Original Article

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Determination of Role of Hyaluronan in Recurrent Renal Stone Formation

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Abstract

Background: Extracorporeal shock wave lithotripsy (ESWL) and percutaneous nephrolithotomy (PCNL) are now widely used to eradicate stones. The present study was conducted to determine role of hyaluronan in recurrent renal stone formation. **Subjects and Methods:** The study was conducted at Department of Urology, Sapthagiri Institute of Medical Sciences and Research Center, Bangalore between November 2019 to August 2020 on 45 patients of both genders were classified into 3 groups. Group I were normal (15), group II (15) were stone formers and group III (15) were post- treated stone formers. The total urinary glycosaminoglycans, hyaluronan and the proportion of HA in total GAGs were recorded. **Results:** There were 8 males and 7 females in group I, 6 males and 9 females in group II and 10 males and 5 females in group III. The total urinary glycosaminoglycans (GAGs) concentration in group I was 235.4 μg hexuronate/ mmol creatinine, in group II was 142.3 μg hexuronate/ mmol creatinine and in group III was 108.2 μg hexuronate/ mmol creatinine. The mean hyaluronan level in group I was 812.4 μg hexuronate/ mmol creatinine, in group II was 1725.4 μg hexuronate/ mmol creatinine and in group III was 672.4 μg hexuronate/ mmol creatinine. The mean HA in total GAGs was 0.26% in group I, 0.74% in group II and 1.04% in group III. The difference was significant ($P< 0.05$). **Conclusion:** Increased HA production during inflammation of renal epithelial cells in SF do enhance the risk of renal stone formation and an higher HA proportion in total GAGs of both SF and Post-SF indicated that they have a higher risk for the occurrence and recurrence of kidney stone disease.

Keywords: Renal Stones, Hyaluronan, ESWL/PCNL, Recurrent Renal Stone Formation

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Introduction

Renal stones have afflicted humans for millennia. Many researchers are attempting to elucidate the mechanism of Calcium Oxalate renal stone formation. Archeological findings give profound evidence that humans have suffered from kidney and bladder stones for centuries.[1] The risk of developing urolithiasis in adults appears to be higher in the western hemisphere than in the eastern hemisphere (1–5%), although the highest risks have been reported in some Asian countries such as Saudi Arabia (20.1%) with lifetime recurrence rates of upto 50%.[2] The interval between recurrences is variable, with approximately 10% within one year, 35% in five years, and 50% by 10 years. However, approximately 75% of stones are primarily calcium oxalate, but up to 50% of these include calcium hydroxyl phosphate (brushite or calcium hydroxyapatite) in trace or greater amounts; 10–20% are composed of magnesium ammonium phosphate (struvite or triple phosphate); 5% are composed of urate; and 1-2% are composed of cystine.[3]

Extracorporeal shock wave lithotripsy (ESWL) and percutaneous nephrolithotomy (PCNL) are now widely used to eradicate stones. However, recurrence rates remain high (up to 60%) over the lifetime of certain patients.[4] These recurrent stone-formers are good models for investigations to establish the important biological markers and mediators of inflammation in the blood and urine of renal stone patients. The fragments of stones remaining after ESWL/PCNL, whether they are clinically insignificant or significant, can pose a long-term risk for patients by serving as a nidus for new stone-formation.[5] There is a need for a reliable biomarker to know about recurrance of stone formation. The present study was conducted to determine role of hyaluronan in recurrent renal stone formation.

Subjects and Methods

The present study was conducted at Department of Urology, Sapthagiri Institute of Medical Sciences and Research Center, Bangalore between November 2019 to August 2020 among 45 patients of both genders. All patients were informed regarding the study and their consent was obtained. Institutional ethics committee approval obtained for the study.

Data such as name, age, gender etc. was recorded. Patients were classified into 3 groups. Group I were normal (15), group II (15) were stone formers and group III (15) were post-treated stone formers. Urine and blood samples were collected from the patients and processed with ELISA and biochemical methods (electrophoresis and HPLC). The total urinary glycosaminoglycans (GAGs) concentration (μg hexuronate/ mmol creatinine), hyaluronan (HA) concentration (ng/ mmol creatinine) and the proportion of HA in total GAGs were recorded. Results thus obtained were subjected to statistical analysis. P value less than 0.05 was considered significant.

Results

Table 1: Distribution of patients

Groups	Group I	Group II	Group III
Status	Normal	Stone formers	Post- treated stone formers
M:F	8:7	6:9	10:5

[Table 1] shows that there were 8 males and 7 females in group I, 6 males and 9 females in group II and 10 males and 5 females in group III.

[Table 2 & Figure 1] shows that the total urinary glycosaminoglycans (GAGs) concentration in group I was 235.4 μg hexuronate/ mmol creatinine, in group II was 142.3 μg hexuronate/ mmol creatinine and in group III was 108.2 μg hexuronate/ mmol creatinine. The mean hyaluronan level in group I was 812.4 μg hexuronate/ mmol creatinine, in group II was 1725.4 μg hexuronate/ mmol creatinine and in group III was 672.4 μg hexuronate/ mmol creatinine. The mean HA in total GAGs was 0.26% in group I, 0.74% in group II and 1.04% in group III. The difference was significant (P< 0.05).

