Semen Quality and Ferritin and Transferrin Seminal Levels

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Background: We proposed to investigate the possible relationship between seminal quality and ferritin and transferrin seminal levels in chronic hemodialysis (CH) patients.

Materials and methods: This is a cross-sectional study in a group of 60 men (case) undergoing CH for more than 6 months, and a group of 30 healthy men (control), aged 18-60 years, without clinical or laboratory signs of infection/inflammation and eugonadic. A spermogram was performed by manual method and measured the ferritin and transferrin seminal levels.

Results: The case and control groups were age-matched (49.47±5.56 versus 47.90±6.22, p = 0.229). Comparison between case and control group, the exception of seminal ferritin levels that were similar (p = 0.136), were significantly lower in the case group (p<0.001) for all constituents of the seminal parameter and seminal transferrin levels. Seminal ferritin does not appear to be associated with seminal parameters and seminal transferrin (p>0.05); but there was an association between seminal transferrin and seminal parameters (p<0.001).

Conclusions: Our results suggest that seminal quality is related to seminal transferrin level and not with seminal ferritin level being useful in the initial evaluation of chronic hemodialysis patients with clinical suspicion of sub / infertility.

BACKGROUND

In the reproductive age, infertility or subfertility affects about 15% of couples.¹ CKD patients, especially hemodialysis patients, have non-depressible changes of fertility manifested by poor seminal quality.² There is no reliable estimate of the percentage accounted for by infertility of this patient group, but it is not depressible. The mechanisms involved in the general etiology of sub or infertility are multiples, but oxidative stress³ stands out, among others, and recognized contribution of uremic factor in CKD / HD⁴. In clinical practice, SQ related biomarkers have been identified over the decades; but by specificity, sensitivity, practicality and operational cost variables, an ideal marker was not identified. In the present study, due to the practicality and low cost of execution, we decided to verify the possible relationship between SQ and of ferritin and transferrin seminal levels, considering its possible anti-oxidative activities in chronic hemodialysis patients.

MATERIALS AND METHODS

RECRUITMENT, INCLUSION AND EXCLUSION

Prospective study of prevalence realized in the Hemodialysis Sector of the University Hospital of the University of Brasilia, between July 2016 and December 2016, after approval by the Research Ethics Committee of the Faculty of Health Sciences of the University of Brasilia under number 53172316.9.0000.0030. Inclusion criteria were age between 18 to 60 years, HD performed for more than 6 months (cases), and absence of acute or chronic liver disease. Exclusion criteria were the presence of hemochromatosis or diseases of iron metabolism. We excluded from the study patients with hypogonadism and clinical conditions that could alter seminal ferritin or seminal transferrin.
levels such as recent history of genitourinary tract infection, clinical signs of acute or chronic infection/inflammation. All participants were subjected to a spermiogram and measured SF and ST levels. Sample consisting of 60 men (cases) in high flow HD by vascular fistula access, 3×week with duration of 4 hours / HD session and 30 healthy men (control) from the health promotion outpatient clinic of the same hospital with renal function (glomerular filtration rate > 90 ml/min per 1.73 m²), sperm without changes.

Semenal analysis: spermiogram was performed by manual method according to the guidelines of the World Health Organization (WHO) laboratory manual for the examination and processing of human semen. The seminal plasma preparation, centrifuging at 3500×g for 20 min after 30 minutes liquefaction. The supernatant was collected into a new tube and held at -20°C for the measurement of SF and ST levels. SF and ST were measured by enzyme immunochemiluminescence using the Immulite 2000 / Siemens automatic analyzer. Specific kits were used for quantification, as well as calibrators and controls recommended by the manufacturer.

Informed consent was obtained from all participants included in the study.

**STATISTICAL ANALYSIS**

After the normal distribution curve of the sample was verified by normality tests, t-test and Pearson’ correlation analysis were used to assess differences between two independent quantitative variables. Statistical significance was set at p<0.05 to reject the null hypothesis. We used SPSS® for Windows, version 20.0.

**RESULTS**

Age was similar between case and control groups (49.47±5.56 versus 47.90±6.22, p = 0.229). Comparison between case and control group, the exception of SF levels that were similar (p = 0.136) were significantly lower in the case group (p<0.001) for all constituents of the seminal parameter and ST levels (Table 1). There was an association between ST level and seminal parameters in case group (p<0.05) (Table 2). SF does not appear to be associated with seminal parameters and ST (p>0.05) (Table 2).

**DISCUSSION**

This study is of great importance because it is the first to verify the possible relationship between QS and of ferritin and transferrin seminal levels. The practicality and low cost of ferritin and transferrin seminal levels measurement can be attractive for the initial evaluation of semen quality in these patients, considering that these proteins (seminal ferritin and seminal transferrin) are routinely measured in the follow-up of these patients.

It is admitted that SQ decreases sensibly with advancing age and by clinical hypogonadism. The similar age (p = 0.136) and eugonadism of the sample is important for spermatogenesis and Leydig, Sertoli and germ cells of the trophism, ensuring reliability greater in the interpretation of the results found here.

