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Analysis of Urinary Calculi by Fourier Transform Infrared Spectroscopy for Patients in Northern Jordan

Barakat M. Shabsoug, Adnan Al-Quraishi, and Hesham Al-Mgassgas

ABSTRACT

The variations in urinary calculi are based on different diameters, which range in the form of sand grain to a urinary collecting system so that it can pass through the urinary tract regardless of any symptoms. To determine the composition of urinary calculi for patients in Northern Jordan by using Fourier Transform Infrared Spectroscopy method, samples of urinary calculi were retrieved from patients at King Abdullah University Hospital and Princess Basma Teaching Hospital. Urinary calculi were surgically removed from patients either arthroscopically (n = 35), or by percutaneous nephrolithotomy (PCNL) (n = 15) over a period of 16 months (2013-2014) and were brought to the laboratory in sterile containers. They were analyzed by Fourier Transform Infrared Spectroscopy method. A total of 39 male patients (78%) aging 23-85 years and 11 female patients (22%) aging 33-69 years were included in the study. The calculi comprised of 40% calcium oxalate and uric acid, 24% calcium oxalate, 18% pure uric acid, 12% magnesium ammonium phosphate, and 6% calcium oxalate and calcium phosphate. Mixed urinary calculi of calcium oxalate and uric acid were predominantly found among the patients with the following characteristic IR bands [1637cm-1 C=C Stretching, 1021 cm-1 N-H stretching, 780 cm-1 aromatic C-N stretching].

Keywords: Brushite, Calcium Oxalate, Fourier Transform Infrared Spectroscopy (FTIR), Struvite, Urinary Calculi.

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I. Introduction

Nephrolithiasis is a pathophysiological condition and one of the most profound health problems globally due to assorted urine compounds. These compounds integrate and impose formed structures toward the formation of the urinary calculi [1]. Due to a biological maladjustment, the event of these formed structures took place when the minerals merge and clump-forming calculi in the form of small rocks [2], [3]. In certain events, these urinary calculi are formed of chemicals, which include uric acid, calcium, ammonium, magnesium, and other dissolved salts found in the normal concentrations of urine [4], [5]. Increase in the concentration of these salts results in crystal formations and they get supersaturated resulting in the formation of urinary calculi [6]. The variations in urinary calculi are due to their different anatomical positions, which include urinary bladder, calyx region, renal pelvis, and ureter in the urinary system [7].

Similarly, the variations in urinary calculi are based on different diameters, which range in the form of sand grain to a urinary collecting system so that it can pass through the urinary tract regardless of any symptoms [8]. Injuries are caused by the urinary calculi at their sites and create blockage to the uretero-pelvic junction. On the contrary, rough injuries cause dysuria and classic rhythmic spasms of pain during their mobility [9], [10]. Due to urinary calculi, the presence of pain is reported as one of the unhealing pains that affect individuals [11]. In the last decades, the recurrence and occurrence of nephrolithiasis have profoundly indicated in previously conducted epidemiological studies [12]. Urinary calculi affect a minimal of 5% of the general population; however, the risk can be documented to 8-10% in the fore coming years [13]. This extent can be reported in both gender groups, different races, and assorted age ranges [14], [15].

Different analytical methods have been used to analyze and classify the constituents of urinary calculi, (e.g.) wet chemical method, X-ray diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR) [16].

FTIR spectroscopy has gained wide acceptance in the analytical community as a qualitative research tool. FTIR is becoming the gold standard for urinary calculi analysis because it allows more sensitive, specific, reliable, and reproducible data than other methods such as the wet chemical method [17].

Considering the compositional analysis of urinary calculi, several investigations have been conducted worldwide due to the increase in the occurrence of urinary calculi disease and the variations in their compositions [18]. Therefore, there is a dire need to understand the mechanism of calculi formation for managing afflicted patients appropriately and to reduce the health care costs and morbidity related with urolithiasis. Additionally, it is important to diagnose different types of urinary calculi due to the available effective treatments for each type of specific calculi [19]. Therefore, this study aims to correlate information concerning the relationship of urinary calculi formation to geographic location and diet. The study has also determined the distribution of each type of urinary calculi among northern Jordan people, which enables preventive treatments by giving fundamental information about the pathogenesis of the disease that prevents recurrent urinary calculi attacks.

