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MICROBIAL AND BIOCHEMICAL ABNORMALITIES IN PATIENTS WITH STRUVITE - STONES

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ABSTRACT

The present study was carried to find out the role of bacteria in struvite - stone formation and the associated biochemical abnormalities in the serum of patients. Thus, 16 struvite stone-formers were categorized into two groups according to the presence or absence of uric acid in the chemical composition of struvite stones. The changes in serum protein and glycoprotein were examined. Isolation and identification of bacteria were carried out for urine and the surface of the stone samples. It was noticed that the high level of α_2-, B-glycoprotein in serum may reflect acute bacterial infection and a sort of inflammation in many cases of the studied groups compared to 17 healthy normal persones. Surgically removed struvite stones were infected with urea-splitting bacteria especially *Proteus mirabilis* which was found in many samples (42.9%) of both urine and stones of patients with Calcium oxalate , Calcium phosphate , Magnesium ammonium phosphate and uric acid stones. This could be explained by the fact that the organism had been participating in the formation of struvite stones. In some cases with struvite stones, associated with chronic urinary tract infection mixed bacteria (more than one organism) were commonly detected in urine samples .

INTRODUCTION

Struvite is a geologic term for crystalline substance composed of magnesium ammonium phosphate ($Mg\ NH_4\ PO_4.6H_2O$). Struvite calculi (triple phosphate stones) are mostly associated with chronic urinary tract infection with urea-splitting bacteria. These organisms alkalinize the urine, produce increased concentration of ammonium ions . In this case the urine becomes supersaturated with the components of struvite.

Common urea-splitting bacteria include *Proteus, Pseudomonas, Klebsiella, Staphylococci , Enterococci* and *Ureoplasma urealyticum* [1,2] . These urea - spliting bacteria are more important than metabolic factors in the growth and the formation of the majority of struvite stones [3].

To form macroscopic struvite stones, the bacterial cells and their secreted products (as glycocalyx or biofilm matrix) appear to provide initial foci for development and aggregation of crystals [4] .

Escheriehia coli does not produce urease enzyme and when associated with this type of stone most likely represents a secondary infection [5] . **Arai et al.** [6] proved that human uropathogenic bacteria play a significant role in the formation of infection stones by inoculation of 5 bacterial species , isolated from patients with urinary calculi, into the bladder of male wistar rates. On the 7th day, their bladders were examined. The investigators found that infection stones were formed in all rates inoculated with *Proteus mirabilis* and 60% of those were inoculated with *Staph. saprophyticus* . The stones were formed in 30% and 25% of the rates inoculated with *Klebsiella pneumoniae* and with *Pseudomonas aeruginosa*, respectively. No bladder stones were formed in the rates inoculated with *E. coli*.

Holmgren et al. [7] reported that struvite stones associated with severe bacterial infection caused recurrent infection in urinary tract. Moroeover, it was revealed that glomerular damage allows serum albumin, alpha macroglobulins, transferrin and IgG to escape in the urine [8] . Values of alpha and gamma globulin, in the serum of patients with uric acid, calcium oxalate and Magnesium ammonium phosphate (MAP) stones, did not significantly differ from the controls [9] . Gamma globulin fraction contains most of the antibodies . When increased or decreased it reflects immunoglobulin aberrations.

Rhman et al. [10] found that there is a significant increase in serum mucoproteins and glycosylated serum proteins in stone formers. The significant increase in the glycoprotein contents of serum have been shown associated with various miscellaneous infections and inflammatory reactions [11] .

Gowenlock and Bell [12] showed that the increment of phosphorus level could be atributed to either hypo-parathyroidism associated with serum calcium iorn to destructive kidney lesions as pyelonephritis due to bacterial infection. The formation of uric acid in stones could be due to the increased level of uric acid in serum [13] .

The present study was designed to find out the role of bacteria in the formation of struvite stones, the types of bacteria which were associated with these stones and the biochemical abnormalities in the serum of stone- formers compared to normal persons.

MATERIAL AND METHODS

The sample of study consisted of 33 male and female persons, divided into : 17 healthy normal individuals (age ranges from 26 to 45 years) and 16

patients (age ranges from 13 to 70 years) with struvite stones . Many of these patients (81.3%) were infected with bilharziasis .

The patients with stone formation were classified according to the chemical composition, of surgically removed stones , into 2 groups :

Group A : Includes 9 cases with calcium oxalate – calcium phosphate + magnesium ammonium phosphate stones (CaOx - CaP - MAP), without uric acid .

