ORIGINAL ARTICLE

Evaluation of seminal zinc levels by atomic absorption in men with spinal cord injury

AP Alexandrino¹, MAF Rodrigues¹, T Matsuo², EP Gregório¹ and JC Santilli³

¹Discipline of Urology, Department of Surgery, Universidade Estadual de Londrina, Paraná, Brazil; ²Department of Mathematics, Universidade Estadual de Londrina, Paraná, Brazil and ³Laboratório Osvaldo Cruz, Londrina, Paraná, Brazil

Study design: A case–control study evaluating seminal zinc level in spinal cord injury (SCI) patients.

Objectives: Patients with SCI have neurological prostate dysfunction. There are only some indications in the literature that seminal zinc level may be lower in these patients. Seminal zinc is mainly produced by the prostate and, therefore, can be considered to be a marker of prostate function. The objective of the present study was to determine whether SCI can induce changes in seminal zinc levels and to compare the results with those obtained for normal men (controls).

Setting: The study was carried out in Brazil.

Methods: A total of 24 men with SCI (mean age $\pm$ s.d. 36.25 ± 10.24 years) and 24 controls (mean age $\pm$ s.d. 36.50 ± 10.31 years) were studied. Blood and semen were collected after 3 days of abstinence from ejaculation. Semen was left at room temperature for 15 min, stored in liquid nitrogen, and lyophilized. Seminal zinc was determined by atomic absorption. Blood was stored at a controlled temperature of -70 to -79°C and later used for the determination of testosterone, prolactin and total prostate-specific antigen using an AxSYM apparatus and Abbott reagents.

Results: Mean seminal zinc concentration was 85.20 mg l^{-1} for the patients, a lower value than that obtained for the controls (147.16 mg l^{-1}) ($P=0.0035$).

Conclusion: Patients with SCI have a significant reduction of seminal zinc.

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Introduction

Men with spinal cord injury (SCI) have neurological dysfunction of the prostate¹ and of the seminal vesicle.² The lack of neurological control of these organs promotes changes that contribute to the low quality of seminal plasma³ and of spermatozoa.⁴ Recent studies have demonstrated that a low prostate-specific antigen (PSA) level in semen is a marker that reflects the prostate dysfunction due to SCI.⁵ There is a consensus in the literature that the main source of seminal zinc is the prostate⁶ and, for this reason, we believe that zinc could also be a marker of the function of this organ. Prostatic zinc is involved in various important enzymatic reactions that may alter the constituents of seminal plasma, such as citrate,⁷ and can also be considered to act as a bactericidal element in semen.⁸ Thus, the quality of seminal plasma interferes with sperm vitality,⁴ which in

turn depends on zinc, a factor considered to be relevant for male fertility⁹ and that may be impaired in patients with SCI. Thus, the objective of the present study was to determine whether SCI causes any alteration in seminal zinc levels and to compare the values obtained for SCI patients with those of control subjects.

Materials and methods

The study was conducted on 24 patients and 24 controls, all of them volunteers. The patients with SCI were selected from the multidisciplinary clinic and the controls were selected from the family planning clinic of the university hospital. The subjects were matched for age, which was 36.25 ± 10.24 years for the patients and 36.50 ± 10.31 years for the controls.

The level and degree of spinal injury were determined according to the classification of the American Spinal Injury Association.¹⁰ The injury was cervical in 5 patients and thoracic in 19. A total of 22 patients had type A (complete) injury according to the ASIA, 1 had type C (incomplete)

Correspondence: Dr MAF Rodrigues, Discipline of Urology, Department of Surgery, Universidade Estadual de Londrina, Av. Bandeirantes, 460 JD Londrilar, Londrina, Paraná 86010-010, Brazil.
E-mail: drmaurelio@gmail.com
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injury and 1 had type D (incomplete) injury. All patients had suffered from SCI more than 6 months before the study. Serum prolactin and testosterone levels were normal in both patients and controls. None of the participants had PSA level or digital rectal examination suspicious of prostate cancer, history of treatment of acute prostatitis in the last 3 months or of chronic prostatitis. None had a clinical history of thyrotoxicosis or a previous prostate surgery. Finally, none of the subjects had presented signs or symptoms compatible with the current urinary infection.

