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Seminal Ferritin and Seminal Parameters in Patients Undergoing Chronic Hemodialysis

ORIGINAL

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Abstract

Background: to verify the association of seminal parameter (SP) and seminal ferritin (SF) levels in patients undergoing chronic hemodialysis (CH), admitting possible antioxidative activity of SF.

Methods: This was a case-control study in group of 60 men (case) in CH with more than 6 months and group of healthy men (control), aged 18-60 years, without clinical or laboratory signs of infection/inflammation. Patients underwent semen analysis, fertility index (FI) calculation, measurement of SF and hormonal profile (follicle-stimulating hormone, luteinizing hormone, total testosterone, and prolactin levels).

Results: There were significant differences between cases and control **(Table 1)** in SP (p = 0.000), sex hormone (p = 0.000) and FI [0.85 (0.57) vs 5.54 (1.3), p=0.000]. There was no difference between cases and control **(Table 1)** in SF levels (226.45 ± 51.03 vs 241.52 ± 30.52, p = 0.137) and age (49.47 ±5.56 vs 47.90 ± 6.22, p=0.229). There was no correlation **(Table 2)** between SF and FI (r = 0.049, p = 0.711) and SP (p> 0.05).

Conclusion: The results suggest that SF is not associated with changes in seminal parameters in patients undergoing chronic hemodialysis, and is not useful singly for initial evaluation of seminal parameters.

Keywords
Chronic Renal Disease; Seminal Parameters; Semen Quality; Male Infertility; Seminal Ferritin; Sexual Hormone.

Introduction

Subfertility and infertility in patients with chronic kidney disease (CKD)/hemodialysis (HD) is frequently encountered, presenting as a reduction in one or more seminal parameters (SP) (concentration, motility, morphology, and vitality of spermatozoa) [1]. Semen quality is the result of the synchronic interaction of several factors involved in the complex spermatogenesis process, including genetic, hormonal, immunological, oxidative, and inflammatory factors [2]. DRC/HD is characterized by an increase in oxidative stress (OS) and inflammation [3]. Is accepted the association between OS and increased synthesis of proinflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor α (TNF-α) induced by the activation of nuclear factors such as kappa nuclear factor B (FN-κB) [3]. Or activator protein-1 (PA-1) [3] and consequent elevation of acute phase reaction proteins such as fibrinogen and C-reactive protein (CRP), suggesting that CKD is a low-grade chronic inflammatory state [4]OS is one of the main factors responsible for subfertility and infertility in humans [5]. Evaluation of seminal SO is usually performed by measuring the total antioxidative capacity of the semen (evaluating the enzymatic and non-enzymatic systems) or individualized lipid peroxidation by-products, such as malonaldehyde (MDA), protein (carbonyls), nucleic acids (oxidized nitrogen bases), and carbohydrates (glycosylation products) [6]. Few studies have separately evaluated seminal OS via the non-enzymatic antioxidant system.

The different isoforms of ferritins, in addition to the intracellular iron ion storage function, they are admitted antioxidant function by controlling the iron ions in the intracellular environment by chelation mechanism [7].

Objective

Considering the low quality of semen observed frequently in CKD/HD patients, it was decided to verify the possible association between seminal ferritin (SF) levels and seminal parameters in patients on chronic hemodialysis, hypothesizing possible SF antioxidative activity.

Methods

Recruitment, inclusion and exclusion

Prospective study of prevalence in the Hemodialysis Sector of the University Hospital of the University of Brasília, between July 2016 and December 2016, after approval by the Research Ethics Committee of the Faculty of Health Sciences of the University of Brasília under number 53172316.9.0000.0030. Inclusion criteria were: age between 18 to 60 years, has been in HD 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. Were not included in the study patients with hypogonadism and clinical conditions that could alter SF levels such as recent history of genitourinary tract infection, clinical signs of acute or chronic infection/inflammation, positive serology for hepatitis B, C and HIV, vascular access infection, leukocytosis, fever, hypoproteinemia. Sample consisting of 60 men (case) in high flow HD by vascular fistula access, 3x week with duration of 4 hours/HD session and 30 healthy men (control) from the health promotion outpatient clinic of the same hospital with normal renal function, this is, above 90mls/min/1.73m^2 and a normal CRP value (≤1 mg/L) considered for a low cardiovascular risk [8].