SF and ST are found abundantly in human seminal plasma, are produced and secreted (80%) by Sertoli

| Parameters evaluated | Case n (60) | Control n (30) | p² - valor |
|----------------------|------------|---------------|------------|
| Seminal volume (ml)  | 01.33±00.36 | 02.77±0.44    | < 0.001    |
| Sperm total motility (MP+NP, %) | 34.00±06.24 | 71.31±7.86 | < 0.001 |
| Sperm vitality (%)    | 47.49±07.31 | 64.41±2.89    | < 0.001    |
| Sperm density (×10⁶/ml) | 14.95±06.18 | 50.21±8.57    | < 0.001    |
| Sperm normal morphology (%) | 25.40±07.87 | 59.76±10.58 | < 0.001 |
| Seminal ferritin (ng/ml) | 226.45±51.03 | 241.52±30.52 | 0.139 |
| Seminal transferrin (ng/ml) | 40.12±08.25 | 73.32±06.81 | < 0.001 |

MP: progressive motility; NP: non-progressive motility; a: t-test; x: average; SD: standard deviation
cells\textsuperscript{7} and are proteins involved iron storage and transport in the systemic or site compartments.\textsuperscript{7}

The high concentrations of these proteins in seminal mammalian plasma are not well understood. It may be due to the great need of iron ion in the different biological processes (ATP generation, DNA synthesis, growth, and development cell) necessary for successive mitosis of testicular germ cells.\textsuperscript{8} Sylvester and Griswold\textsuperscript{9} devised an ingenious process of iron ion supply to germ cells with participation of SF and TS proteins. On the other hand, the toxic effect of excess iron ion in the cellular environment is recognized.\textsuperscript{9}

It is hypothesized that anti-oxidative activity of SF and ST proteins are attributed to their ability to control iron ion concentrations in the intracellular by chelation mechanism, controlling the reactions of free radical generators (Fenton and Haber-Weiss).\textsuperscript{10}

Our results suggest that only ST levels were significantly lower in the seminal plasma of uremic patients (p <0.001). This result is corroborated by other previous studies conducted by different researchers in non-uremic populations with suspected sub/infertility as Koşar et al.\textsuperscript{11} (16, 58.1±14.4 µg/ml vs 108.4±17.5 µg/ml); Bharshankar and Bharshankar\textsuperscript{12} (2.63±1.76 mg/dl vs 5.35±2.07 mg/dl, 91±51 µg/ml), and Saeed et al.\textsuperscript{13} [17, 54.0 µg/ml (50.0-60.0) vs 74.0 µg/ml (69.0-80.0)]. The ST maintained relationship with SQ.

The explanation for a decrease in mean ST levels in unhealthy men and its correlation with SQ observed in this and other sub/infertility studies are not well known. However, it is speculated that it is multifactorial and by various mechanisms, with participation of immunological, hormonal, uremic, oxidative, and inflammatory factors.\textsuperscript{14,15} The inhibitory effect of interleukin 6 on ST synthesis by Sertoli cells is also hypothesized.\textsuperscript{15}

The SF levels was indifferent to SQ. Little is known about SF behavior in seminal plasma. Silva et al.\textsuperscript{16} did not find a change in the SF seminal level in uremic and non-uremic patients in a sample of 60 hemodialysis patients. On the other hand, Wan et al.\textsuperscript{17} found higher concentrations of SF in seminal plasma of 62 men with infertility infected by Chlamydia trachomatis.

Despite the supposed ant-oxidative activity of the proteins studied, it is not possible to affirm with high probability the relation of these proteins with SQ. It is consensus to admit multifactorial cause for low seminal quality, highlighting oxidative stress, hypercytokinemia and uremia which are responsible for sperm dysfunction.\textsuperscript{18,19}

This would cause profound and direct changes in the physiology of the hematotesticular barrier modifying the local paracrine/autocrine systems and other mechanisms which are responsible for maintaining the immune privileged condition of the testes.\textsuperscript{19,20}

This study has two limitations: the lack of measurement of total seminal antioxidant capacity and a small sample size.

**CONCLUSIONS**

The results suggest that isolated analysis of ferritin and transferrin seminal levels, only the last showed relation with seminal quality that can be used in the initial investigation of sub/infertility in chronic hemodialytic patient.
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Качество спермы и уровни ферритина и трансферрина в семенной жидкости

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Введение: Мы исследуем возможную взаимосвязь между качеством спермы (КС) и уровнями ферритина и трансферрина в семенной жидкости пациентов с хроническим гемодиализом (ХГ).

Материалы и методы: Это исследование текущего состояния группы из 60 мужчин (случаи), которые проходили ХГ в течение более 6 месяцев, и группы из 30 здоровых мужчин (контроль) в возрасте между 18 и 60 годами без каких-либо клинических или лабораторных признаков инфекции / воспаления и с сохраненной функцией гонад. Спермограмма была осуществлена мануальным методом и были измерены уровни ферритина и трансферрина в семенной жидкости.

Результаты: Группы контроля и случаев сопоставимы по возрасту (49.47 ± 5.56 по сравнению с 47.90 ± 6.22, р = 0.229). При сравнении групп контроля и случаев, за исключением уровней ферритина в семенной жидкости, которые были сходными (р = 0.136), все показатели параметров спермы и уровней трансферрина в семенной жидкости были значительно ниже в группе случаев (р <0.001). Ферритин в сперме, похоже, не связан с семенными параметрами и семенным трансферрином (р > 0.05); но существует связь между семенным трансферрином и семенными параметрами (р <0.001).

Выводы: Наши результаты показывают, что качество спермы связано с уровнем семенного трансферрина, но не с семенным ферритином, что является полезным для первоначальной оценки пациентов с хроническим гемодиализом при клиническом подозрении на суб / бесплодие.