II. Experimental

The data has been collected from the patients admitted to the Princess Basma Teaching Hospital and King Abdullah University Hospital in Irbid, Jordan for the urinary calculi samples. A total of 50 urinary calculi were collected on a random procedure for investigating their calculi in the urology in the surgery department of Princess Basma Teaching Hospital and King Abdullah University Hospital in Irbid, Jordan. Over a period of 16 months, these samples were surgically removed either by percutaneous nephrolithotomy (n = 15) or by arthroscopically (n = 35). Percutaneous Nephrolithotomy is a minimally invasive process for removing calculi from the urinary by a small puncture wound using the skin. Moreover, these samples were brought to the laboratory in sterile containers. Both male and female patients (23-84 years) with urinary calculi were included in this study.

The study has collected data from patients related to gender, age, geographic location, weight, water source, intake of coffee and tea, and type of diet of patients using the close-ended questionnaire. Additionally, the study has documented and investigated complete hospital records and the health status of patients for fulfilling the objective. For eliminating blood and other residuals, calculi were washed carefully with deionized water before conducting compositional analysis. Afterwards, the calculi were dried completely for 2 hours at 100 °C. Furthermore, one-dimensional measures were used to examine the diameter of each calculus. The examination has reported that these calculi were hard; therefore, grinded and crushed through mortar and a clean pestle for extracting fine ground powder. The extracted powder was weighed and incubated at 70 °C overnight in an incubator.

The potassium bromide (KBr) pellets (Beam splitters) were obtained by mixing 3 mg of powdered renal calculi of each sample with 300 mg of dried IR grade KBr, which were transferred to a mortar and again grounded together. These pellets are used as the window material they are transparent in the IR, between 4000-400 cm^{-1}. Using two stainless steel disks specialized for pellets preparation, the sandwich of powdered samples was transferred onto the pistil in the hydraulic press. The hydraulic pump handle was moved downward with a pumping movement. The film was removed, which was homogenous and transparent in appearance. The obtained pellets were ready for FTIR analysis. Urinary calculi analysis for the composition was done using Shimadzu IR Affinity-1 Fourier Transformer Infrared Spectrophotometer (FTIR); model IR-460 with a scan range of 4000 to 400 cm^{-1}. FTIR is a technique, which is used for obtaining an infrared spectrum of emission or absorption of a solid, liquid or gas. The device was purchased from Kyoto, Japan. This technology provides a more accurate urinary calculi diagnosis because it has an improved sensitivity level of measuring [20]. The obtained urinary calculi pellets were transferred and inserted into the FTIR sample holder.

III. Results and Discussion

Patients aged between 23-84 years with mean age of 51.72 years and standard deviation of 12.44. Urinary calculi were reported among male patients (78%) as compared to 22% female patients aging 23-84 years and 33-69 years respectively. In this regard, the proportion computed for the specific presence of urinary calculi was 3.54:1 as compared to a male to female ratio 4.6:1 in a study carried out by Khan *et al.* [21]. Table I has presented average age of female patients as compared to male patients where female patients represent higher average age than their male counterparts. It has been reported that the occurrence of urinary calculi reached among male patients was peaked at age group 51-50 years and then reverted. On the contrary, a peak occurrence of urinary calculi was found among female patients at age group 61-70 years, which was comparatively higher than their male counterparts.

TABLE I: DISTRIBUTION OF URINARY CALCULI IN RELATION TO PATIENT'S AGE AND SEX

Age Group	Male	Female	Total	Percentage
21-30 years	3	Nil	3	6%
31-40 years	5	1	6	12%
41-150 years	11	1	12	24%
51-60 years	13	3	16	32%
61-70 years	5	6	11	22%
Above 70 years	2	Nil	2	4%
Total	39 (78%)	11 (22%)	50	100%

