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Is Free Testosterone Concentration a Prognostic Factor of Survival in Chronic Renal Failure (CRF)?

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Background: Lowered testosterone level in CRF patients is associated with elevated risk of death due to cardiovascular reasons, and is influenced by many factors, including acid-base balance disorders. Aims: evaluation of testosterone concentration (TT) and free testosterone concentration (fT) in pre-dialysis and dialysis patients; assessment of TT and fT relationships with biochemical parameters; evaluation of prognostic importance of TT and fT in predicting patient survival.

Material/Methods: 4 groups of men: 14 – on hemodialysis (HD), 13 – on peritoneal dialysis (PD), 9 – with chronic renal failure (CRF) and 8 – healthy (CG), aged 56±17, 53±15, 68±12, 43±10 years, respectively. TT and biochemical parameters were measured; fT was calculated.

Results: The lowest TT and fT were observed in HD and CRF, the highest – in CG ($p=0.035$ for TT; $p=0.007$ for fT). fT in CRF and CG were different ($p=0.031$). TT and age was associated in HD ($p=0.026$). Age and fT was strongly associated in PD ($p<0.001$). After adjustment for age, TT was negatively associated with BMI ($p=0.013$) and fT was positively associated with HCO₃ level ($p=0.007$). fT was lower in those who died during 5 years of observation than in survivors ($p=0.009$). We have found that, opposite to TT, fT appeared to be a better predictor of 5-year survival than age. After combining pH and HCO₃ levels into a single variable – no acidosis, acidosis with HCO₃ normal serum level, acidosis with low concentrations of HCO₃ and adjustment for age and the study group – a trend toward the lowest values of free testosterone in decompensated acidosis was observed ($p_{\text{trend}}=0.027$). Such a trend was not seen for testosterone concentrations ($p_{\text{trend}}=0.107$).

Conclusions: Total and free testosterone levels were lower in HD and pre-dialysis than in healthy patients. Free testosterone level may predict long-term survival better than age. Total and free testosterone levels are lower in metabolic acidosis and total and free testosterone levels were positively associated with HCO₃ level.

MeSH Keywords: **Acidosis • Kidney Failure, Chronic • Quality of Life • Survival Analysis • Testosterone**

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Background

The number of patients with chronic renal failure (CRF) in Poland and in the world increases. The disease concerns especially elderly patients and is the reason of excessive mortality. CRF influences the frequency of cardiovascular diseases, the development of atherosclerosis, hypertension, lipid disorders, insulin resistance, malnutrition and cachexia. It becomes important to improve quality of life of CRF patients and to prolong their lives [1,2].

In recent years it has been found that testosterone is an important hormone affecting the quality of life, both general physical and mental state. Lowered testosterone – total and free concentration is also one of the endocrinological disorders present in CRF [1,3,4].

The same observations apply not only to patients with CRF, but also the elderly [1,3]. In healthy men, testosterone level declines at the rate of 1% per year. This decrease relates more to free than total testosterone due to gradual increase of sex hormone binding globulin (SHBG) with age [5]. After 70 years of age, circadian rhythm of testosterone secretion may be disturbed as well [6]. However, not all investigators confirm this dominant opinion about serum testosterone reduction with age [7].

The rate of decline in testosterone levels is higher in chronic kidney disease, including patients on dialysis [3,8]. The concentration of testosterone is lowered in 2/3 of men undergoing dialysis [4,9,10]. It is not clear what causes the decrease of testosterone concentration in patients with CRF. Lowered concentration of testosterone is associated with elevated risk of death due to cardiovascular reasons and also increases anemia. Renal transplantation increases the concentration of testosterone and reduces the risk of cardiovascular complications [2,4,11–14]. Obesity negatively affects testosterone concentrations in patients with and without renal failure [15,16]. The effect of acidosis on the concentration of total and free testosterone in patients with CRF requires further study because it is not clear [17,18]. The aims of this study were: 1) evaluation of testosterone and free testosterone concentrations in pre-dialysis patients and patients treated with different methods of dialysis 2) assessment of relationship of testosterone and free testosterone concentrations with biochemical parameters, which are sensitive to renal failure 3) evaluation of prognostic importance of testosterone and free testosterone concentrations in predicting long-term survival of CRF patients.