Group B : Includes 7 cases with calcium oxalate + calcium phosphate + magnesium ammonium phosphate + uric acid stones (Ca Ox - CaP -MAP - U).

All patients were seeking medical care in the Urology Department, Faculty of Medicine , Cairo University.

The clinical diagnoses were evaluated before the surgical operation and the initiation of any treatment.

(A) Biochemical analysis :

1- Determination of serum total protein by the biuret method as described before (14) .

2- Evaluation of protein and glycoprotein fractions in serum by Agraose Simple Electrophoresis . In addition the serum protein was E;ectr[jpresed according to previous reports (15,16) .

3- Determination of serum uric acid, calcium, magnesium and inorganic phosphorus

Serum uric acid (17), calcium (18) and magnesium(19) were determined by colorimetric test. Reagents were obtained from Boehringer Mannheim/ Germany, Bio-Merieux / France and Quimica Clinica Aplicada S. A/ Spain , respectively

Serum inorganic phosphorus was determined by photometric UV test (20) , and the reagents were obtained from Human / Germany.

(B) Analysis of calculi constituents after being removed by surgical operations as reported by Wooton (14) :

(C) Microbial isolation and identification :

Under aseptic conditions , clean mid-stream urine specimens were collected early morning before the operation. The stone samples were collected in sterile saline , after the operation.

The urine and pieces of crushed surface of stones were cultured on Nutrient agar, Baird Parker plates, Mannitol salt agar, CLED agar (for urine), MacConkey agar, Shigella- Salmonella medium (S.S.), Endo agar , E M B agar and XLD agar, the selected colonies were kept on nutrient agar and T.S.I stants. The procedures for isolation and identification were previously established(21-23) .

RESULTS AND DISCUSSION

Egypt is considered one of the areas in which stone formation is a health problem. Usually when renal calculi are existing, infection is superimposed and various lesions may develop, such as calculus pyelonephritis and perinephritis.

It has been reported that, with the introduction of infection, multiple stones may be formed (24) , specially with urea- splitting organisms which are associated with alkaline urine and a high incidence of magnesium ammonium phosphate calculi

In our groups, the stones were composed of a mixture of CaOx, CaP, MAP with or without Uric acid, these are called " mixed stones " . Most of the patients (81.3%) were suffered from bilharziasis .

Figure (1) showed the mean values of serum total protein and their fractions in the group compared to the normal healthy persons. It has been found that the serum protein was significantly increased in the two groups. This elevation could be attributed to acute or chronic bacterial infection. The data are in agreement with the corresponding data reported before (25). In contrast, serum albumin showed a significantly decreased level in the stone formers as compared to the healthy subjects.

The observed hypo-albuminemia in these urolithiatic groups was associated with hyper-albuminuria due to a decreased hepatic synthesis specially in bilharziasis and / or associated with renal leakage due to renal tissue involvement under the effect of calculus formation.

The high observed elevation of α_1- α_2-, β- and γ-globulin in the studied groups could be attributed to chronic inflammatory states. Furthermore, chronic or acute urinary tract infection also leads to increment of globulin fractions. Consequently, serum albumin was decreased simulatneously α_1-, α_2 - globulin increased in cases with active tisue damage (acute phase reaction) or in cases with infection as reported before (11) .

In comparison to the normal group, α_1-glycoprotein (seromucoid) and Gama - glycoprotein fractions showed a significant decreased value in the patients (Fig. 2) . The marked decrease in seromucoid was observed in infectious conditions by bacteria. Alpha 2- and β- glycoprotein revealed a significantly increased level. This elevation may reflect processes of tissue destruction and inflammation . Albumin fraction was absent in both the healthy subjects and the studied groups of struvite stone-formers. This observation had been confirmed (26) .

The results obtained from Fig.(3) , for the values of calcium, magnesium phosphorus and uric acid in the sera of patients and healthy group are in agreement with

