Effects of Hemodialysis on Testicular Volume and Oxidative Stress in Humans

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Purpose: Male infertility is a serious problem in patients on hemodialysis. Our understanding is that end stage renal disease or hemodialysis causes poor semen quality but the mechanism leading to impaired spermatogenesis is largely unknown.
Materials and Methods: Testicular volume in 120 patients on maintenance hemodialysis was compared with that in age matched healthy controls. Volume was correlated with clinical findings. In 10 testicular biopsy specimens from patients on hemodialysis who visited our infertility clinic Western blotting was performed to examine the generation of 4-HNE modified proteins, which are markers of oxidative stress, and the expression of proliferating cell nuclear antigen. Interstitial fibrosis was determined by Masson's trichrome staining.
Results: Mean bilateral testicular volume in patients on hemodialysis was significantly smaller than that in healthy controls (31.7 vs 36.4 ml, $p < 0.01$) in a hemodialysis duration dependent manner ($r = -0.32$, $p < 0.01$). The increase in serum ferritin correlated inversely with testicular volume ($r = -0.25$, $p < 0.01$). The generation of 4-HNE modified proteins was significantly increased 3.1-fold in patients on hemodialysis, following the 60% decreased expression of proliferating cell nuclear antigen. Quantitative analysis of Masson's trichrome staining revealed increased interstitial fibrosis in patients on hemodialysis compared with that in controls (41.5% vs 14.8%, $p < 0.01$). Serum ferritin, proliferating cell nuclear antigen expression and interstitial fibrosis correlated with the generation of 4-HNE modified proteins ($p < 0.05$).
Conclusions: Testicular volume, which is a parameter of spermatogenesis, is impaired in patients on hemodialysis and oxidative stress is considered to be involved in the process. Serum ferritin is a useful parameter for predicting oxidative stress in the testis.

Key Words: testis; renal dialysis; infertility, male; oxidative stress; spermatogenesis

The primary objective of therapy for ESRD, that is good longevity, has been achieved with the development of HD technologies. Male infertility is one of the most serious concerns, especially in young patients. However, the exact mechanism leading to impaired spermatogenesis by ESRD and HD is largely unknown. Cross-sectional studies of spermatogenesis and fertility in men with ESRD have been reviewed, showing that semen quality in men undergoing HD is reported to be poor and only successful renal transplantation can restore spermatogenesis.[1,2]

ESRD and HD have detrimental effects, influencing all levels of the hypothalamic-pituitary-testicular axis.[1,2] Several lines of evidence indicate that ESRD is a state of microinflammation with increased cytokine activation and augmented oxidative stress.[3,4] The contact of blood with the artificial dialysis membranes during HD also results in the production of ROS by leukocytes.[4] Oxidative stress causes peroxidative damage to sperm[5] and testis,[6] resulting in male infertility. Under normal conditions antioxidants maintain ROS at a low level. A stable end product of lipid peroxidation, the aldehyde 4-HNE, also causes oxidative stress as well as ROS.[7] A potent alkylating agent, 4-HNE reacts with DNA and proteins, generating various forms of adducts[7] that are capable of inducing specific cellular stress responses, such as cell signaling and apoptosis.[8] Increased 4-HNE has been noted in testes with varicocele, seminal obstruction and idiopathic infertility.[6]

We examined spermatogenic function in patients on HD by measuring the testicular volume, which is a reliable and easy method to evaluate spermatogenesis. Oxidative stress, detected as 4-HNE modified proteins, in the testis was also investigated using testicular biopsy specimens from patients on HD.

PATIENTS AND METHODS

Patients

This study involved 120 male patients with a mean ± SE age of 60.0 ± 1.1 years (range 30 to 79) who had ESRD and were on 3 times per week maintenance HD with a mean dialysis duration of 4.24 ± 0.3 years (range 0.5 to 17). The etiology of ESRD was glomerulonephritis in 44 patients (37%), diabetic nephropathy in 37 (31%), hypertensive nephropathy in 9 (8%), autosomal-dominant polycystic kidney in 5 (4%), interstitial nephropathy in 4 (3%) and other, including posterior urethral valves, iatrogenic and collagen disease, and an unknown origin. Patients with malignancy, active inflammation/infection and uncontrolled calcium/phosphate status were excluded from study.

Submitted for publication December 3, 2007.
Study received institutional review board approval.
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DOI:10.1016/j.juro.2008.04.010