Routine collection of blood and semen

The blood sample for analysis was collected from the arteriovenous fistula immediately before the first weekly hemodialysis session in the case group and on a previously scheduled day for the control group, always between 8:00 and 10:00 a.m. in the clinical laboratory of the same hospital. Were measured SF, follicle-stimulating hormone (FSH); luteinizing hormone (LH); prolactin (PRL) and total testosterone (TT). On the same day of blood collection,

the semen was collected by voluntary masturbation in an appropriate environment at 37° C to perform spermogram by manual method according to the guidelines of the WHO laboratory manual for the examination and processing of human semen 5th ed [9] and 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 SF. SF and sex hormones (SH) were measured by enzyme immuno-chemiluminescence using the Immulite 2000/Siemens automatic analyzer. Specific kits were used for quantification, as well as calibrators and controls recommended by the manufacturer.

Fertility Index (FI)

IF was calculated according to Harvey [10] as follows: FI = sperm concentration (x 10^6/ml) x sperm motility x percentage of spermatozoa with normal morphology.

Statistical analysis

After the normal distribution curve of the sample was verified by normality tests, for differences between two independent quantitative variables was used t-test and Pearson correlation analysis. Statistical significance was set at p <0.05 to reject the null hypothesis. Used SPSS® for Windows, version 20.0

Results

There were significant differences between cases and control **(Table 1)** in SP (p = 0.000), sex hormone (p = 0.000) and FI [0.85 (0.57) vs 5.54 (1.3), p=0.000]. There was no difference between cases and control **(Table 1)** in SF levels (226.45 ± 51.03 vs 241.52 ± 30.52, p = 0.137) and age (49.47 ± 5.56 vs 47.90 ± 6.22, p=0.229).There was no correlation **(Table 2)** between SF and FI (r = 0.049, p = 0.711) and SP (p> 0.05).

Table 1. Comparative evaluation of age, semen and hormone parameters, index and ferritin seminal levels among groups case and control (x ± sd).

Observational parameters	Case n (60)	Control n (30)	p[a] -valors
Age (y)	49.47 ± 5.56	47.90 ± 6.22	0.229
Semen volume (ml)	01.33 ± 00.36	02.77 ± 0.44	0.000
Total motility (PR + NP, %)	34.00 ± 06.24	71.31 ± 7.86	0.000
Sperm vitability (%)	47.49 ± 07.31	64.41 ± 2.89	0.000
Sperm density (x 10^6/ml)	14.95 ± 06.18	50.21 ± 8.57	0.000
Morphology sperm (%)	25.40 ± 07.87	59.76 ± 10.58	0.000
FSH (mIU/ml)	06.30 ± 01.21	03.40 ± 00.48	0.000
LH (mIU/ml)	15.91 ± 02.62	02.84 ± 00.54	0.000
Testosterone (ng/dl)	411.03 ± 58.88	510.60 ± 92.56	0.000
Prolactin (ng/ml)	16.38 ± 02.92	05.86 ± 01.93	0.000
Fertility index	0. 85(0.57)	5.54(1.3)	0.000
Ferritin seminal (ng/ml)	226.45 ± 51.03	241.52 ± 30.52	0.137

PR: Progressive motility; NP: non-progressive motility; FSH: follicle-stimulating hormone; LH: luteinizing hormone; [a]: t tests

Table 2. Correlational evaluation Pearson` between seminal ferritin and seminal parameters in group of case (p < 0.05 significative).