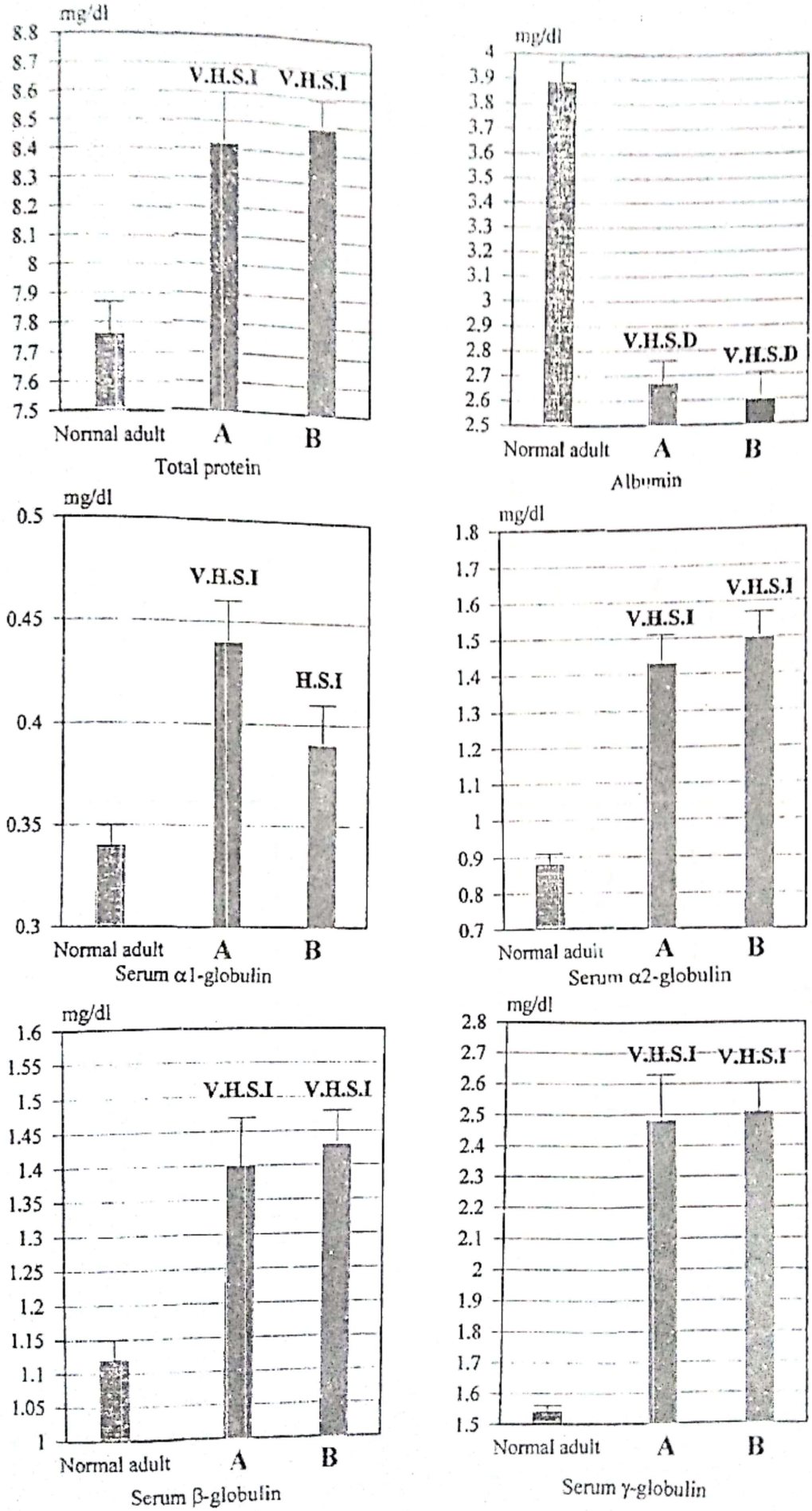
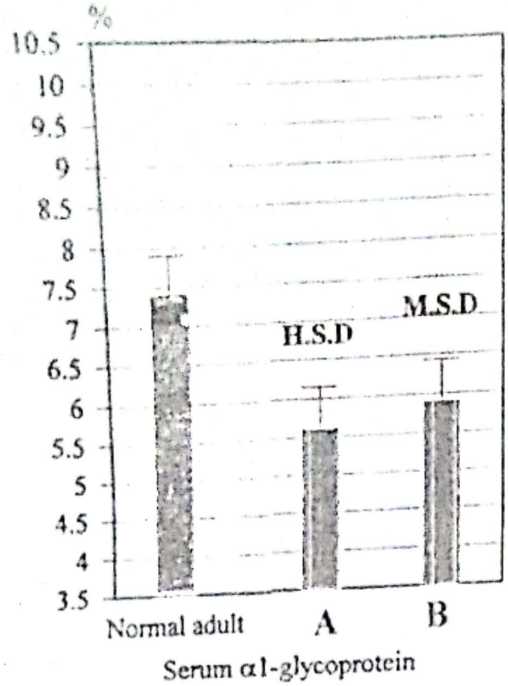
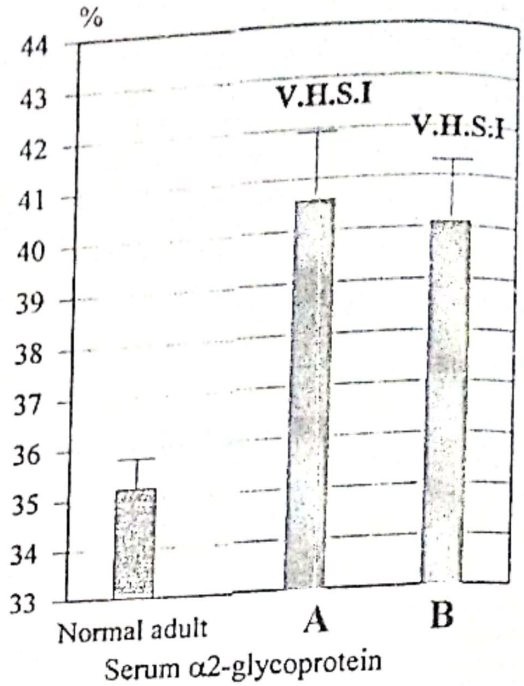