Blood and semen samples were collected from all participants after at least 3 days of abstinence from ejaculation, with blood being collected before the seminal fluid. Control subjects collected semen by masturbation and in the patient group, 21 collected semen by electroejaculation, 2 by masturbation and 1 by vibroejaculation (VE). The semen samples of the patients were obtained after complete emptying of the bladder with a number 12 urethral catheter.

A 2-ml serum aliquot from each participant was stored in a freezer at a controlled temperature of -70 to -79°C . According to the volume obtained, semen was fractionated into 0.5-ml aliquots in propylene tubes that were wrapped in plastic and stored in liquid nitrogen 15 min after collection at room temperature. The semen samples were then lyophilized and stored in a freezer until the time for analysis. For the measurement of seminal zinc, the samples were placed in specific tubes, diluted in 0.5 ml of water-reagent grade type 1 and analyzed by atomic absorption using an Analyst 100 apparatus (Perkin-Elmer Life and Analytical Sciences, Shelton, CT, USA).

The blood samples were tested for testosterone, prolactin and total PSA by the microparticle enzymatic immunoassay using the AxSYM System apparatus and the AxSYM testosterone, AxSYM prolactin and AxSYM total PSA reagents of Abbott Laboratórios do Brasil (São Paulo, SP, Brazil).

The study was approved by the Bioethics Committee of the State University of Londrina and all volunteers gave written informed consent to participate in the study.

Data are reported as median and mean $\pm$ s.d. and are presented graphically by quartiles. The patient and control groups were compared by the Mann-Whitney test and the correlation between time since the accident and seminal zinc levels was determined by the Spearman correlation test.¹¹ The level of significance was set at $P \leq 0.05$ in all analyses.

Results

Median seminal zinc concentration was 71 mg l^{-1} (range 13.2–260, mean 85.20 ± 65.52) for patients and 142 mg l^{-1} (range 20–340, mean 147.16 ± 72.17) for controls, with a significant difference ($P = 0.0035$) between groups (Figure 1). Seminal zinc concentration was lower in patients compared with controls. The time since the accident ranged from 0.6 to 20 years (mean $\pm$ s.d. 9.5 ± 5.8) and did not show a significant correlation with seminal zinc levels ($r = -0.056$ and $P = 0.769$).

Figure 1 Box and Whisker plot of seminal zinc (mg l^{-1}) in patients with spinal cord injury and controls.

Discussion

Studies have demonstrated that SCI leads to neurological dysfunction of the prostate, as shown by the low seminal levels of its function markers such as PSA⁵ and citrate (data accepted for publication in *Spinal Cord*). There is strong evidence for neurological changes of the prostate in these patients.¹² Some findings suggest that prostate dysfunction may be secondary to the lack of neurological control of the organ. In 1998, Benaïm *et al.*,¹² in a comparative study of prostate biopsies from SCI patients and controls, did not detect significant differences in prostate architecture, but observed a smaller number of adrenergic nervous cells. Therefore, prostate atrophy¹³ and prostate dysfunction due to neurological lack of control of the prostate caused by SCI¹ may possibly be the major features that favor alterations of the seminal fluid. Most of these changes are still unknown, but they could certainly explain some disorders of the urogenital tract of these patients. The present comparative study of seminal zinc was conducted in order to obtain a better understanding of the impact of SCI on the prostate of these individuals.

In a literature report, seminal zinc concentration was low in six patients with SCI, although without statistical significance.¹⁴ In the present study, the seminal zinc concentration of patients with SCI was significantly lower compared with the controls regardless of the time since the accident. This demonstrated that the lack of neurological control caused by the injury leads to prostatic dysfunction and that seminal zinc could act as a marker of the damage to the prostate of these patients.