Material and Methods

The research was conducted in two cooperating centers (Medical University of Warsaw and Military Institute of Medicine). Forty

four male patients: 14 – on hemodialysis (HD), 13 – on peritoneal dialysis (PD), 9 – with chronic renal failure (CRF) (Glomerular Filtration Rate <30 ml/min/1.73 m²) and 8 – healthy (control group – CG), aged respectively: 56 ± 17 , 53 ± 15 , 68 ± 12 , 43 ± 10 years were included to the study. Serious organ diseases, severe infections and organ failure, as well as poor prognosis and lack of consent to participate in the study constituted exclusion criteria. In each subject the concentrations of testosterone, sex hormone binding globulin (SHBG), thyroxine-binding globulin (TBG), prealbumin, albumin, blood gases, C reactive protein (CRP), parathyroid hormone (PTH), prolactin (PRL) and Hb level were measured. Free testosterone levels were calculated using The International Society for the Study of the Aging Male (ISSAM) calculator available at <http://www.issam.ch>.

For the laboratory determination of examined parameters the following methods were used: testosterone, SHBG, PTH, PRL – electrochemiluminescence method – Roche Elecsys 2010 analyser, blood gases – blood gases method – ABL Radiometer, prealbumin, CRP – immunoturbidimetric method – Roche cobas c system, TBG – radioimmunoassay (RIA) method, and albumin, hemoglobin were performed by routine methods. Tests were performed in Laboratory Department of Clinic of Endocrinology and Internal Diseases of the Medical University of Warsaw. In each patient Body Mass Index (BMI) was also determined.

Five years from the beginning of the study, the state of the patients was checked in telephone survey, the ultimate cause of death has not been determined.

Statistical analysis

Kruskall-Wallis non-parametric test with adjustment for multiple comparisons was applied for analysis of variation of continuous variables between study groups. For analysis of bivariate relationships between continuous variables Spearman correlation coefficient was used. In multivariate analyses linear regression and Cox proportional hazard models were estimated. Survival time of patients who underwent transplantation was treated as censored at the date of operation. Statistical significance was assessed at the level of $p < 0.05$.

Results

The lowest testosterone and free testosterone concentrations were observed in HD and CRF patients, the highest – in the control group (Table 1). In multiple pairwise comparisons there was a statistically significant difference in free testosterone concentration between CRF patients and healthy controls ($p=0.031$). Also the difference in free testosterone concentration between HD patients and healthy controls was at the border of significance ($p=0.059$). All other remaining comparisons

Table 1. Testosterone and free testosterone concentrations in the study groups.

	HD			PD			CRF			Control group		p-value*	
	n	Mean ±SD	Median	n	Mean ±SD	Median	n	Mean ±SD	Median	n	Mean ±SD		Median
Testosterone (ng/mL)	14	3.98±1.76	3.79	13	5.46±1.95	5.18	9	4.02±1.26	4.26	8	6.40±2.43	6.11	0.035
Free testosterone (ng/mL)	10	0.076±0.033	0.069	13	0.112±0.054	0.094	9	0.069±0.018	0.065	8	0.139±0.065	0.117	0.007

* Overall comparison in Kruskal-Wallis test.

Table 2. Selected biochemical parameters in the study groups.

	HD			PD			CRF			Control group		
	n	Mean ±SD	Median	n	Mean ±SD	Median	n	Mean ±SD	Median	n	Mean ±SD	Median
BMI (kg/m ²)	14	23.8±3.2	23.0	13	23.9±5.2	23.3	9	26.4±2.5	26.8	8	24.1±4.8	23.5
Albumin (g/dL)	10	3.95±0.51	3.90	13	3.51±0.31	3.57	9	4.16±0.41	4.10	8	4.70±0.31	4.55
Prealbumin (g/L)	14	0.33±0.10	0.33	12	0.39±0.08	0.36	9	0.31±0.008	0.33	8	0.28±0.04	0.28
TBG (mg/L)	14	21.7±4.3	21.4	13	26.0±3.5	26.8	9	22.1±2.8	21.8	8	21.9±2.7	21.9
SHBG (nmol/L)	14	46.5±28.1	40.6	13	45.8±25.1	43.9	9	43.9±16.2	48.9	8	36.6±8.2	35.1
CRP ≥5.00 (mg/L)	10	20.0%	13	46.2%	9	33.3%	8	12.5%				
Hemoglobin (g/dL)	14	11.3±1.6	11.0	13	12.0±1.1	12.2	9	12.6±2.4	12.5	8	15.3±1.1	15.0
HCO ₃ (mmol/L)	12	21.1±2.1	20.7	13	27.5±1.0	27.9	9	22.6±6.4	24.5	8	28.5±2.1	28.5
pH	12	7.37±0.10	7.35	13	7.32±0.04	7.34	9	7.32±0.08	7.36	8	7.33±0.44	7.33
PTH (pg/mL)	14	397.6±333.5	259.3	13	190.8±113.6	202.1	9	200.1±216.5	134.2	8	20.8±9.3	20.8
PRL (ng/mL)	14	37.0±39.7	19.9	13	19.1±10.1	14.5	9	23.2±38.0	11.1	8	10.1±4.9	8.8

of free testosterone concentration and comparisons of testosterone concentration were neither significant nor at the border of significance.