Fig. (1): The mean values ± S.E of serum protein concentration (mg/dl) in the different studied groups.



Serum α1-glycoprotein



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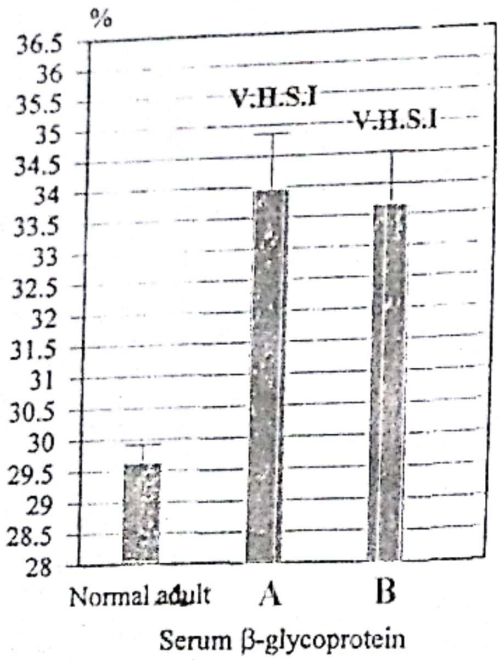
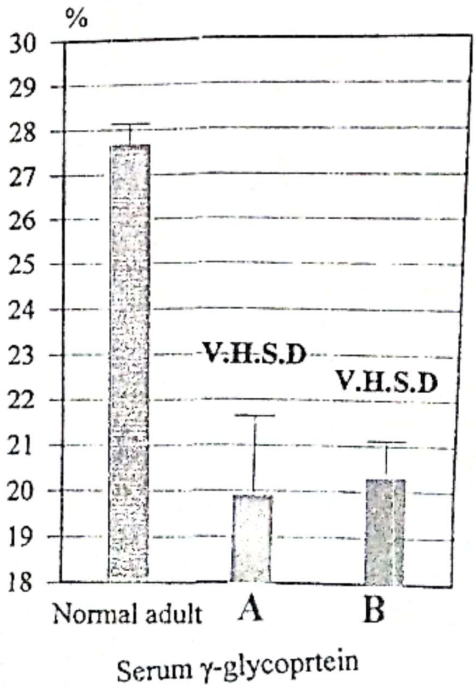
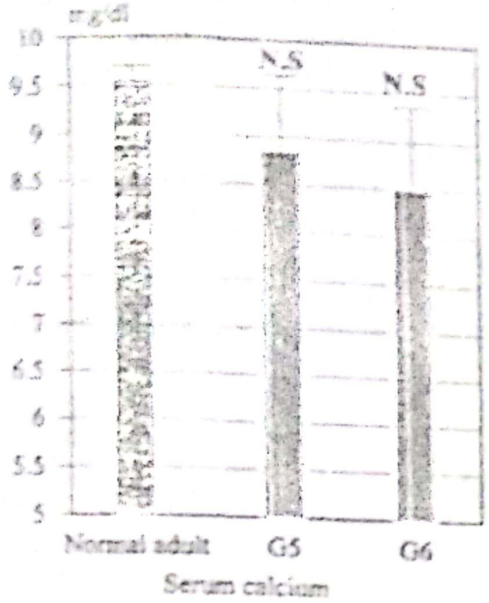