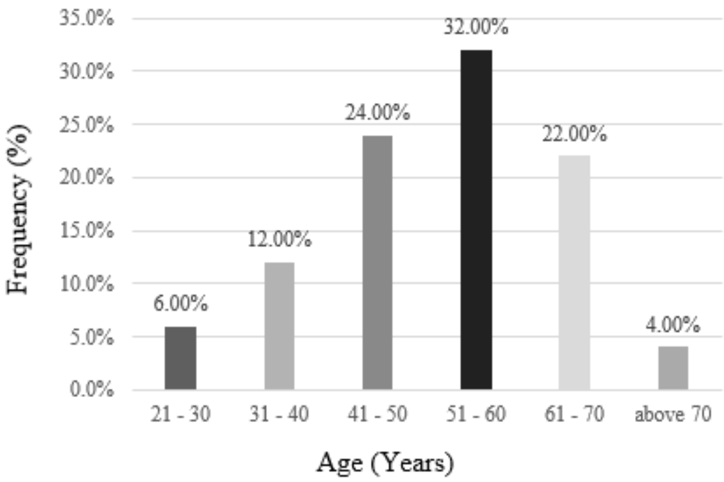

Fig. 1. Frequency of age distribution in urinary calculi patients.

Limited age distribution among females has been shown since no urinary calculi were reported in either 21-30 years age group or above 70 years age group. This occurrence illustrates that male patients might form urinary calculi in different age groups, and this can show that male patients have a higher occurrence rate as compared to their female counterparts. Among males, only 4% of urinary calculi were found above age of 70 years, while only 6% of patients were found in the age group of 21-30 years. The age distribution has been shown in Fig. 1. Mean age was significantly different between males and females which was 50.18 ± 12.60 and 57.18 ± 10.62, respectively (mean ± S.D).

A total of 40 patients (80%) were living in villages located near Irbid city out of 50 urinary calculi patients while 10 patients (20%) were living in Irbid City out of 50 urinary calculi patients. The mean weight of patients (91.6 kg) was recorded with a range of 81 to 98 kg. Furthermore the results have reported the minute extent of several common diseases among the patients with hypertension (10%) and diabetes (8%). In contrast, the study has reported insignificant and indirect relationship between urinary calculi diseases and these common diseases among targeted patients. The occurrence of urinary calculi was adhered for each sample and reported uniqueness in every sample with different diameters. Colors of urinary calculi were reported from yellowish white to dark grey due to the difference in diameters range from 4-13 mm.

The frequency of different subtypes of the 50 urinary calculi analyzed by FTIR spectroscopy have been presented in Fig. 2. The highest percentage among 50 samples comprised of mixed calculi containing calcium oxalate and uric acid (40%), followed by pure calcium oxalate calculi (24%), pure uric acid calculi (18%), and then magnesium ammonium phosphate calculi (12%). Another type of less common urinary calculi were the calculi containing calcium oxalate and calcium phosphate (6%). These findings showed the specific combinations of constituents found in those samples. The calculi formed of a single element that is pure uric acid calculi were present among 27 individuals (18%).

In the present study, all FTIR spectra of urinary calculi were compared with FTIR bands observed in standards and typical FTIR spectra, and then identified [22]. The Characteristic bands for CaOX included five grouping bands between 2070-3385cm^{-1} (Symmetric and asymmetric OH stretch), 1621 cm^{-1} (C=O Stretch), 1317 cm^{-1} (C-O stretch), 950 cm^{-1},885 cm^{-1} (C-C stretch), 780 cm^{-1} (O-C-O bending), 664 cm^{-1} (Out of plane O-H and C-H bending), and 517 cm^{-1} (O-C-O in plane bending). For pure uric acid calculi, band intensities were around 780 cm^{-1} (aromatic C-N stretching), 1637cm^{-1} (C=C stretching), and 1018 cm^{-1} (N-H stretching). While the diagnostic bands identified for calculi containing uric acid and calcium oxalate were around 1637 cm^{-1} (C=C Stretching), 1021 cm^{-1} (N-H stretching), and 780 cm^{-1} (aromatic C-N stretching).

