group *P*-value	.775	.892	.769	
Potassium (mEq/L)				
Synbiotic	4.0±0.2	4.1±0.2	0.1±0.2	.196
Placebo	4.1±0.3	4.2±0.2	0.1±0.4	.150
Between-group p value	.576	.115	.526	
PTH (pg/mL)				
Synbiotic	33.0±11.0	31.5±10.0	-1.5±4.5	.106
Placebo	29.0±11.5	28.0±9.0	-1.0±8.0	.631
Between-group *P*-value	.260	.287	.703	
25-OH-D3 (ng/mL)				
Synbiotic	29.0±10.0	29.5±9.0	0.5±3.0	.437
Placebo	33.0±10.0	33.0±11.0	0.0±3.0	.722
Between-group *P*-value	.207	.301	.423	

[a] N=22 for the synbiotic group and N=20 for the placebo group.

[b] All values are presented as mean ± SD

[c] Within-group comparisons were performed by paired t-test

[d] Between-group comparisons were performed by independent samples t-test

[e] Changes reflect week 12 – baseline values.

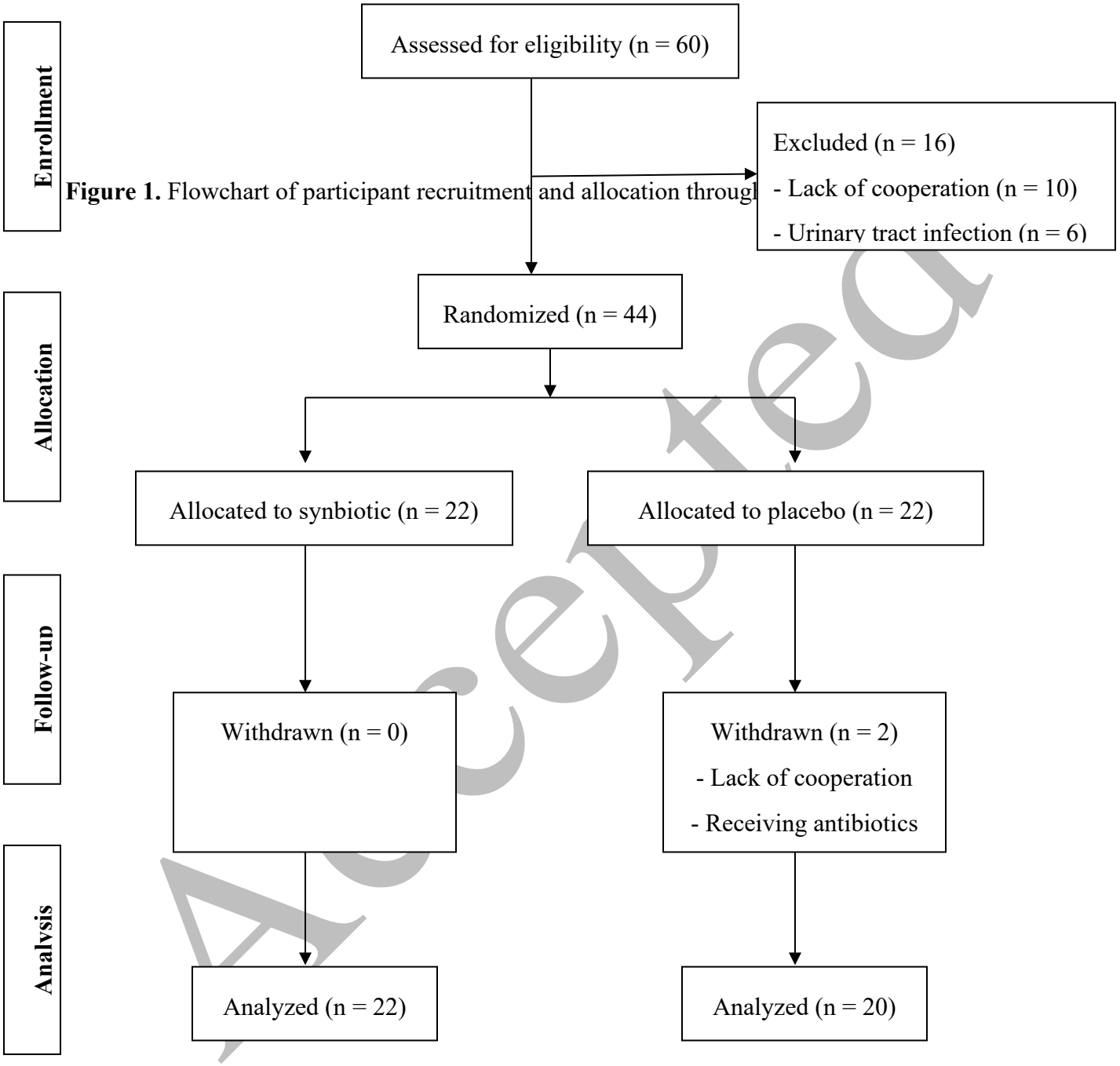

Figure 1. Flowchart of participant recruitment and allocation throughout the study

Figure 1- Summary of the patient flow diagram.