The influence of different methods of sperm retrieval on seminal zinc has not been studied in men with SCI. However, most authors agree that the best motility is obtained when semen is collected by VE than by electroejaculation.¹⁵

Brasso *et al.*¹⁶ have studied seminal PSA using either VE or electroejaculation for collecting semen. They have not found statistical difference between the two methods. In the present study, the majority of patients collected semen by electroejaculation, two by masturbation and one by VE. Even if there were differences in the seminal zinc concentration depending on the method, the fact that a single patient collected his semen by VE would not compromise our results.

The prostate is the major source of human seminal zinc. This component has been reported as a good marker of its function.^{6,17} In healthy adult men, its median semen concentration is 133 mg l^{-1} ,⁶ because prostatic epithelial cells have the ability to accumulate high zinc levels, a property regulated by prolactin and testosterone.¹⁸ Testosterone is reduced during the first 2 months after a cervical SCI, but later normalizes.¹⁹ The mean time of injury was 9.5 ± 5.8 for the patients and was less than 1 year in only one of them, but testosterone levels were normal in all participants. Serum zinc normalizes within 2–7 weeks after SCI, with its rate of urinary excretion remaining high due to bone demineralization.²⁰ In the present study, serum zinc levels did not differ between patients and controls, but this was not reported because of lack of statistical power of the test owing to the small number of patients.

In the normal prostate, high zinc levels inhibit mitochondrial aconitase, a citrate-oxidizing enzyme, permitting the epithelial cells of the prostate to accumulate this substance.⁷ In a previous study by our group, we observed that patients with SCI had a lower seminal citrate concentration. In the present study conducted on patients of the same group, seminal zinc concentration was also lower. This fact may be related to the previous finding for seminal citrate. We may perhaps infer that seminal zinc concentration is low because its intracellular concentration may also be reduced, resulting in lower inhibition of mitochondrial aconitase. Thus, citrate may be more oxidized, with a reduction in its cellular accumulation and a consequent reduction of its seminal concentration, as actually observed in the present study.

It also seems correct to state that zinc levels in prostate tissue are much lower in the presence of prostate cancer than those in normal and hyperplastic glands.²¹ Patients with SCI have cancer of the prostate of higher grade and more advanced stage than that of normal men.²² Zinc has the property of inhibiting the growth of prostatic tumor cells by its ability to induce apoptosis²³ and, therefore, may be a probable protective factor against cancer. Thus, we may wonder if low seminal zinc levels in patients with SCI would make them more susceptible to more aggressive prostatic tumors.

Since the studies by Brackett *et al.*,⁴ it has been known that the seminal plasma of patients with SCI causes worsening of the sperm motility. It was later demonstrated that low seminal fructose² and high levels of reactive oxygen compounds in the semen of these patients contribute to low sperm vitality.³ Indeed, it has been confirmed that semen samples with a greater potential for fertility are those with the highest zinc concentrations, a fact that is also related to lower teratogenicity and to a larger number and better motility of spermatozoa.⁹ In addition, the fact that the zinc concentration in spermatozoa is $2.5 \mu\text{g}$ for every 10^8 cells, exceeding the concentration in seminal plasma,²⁴ demonstrates how vital this trace element is for sperm survival. Thus, in view of the importance of zinc for male fertility, low zinc concentrations in patients with SCI are consistent with the notion of a possible impairment of their potential for fertility. The control group studied, regarding fertility, served

as a good sample of normality as it mostly consisted of individuals who already had at least two children per couple.

Patients with SCI and neurogenic bladder have a higher incidence of urinary infection and it has been well defined that prostatitis courses with low seminal zinc levels.⁹ In addition, William *et al.*⁸ demonstrated that zinc is a bactericidal factor of semen,⁸ a property that may cause it to be a protective factor against infection. Thus, lower zinc levels may facilitate the occurrence of focal points of urinary infection.

Finally, the present results suggest that prostatic dysfunction is secondary to the lack of neurological control of the gland. Further studies in this direction are needed to better elucidate this question.

Conclusions

The seminal zinc level measured by atomic absorption is lower in SCI patients compared with controls. The implication of the results must be better studied.

Conflict of interest

The authors declare no conflict of interest.

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