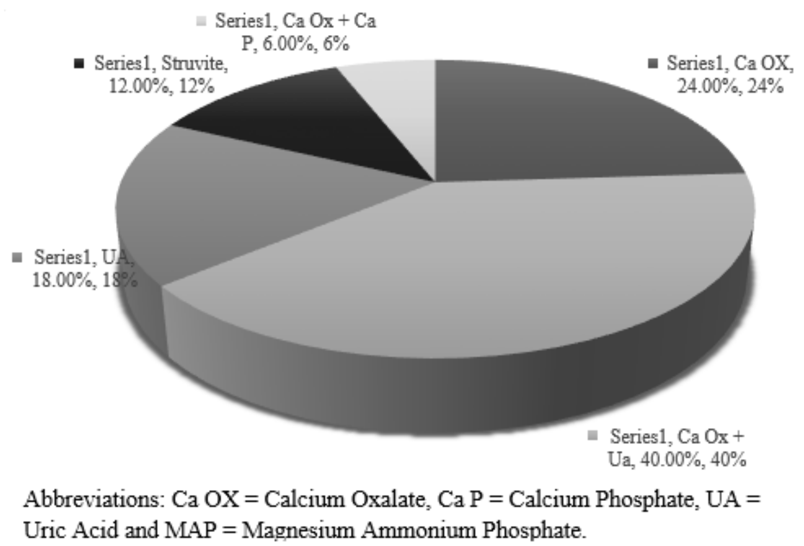

Fig. 2. Types of calculi recovered from urinary calculi patients assessed by FTIR.

Magnesium ammonium phosphate calculi (Struvite) had a characteristic infrared spectrum and was easily recognized by the position of the strong band at 1010 cm^{-1}. Moreover, the phosphates containing PO_4^{-3} ion revealed that the stretching and bending vibrations arise in the mid-IR region (900-1200 and 300-600 cm^{-1}), respectively. A clear broad IR band occurs at 985-1100 cm^{-1} with an intense feature at 1038 cm^{-1} due to PO_4^{-3} stretching vibration, while a sharp and intense band present at 605 cm^{-1} is due to the bending vibrations of PO_4^{-3} (Table II). Fig. 3, Fig. 4, and Fig. 5 show three FTIR spectra based on the present study.

The most common calculi compositions were CaOx + Urate, CaOx, Urate, Struvite, Brushite in turn (Table III).

TABLE II: Detailed Infrared Absorption Bands of the Characteristic Group Observed for Different Types of Urinary Calculi

Type of Calculi	Principle IR band observed	Characteristic Group
CaOX (pure)	Five groups of bands between 2070-3385 cm^{-1}	Symmetric and O-H stretch
	1620 cm^{-1}	C=O stretch
	1318 cm^{-1}	C=O stretch
	950,890 cm^{-1}	C-C stretch, out of the plane
	662 cm^{-1}	O-H bending
	781 cm^{-1}	Out of plane C-H bending
	517 cm^{-1}	In-plane O-C-O
UA (pure)	1637 cm^{-1}	C=C stretching
	1018 cm^{-1}	N-H stretching
	780 cm^{-1}	C-N stretch of aromatic
CaOX + UA	IR band observed in both CaOX UA	Same as calcium oxalate and uric acid functional groups stretching
MAP	2362 cm^{-1}	N-H and C-H Stretch
	1469 cm^{-1}	NH_3 symmetric bending
	970 cm^{-1}	P-O-C aliphatic stretching
	1010 cm^{-1}	PO_4^{-3}
CaOX + CaP	Same as calcium oxalate and 985-1100 cm^{-1}, 1038 cm^{-1}, 605 cm^{-1}	PO_4^{-3} stretching vibration, PO_4^{-3} bending vibration

TABLE III: Frequency of Compositions Identified in Urinary Calculi (n= 50)

Constituent Frequency	(%)
Calcium oxalate + Urate	40
Calcium oxalate	24
Urate	18
Magnesium ammonium phosphate calculi (Struvite)	12
Calcium oxalate + Calcium phosphate (Brushite)	6