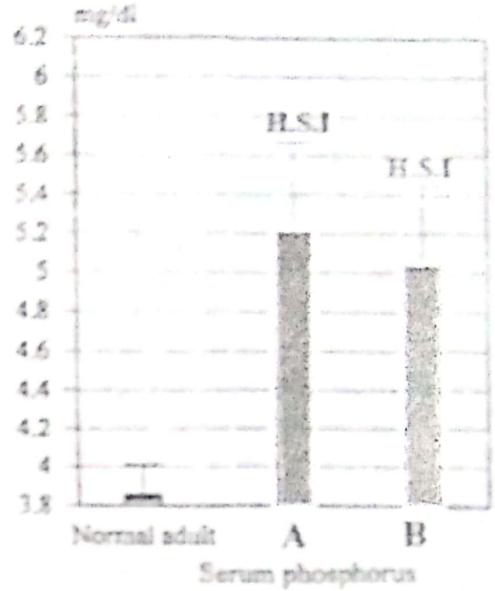
Serum β-glycoprotein

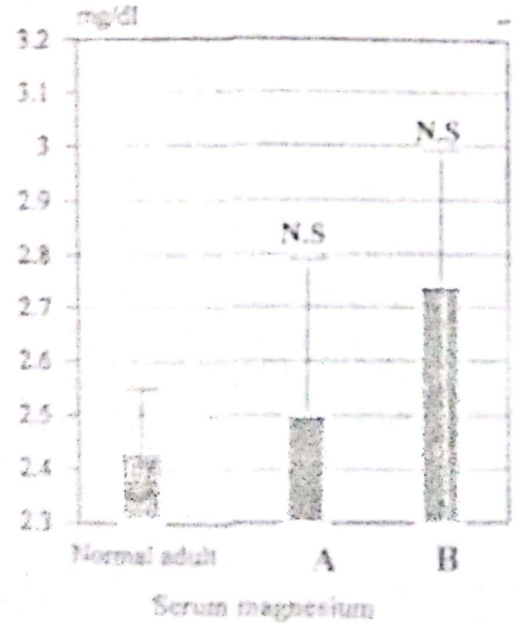


Serum γ-glycoprtein

Fig. (2): The mean values ± S.E of serum glycoprotein concentration in the different studied groups.







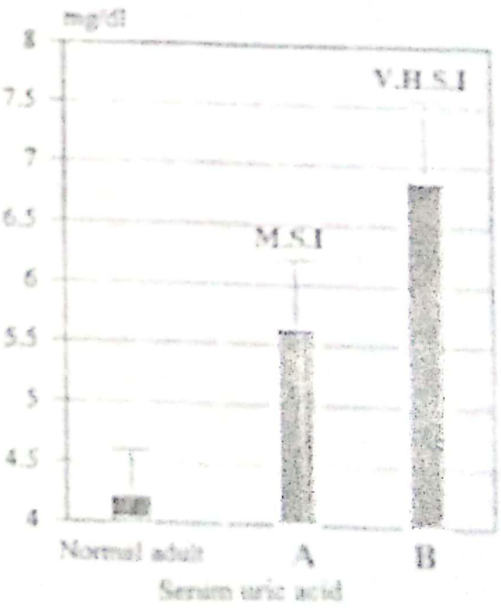


Fig. (3): The mean values ± S.E of serum calcium phosphorus magnesium and uric acid concentration (mg/dl) in the different studied groups.

the corresponding data reported before [25] . The mean values of serum calcium and magnesium showed insignificant changes in the patients. While inorganic phosphorus and uric acid showed a highly significant increased level in all patients compared with the normal group. The increment of phosphorus level could be attributed to either hypo parathyroidism associated with a low serum calcium or to the destructive kidney lesions as pyelonephritis due to bacterial infection. The formation of uric acid in stones, as in group B, could be due to the high increases level of uric acid in serum [13].

Crystalluria alone is not sufficient to cause a stone to form , but there are other factors that affect stone formation.

In group A , most of the stones were located in kidney (44.4%) . All patients had bilharziasis . Thus , a high incidence rate of bacterial infection was recorded in all urine samples and in 77.8% of the surface of stones . Thus , the stone recurrence was observed in all cases (Table 1).

In group B, stones in kidney were observed in 42.8% of the patients . The rate of bacterial infection was 71.4% in both urine and stone samples.

