The effect of zinc sulfate on the bacterial strains isolated from patients with chronic prostatitis

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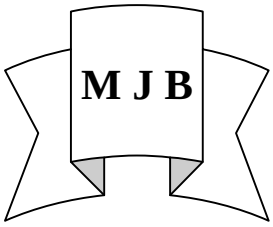

Abstract

This study included (150) patients with benign prostatic hyperplasia(BPH) & or chronic prostatitis (CP) visited Al-Hilla Teaching Hospital-Department of Urology from (October) 2004 to (May) 2005. One hundred seventeen (117) had (BPH), (20) had chronic prostatitis CP and (13) had both BPH and CP. Prostatic secretion was taken from (33) patients had CP selected for this study.

Prostatic secretions cultures are concerned, (45.5%) had positive results for bacteria. The most common isolates were *Staphylococcus aureus* were (12.1%). *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *E.coli* and *Klebsiella pneumoniae* represented (6.1%) each. *Enterobacter* spp., *Acinetobacter* spp. and *Corynebacterium* spp. represented (3%) each.

Zinc had inhibitory effect on the growth of bacteria especially at concentrations (1.05) mg/ml in which there was more effect on the Gram-negative bacteria than Gram-positive bacteria and it increased the bactericidal effect of antibiotics. In addition, it had an effect in the loss of the capsule of the bacteria.

From this study we can concluded that zinc sulfate had inhibitory effect on the growth of Gram-negative and Gram-positive bacteria especially when mixed with antibiotics.

الخلاصة

تضمنت هذه الدراسة (150) مريضاً يعانون من تضخم البروستات الحميد أو التهاب الغدة المزمن راجعوا استشارية الجراحة البولية في مستشفى الحلة التعليمي خلال الفترة من نيسان (2004) الى أيار (2005). وقد ظهر ان(117) يعانون من تضخم البروستات الحميد فقط بينما (20) كانوا يعانون من تضخم البروستات الحميد و التهاب البروستات المزمن و(13) مريض كانوا يعانون فقط من التهاب البروستات المزمن. تم أخذ سوائل البروستات من (33) مريضا فقط كانوا يعانون من التهاب البروستات المزمن اختيروا ليلتحقوا بهذه الدراسة.

وقد أظهرت نتائج الزرع لسوائل البروستات إن (45.5 %) من العينات كانت حاوية على نمو البكتيري وكانت *Staphylococcus aureus*هي الأعلى من بين العزلات حيث مثلت (12.1 %) ، تلتها بكتيريا *Staphylococcus epidermidis* و*Staphylococcus saprophyticus* و*E. coli* و*Klebsialla pnumoniae* كل واحدة مثلت (6.1 %) بينما انواع *Enterobacter* وأنواع *Acinetobacter* وأنواع *Corynebacterium* كل واحدة مثلت (3 %).

بالنسبة لتأثير الزنك على نمو هذه العزلات فقد أظهرت النتائج بأن الزنك بتركيز (1.05ملغم/مل) كان هو الأكثر في تثبيط النمو البكتيري وخصوصا على البكتيريا السالبة لصبغة كرام أكثر من البكتيريا الموجبة لصبغة كرام ، ووجد أنه يزيد من التأثير القاتل للمضادات الحيوية على العزلات بالإضافة إلى تأثيره في اختزال محفظة البكتريا .

Introduction

Chronic bacterial prostatitis usually is the result of partial blockage of male lower urinary tract, which occurs with benign prostatic hyperplasia. Such blockages promote the harboring of bacteria from a previous infection and reduce circulation, thereby preventing both the body's natural immune mechanisms and medication from getting to the site (1).

Benign prostatic hyperplasia and prostatitis cannot be distinguished according to symptoms, and some believe that they may be the same disease (2). The line between them is blurred; the prevalence of histological signs of prostatitis increased with age and was highest when BPH was also present, so prostatitis as a histological lesion was found in 98% of patients with benign prostatic hyperplasia (3).

Zinc is important to maintain prostatic health by reducing the blood level of circulating prolactine which promotes the uptake of testosterone by the prostate. This in turn leads to activation of the enzyme 5-alpha-reductase, which catalyzes the formation of dihydrotestosterone so a high concentration of zinc will tend to reduce DHT and reduce BPH.The studies support the concept that the prostate cells express a unique hormone-responsive, plasma membrane-associated, rapid zinc uptake transport gene associated with their unique ability to accumulate high zinc levels (4).