Discussion

The formation of renal stones is a consequence of increased urinary supersaturation with subsequent formation of crystalline particles. Supersaturation is the driving force for crystallization in solutions like urine. When a salt is added to a solvent it dissolves in the solvent until a particular concentration is reached, beyond which no further dissolution is possible. At this point, the solvent is said to be saturated with the salt.[6] If more salt is added it crystallizes in solution, provided the temperature and pH are unchanged. The concentration at which saturation is reached and crystallization begins is called the thermodynamic solubility product (Ksp).[7] If inhibitors of crystallization were not able to act, the final result will be nephrolithiasis. Inhibitors allow higher concentration of calcium salts to be held in solution than in pure solvents. Urine is thus metastable with respect to calcium salts.[8] The present study was conducted to determine role of hyaluronan in recurrent renal stone formation.

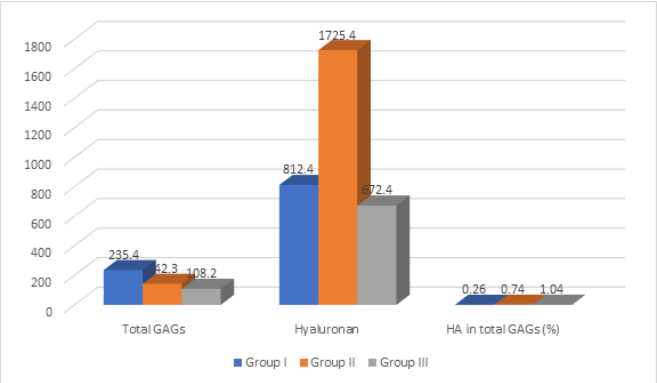

Figure 1: Assessment of urinary glycosaminoglycans (GAGs) and hyaluronan level in groups

In present study, there were 8 males and 7 females in group I, 6 males and 9 females in group II and 10 males and 5 females in group III. Mayur et al,[9] studied concentrates on idiopathic stone-formers (SF) and 6 groups of subjects were recruited with Active SF (pre- and post- treatments), Non-SF (with and without infection) for comparisons. 120 samples were collected amongst the 3 groups. The following demographics were obtained: Age-range (32 – 63 years old); Male: Female ratio (58: 42); Mean urinary pH 6.33 $\pm$ 0.23 (though in each group there are differential mean pH) and urinalysis done for all samples to verify the integrity of the samples. The first biomarker studied was the excretion of urinary glycosaminoglycans (GAGs). Chondroitin sulphate A/C (CS), dermatan sulphate (DS), heparin sulphate (HS) and hyaluronan (HA) were extracted and quantified. Active SF (prior treatment) had 70% positive indicator for GAGs and those SF (post treatment) had over 90% compared to the Normals. Other biomarkers (not reported here) under investigations are cytokines including NAG and MIP-1α.

We found that the total urinary glycosaminoglycans (GAGs) concentration in group I was 235.4 μg hexuronate/ mmol creatinine, in group II was 142.3 μg hexuronate/ mmol

Table 2: Assessment of urinary glycosaminoglycans (GAGs) and hyaluronan level in groups

Groups	Group I	Group II	Group III	P value
Total GAGs	235.4	142.3	108.2	0.01
Hyaluronan	812.4	1725.4	672.4	0.02
HA in total GAGs (%)	0.26	0.74	1.04	0.05

creatinine and in group III was 108.2 μg hexuronate/ mmol creatinine. The mean hyaluronan level in group I was 812.4 μg hexuronate/ mmol creatinine, in group II was 1725.4 μg hexuronate/ mmol creatinine and in group III was 672.4 μg hexuronate/ mmol creatinine. The mean HA in total GAGs was 0.26% in group I, 0.74% in group II and 1.04% in group III, thereby indicating that the levels of Hyaluronan are higher in stone formers and post-treatment stone formers which is also statistically significant ($p<0.05$).

Hyaluronan (HA) is a non-sulfated GAGs involved in several fundamental cell biological processes such as regulation of cell-cell adhesion, development, proliferation, migration, differentiation, metastasis, inflammation and wound healing.[10] HA fragments are released into urinary tract as a consequence of active turnover of renal tissue in the diseased state. Studies showed that migrating cells produce large amounts of HA during repair of damaged renal epithelial cells. Up-regulation of HA was observed in human kidney proximal epithelial (HK-2) cells during CaOx crystals induced cell injury for mediating repair of an injured epithelium.[11]

Conclusion

Authors found that increased HA production during inflammation of renal epithelial cells in SF do enhance the risk of renal stone formation and an higher HA proportion in total GAGs of both SF and Post-SF indicated that they have a higher risk for the occurrence and recurrence of kidney stone disease. The drawback of study is it was a smaller study and for a short period of time. Larger studies at multiple centres can throw more light on the role of hyaluronan in recurrent renal stone formation.

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