Statistically significant association between testosterone concentration and age was observed in the HD group ($r=-0.590$, $p=0.026$). In the PD and the control group the strength of the relationships was virtually similar although not significant ($r=-0.505$, $p=0.078$ and $r=-0.524$, $p=0.183$, respectively). In the CRF group testosterone concentration did not depend on age ($r=0.250$, $p=0.516$) but this group did not include younger patients.

The strongest association between age and free testosterone concentration was seen in the PD group ($r=-0.880$, $p<0.001$). In the HD and the control group the relationships were also relatively strong, but not significant ($r=-0.543$, $p=0.105$ and $r=-0.683$, $p=0.062$). In the CRF group free testosterone

concentration did not depend on age ($r=-0.200$, $p=0.606$), similarly to testosterone concentration.

The characteristics of the study group according to selected biochemical parameters, which are usually altered in renal failure, are presented in Table 2. The relationship of these parameters with testosterone and free testosterone concentrations were examined in multivariate linear regression as well.

After adjustment for age and the study group, testosterone concentration was negatively associated with BMI and free testosterone concentration was positively associated with HCO₃ level (Table 3). Total testosterone concentration was also positively associated with HCO₃ level and free testosterone concentration – with pH level (at the border of significance), which suggests possible influence of acidosis on testosterone concentrations.

Table 3. Association of testosterone and free testosterone concentrations with selected biochemical parameters after adjustment for age and the study group.

	Testosterone			Free testosterone*		
	β	95% CI for β	p-value	β	95% CI for β	p-value
BMI	-0.160	-0.284; -0.035	0.013	-0.008	-0.034; 0.017	0.513
Albumin	-0.353	-1.901; 1.194	0.646	-0.128	-0.418; 0.161	0.374
Prealbumin	5.572	-0.940; 12.085	0.091	0.632	-0.654; 1.918	0.324
TBG	0.039	-0.120; 0.198	0.623	-0.0001	-0.032; 0.032	0.996
SHBG	0.020	-0.004; 0.045	0.100	-0.003	-0.009; 0.003	0.324
CRP ≥ 5.00	-0.422	-1.717; 0.874	0.513	-0.048	-0.286; 0.191	0.685
Hemoglobin	0.203	-0.139; 0.545	0.237	0.055	-0.014; 0.123	0.114
HCO ₃	0.149	-0.022; 0.319	0.085	0.043	0.013; 0.073	0.007
pH	5.426	-2.871; 13.722	0.193	1.404	-0.257; 3.065	0.095
PTH	-0.001	-0.003; 0.002	0.554	-0.0002	-0.001; 0.0003	0.357
PRL	-0.003	-0.022; 0.017	0.791	-0.001	-0.005; 0.002	0.438

* In transformed.

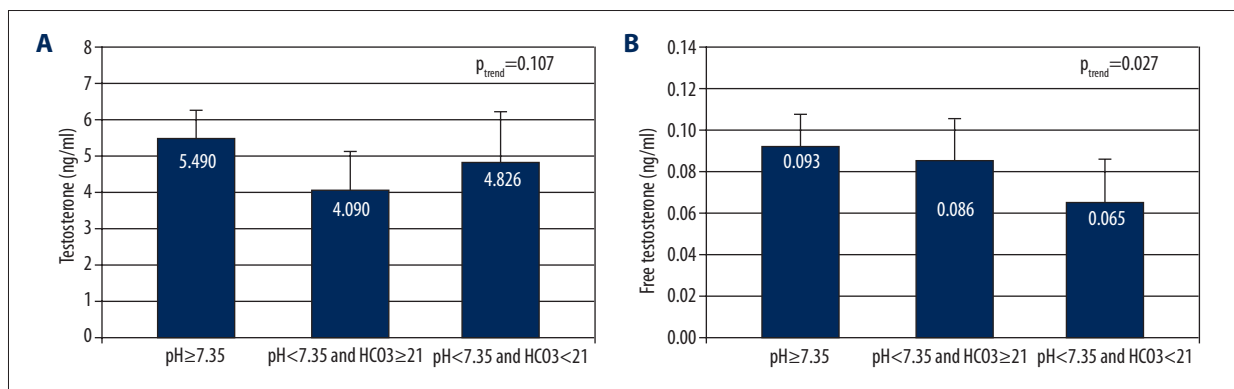


Figure 1. Testosterone (A) and free testosterone concentrations (B) in participants in the study by pH and HCO₃ levels (estimates from the linear regression models adjusted for age and study group evaluated at age equal to 55.5 years).