Stone recurrence was also observed in 71.4% of the patients. It was noticed that the stones, containing MAP , were associated with severe bacterial infection exceeded the other types of stones. *Staphylococcus epidermidis* , *E. coli* and *Citrobacter* (all had negative urease test) were isolated from urine and from the stone samples of group A. **Nickel et al.** [4] isolated *Staphylococcus epidermidis* from urine, but found heavy growth of *Staphylococcus faecalis* (non - urease producer) in crushed fragment of struvite stone **Hess**[27] found that the urinary infection with non- urease producing *E. coli* , probably promoted struvite particle formation.

High frequencies of mixed bacterial infection (more than one organism) in urine samples of our patients with struvite stones, especially group A (44.4%), are mostly due to chronic urinary tract infection accompanied with inflammation , which leads to the presence of more than one organism as secondary infection (Fig.3 A) The most common organisms encountered in mixed infection were *E. coli, Enterobacter,Citrobacter, Pseudomonas* and *Klebsiella*. It was observed that high frequency MAP and CaP calculi was found in patients with mixed infection [7]. *Enterobacter* was found in 22.2% of stone samples .

Figure (3 B) showed that *Proteus mirabilis* was the major strain (42.9%) isolated from both urine and the surface of the stone samples of group B. It was previously reported that a high frequency of MAP calculi was found among patients with *Proteus* infections [6, 28] . They also reproted that *P. mirabilis* was the predominant organism in the struvite stones plays a significant role in the formation of infection stones.

Pseudomonas was the secondary organism present in 14.3% of the stone samples but it was not isolated from the urine samples of this group. **Duralch et al.,** [29] isolated *Pseudomonas* from urine samples of patients with struvite stones. In our study, *Enterococci spp.* were also isolated from 14.3% of the urine samples.

In conclusion , most of the stones , containing magnesium ammonium phosphate , were found to be large in size and were located in kidney. The bacterial infection was found in all urine and in most stone samples in cases with Ca Ox - CaP- MAP stones. In this group, mixed bacteria were the prevalent organisms found in urine samples. while *enterobacter* was found on the surface of the stone samples. In the patients with Ca Ox - CaP - MAP - U stone , *P. mirabilis* was the common bacteria isolated from urine and from stone samples.

The increased level of serum α_2 and β-glycoprotein, in patients with struvite stones , was associated with high bacterial infection and incidence of acute inflammation and repreated stones.

The high incidence of stones was found in patients who suffering from bilharziasis and

Table (1) : Type of stone , its location and recurrence in patients with or without Bilharziasis .

Group	Kidney stone (n=9)		Urter stone (n=4)		Bladder stone (n=3)		Rate of recurrence (%)
	Bilharzial casses (%)	Non bilharzial casses (%)	Bilharzial casses (%)	Non bilharzial casses (%)	Bilharzial casses (%)	Non bilharzial casses (%)	
Group A (9 stones)	44.4	-	22.2	-	33.4	-	100
Group B (7stones)	42.8	28.6	14.3	-	-	14.3	71.4