Observational	Case (n=60)	
Parameters	r	p
Seminal ferritin		
Fertility index	0.049	0.711
Sperm motility	-0.005	0.971
Sperm viability	-0.088	0.504
Sperm density	-0.007	0.960
Sperm morphology	0.003	0.980

Discussion

The present study intends to add knowledge in the understanding of the changes observed in the SP of patients with CKD, especially in HD, studying the possible association of SF levels with these alterations, considering possible antioxidative activity attributed to SF. This activity is due to its physiological function of regulating intracellular iron ion concentration, avoiding excessive formation of Fe^{++} by the reduction of free iron (Fe^{+++}) by anion (O_2^-), not favoring the biochemical reactions of Fenton and Haber-Weiss generating the hydroxyl radical (• OH), most reactive of ROS responsible for peroxidation of lipid, protein, glycides and fragmentation of DNA [11].

SF is an isoform of the different ferritins found in humans tissues, abundant in human seminal plasma; it is primarily produced and secreted (80%) by Sertoli cells [12] sensitive to systemic or local inflammatory changes [13].

On the other hand, Spermatogenesis is an iron-dependent process, because developing male germ cells undergo many mitotic divisions and require iron for DNA synthesis and cell growth, in particular for mitochondriogenesis. In addition, maturing spermatids and spermatozoa are extremely vulnerable to oxidative stress, implying a need for a well-balanced iron homeostasis in the seminiferous tubule [14]. This may partially justify the high concentrations of this protein in seminal plasma.

The similar age between the groups studied ($p > 0.05$) ensures greater reliability in the interpretation of the results and in the comparative statistical analysis of the impact of age on outcome variable [15].

Sex hormones (SH)

The most commonly observed male sex hormones (SH) profile in patients with CKD/HD is elevated serum levels of follicle stimulating hormone, luteinizing hormone, and prolactin and decreased serum TT levels, in response to the inhibitory action in one or more sites of the hypothalamic-pituitary-gonadal (HPG) axis promoted by factors such as uremia, OS, and pro-inflammatory cytokines generated by CKD/HD [16]. The SH profile **(Table 1)** between case and control follows the previously described pattern, with significant differences between them ($p=0.000$). These results are corroborated by previous reports [1, 17]. The absence of clinical hypogonadism in the sample, in principle, removes the interference of the hormonal factor on SP, FI, and SF levels.

Fertility Index (FI) and Seminal Parameters (SP)

The FI was significantly lower in the case group than in the control group ($p = 0.000$). Our results are in agreement with results obtained by other authors in similar studies. Xu et al [18] in the study of the semen quality in (40) uremic patients, (40) transplanted renal patients, and (40) healthy and fertile men, found higher IF for healthy patients in relation to transplanted groups A and B and uremic, respectively, 13.02 (14.26); 5.53 (8.30); 9.27 (22.49) and 0.23 (0.76). Xu et al. [19], evaluating the effect of uremia on semen quality and reproductive function in 53 terminal uremic patients, comparing them with controls of 46 fertile and 46 infertile men. They found that the motility, survival rate, density and morphology of the remaining 46 uremic patients were significantly lower than controls. The uremic patients presented IF 0.68 (2.08) significantly lower than the fertile 7.7 (13.51) and infertile 4.13 (5.77). **(Table 1)**

Although the antioxidative activity of ferritin is admitted in thesis, its evidence in clinical practice is questioned, considering the limited number of studies about the theme. Kwenang et al. [20] analyzed levels of iron, copper, and ferritin in seminal plasma from young healthy students (n=30) and infertility (n=30) with severe teratospermia, and no significant differences were found between them. However, Ferreira et at. [21], analyzing the seminal plasma

oxidative/antioxidative capacity of 69 men with suspected infertility and their association with iron and SF levels, showed a positive correlation between SF and total antioxidant capacity ($p = 0.005$) and a negative correlation with OS index ($p = 0.02$, $r = -0.329$). They concluded that the measurement of SF can be useful in the evaluation of seminal oxidative/antioxidative status in studies of fertility and seminal oxidative stress.

