

ALBUMIN AND ITS ROLE IN UROLITHIASISDEVESH RAIZADA¹, PRAGYA KUMAR¹, TANYA SINGH¹, TRISHA PRUTHI¹, PRIYADARSHINI^{1*}¹Department of Biotechnology, Jaypee Institute of Information Technology, Noida, Uttar Pradesh, India.

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ABSTRACT

Urolithiasis is a multifactorial disease with an incidence rate of more than one million cases reported annually in India. Various forms of the calculus have been reported to have 90-95% inorganic and 5% organic matter. Out of the major proteins that comprise this organic component of the matrix, albumin, and uromodulin are found to be the most abundant. Albumin is also the most abundant protein in the human blood serum where it plays the role of a transporter of hormones, fatty acids, and other compounds. The increased concentrations of albumin may significantly affect a patient's susceptibility to kidney stone formation. The study of the role of albumin in urolithiasis could give us useful insights on its potential role in this disease and may add to the therapeutic repertoire of albumin.

Keywords: Urolithiasis, Calculus, Albumin, Albuminuria, Therapeutic agent.© 2017 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2017.v10i10.20059>**INTRODUCTION**

Urolithiasis is a widespread disease which has been seen to be affected by age, sex, and race. The problem is more common in males than in females and varies with age. It is more frequent in the middle-aged males and in women in their late 20's [1]. Furthermore, whites are more prone to the disease with its highest occurrence in middle-aged white men [2,3].

Stone formation occurs in the urinary tract including the tubular region of the kidney (nephrolithiasis), ureter (ureterolithiasis) or bladder (cystolithiasis) and is thus in constant contact with the urine and its various components [4]. Kidney calculi are classified into five major types: Calcium oxalate (CaOx) - most commonly occurring, calcium phosphate (CaP), uric acid, struvite, and cysteine stones [5,6]. Besides, the fact that these urinary risk factors that are excreted in the urine, such as calcium, uric acid, and cysteine, are higher in men might actually be one major reason hypothesized to be bolstering the demographic findings [7]. Thus, digging further deep into the causative and the modulating factors that affect the disease, the role of the various components of the urine seems one regulatory aspect that cannot be ignored.

Albumin, a commonly occurring protein found in the kidney stone matrix is one of the most abundant proteins in the blood plasma as well as the urine [8]. It is small in size and is easy to pass through the glomerulus under slight stress [9]. Under metabolic aberrations and diseased conditions, the concentration of albumin can be abnormally high in the urine; it may be possible that the increased concentrations of albumin may significantly affect a patient's susceptibility to kidney stone formation once its true role in the process is identified.

HUMAN SERUM ALBUMIN (HSA)

HSA is an abundant protein in the blood and is synthesized in the liver. It is negatively-charged and supposedly comprises 50% of the total protein content of the plasma. Moreover, it has a molecular weight of 66 kDa and is made up of 565 amino acids and three homologous domains, each containing of two sub-domains [10]. HSA can exist in monomeric, oligomeric as well as polymeric forms in solution and can undergo conformational changes when the pH changes [11].

The protein has several important functions in the body. HSA is most notably known for its several ligand binding sites: Sites I and II are responsible for the binding of most pharmaceuticals that interact with the protein. Different large and bulky endogenous substances bind with high affinity to Site I of HSA indicating its adaptable nature while Site II is smaller and less flexible as the binding is more stereospecific. Apart from Sites I and II HSA has other binding sites for drugs and compounds that do not bind to either of the two [12,13].

Ligands binding to HSA can be broadly divided into two categories - The endogenous ligands and the exogenous ligands. The endogenous category comprises free fatty acids, bilirubin, free radical species, hormones, etc. Bilirubin, almost all of it, binds to albumin and is transported to the liver for further processing whereas free fatty acids also bind to albumin and are thus converted to a nontoxic form. Exogenous ligands like drugs show their affinity for HSA. Warfarin, an anticoagulant drug shows an affinity for Site I while general anesthetics such as propofol and halothane are known ligands binding to Site II [14]. Antibiotics, e.g., penicillin, cephalosporin, anti-inflammatory drugs such as ibuprofen, salicylic acid, and central nervous system drugs such as thiopental and chlorpromazine are some other examples and broad categories of drugs that have shown their affinity for HSA [14].