In healthy men, prostatic secretions contain a significant amount of zinc, which has antibacterial activity and is a key factor in the natural resistance of the male urinary tract infection. In vitro studies of free zinc ions at concentrations normally found in prostatic fluid have confirmed the bactericidal activity of zinc to most common pathogens that commonly cause genitourinary tract infections (5).

The aim of this study was to show the effect of zinc at various concentration. On the bacterial strains isolated from prostatic secretions obtained from patients with chronic prostatitis.

Patients & Materials

A-Patients

Thirty three (33) prostatic secretions were taken from male patients with ages ranged between (30-90) years were considered during the period from (October) 2004 to (May) 2005; these patients visited Hilla teaching Hospital-Department of Urology.

All patients underwent full history and complete physical examination. Expressed prostatic secretion examination, culture and sensitivity and abdominal ultrasound.

B-Specimens collection

Expressed prostatic secretion sample

Samples were taken after prostatic massage in classical way under sterile condition.

C-Isolation of bacteria

All specimens were subjected for cultivation on different types of media. The bacterial isolates were identified according to (6, 7).

D-Effect of zinc on bacterial growth

The effect of zinc sulfate was tested by the following method (8).

1. Nutrient broth was prepared in tubes and zinc sulfate was added to each tube at various volumes to gain the final concentration (0.15, 0.3, 0.45, 0.6, 0.75, 0.9, 1.05, 1.2, 1.35, and 1.5) mg/ml.
2. Positive control was prepared by using nutrient broth free from zinc sulfate.
3. The tubes were inoculated with 0.1ml of freshly grown bacterial suspension, then serial of tenth fold dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}) were used in sterile normal saline.
4. 0.1 ml of of the bacterial growth were spreaded on the supplemented nutrient agar and incubated for 24 hr. at 37 C°.
5. After the period of incubation, the viable count of bacteria was estimated (6).

E- Effect of combination of some antibiotics with zinc sulfate on bacterial growth.

The effect of combination of antibiotics with zinc sulfate was tested by the following (8).

1. Nutrient broth was prepared in tubes as 2ml in each tube.
2. The antibiotics solutions were added to each tube as 0.2mg of each of amoxicillin and cefotaxim, 0.1mg of amikacin, lastly 0.04 mg of ciprofloxacin.
3. A different volume of zinc sulfate was added to each tube to get final concentration (0.9, 1.05, 1.2, 1.35, and 1.5) mg/ml.

4. A positive control was prepared by using nutrient broth with antibiotics only.
5. The tubes were inoculated with 0.1ml of freshly grown bacterial suspension, then a serial tenth fold dilution (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}) in sterile normal saline in relation to antibiotics alone and antibiotics with zinc sulfate.
6. The colonies were streaked on the supplemented nutrient agar and incubated for 24 hr. at 37C°.
7. After incubation the viable count of bacteria was estimated (6).

Results and Discussion

Isolation and identification of bacterial isolates from expressed prostatic secretions.

Thirty three patients had chronic prostatitis in which expressed prostatic secretions cultures were examined.

In this study, from (15) isolates, (6) isolates from expressed prostatic secretions of patients whose ages ranged from (30-50) years and the remainder isolates from those aged more than 50 years and presented with benign prostatic hyperplasia. The different number of EPS which were taken from lesser number of patients because most of them refused to submit to this test.

According to (Table 3.1), the most isolates were *S.aureus* which represented 12.1% followed by *S. epidermidis*, *S. saprophyticus*, *E. coli* and *K. pneumoniae* represented 6.1% each. *Enterobacter* spp., *Acinetobacter* spp. and *Corynebacterium* spp. represented 3% each.

The identification of Gram-positive bacteria from expressed prostatic secretion and/or prostate tissue has also sparked debate. Some believe that these bacteria are involved in the pathogenesis of chronic pelvic pain syndrome(9). On histology, the Gram-positive *Staphylococci* were identified in focal colonies adherent to the prostatic duct walls[3]. In addition, (10)suggested that patients with chronic pelvic pain syndrom have had multiple series of long-term antibiotics, the bacterial flora of the prostate was disturbed, allowing the growth of Gram-positive bacteria, they treated these patients and found symptom eradication in 40% and symptom improvement in 25%, suggesting that these Gram- positive bacteria were pathogenic.

Effect of zinc sulfate on the bacterial growth

The effect of zinc sulfate at different concentration ranges between (0.15-1.5) mg/ml of broth culture (according to normal value of zinc concentration in prostatic

secretion range of 150-1000µg/ml) on some bacterial growth was investigated. It was observed that the addition of zinc sulfate to the culture media of isolates decreased the viable count of Gram-negative bacteria particularly at concentrations more than 1.05 mg/ml of broth culture (Figure 1).