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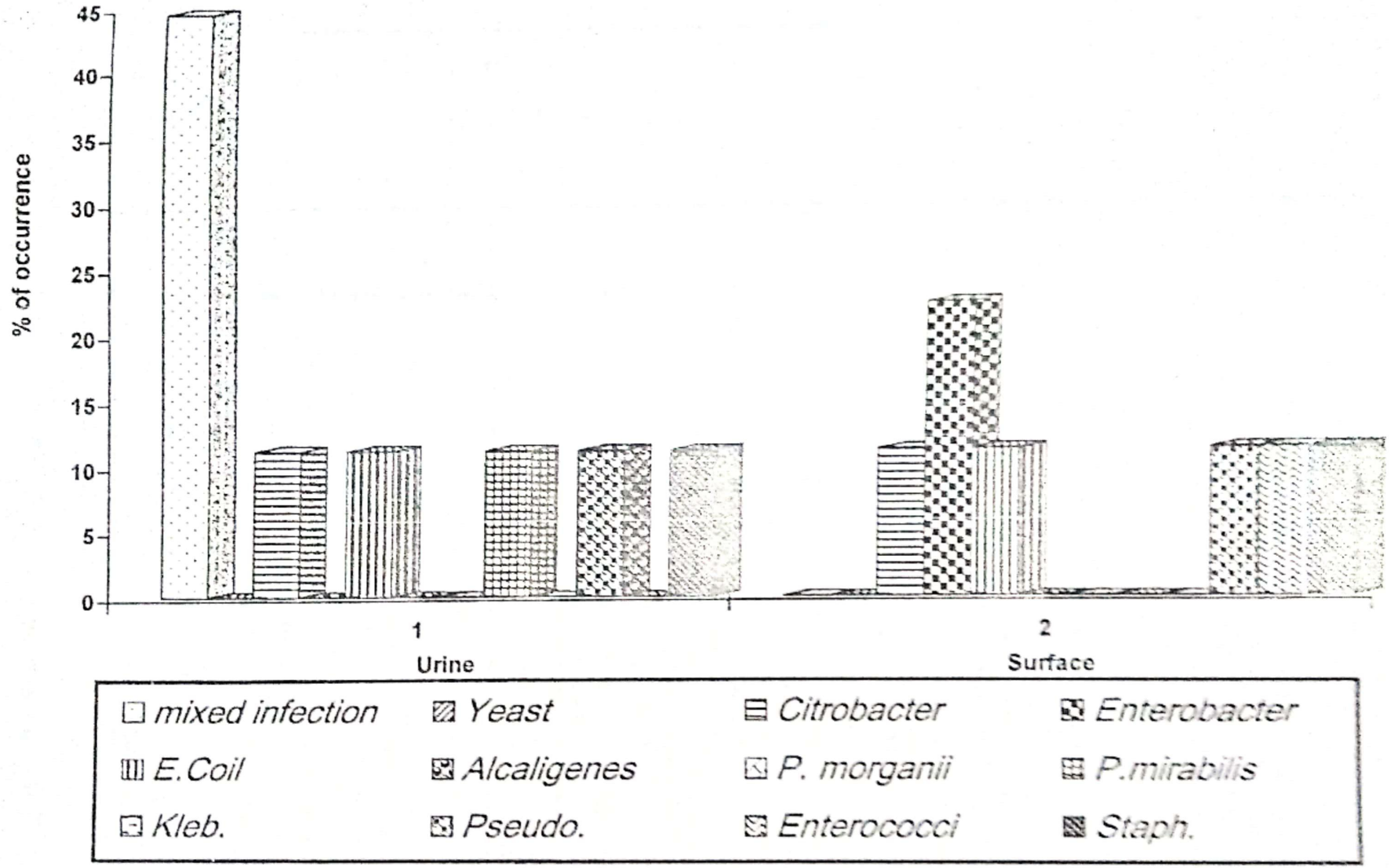

Fig (3a) : Percentage occurrence of different microorganisms in urine and stone (surface) samples with Ca- Ox + Ca-P + MAP