The pharmacokinetics of drugs is affected by the binding phenomenon discussed above. The therapeutic level of a drug depends on the binding activity of other drugs as well as on plasma concentrations of endogenous HSA ligands. Hence, if the concentration of endogenous ligands increases unexpectedly then this might lead to a massive release of the bound drugs and in turn lead to intoxication, whereas the opposite case in which drug-binding affects HSA binding of endogenous ligands; it may lead to an alteration in their homeostasis [14]. Bilirubin is known for competing with several drugs for the same binding site, hyperbilirubinemia - A state in which there are increased amounts of bilirubin in the blood, the level of unbound drugs in the circulatory system can be high and it can be damaging [14,15]. Aspirin, warfarin, etc., are some drugs that compete with bilirubin for the same binding site [14]. These ligand-binding activities of HSA also contribute toward its antioxidant properties [16-18].

One of the other important functions of HSA includes the maintenance of oncotic pressure, i.e., the ability to be able to balance out the

tendency of fluids to leak out of the vascular system and into the tissues [10,19]. The capillary pore size in vascular endothelium of non-hepatic capillaries is 6-7 nm in width which is slightly less than the size of an HSA molecule and which allows for the retention of albumin in the vascular system [20]. Approximately, 3.3 g of HSA is filtered daily in the kidneys and subsequent tubular reabsorption provides for recovering most of it, 71% in the proximal convoluted tubule, 21% in the loop of Henle and the distal convoluted tubule, and 3% in the collecting ducts [21]. Size selectivity is thought to be the only criteria for glomerular albumin filtration [22].

As a therapeutic agent, albumin has been previously used in response to vascular collapse for maintaining colloidal pressure and fluid balance. It is majorly used in the treatment of liver diseases like cirrhosis for vascular volume maintenance. The molecular adsorbent recirculating system treatment for liver dialysis utilizes albumin as it readily binds toxins, protein breakdown products, copper ions, etc., that accumulate during diseases like cirrhosis. Not only this, nitric oxide (NO) production is reported in cirrhosis that further leads to renal impairment and organ failure, HSA can specifically bind to NO and regulates its amounts helping preclude detrimental downstream effects [10].

HSA IN UROLITHIASIS

HSA was one key component of the protein species that were adsorbed on the surface of hydroxyapatite (HAP) crystals when these crystals were incubated in whole human serum [23]. Bolstering the aforementioned findings, Atmani *et al.* reports albumin to be the major component of the organic matrix of both CaOx as well as CaP crystals when the crystals were induced in the urine of stone formers, while Kaneko *et al.* and Dussol *et al.* reported albumin as a commonly occurring protein in all forms of kidney stone formations [8,24,25]. Henceforth, the implication that albumin, given its prominence in the calculi, can be a major regulator of nephrolithiasis seems relevant and forms a significant aspect of the investigations into the disease.

In vitro studies of albumin in urolithiasis show that it was able to promote the process of nucleation in CaOx crystals in both an immobilized phase as well as in solution. Both monomeric and polymeric forms of albumin can produce this effect even though the polymeric form nucleates larger crystals than its monomeric counterpart, also hinting at the significant role that the aggregation of the protein might play in the nucleation process. Furthermore, quite contradictory to its nature as a promoter of nucleation in CaOx crystals, albumin inhibits aggregation in a concentration-dependent manner. Although, this involvement in the nucleation process might just be benign [26].

Albumin preferentially leads to the formation of COD (CaOx dehydrate) crystals over COM (CaOx monohydrate), a similarly favorable formation of COD has also been reported with the use of (BSA), a homolog of HSA [26,27]. These crystals are small and are easily removed in comparison to their larger counterparts (COM crystals). The fact that healthy subjects have smaller crystals in their urine than stone formers supports these findings [28-30].