There was a relationship between zinc sulfate concentration and bacterial growth. The effect of zinc sulfate on the growth of *S. aureus* which represents Gram-positive bacteria was lesser than that on gram-negative bacteria in which the viable count reached to 9×10^7 cfu/ml at concentration 1.5mg/ml. The zinc affected the capsule of gram negative and Gram positive bacteria which examined by capsular stain and demonstrated the loss of capsule. These findings agreed with [11] who established that the presence of zinc in the prostatic secretion in high concentration which considered as antibacterial polypeptides is effective against Gram-negative and Gram-positive bacteria.

This work agreed with (12) who revealed that in vitro studies of free zinc ions at concentrations normally found in the prostatic fluid have confirmed the bactericidal activity of zinc against a variety of Gram-positive and Gram-negative bacteria.

Zinc is the mineral ion present in the prostate and prostatic fluid is higher than any organ. Zinc is bound to high molecular weight lignads such as metalloproteins (13). In Gram-negative bacteria, zinc may bind to proteins inserted in lipopolysaccharide layer that act as water filled channels (porins) and its effect may be related to increasing diffusion of zinc into the bacteria through these channels, bind to the plasma membrane and disrupts its structure and permeability properties. The high concentration of zinc may lead to morphologic changes or lyses of susceptible bacteria. In Gram-positive bacteria, which have thicker cell wall (thicker murein coat), compared to Gram-negative bacteria, so the effect of zinc may lesser than the Gram negative bacteria. The presence of zinc in the prostatic fluid is very important to protect the prostate from many invaders. However, it's concentration in the prostatic fluid is important to carry out such task.

Effect of combination of some antibiotics with zinc sulfate on bacterial growth

The results of present study evaluated the activity of some antibiotics combined with zinc sulfate. The addition of zinc sulfate to antibiotics in different concentration (0.9, 1.05, 1.2, 1.35 and 1.5)

mg/ml of broth culture increased the zinc-binding-antimicrobial activity of these antibiotics.

In fact, this experiment showed enhancement of susceptibility of *S.aureus* which exhibited resistance to amoxicillin and *E. coli* isolates which exhibited resistance to some antibiotics such as ciprofloxacin, cefotaxim, and amikacin. It was observed that the viable count of bacteria was 120×10^6 cfu/ml for (AMX), 165×10^6 cfu/ml for (CF), 154×10^6 cfu/ml for (CTX) and 98×10^6 cfu/ml for (AK). The combination of each of those antibiotics with zinc sulfate affected on the bacterial growth which was characterized by decreasing the viable count of bacteria when zinc sulfate added at a concentration 0.9 mg/ml and more (Figure 3.2).

In vitro, zinc sulfate appeared to enhance the bactericidal ability of β-lactam, fluroquinolones, and aminoglycosides. These results matched those of (14) who revealed that the number of *E. coli* isolates(CFU) in samples with either cefotaxim or ampicillin significantly decreased when zinc was added to the samples. ß-lactams are known to display bactericidal activity that is dependent on the rate of growth. Thus, it might be expected that this class of antibacterial agent would be more effective in vitro than in vivo (15). The antibacterial effect of zinc may be synergistic with β-lactam, flouroquinolones and aminoglycosides antibiotics. Zinc may alter the permeability of the bacterial cell, so it can facilitate entry of antibiotics that might not ordinary gain access to target sites, thus resulting in enhanced antimicrobial activity. Presumably, the addition of zinc made the organisms more susceptible to the microbicidal activity of antibiotics.

It was theorized that the substance responsible for antimicrobial effect is the calcium-and zinc binding protein called calprotectin, it appears to originate in cytoplasm of neutrophils and is then released at sites of infection so the cells die and lyse; this protein has bacteriostatic activity against a variety of bacterial and fungal microorganisms (16).

Some antibiotics, particularly those of β-lactams, are most active against rapidly growing organisms and the microbistatic activity of calprotectin could interfere with the microbicidal activity of these antibiotics when they are used to treat complicated infections(17). Indeed, (18) have demonstrated that prostatic fluid interferes with the ability of cefalozolin to kill *S. aureus* and *E. coli* but if zinc is added to reverse the growth-inhibitory effect of calprotectin, then killing proceeds normally.

Thus, calprotectin appears to compromise the microbicidal effect of β-lactam antibiotics by suppressing growth of the target organisms. Quinolone antibiotics may relatively be more effective in killing microorganisms in bacterial prostatitis and its effect with zinc in microbial killing similar to β-lactam effect (19).

