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The relationship between total urinary testosterone and renal calculi

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Summary. It is generally known that age and sex are risk factors of urolithiasis. Therefore the total urinary testosterone concentrations of persons with and without renal stones were investigated by means of radioimmunoassay. The total testosterone level of the first morning midstream urine was comparable with 24 h urine samples of 16 healthy persons ($r_s = 0.9618$). Investigation of the total urinary testosterone confirmed that the concentration is age dependent. A distinct decrease in total testosterone was observed in elderly persons. Therefore the total testosterone concentrations of the two groups, with and without stones, were studied within the same age interval ($P = 0.8292$). The total testosterone level differed significantly for these two groups ($P = 0.0006$). In general, the testosterone level of the kidney stone patients was lower than that of their healthy counterparts. In order to determine whether this variation in testosterone concentration would affect the urinary urokinase activity, a correlation study was undertaken. A positive correlation was found between the total urinary testosterone concentrations and the activity of urokinase ($r_s = 0.7305$). It therefore seems that the total urinary testosterone concentrations may play a role in the pathogenesis of the multifactorial disease, urolithiasis.

Key words: Renal calculi – Total urinary testosterone

Introduction

Urolithiasis is a multifactorial disorder of some complexity. To explain lithiasis, various theories have been postulated [1, 9]. According to the matrix theory, a protein such as uromucoid activates the initial crystallisation process by promoting calcium oxalate and calcium phosphate crystal formation and clumping in whole urine [3, 10]. Daily excretion of uromucoid is also greater in stone formers than in control subjects [6]. The concentration of urinary glycoproteins may therefore play an important role in stone formation. We therefore postulated that low activities or decreased production of urinary urokinase or plasmin may increase the urinary uromucoid concentration, inducing stone formation. Increased levels of urokinase and plasmin inhibitors in the urine of stone formers are well established [12, 14]. Recently it has been shown in vitro that low concentrations of steroids decrease the activity of the urokinase system [13]. Because it is generally known that sex and age are risk factors of urolithiasis [9], we investigated the total testosterone concentrations in the urine of male subjects of different ages with and without renal stones. A positive relationship was obtained between urinary total testosterone concentration and kidney stones.

Materials and methods

Reagents and chemicals

All reagents were of the "Analar" grade. Merck and BDH supplied the reagents sodium phosphate, EDTA, sodium azide and Triton X-100. Sigma Chemical Co. supplied the substrates plasminogen (human plasma) and D-valyl-L-leucyl-L-lysine p-nitroanilide, as well as the enzyme urokinase from human kidney (U 5628). The Coat-A-Count Total Testosterone radioimmunoassay kit was obtained from Diagnostic Products Co. The LKB 1271 Riagamma automatic gamma counter was used.

Urine specimens

First morning urine was collected in plastic tubes from adult male patients in whom calcium oxalate stones dominated and from healthy male subjects without stones. Urinary tract infections or injury were also absent in the stone patients. Glass bottles were used for the 24 h urine collection. One gram of boric acid per 100 ml was added to the urine collected for the testosterone assay and was frozen (−15°C) immediately. Morning urine collected for the urokinase activity assay had no boric acid added and was also kept frozen. The urokinase assays were performed within 7 days of collection [14].

Standard curve of urokinase activity

A standard activity curve of urokinase (UK) coupled to plasmin was obtained by incubating different amounts of urokinase in a stirred waterbath for 90 min at 37°C. This assay was done according to a modified method of Wiman et al. [15]. 400 µl activator reagent was added to 418 µl of 0.1 M sodium phosphate buffer, pH 7.3,

containing 10 mM EDTA, 0.1 g/l sodium azide and 0.1 g/l Triton X-100. The activator reagent consisted of 1 μM plasminogen and 0.6 mM D-valyl-L-leucyl-L-lysine p-nitroanilide, which was dissolved in 0.1 M sodium phosphate buffer. The blank consisted of a buffer with an activator reagent. The total volume for each was 828 μl. After the desired incubation time, the reaction was stopped immediately by inserting the glass tubes in ice and adding 0.1 ml of a 50% acetic acid solution to all the tubes [4]. Enzyme activity was estimated from the product concentration recorded at 405 nm, on a Hitachi 150-20 spectrophotometer connected to a data processor. The urokinase activity was expressed in International Units (IU). One IU equals 0.5 nmoles [2].

Determination of urokinase activity in the presence of urine

The effect of urine on urokinase was assayed according to the method described under *Standard Curve of Urokinase Activity*. This assay consisted of a blank, control, blank plus 10 μl urine and control plus 10 μl urine. The same amount of urokinase was added to all the controls (av. 0.41 IU). After 90 min at 37°C the reaction was stopped by adding 50% acetic acid and the absorbance was read at 405 nm. The blank rate value was substracted from the rate value obtained in the presence of the enzyme to obtain the real control value. This procedure was repeated with the blank and the control containing urine, which allowed the effects of urinary urokinase to be eliminated. Urine specimens from 16 subjects were used for the statistical analysis.

Statistical analysis

The Spearman Correlation was used to calculate the correlation between morning and 24 h urinary total testosterone values as well as the correlation between urokinase activity and total testosterone concentration in urine. Statistical comparisons of the urinary data obtained from black and white subjects without stones was done according to the Analysis of Covariance. Analysis of Variance was used to analyse the data obtained from subjects with and without stones.

Results

To determine whether experiments should be done using first morning urine or 24 h urine samples, these two parameters were studied first. Total testosterone concentrations of 16 morning midstream urine specimens and 24 h urine specimens were statistically correlated ($r_s = 0.9618$, $P = 0.0001$). Therefore most of the following assays were done on first morning midstream urine samples.