Furthermore, the presence of COM crystals is associated with stone formers rather than healthy individuals as they are found absent in the latter that have more of COD. The inhibitory nature during aggregation can be attributed to the fact that COD crystals have a high positive charge and heavy repulsion between crystals might result in a decrease in the formation of aggregates; further, the less negative charge might affect crystal retention [25]. In HAP - CaP crystals, a concentrated albumin-rich extract showed equal potency in inhibiting HAP crystal growth when compared to an albumin-free serum showcasing the protein's efficacy in comparison to other serum components [31]. Furthermore, albumin, a known drug binding agent has been seen to bind other urinary macromolecules as well [26].

Although these studies suggest a tentatively benign role of the protein in urolithiasis, drawing more practical and applicable conclusions require

further investigations especially regarding the mechanism through which albumin interacts with the crystal. The binding of albumin has been suggested to be face-specific and competitive with protein showing a penchant for the side faces in inorganic COM crystals, whereas the carboxyl groups of albumin have been suspected to be preferentially involved in the nucleation process in CaOx crystals [26,32]. Moreover, the forms of albumin used for *in vitro* studies are not the exact representations of the albumin present in the urine as it is a transport protein and is present in a complexed form with other molecules, dubiousness over the present data further prevails when one reaches farther out to take into consideration the complex environment of the urine. Not only this, the protein has been found to aid crystal invasion of the extracellular matrix which is a significant process in kidney stone formation [33].

ALBUMINURIA, METABOLIC SYNDROME, AND UROLITHIASIS

Numerous studies have been put forward the possible association of metabolic syndrome and urolithiasis [34,35]. The metabolic syndrome refers to an array of risk factors for heart diseases and other health issues, such as stroke and diabetes. The five conditions or traits of metabolic syndrome are abdominal obesity, high blood pressure, insulin resistance, dyslipidemia, and cardiovascular risk [36,37]. Cupisti *et al.* observed the association of insulin resistance with calcium nephrolithiasis by assessing insulin resistance via the homeostatic model assessment-insulin resistance (HOMA-IR) value and drawing association between HOMA-IR value lowered citrate excretion in calcium stone formers. The levels of HOMA-IR value were higher in hypocitraturic patients than ones with normal citrate excretion [34]. Insulin resistance has been seen to be specifically linked to the lithogenesis of uric acid crystals as well. It induces defective ammoniogenesis in kidneys resulting in the production of acidic urine. The most prevalent manifestation of metabolic syndrome is abdominal obesity which further causes insulin resistance [38]. There is an increase in incidences of urolithiasis up to 75% in patients who are overweight or obese [34]. Kohjimoto *et al.* studied that patients with four metabolic traits have 1.8 times greater chances of recurrent stone formation as well as the formation of multiple stones compared to patients that have no traits of the same [35].

Under certain physiological conditions such as inflammation or swelling of kidney filters and disorders such as diabetes and hypertension, the level of albumin in the urine can get altered leading to increased concentrations of the protein, a condition called albuminuria [39,40]. The kidney performs the function of filtering the blood to remove waste products and preventing large molecules such as proteins from passing through it. If the glomerulus is impaired or its functioning altered, proteins such as albumin pass from the blood into the urine [41]. If albumin leaks into the urine in a very small quantity, it is known as microalbuminuria, i.e., 30-300 mg/day. If the glomerulus is severely damaged, then greater amounts of albumin may be present in the urine, and this stage is called macroalbuminuria, i.e., >300 mg/day [42]. Microalbuminuria is recommended under the World Health Organization criteria as one of the indicators to diagnose the metabolic syndrome [39]. A study was conducted by Cho *et al.* to evaluate the association between serum albumin levels and the presence of metabolic syndrome among a sample of healthy Korean

