Review

Revisiting Randall’s plaque

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Abstract
Kidney stones have probably affected mankind for ages with early reports in an Egyptian mummy. While prevalence of stone disease is increasing, its pathogenesis remains elusive. Randall, after his study on more than 1100 cadaver kidneys, gave hypothesis of subepithelial plaque acting as a nucleation site for kidney stones. His plaque hypothesis met with criticism because he proposed a unified theory for all types of stones. However, recently Randall’s plaque has been reinvestigated. This review discusses their role in stone formation and current understanding about their pathogenesis. Randall’s plaques begin in the basement membrane of thin segment of loop of Henle. Low urine volume, hypercalciuria, low urine pH are now implicated as important urinary risk factors. Plaque–stone association is best described in the idiopathic calcium oxalate stone formers. Pathogenesis of plaque itself involves interaction of multiple factors including gene polymorphism, oxidative stress, inflammatory mediators, matrix proteins, and urinary solute supersaturation.

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Introduction
The history of kidney stones probably dates back to the history of medicine itself with earliest reference of kidney stone in the Oath of Hippocrates. However, pathogenesis of kidney stones still remains an unsolved mystery. Alexander Randall, from his observations on cadaver kidneys, proposed that suburothelial apatite plaques are the nucleating sites for stone formation. Randall’s idea was not immediately realized due to unavailability of the modern technology to prove it. But recent advances in the field of endourology, radiology and molecular biology have revived the interest in Randall’s plaque (RP).

The purpose of this review is to present evolution of RP, hypothesis of plaque formation, and its role in the pathogenesis of renal stone. Pubmed search was conducted with keywords “Randall plaque”, “Randall’s plaque”, and “Randall’s plaque pathogenesis” till November 2013. After excluding duplicate records all the abstracts were screened and suitable studies were selected for review (Fig. 1).

Abbreviations: RP, Randall’s plaque; SEM, scanning electron microscope; CaOx, calcium oxalate; ICSF, idiopathic calcium oxalate stone formers; FTIR, Fourier transform infrared microspectroscopy; TEM, transmission electron microscopy; HA, hydroxyapatite; LH, loop of henle; IHC, immunohistochemistry; THP, Tamm–Horsfall protein; ROS, reactive oxygen species; CaP, calcium phosphate.

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Origin of Randall’s plaque concept

Randall’s renal stone research is a result of his observations on a lady who presented to him in 1934 for left side flank pain. A small radio opaque shadow was seen on plain X-ray abdomen in the right renal area. Dr. Randall followed this lady with plain X-ray abdomen. The shadow remained stationary and she remained asymptomatic for three years. After three years she presented with acute colic and the shadow was seen to have moved lower down in the ureter. After few days she passed out the stone in urine. It was a small stone with smooth convex surface on one side and concave surface on opposite side representing attachment site. Randall thought that stones like this could grow attached to some area of minor calyx for years until they detach and cause symptoms. He called these stones as papillary stones.

In order to confirm his idea, he dissected 1154 pairs of kidneys in autopsy lab and observed collecting system with hand held magnifying glass. He found papillary plaques of calcium in 237 (20.5%) individuals and named them as type I plaques [1,2]. In 65 individuals he actually observed small papillary stone attached to the site of plaque [1,2]. Randall also observed type II plaques in 23 individuals due to intra-tubular calcium salt deposits. Most important and consistent observation was that plaque was made of calcium phosphate while stone attached to it was made of calcium oxalate [1,2].

Early criticism of Randall’s plaque

Based on his research and observations Randall hypothesized that plaque of calcium phosphate remains innocent when it is suburothelial. Due to unknown reasons there is loss of urothelium at one site and plaque then acts as a nidus for different crystal growth driven by urinary super-saturations [2]. However, this hypothesis met with criticism for it was based on the cadaver kidneys. Randall could not present data on the urinary milieu and history of clinical stone events in the cadavers. Also Randall made a generalized statement proposing plaque as the nucleating site for all form of urinary stones. In order to test Randall’s hypothesis, Cifuentes Delatte et al. studied eighty-seven papillary stones with SEM and X-ray dispersive energy analysis [3]. They found evidence of plaque on sixty-three stones. The fact that, not all papillary stones had plaque and presence of papillary plaques in patients who passed stones covered with plaque was not confirmed; stone–plaque association could not be established.