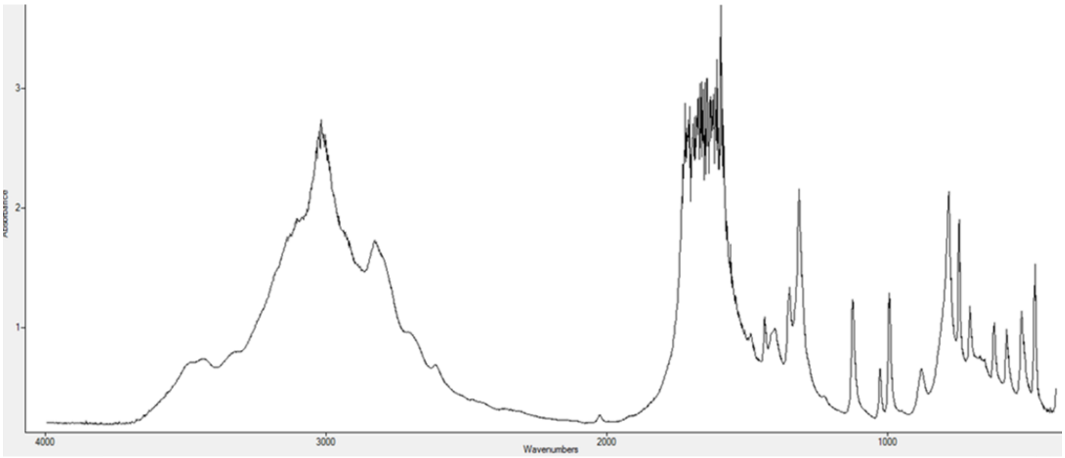

Fig. 3. FTIR Spectrum of mixed uric acid and calcium oxalate calculi.

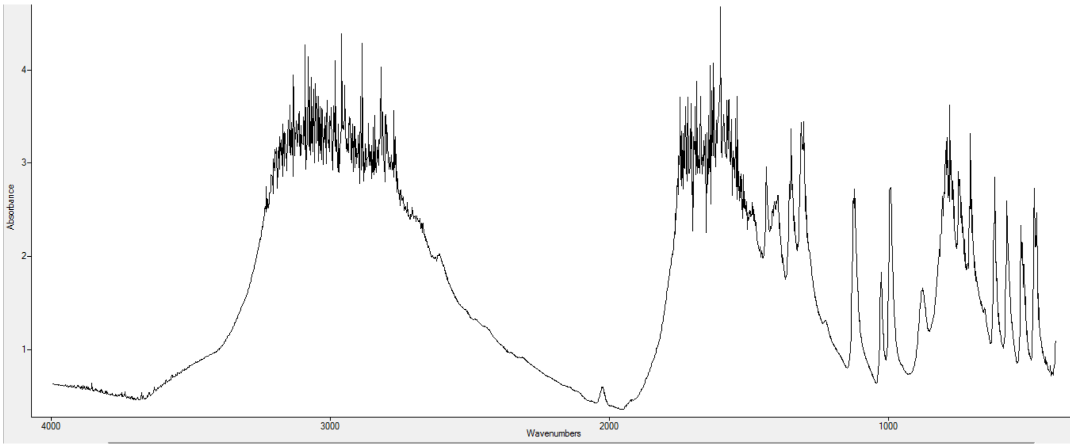

Fig. 4. FTIR spectrum of calcium oxalate (pure).

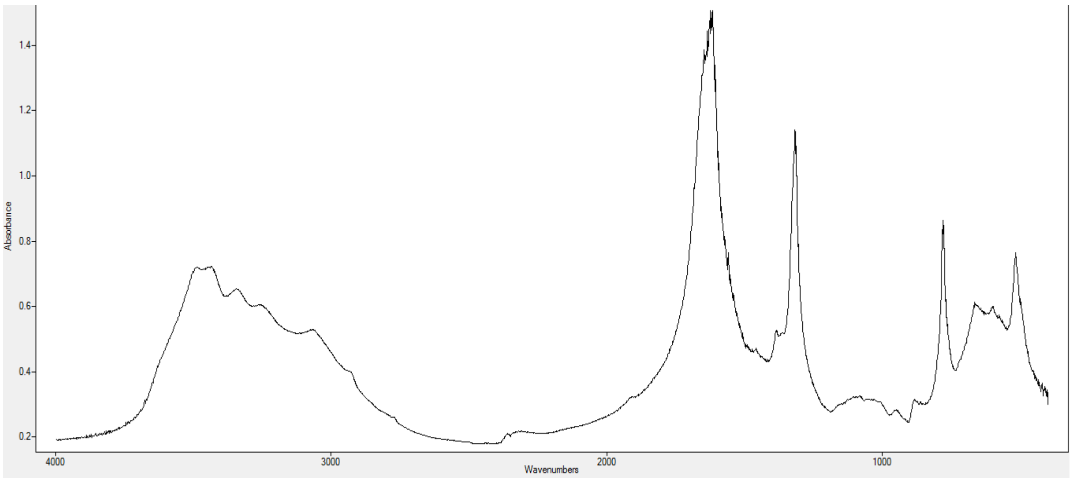

Fig. 5. FTIR spectrum of uric acid stones (pure).