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Table (1): Number and percentage of bacterial isolates from expressed prostatic secretions in patients with chronic prostatitis

Types of bacterial isolates	No. of isolates	%
Staphylococcus aureus	4	12.1%
E. coli	2	6.1%
Klebsiella pneumoniae	2	6.1%
Staphylococcus epidermidis	2	6.1%
Staphylococcus saprophyticus	2	6.1%
***Enterobacter* spp.**	1	3%
***Acinetobacters* spp.**	1	3%
***Corynebacterium* spp.**	1	3%
Culture positive	15	45.5%
Culture negative	18	54.5%
Total	33	100%

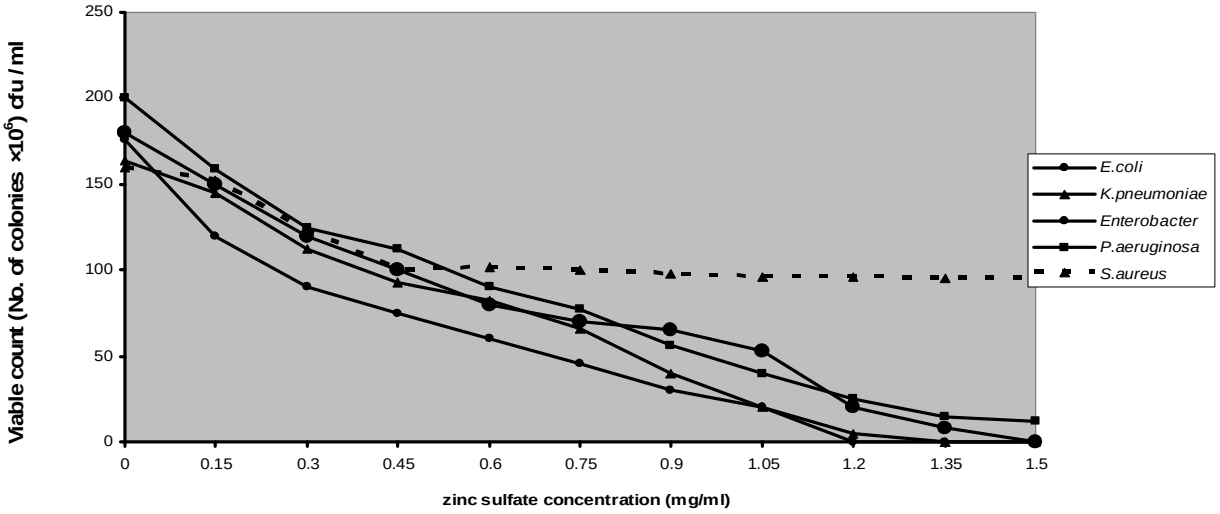

Figure (1): Effect of zinc sulfate on bacterial growth

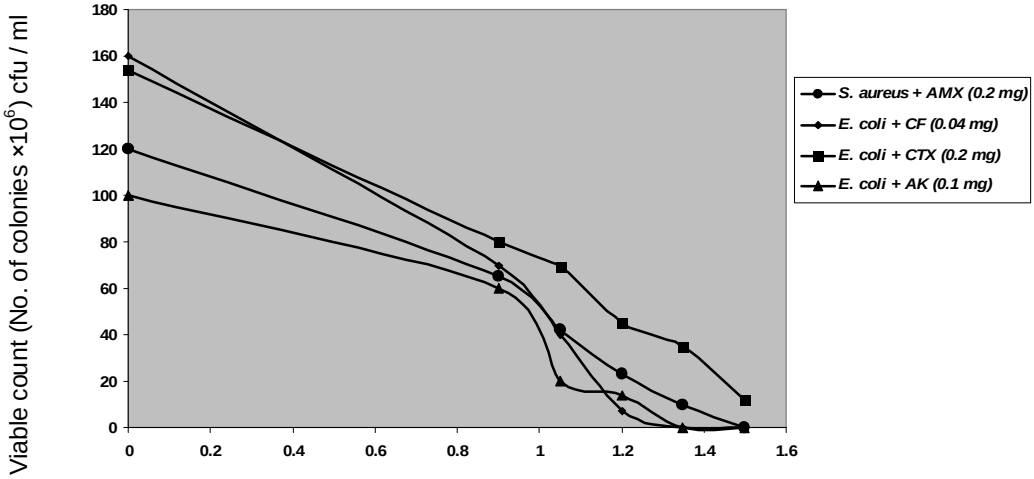

Figure (2): Effect of some antibiotics with zinc sulfate on bacterial growth