Reinvestigating idea of Randall’s plaque

Authors from University of California conducted few studies to reinvestigate the Randall plaque hypothesis. In their first of three studies on RP, they observed cadaver kidneys for papillary micro-calculifications with high resolution radiography [4]. Unlike Randall, they gathered epidemiological data on patients about their
detailed medical history with special emphasis of cardiovascular system and stone disease. Radiologically, linear and punctate calcifications were observed in 57% of renal units [4]. Linear calcifications were radiating peripherally from the tip deep into the papillae. On microscopy these calcifications were in the interstitium, in the basement membrane of collecting tubules, and vasa recta. Hypertension was the only clinical finding correlating with plaques [4]. High resolution radiography though showed a higher incidence of plaque than described by Randall, exact correlation between stone and plaque formation remained elusive.

In their second study they mapped papillae with flexible nephroscopy, in vivo, in patients undergoing endoscopic removal of stones (n = 57) and compared the findings with group undergoing endoscopy for indications unrelated to stones (n = 7) [5]. They were first to characterize appearance and prevalence of plaques in patients with renal stones. Prevalence of RP was 74% and 43% in stone formers and non stone formers respectively [5]. Important finding of the study was low incidence of RP in struvite and cystine calculi and high incidence in calcium calculi. Authors hypothesized that RP may have role in formation of calcium calculi. For testing their hypothesis they designed third study and evaluated relation between the urinary risk factors for calcium stone formers and plaques [6]. Plaques were most common in CaOx stone formers. There was trend toward increased urinary risk factors (Hypercalcuria) in patients with plaques, though no statistic significance was found [6].

Following the observations made by Stoller et al., Evan et al. designed a set of experiments. The group initially demonstrated the safety of endoscopic papillary biopsy during percutaneous procedure [7]. Safety, feasibility, and adequacy of retrograde transurethral papillary biopsies have also been demonstrated [8]. Examination of papillary tissue from living stone formers, using modern imaging and histopathology techniques, advanced knowledge on the RP role in stone pathogenesis. In their first study Evan et al. examined papillary tissues from fifteen ICSF and four CaOx stone formers post intestinal bypass procedure for morbid obesity with light microscopy, FTIR, X-ray diffraction, and TEM [9]. Subsequently, similar studies were done on other stone groups and about ten distinct phenotypes of stone formers have been described [10–13]. Plaques of HA were exclusively seen in ICSF. These plaques were beginning in the basement membrane of thin limb of LH without any evidence of cell injury, inflammation, intratubular crystal deposits [9]. Intestinal bypass patients universally lacked interstitial plaques but had intra-tubular HA crystals plugging deep papillary collecting ducts, similar to type II plaque described by Randall [9]. Other phenotypes, as brushite stone formers, formed both interstitial plaques as well as tubular plugging [10,12]. Basement membrane of LH as the origin of plaque has also been mentioned in an early study that examined sixty-two cadaver kidneys for papillary calcification with electron microscopy [14]. However, no reference to RP was made and study did not examine the mineral composition of calcifications.

For studying stone–plaque association, Kuo et al. devised a novel method of quantifying plaque using digitized imaging and Adobe Photoshop [15]. Using the technology most accurate plaque area estimation could become possible, whereas previous studies reported just absence or presence of plaque [6,15]. Plaque coverage was inversely proportional to urine volume and directly proportional to the urinary calcium and urine pH [15]. Plaque coverage also directly correlated with the number of stones formed by a patient even after correcting the duration of stone disease [16], 91% papillae in ICSF had RP and attached stone were observed in 50% kidneys [17]. These rates were much higher than earlier reported rates on unselected cadavers or unselected stone formers. Williams et al. studied papillary stones with micro computed CT. With micro computed CT, they could study stone composition and morphology simultaneously [18]. On micro CT analysis stone was composed of CaOx and there was evidence of HA on concave surface corresponding to the attachment site on RP [18]. Study by Williams et al. provided evidence to establish the association between RP and CaOx stones. Miller et al. have shown that majority of stones in ICSF grow attached to the plaques and even stones found free have evidence of their origin on the plaque [19,20].