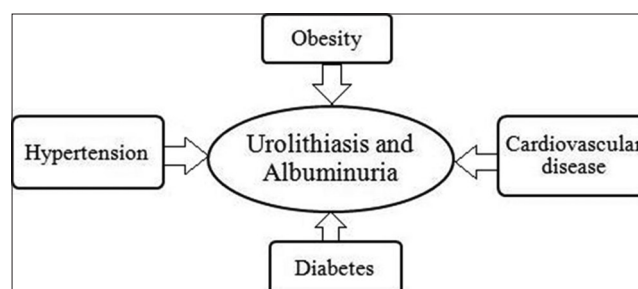


Fig. 1: Conditions associated with urolithiasis and albuminuria

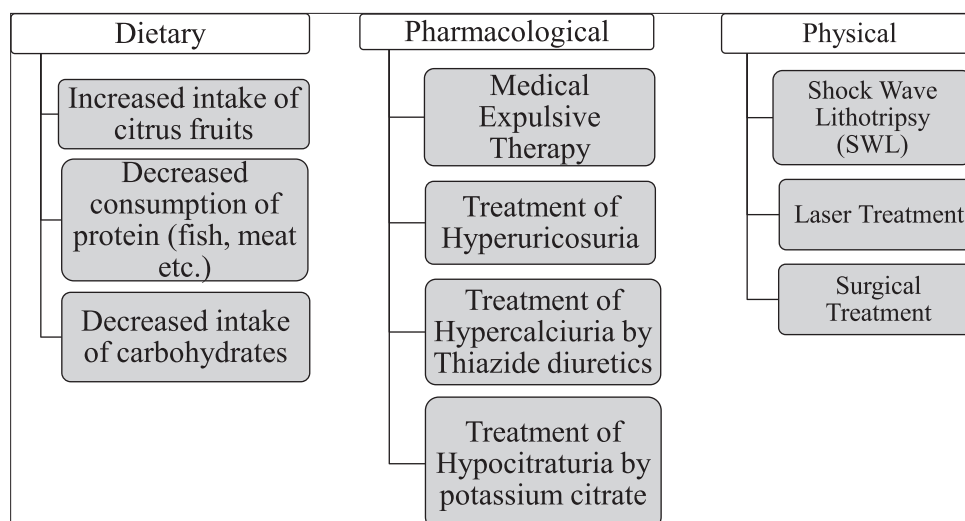


Fig. 2: Methods for treatment of urolithiasis

adults. The results showed a positive association between serum albumin levels, and the occurrence of metabolic syndrome in the cohort even though related covariates such as age, body mass index, smoking status, alcohol consumption, and physical activity were controlled [43].

The aforementioned studies have identified (1) albumin to be a prominent macromolecule in the renal calculi and (2) its involvement in urolithiasis as a modulator in the process of stone formation. Besides, the increase in susceptibility to urolithiasis as a downstream effect of metabolic syndrome, a condition seen to be associated with the development of albuminuria, also directs attention to the role of albumin and the extent to which it affects the process of stone formation which needs to be further investigated for a better understanding (Fig. 1).

THERAPEUTICS IN UROLITHIASIS

Therapeutics in urolithiasis today harbors a wide range of possibilities ranging from physical to pharmacological to dietary preventive as well as curative treatment. Dietary and pharmacological treatment is easier and less invasive while in severe conditions where the stone formations can no longer be treated by the former methods, physical treatment is implemented. Fig. 2 shows some of the more commonly used physical, dietary as well as pharmacological measures [44-46].

CONCLUSION

Urolithiasis has been found to be a consequential risk in various metabolic disorders, such as diabetes and obesity, that are often associated with albuminuria, something that is also suggestive of a possible effect of the increased concentrations of albumin in the urine which might or might not be a positive contribution toward stone formation. Albumin has also been found to be one of the most commonly occurring proteins in the calculi. Furthermore, *in vitro* studies have shown that it might actually play a beneficial role in the prevention of CaOx stone formations by forming COD crystals that are supposedly less damaging than COM. A potent role as an inhibitor of CaP crystal growth has also been reported.

It may be that the imbalance in the concentration of albumin present in the urine is affecting stone formation in the patient. If the increased concentrations of albumin in the urine are actually promoting stone formations, then the aberrant state of albuminuria might just add to the susceptibility of a person to urolithiasis.

Albumin is currently being used in therapeutics for liver cirrhosis, malnutrition, nephrotic syndrome, etc. [47]. Therefore, if the true nature of albumin as a modulator of urolithiasis is further investigated then its regulation in urine might help in the medical therapies to combat this

disease. However, the practicability of these studies is also a question considering the complexity of the urine and the fact that albumin *in vivo* occurs in a complex with other molecules and might be involved in a more complicated mechanism in its interaction with the crystals.

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