The complex systemic inflammation and oxidative stress affecting 40-60% of patients with CKD in HD [22] is a clinical condition that can alter SF levels. It is accepted that SI is associated with increased synthesis and secretion of intratesticular proinflammatory cytokines by stimulation of macrophages, Sertoli cells, and Leydig cells with nonpermanent alterations in SP (subfertility and infertility), evidenced in animal models and human studies [23].

Study experimental in rodents demonstrated that Il-6 secreted by the Sertoli cell stimulates the production of intratesticular SF [24]. In the animal model, the induction of systemic inflammation by lipopolysaccharide injection (LPS) promoted unsustainable elevations in proinflammatory cytokines (TNFα, interferon gamma [IFNγ], IL-6, IL-12, IL-17, and IL-23). Decreases in SP and elevations of a lipid peroxidation marker (MDA) lasted up to 30 days after the inflammatory induction, with values returning to baseline values later [25]. In humans, Qian et al [26] verified the relationship between different cytokines in the semen samples of 57 patients with infertility, identifying a decrease in SP and a significant difference in IL-23 levels of semen in the normal (5.87 pg/mL) and abnormal (11.14 pg/mL) groups ($p < 0.001$), with positive correlations between IL-6, IL-8, and TNF-α. Kokabet et al [27] evaluated whether IL-6 and IL-8 concentrations and numbers of seminal leukocytes were related to chlamydial infection in a population of 255 men with infertility. Significantly, increased levels of IL-8, but not IL-6, were found in patients with trachomatis infection when compared with uninfected patients, and there was a correlation between trachomatis infection and lower progressive motile sperm, as well as an increase in seminal leukocytes.

Silva et. al [28] studying effect of systemic inflammation on level of ferritin seminal in chronic renal male patient undergoing hemodialysis, using similar methodology, examined a sample of 60 patients on HD and did not find differences or correlations of SF levels among patients with inflammation, without inflammation, and controls ($p > 0.05$). Wan et al. [29] investigated the association between chlamydia trachomatis infection and the contents of seminal plasma ferritin, β-2-microglobulin, and total protein in seminal plasma of 30 fertile men (normal group) and 62 men with infertility infected by chlamydia trachomatis. They found higher concentrations of SF level in the infected group compared to healthy controls. It is plausible to hypothesize that disagreement is apparent for the behavior of SF levels of inflammation in the two previous studies. Silva et. al [28] evaluated patients with chronic systemic inflammation with consolidated adaptive process; Wan et al. [29]evaluated patients with acute local or subacute infection. With resolution of an acute infectious stimulus, proinflammatory cytokines and SF levels tend to normalize [30]. **(Table 2)**

Considering little known in the mechanism that causes of the low quality of semen and without correlation with SF levels in the group of patients studied, it is plausible to hypothesize that other factors are associated with the DRC/HD condition, besides the uremic factor, contribute to decrease the seminal quality as inflammation systemic (IS), OS or both by diverse mechanisms [2].

The similar level of SF in the case and control group identified in the present study may be partially justified by local immunosuppressive milieu and systemic immune tolerance are involved in maintaining testicular immune privilege status in response to uremic, IS, OS, isolated or associated factors. The

testicular immune privilege is a complex mechanism developed in the testicular compartment to maintain the balance local of pro/anti-inflammatory cytokine levels [31], involved in regulation the synthesis of SF [32] and intracellular iron ion level via the ion regulatory protein (IRP) [33]. Mechanisms of functional interactions between germ cells and Sertoli cells; Sertoli cells and Leydig cells, known as autocrine/paracrine regulation and hematopoietic barrier, are also part of the mechanism of testicular immune privilege protecting germ cells from effect inflammatory/oxidative/ionic toxicity [34]. The breakdown of homeostasis of one of these mechanisms may explain in part the genesis of changes in the seminal parameters of these patients. The present study presents the following limitations: absence of complementary analysis of seminal of OS and reduced sample.