The chemical examination of urinary calculi represented a pathological biomineral analysis, which is a crucial step for the etiology of this disease because it provides a better understanding of the genesis of individual calculi. This is helpful while prescribing a diet or therapy intended to reduce the risk of recurrence and patient follow-up [23]. Different methods were used to reveal these purposes. Among these methods, dry analysis by FTIR was recently recommended to be used because it gives an accurate characterization of the calculi compositions [24]. In addition, the average age of affected individuals (51.76 years) was higher than the age averages of previous studies [25], [26]. Urinary calculi were removed either arthroscopically (n = 35), or by PCNL (n = 15) over a period of 16 months (2013-2014).

Difficulties and obstacles were faced to get more detailed information from patients about the history of calculi formation, along with other obstacles regarding daily intake of calcium (including supplements and salts), oral intake of fluids, personal privacy, and other restrictions. Urinary calculi were more common among males as compared to females by 3.54:1 ratio. This finding was near a previous study [26], which showed that the formation of urinary calculi was based on the clear male predominance in the overall population. However, there are still unclear justification present regarding the marked difference and gender-related factors. A contributing factor for this justification can be observed from the differences in diet between both gender groups. Another important reason reported was the complications in the male urinary tract as compared to female urinary tract. Obesity and overweight of patients can increase the risk factors due to the presence of their average body weight. Furthermore, it shows the contribution in the formation of urinary calculi by increasing the uric acid, calcium, and oxalate. In addition, higher risk has been noticed among individuals with obesity as compared to individuals who are slimmer. These findings have been supported by previous studies and revealed a clear association between the persons being overweight and urinary calculi formation. Moreover, the current study has indicated that 78% of patients were ranged between 40-70 years of age and therefore, the symptoms of disease are commonly found among middle aged and older individuals as compared to younger people. Therefore, it is important to implement more than one approach to acquire additional composition details of all samples and for the confirmation of results.

Calcium oxalate calculi were reported to be the second most risk factor following to the calcium mixed calculi and uric acid. These calculi have been associated with the increased levels of acidity and hyperuricemia. The complementary findings have been reported for the profound urinary calculi, supporting the clear stance in uric acid calculi and calcium oxalate occurrence in Jordan. The consumption of large quantities of milk and dairy products, the consumption of large quantities of red meat and increasing calcium levels are associated with the presence of urinary calculi. Personal communication with patients was confirmed with this dietary habit. The patients living in the northern Jordan villages were having sedentary lifestyles. This is correlated with statistics done on the patients' population, which revealed that 80% of patients were villagers. The recurrence of calculi was reduced by increasing the fluid intake and found to be a well-accepted method. Additionally, the risk of calculi formation can be increased from daily consumption of tea. An improvement in hygiene conditions and the treatment of urinary tract infections have been indicated from the slight increase in urinary infections calculi. Cysteine calculi are formed due to the presence of genetic disorder among male patients. The amenities and transport resulting in reduced physical activity has been reported from the scientific and technological progress in recent years, leading to an increase in the ratio of urinary calculi, obesity, and other associated diseases.

IV. CONCLUSION

Different chemical constituents have been reported in the formation of urinary calculi. Different etiologies have been reflected in Jordan specifically comprising uric acid and calcium oxalate that were composed of predominant chemicals. The development of urinary calculi is variable at different ages. This study has concluded that the presence of urinary calculi is more likely among male patients as compared to female patients, also it revealed different calcium oxalate calculi composition of (24%) as compared to those reported by Ye *et al.* [27] CaOx (65.9%) and Sekkoum *et al.* [28] CaOx (58.08%). The most common calculi compositions were CaOx + Urate, CaOx, Urate, Struvite, Brushite in turn.

Therefore, it has been suggested that additional information should be required to examine the prevalence of specific chemical constituents, its association with environmental, genetic, and dietary factors and its type. This might lead to appropriate diagnostic information of this disease and more comprehensive investigations related to this problem.

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