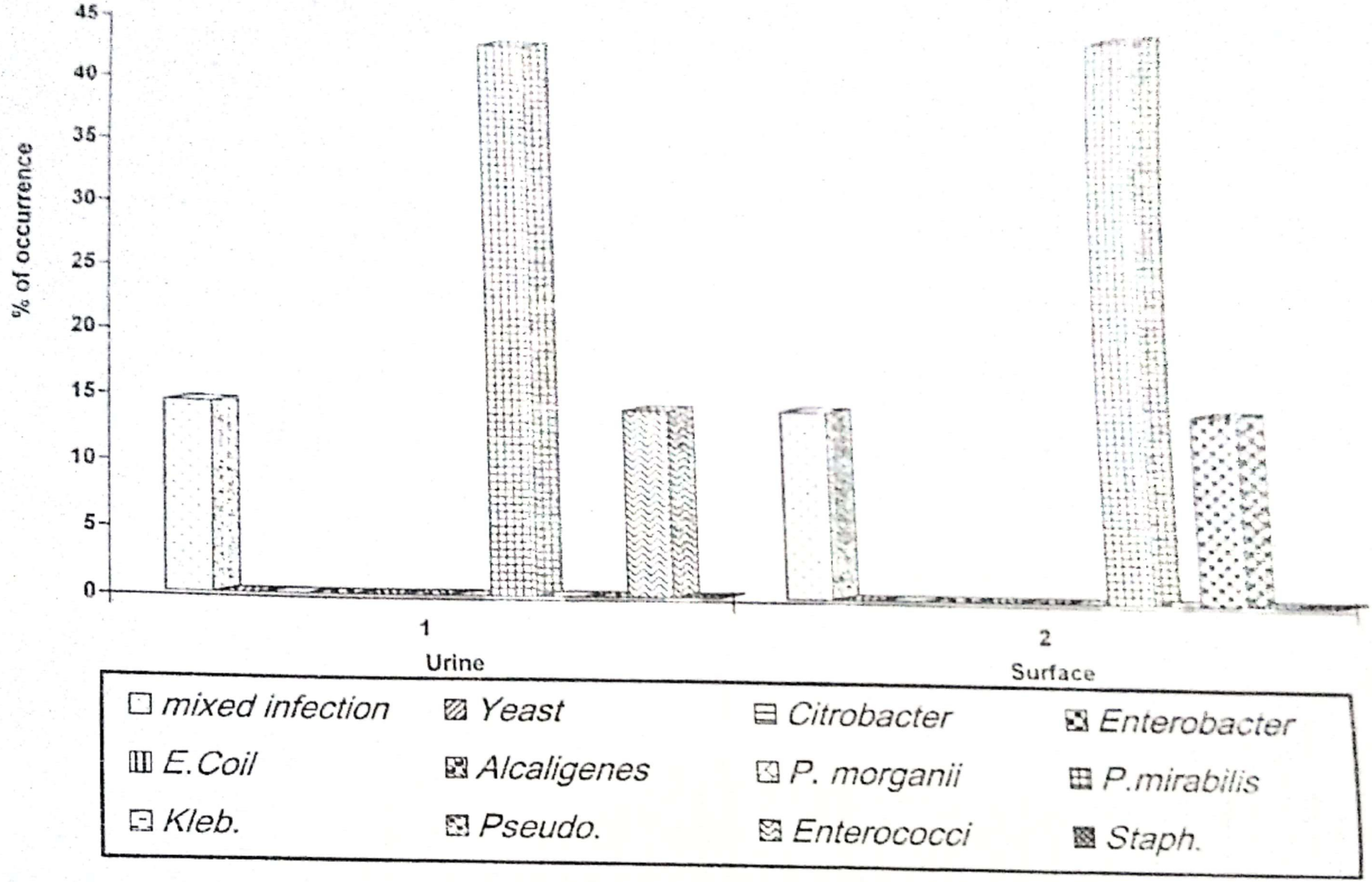

Fig (3b) : Percentage occurrence of different microorganisms in urine and stone (surface) samples with Ca- Ox + Ca-P + Uric acid

subsequently with bacterial infection. The recurrence rate was frequently high in the patients with chronic urinary tract infection, referring to the fact that bacterial infection plays an important role in the formation of stone.

In order to prevent recurrence , the stone should be removed and the patient also must be also observed carefully to keep his urinary totally free from infection.

The struvite stones , containing MAP - CaP - U , were associated with urea spliting bacteria, especially *Proteus mirabilis* , in both urine and stone samples This explained the fact that these organisms had been participating in the formation of stones.

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ميكروبية وبيوكيميائية غير طبيعية فى مرضى حصوات الأستوفيت

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أجريت هذه الدراسه لإيجاد دور للبكتريا فى تكوين حصوات الأستوفيت (المحتوية على كبريتات الماغنسيوم والأمونيوم) والمصاحبة بظواهر بيوكيميائية غير طبيعية فى سيرم دم المرضى . لذلك فإن ١٦ مريض بحصوات الأستروفيت قد قسموا إلى مجموعتين تبعاً لوجود أو عدم وجود حامض اليوريك في التركيب الكيميائى للحصوات. التغيرات في بروتين وجليكوبروتين السيرم قد قدرت . كما تم تم عزل وتصنيف البكتريا الملوثة للبول وسطح الحصوة .

لوحظ أن هناك ارتفاع فى مستوى الفا٢ - ، بيتا - جليكوبروتين يعزى هذا إلى العدوى البكتريا الحادة وإلى نوع من الالتهابات الموجودة في حالات كثيرة من مجموعة البحث - مقارنه مع ١٧ من الأشخاص العاديين الاصحاء. وكانت حصوات الأستروفيت المعزولة جراحياً، ملوثه ببكتريا محلله للبرويا . خاصة بروتيوس ميرابيلس التى وجدت فى عينات كثيرة (٩ر٤٢٪) من بول وسطح حصوات المجموعة ب (ذات الحصواب المتكونه من أكسلات الكالسيوم + فوسفات الكالسيوم + فوسفات الماغنسيوم والأمونيوم + حامض اليوريك) . هذا يفسر أن هذا الكائن يشار في تكوين حصوات هذه المجموعة. فى بعض الحالات ، ذات حصوات الأستروفيت ، المصحوبة بتلوث مجرى البول المزمن ، كان التلوث بأكثر من نوع ميكروبى موجود بكثرة فى بول المرضى.