Conclusion

The results suggest that SF levels are not associated with changes of seminal parameters in patients undergoing chronic hemodialysis and is not useful singly for initial evaluation of seminal parameters.

Abbreviations

Activator protein-1 (AP-1); c reactive protein (PCR); chlamydia trachomatis (CT); chronic kidney disease (CKD); factor-kappa B (NF-κb); seminal ferritin (SF); follicle stimulating hormone (FSH);hemodialysis (HD); highly sensitive C-reactive protein (hs-PCR); hypothalamic-pituitary-gonadal (HPG); interleukins (IL); iron regulatory proteins (IRP); lipopolysaccharide (LPS); luteinizing hormone (LH); malonaldehyde (MDA); oxidative stress (OS); oxygen reactive oxygen Species (ROS); prolactin (PRL); seminal parameters (PS); sex hormones (SH); total antioxidative capacity (TAC); total testosterone (TT); tumor necrosis factor α (TNF-α)

Compliance with Ethical Standards

Competing interests

The authors declare that they have no competing interests.

Ethical approval

Approved by the Research Ethics Committee of the Faculty of Health Sciences of the University of Brasília under number 53172316.9.0000.0030.

Informed consent

Informed consent was obtained from all individual participants included in the Study.

Authors' contribution

GPS drafted the manuscript. and FPC and VPXG critically reviewed it and makes addition.

References

1. Zedan H, Kamal EE, El Shazly A, El Rahman MZA, Shawky A: Impact of renal failure and haemodialysis on semen parameters and reproductive hormones. Human Andrology 2013; 3:16-20.
2. Neto FTL, Bach PV, Najari BB, Li PS, Goldstein M: Spermatogenesis in humans and its affecting factors. Semin Cell Dev Biol 2016; 59:10-26.
3. Closa D, Folch-Puy E: Oxygen free radicals and the systemic inflammatory response. IUBMB Life 2004; 56:185-191.
4. Tucker PS, Scanlan AT, Dalbo VJ: Chronic kidney disease influences multiple systems: describing the relationship between oxidative stress, inflammation, kidney damage, and concomitant disease. Oxid Med Cell Longev 2015; 2015:806358.
5. Aitken RJ: Oxidative stress and the etiology of male infertility. J Assist Reprod Genet 2016; 33:1691-1692.
6. Pinchuk I, Shoval H, Dotan Y, Lichtenberg D: Evaluation of antioxidants: scope, limitations and relevance of assays. Chem Phys Lipids 2012; 165:638-647.
7. Bartosz G: Non-enzymatic antioxidant capacity assays: Limitations of use in biomedicine. Free Radic Res 2010; 44:711-720.
8. Willerson JT, Ridker PM: Inflammation as a cardiovascular risk factor. Circulation 2004; 109:Ii2-10.
9. Organization WH: WHO laboratory manual for the examination and processing of human semen. 2010.
10. Harvey C: A fertility index derived from semen analysis. J Clin Pathol 1953; 6:232-236.