Non-invasive mapping of Randall’s plaque

From the above studies it appears that RP definitely have role in the pathogenesis of idiopathic CaOx stone. Number of stones a patient form is directly proportional to the plaque area [16]. Attempts have been made to noninvasively investigate the formation of RP and subsequent stone formation in a number of radiology studies with conflicting results [21–24]. RP containing apatite should have higher attenuation value on non-contrast CT than normal papillae. Various cut-off values have been described [21,23]. Currently, there is no radiological technique sensitive enough to detect plaques noninvasively [24]. Size and attenuation value of RP falls below the detection threshold of current CT scanners [24].

Ultrastructure of stone–plaque interface

Correlative evidence and even actual demonstration of stone attached to the plaque does not provide any information on the mechanism by which apatite plaque nucleates stone of different mineral composition. During their experiments Evan et al. were once able to biopsy 1 mm small stone still attached to the papilla [25]. This permitted them to conduct ultrastructural studies on the stone–plaque interface using light microscopy, FTIR, micro CT, TEM, and IHC for osteopontin and THP [25]. There was loss of urothelial cells at the attachment site. The organic matrix covered surface of the plaque. Amorphous apatite in plaque did not come in direct contact with CaOx. Randall plaque at the attachment site had sharp demarcation boundary to the urinary space and showed nine alternating layers of dark organic matrix and light mineral phase. Osteopontin was seen in the urine, renal tissue, and plaque. THP was seen in the urine and at plaque outer border and stopped abruptly at plaque–tissue interface. Proteins in the organic layer favored apatite deposition from urine. Another organic layer containing urinary proteins was deposited on newly formed apatite. These layers showed gradual transition of mineral from amorphous apatite to biologic apatite and finally to CaOx in stone (Fig. 2).

Ultrastructure of Randall’s plaque

Polymer induced liquid like precursor (PILP) process

Crystallization of the amorphous HA is facilitated by anionic polymers (osteopontin, proteins, glycolipids, other organic molecules). Initially amorphous HA crystals are in liquid precursor phase having high levels of organic polymers [26]. Over time crystallization process undergoes pseudomorphic transformation where water and organic additives are excluded and crystal assumes spherical morphology. Transformation proceeds from core to the periphery.
Randall’s plaque

plaque is composed of small spherical units mixed with long thin fibers running between them. These units lose their identity in the interior of plaque. Spherical units in the basement membrane are closely associated with membrane bound vesicles and those in the interstitium with collagen. Crystal process may be initiated by membrane bound vesicles in the presence of supersaturation and further growth of plaque can occur by addition of crystals at the periphery in the framework of collagen, leading to biomineralization of collagen [31].

Membrane bound vesicles are secreted by different cell types in response to the disease states [32]. These are phospholipid bound vesicles containing albumin, fetuin, apolipoprotein A1 and have been shown to induce precipitation of apatite mineral–protein complexes resembling calcifying nanoparticles and apatite spherules [32]. On analysis of calcium phosphate stone with nuclear magnetic resonance spectroscopy using P [31] and C [13] interatomic distance between apatite and biomolecules is of order of 0.5 nm [33]. This distance represents intermolecular physicochemical interactions such as ion pairing or hydrogen bond. So biomolecules as proteins and glycosaminoglycans play key role in apatite formation.