TABLE 1. *Patient demographic and clinical characteristics*

	Control	Hemodialysis	p Value
Mean ± SEM age (years)	60.2 ± 1.1	60.0 ± 1.1	0.900 (Mann-Whitney U test)
No. paternity (%)	114 (95)	106 (88)	0.100 (chi-square test)
Mean ± SEM dialysis duration (yrs)	Not applicable	4.24 ± 0.30	
No. hypertension (%)	11 (9)	50 (42)	<0.001 (chi-square test)
No. diabetes (%)	9 (8)	37 (31)	<0.001 (chi-square test)
Mean ± SEM body wt (kg)	67.7 ± 0.7	64.0 ± 0.6	<0.001 (Mann-Whitney U test)
Mean ± SEM % hematocrit	41.7 ± 0.4	33.1 ± 0.3	<0.001 (Mann-Whitney U test)
Mean ± SEM albumin (gm/dl)	3.71 ± 0.02	3.64 ± 0.03	0.078 (Mann-Whitney U test)
Mean ± SEM BUN (mg/dl)	14.2 ± 0.3	75.1 ± 1.2	<0.001 (Mann-Whitney U test)
Mean ± SEM creatinine (mg/dl)	0.9 ± 0.01	10.1 ± 0.1	<0.001 (Mann-Whitney U test)
Mean ± SEM serum uric acid (mg/dl)	6.7 ± 0.1	7.0 ± 0.1	0.09 (Mann-Whitney U test)
Mean ± SEM glucose (mg/dl)	103.4 ± 2.6	108.6 ± 2.6	0.124 (Mann-Whitney U test)
Mean ± SEM total cholesterol (mg/dl)	172.5 ± 4.0	165.8 ± 4.7	0.192 (Mann-Whitney U test)
Mean ± SEM triglyceride (mg/dl)	141.9 ± 6.3	151.1 ± 4.9	0.073 (Mann-Whitney U test)
Mean ± SEM C-reactive protein (mg/dl)	0.34 ± 0.06	0.36 ± 0.09	0.500 (Mann-Whitney U test)
Mean ± SEM serum calcium (mg/dl)	9.00 ± 0.10	8.73 ± 0.08	0.075 (Mann-Whitney U test)
Mean ± SEM serum phosphorous (mg/dl)	5.26 ± 0.06	5.51 ± 0.07	0.063 (Mann-Whitney U test)
Mean ± SEM serum iron (μg/dl)	78.0 ± 2.6	76.7 ± 4.0	0.10 (Mann-Whitney U test)
Mean ± SEM % iron saturation	36.8 ± 3.6	34.5 ± 6.6	0.184 (Mann-Whitney U test)
Mean ± SEM serum ferritin (ng/ml)	71.3 ± 3.9	194.7 ± 14.1	<0.001 (Mann-Whitney U test)

Total of 120 patients per group.

HD was performed with hollow fiber hemophane dialyzers at a dialysate flow rate of 500 ml per minute and a blood flow rate of 150 to 200 ml per minute for 3 or 4 hours. All patients underwent adequate dialysis, as evidenced by a urea reduction ration of 72.0% ± 1.5%. A regular dose of erythropoietin was used to maintain hematocrit at 32% and a minimum dose of iron was infused at the end of HD when iron saturation was less than 20%. A total of 70 patients (58%) were on erythropoietin therapy for renal anemia and no patient received another hormonal therapy. A total of 92 patients (77%) were on oral vitamin D treatment.

Blood samples were collected before HD on Monday or Tuesday. Testicular volume measurement was done at the time of annual urological assessment, including digital rectal examination. Informed consent to measure testicular volume was obtained from all patients. Measurement was done using a punched out orchidometer and total testicular volume, representing spermatogenesis in each patient, was calculated. All measurements were performed by 1 urologist (KS) at Yamaguchi University and affiliated hospitals. A total of 120 consecutive patients between ages 30 and 80 years who visited our multiphasic health screening center or urological clinic with nontesticular and nonrenal disease served as controls.

Testicular Biopsy

Ten patients with a mean age of 42.3 years (range 34 to 63) undergoing 3 times per week maintenance HD for a mean of 6.5 years (range 2 to 12) had a history of infertility. Four patients with nonobstructive azoospermia underwent testicular sperm extraction for intracytoplasmic sperm injection. Two patients with azoospermia and 4 with oligo-asthenospermia underwent diagnostic biopsy to evaluate impaired spermatogenesis. There was no patient with diabetes mellitus. In patients with oligo-asthenospermia sperm concentration was $4.5 \pm 1.9 \times 10^6$ and motility was 29.3% ± 8.7%. Testicular biopsy specimens from 10 age matched fertile adults with a mean age of 39.5 ± 5.2 years (range 33 to 50) and normal semen parameters served as controls, including 7 with scrotal hydrocele and 3 who underwent orchiopexy after contralateral testicular torsion. A biopsy specimen was treated as described previously.[6] This study was started after receiving approval from institutional review boards and informed consent was obtained from all patients after explaining the purpose of this study.

Western Blotting

One of the most toxic aldehydes formed during oxidant induced lipid peroxidation is 4-HNE, which reacts primarily with Cys, Lys and His amino acids, altering protein function and forming protein adducts. These protein adducts, which are detected by Western blotting using the specific antigen that recognizes 4-HNE modified proteins, have been used as an index of oxidative stress.[6,8] Western blot analysis for 4-HNE modified proteins and PCNA were performed as previously reported.[6] After electrophoresis the membranes were incubated with mouse monoclonal anti-4-HNE modified proteins (Japan Institute for the Control for Aging, Shizuoka, Japan) (1:1,000) or PC10 mouse monoclonal anti-PCNA antibody (Santa Cruz Biotechnology, Santa Cruz, California) (1:2,000) in 1% bovine serum albumin overnight at room temperature. Negative controls were similarly processed with mouse IgG instead of primary antibody. Data are

TABLE 2. *Demographic and clinical characteristics of 120 patients on HD by testicular volume*

	r	p Value (Spearman rank correlation coefficient)
Age	−0.002	0.980
Dialysis duration	−0.262	0.004
Body wt	0.093	0.311
Hematocrit	0.010	0.910
Albumin	−0.065	0.479
BUN	0.024	0.800
Creatinine	0.023	0.800
Serum uric acid	−0.028	0.763
Glucose	0.118	0.200
Total cholesterol	0.015	0.874
Triglyceride	0.022	0.812
C-reactive protein	−0.08	0.383
Serum calcium	0.084	0.364
Serum phosphorous	0.093	0.310
Serum iron	0.107	0.243
Serum ferritin	−0.247	0.007