The total testosterone concentration in the urine samples of 14 subjects with stones and 21 subjects without stones is illustrated in Fig. 1. According to the Analysis of Covariance, the total testosterone level in each group was dependent upon age ($P < 0.001$). However, age did not play the same role between the two groups. As the age interval between the two groups did not differ significantly ($P = 0.8292$) the total testosterone concentrations were compared statistically by applying the Analysis of Variance. It was then found

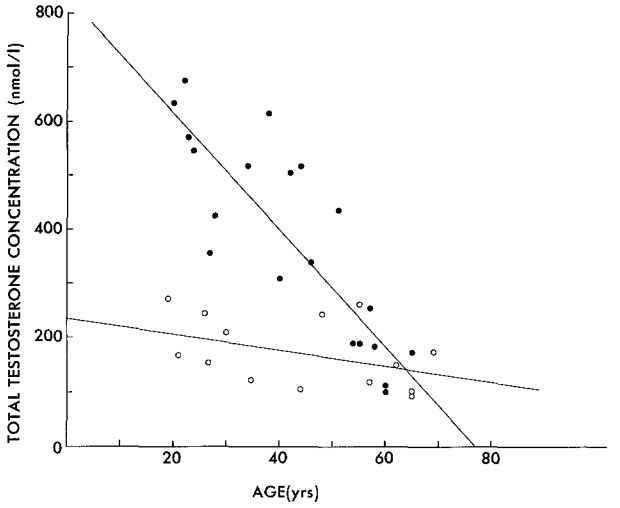

Fig. 1. Total testosterone concentration of morning urine specimens collected from subjects with (○) without (●) stones

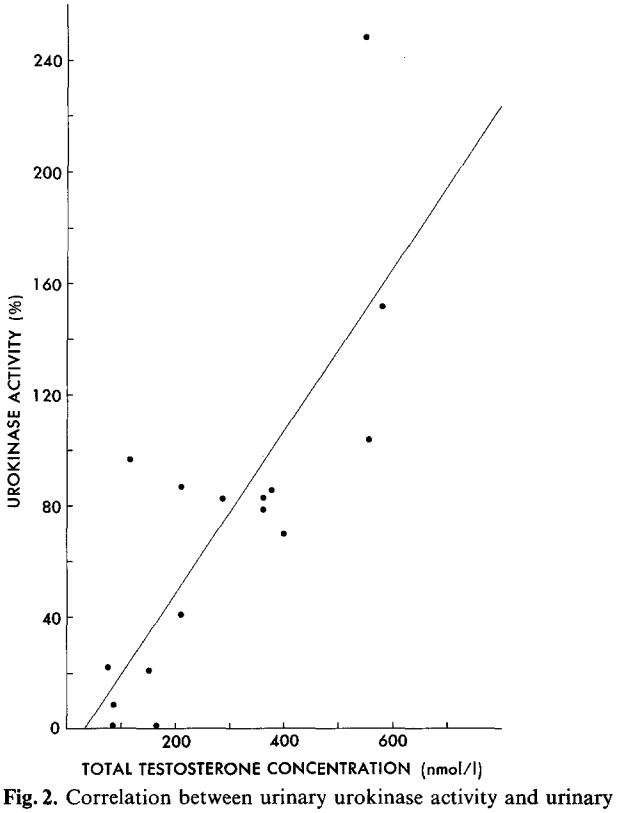

Fig. 2. Correlation between urinary urokinase activity and urinary total testosterone concentration ($r = 0.7305$)

that the total testosterone concentration differed significantly ($P = 0.0006$), but in general, the total testosterone concentration of kidney stone patients was lower than that of their healthy counterparts.

Because it is well known that black people seldom form stones [5, 7], the total testosterone concentration in the morning urine of 21 healthy white persons was compared with the total testosterone values in the urine of 10 healthy black persons. No significant differences

were observed ($P=0.524$). The two groups were also within the same age interval ($P=0.291$).

It has been reported that the testosterone concentration affects the urokinase activity in vitro [13]. Therefore this phenomenon was investigated in urine (Fig. 2). A positive correlation was found between urinary urokinase activity and urinary total testosterone concentration ($r_s=0.7305$).

Discussion

In the present study the total testosterone concentration in the urine of persons with and without stones was investigated. Initially the total testosterone concentrations in morning urine were compared with the concentrations in 24 h urine samples. Statistically no difference was observed when the total testosterone concentration was expressed as pmol/ml. Morning urine specimens were obtained for further studies as they were more convenient to collect.

The total testosterone concentrations in the urine collected from subjects with and without stones, belonging to the same age groups, differed significantly. The urine of stone patients contained lower total testosterone concentrations than that of their healthy counterparts. This was especially noticeable in the younger age groups. The total urinary testosterone values in both groups were higher at a young age than at an old age (50–70 years). In the groups without stones the total urinary testosterone concentration differed markedly between young and old persons. This finding is in accordance with published data that older men suffer more from stone disease than younger men [8, 11]. The same trend was observed in healthy black and white subjects. Therefore total urinary testosterone concentrations did not supply the answer as to why black people seldom get kidney stones. Urolithiasis remains a complex multifactorial disease.

One theory as to why people with a low total urinary testosterone concentrations do have stones may be that if these subjects have low urinary urokinase activities, this would in turn be responsible for higher urinary uromucoid concentrations. The uromucoid would induce stone formation according to the matrix theory. Previous work concerning the activity of the urokinase system revealed a decrease in the enzyme activity with low concentrations of testosterone. Therefore a study was undertaken to correlate urinary urokinase activity and total testosterone concentration, and a positive correlation between these two parameters was obtained. Therefore it can be concluded that total urinary testosterone may, amongst other things, play an important role in the pathogenesis of renal stones, in the sense of stimulating the urokinase system.

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