Role of reactive oxygen species (ROS)

ICSF show higher urinary excretion of gamma-glutamyltranspeptidase, angiotensin I converting enzyme, beta-galactosidase and N-acetyl-beta-glucosaminidase [34]. These enzymes are markers of renal tubular damage. However, in the absence of any histologic evidence of inflammation and tubular cell injury in ICSF, this may not be the primary event. Calcium crystals when come in contact with tubular cells or interstitial cells induce inflammation. CaOx crystals are most reactive [35]. Molecular analysis of THP from hypertensive and ICSF shows altered kinetics due to increased carbonyl and decreased thiol contents signifying ROS damage [36]. THP with altered kinetics exhibits enhanced nucleating properties. THP kinetics and nucleating properties are nearly normalized after nine months of regular vitamin E supplementation further supporting the role of ROS in the pathogenesis of RP [36]. Nephrolithiasis patients also show higher than normal urinary excretion of 8-hydroxydeoxyguanosine (8-OHdG), a marker of oxidative DNA damage [37].

Exposure to oxalate, CaOx, CaP results in increased gene expression and increased synthesis of molecules involved in inflammation and biomineralization [38]. Almost all modulators of crystallization; like THP and osteopontin; are also active participants in inflammation [38]. However, this inflammation may be a protective response. After formation of apatite crystal nearby tissue cells may respond by producing ROS. Macrophages thus recruited may engulf crystal and are protective. However, if conditions for crystallization persist, localized inflammation and fibrosis sets in and biomineralization progresses by mineralization of collagen [38].

Hypothesis of Randall’s plaque formation

It is evident that stone formers exist as distinct phenotypes with characteristic endoscopic, physiologic, and histologic features. Role of RP has been best described in the pathogenesis of CaOx stones in ICSF. The pathogenesis of RP appears multifactorial. We have
generated a hypothesis on RP formation based on the review of literature (Fig. 3).

Low urine volume and high calcium excretion leads to high calcium concentration in the thin limb of LH. Though this segment is impermeable to ions, constant presence of high amounts of calcium can lead to its efflux into basement membrane. At the same time low urine pH leads to high bicarbonate absorption increasing pH of interstitial fluid. Countercurrent mechanism further concentrates the minerals in the interstitium near LH. Basement membrane is rich in anionic proteins and glycosaminoglycans having affinity for calcium. Due to some unknown reason LH cells produce membrane bound vesicles in the basement membrane. This may be in response to oxidative stress. These vesicles act as initial nuclease sites for amorphous apatite in the presence of high concentration of calcium, phosphorus, and favorable pH conditions.

Once formed apatite acts as a foreign body and sets in subtle inflammatory response with release of ROS and proteins like osteopontin, fetuin. These proteins have apatite binding sites and act as inhibitors of mineralization. In the presence of continuous stimulus for apatite formation, binding sites on fetuin and osteopontin are saturated. ROS itself can alter kinetics of these proteins limiting their inhibitory properties. This causes precipitation of mineral protein complexes, described as calcifying nanoparticles. These initial complexes are in liquid phase with high content of anionic organic molecules. Later, these undergo pseudomorphic transformation with exclusion of water and organic molecules and formation of solid phase apatite spherules.

These spherules grow in size by addition of more crystals at the periphery and bio-mineralization of collagen in the interstitium. Mineralization proceeds in the framework of collagen fibers. Spherules coalesce with each other and lose their identity in the interior of plaque. For sometime plaque remains subepithelial. In response to unknown stimulus there is loss of epithelium at one site and plaque is exposed to urine. Here urinary proteins like osteopontin and THP form covering over plaque.

Conclusion

Randall’s plaques, once forgotten, have been revisited with the advancement in the fields of endourology, optics, radiology, and molecular biology. The role of RP in the pathogenesis of ICSF appears convincing. Pathogenesis of Randall’s plaque itself is not clearly known till date. However, experimental studies suggest multiple factors like genetic influences, urinary mineral supersaturation, oxidative stress, interstitial organic molecules (collagen, glycosaminoglycans), modulators of mineralization (osteopontin, THP, fetuin), crystal induced inflammation. Further research in molecular biology and advanced imaging may help to study the mineral–organic interactions and may place RP in its correct perspective.
Conflicts of interest

No conflicts of interest.

References