11. Silva B, Faustino P: An overview of molecular basis of iron metabolism regulation and the associated pathologies. Biochim Biophys Acta 2015; 1852:1347-1359.
12. Griswold MD: Sertoli Cell Biology. Academic Press, 2014.
13. Watt RK: The many faces of the octahedral ferritin protein. Biometals 2011; 24:489-500.
14. Chen H, Mruk D, Xiao X, Cheng CY: Human Spermatogenesis and Its Regulation; Male Hypogonadism, Springer, 2017, pp 49-72.
15. Somigliana E, Paffoni A, Busnelli A, Filippi F, Pagliardini L, Vigano P, Vercellini P: Age-related infertility and unexplained infertility: an intricate clinical dilemma. Hum Reprod 2016; 31:1390-1396.
16. Palmer BF, Clegg DJ: Gonadal dysfunction in chronic kidney disease. Rev Endocr Metab Disord 2016.
17. Lehtihet M, Hylander B: Semen quality in men with chronic kidney disease and its correlation with chronic kidney disease stages. Andrologia 2015; 47:1103-1108.
18. Xu LG, Xu HM, Zhu XF, Jin LM, Xu B, Wu Y, Lu NQ: Examination of the semen quality of patients with uraemia and renal transplant recipients in comparison with a control group. Andrologia 2009; 41:235-240.
19. Xu L, Xu H, Zhu X, Zhang J, Ma M, Shi X: Effect of uremia on semen quality and reproductive function in humans. Cell Biochem Biophys 2012; 62:29-33.
20. Kwenang A, Kroos MJ, Koster JF, van Eijk HG: Iron, ferritin and copper in seminal plasma. Hum Reprod 1987; 2:387-388.
21. Ferreira J, Pio C, Lima ES: A dosagem da ferritina no plasma seminal pode ser um marcador do estresse oxidativo no sêmen. News Lab 2009.
22. Maeda Y, Inoguchi T: [Oxidative stress and chronic inflammation]. Nihon rinsho Japanese journal of clinical medicine 2016; 74 Suppl 2:73-76.
23. Fraczek M, Kurpisz M: Cytokines in the male reproductive tract and their role in infertility disorders. J Reprod Immunol 2015; 108:98-104.
24. Elhija MA, Potashnik H, Lunenfeld E, Potashnik G, Schlatt S, Nieschlag E, Huleihel M: Testicular interleukin-6 response to systemic inflammation. European cytokine network 2005; 16:167-172.
25. Collodel G, Moretti E, Brecchia G, Kuželová L, Arruda J, Mourvaki E, Castellini C: Cytokines release and oxidative status in semen samples from rabbits treated with bacterial lipopolysaccharide. Theriogenology 2015; 83:1233-1240.
26. Qian L, Sun G, Zhou B, Wang G, Song J, He H: Study on the relationship between different cytokines in the semen of infertility patients. Am J Reprod Immunol 2011; 66:157-161.
27. Kokab A, Akhondi MM, Sadeghi MR, Modarresi MH, Aarabi M, Jennings R, Pacey AA, Eley A: Raised inflammatory markers in semen from men with asymptomatic chlamydial infection. J Androl 2010; 31:114-120.
28. Silva GP, De La Vega Elena CD, Carneiro FP, Veiga JPR: Effect of systemic inflammation on level of ferritin seminal in chronic renal male patient undergoing hemodialysis. Int Arch Med 2014; 7:23.
29. Wan Changchun WZHAOBRC: The relation between chlamydia trachomatis infection and the contents of seminal plasma ferritin, β_2-Microglobulin and total protein (Chinese Journal of Andrology) 2003, 01. Chinese Journal of Andrology 2003; 1:37-38.
30. Brecchia G, Menchetti L, Cardinali R, Castellini C, Polisca A, Zerani M, Maranesi M, Boiti C: Effects of a bacterial lipopolysaccharide on the reproductive functions of rabbit does. Anim Reprod Sci 2014; 147:128-134.
31. Fijak M, Bhushan S, Meinhardt A: The Immune Privilege of the Testis; Immune Infertility, Springer, 2017, pp 97-107.
32. Chen Q, Deng T, Han D: Testicular immunoregulation and spermatogenesis. Semin Cell Dev Biol 2016; 59:157-165.
33. Zhang D-L, Ghosh MC, Rouault TA: The physiological functions of iron regulatory proteins in iron homeostasis - an update. Front Pharmacol 2014; 5.
34. Meinhardt A, Hedger MP: Immunological, paracrine and endocrine aspects of testicular immune privilege. Mol Cell Endocrinol 2011; 335:60-68.