After combining pH and HCO₃ levels into one variable: no acidosis (pH ≥ 7.35), acidosis (pH < 7.35) with HCO₃ normal serum level (≥ 21), acidosis with low concentrations of HCO₃ (< 21) and adjustment for age and the study group, a trend toward the lowest values of free testosterone in decompensated acidosis was observed ($p_{\text{trend}}=0.027$) (Figure 1B). Such trend was not seen for testosterone concentrations ($p_{\text{trend}}=0.107$) (Figure 1A).

The values of total and free testosterone concentrations presented in figures were estimated from the linear regression model evaluated at age equal to 55.5 years and 55.5 years of age.

The results of observation of the studied subjects within five years from the baseline are presented in Table 4. In the HD group 5 patients died, 4 underwent transplantation and 5

survived. In the PD group 5 patients died, 1 underwent transplantation and 7 survived. In the CRF group 3 patients died, 2 underwent transplantation and 3 survived. The state of 1 patient is unknown. In the control group all patients were alive at the end of the observation period.

The mean age at the start of follow-up was 65 ± 14 years among those who died within 5 years, 45 ± 14 years among those who underwent transplantation and 51 ± 14 years among survivors (Table 4). The patients who died were older at the beginning of the study than patients who underwent transplantation ($p=0.029$) and – at a border of significance – survivors ($p=0.058$).

The mean concentration of testosterone at the beginning of the study was not statistically different between those who underwent transplantation, died or survived.

Table 4. Age, testosterone and free testosterone concentrations at the baseline in participants in the study.

	Undergoing transplantation			Dead		Survivors			p-value*	
	n	Mean ±SD	Median	N	Mean ±SD	Median	n	Mean ±SD		Median
Age	7	45±14	53	13	65±14	65	23	51±14	55	0.016
Testosterone (ng/mL)	7	5.03±1.80	4.43	13	4.24±2.01	3.75	23	5.24±2.14	4.97	0.303
Free testosterone (ng/mL)	5	0.092±0.043	0.065	13	0.074±0.037	0.065	21	0.114±0.055	0.095	0.010

* Overall comparison in Kruskal-Wallis test.

Table 5. Survival analysis of participants in the study with available free testosterone measurements and age less than 86 (n=39).

	Univariate analysis			Multivariate analysis		
	Exp(β)	95% CI for β	p-value	Exp(β)	95% CI for β	p-value
Age	1.058	1.016; 1.101	0.007	1.049	0.995; 1.107	0.078
Testosterone	0.686	0.466; 1.010	0.056	0.903	0.562; 1.450	0.673
Age	1.058	1.016; 1.101	0.007	1.027	0.974; 1.084	0.322
Free testosterone*	0.092	0.020; 0.418	0.002	0.165	0.023; 1.185	0.073

* In transformed.

The concentration of free testosterone was significantly lower in those who died than in survivors ($p=0.009$ in multiple pairwise comparisons). In the Cox proportional hazard model, age appeared to be a better predictor, at the border of significance, of 5-year survival than testosterone and free testosterone appeared to be a better predictor of 5-year survival than age, also at the border of significance (Table 5). In these analyses only persons with available free testosterone concentrations were taken into account. Additionally, one 86-year old man was excluded as his predicted survival time based on Polish life tables for 2007 was shorter than 5 years.

Discussion

In patients with chronic renal failure (CRF) endocrinological disorders occur very often. The most frequent disorder concerning gonads in men with CRF is lowered testosterone concentration. It occurs even in 2/3 of patients with CRF. The reason of hypogonadism in CRF patients is not fully understood, however disturbed metabolism of sex hormones on different levels is confirmed [4]. Both, testosterone synthesis and its secretion decrease with GFR reduction. Increased concentration of PRL and PTH may play an unfavorable role in concentration of testosterone [19,20]. Increased body weight (obesity), lipid disorders, inflammation and age of patients may affect concentrations of testosterone [1,3,14,21]. In order to recognize low testosterone concentration, two low measurements in the morning are needed. At present, testosterone is

estimated most often and in most of the laboratories determination of free testosterone is not possible [20].

Hemodialysis (HD) does not normalize lowered testosterone concentrations [14,22]. Kidney transplant restores correct function of the hypothalamic-pituitary-gonadal axis, lowers concentrations of FSH, LH as well as PRL and in most of the cases normalizes concentration of testosterone. After transplantation, steroidogenesis returns to normal, but spermatogenesis does not fully improve [2,23].

In the course of CRF, primary hypogonadism is developed. The lowered testosterone concentrations in the studied groups of patients are related to age, which is similar to the general population. Metabolic acidosis is not dependent on age in general population but is often present in CRF. In our study there was a normal concentration of bicarbonates only in the PD patients and the control group (data not shown). In the PD patients the concentrations of testosterone and free testosterone were higher than in the groups with renal failure and similar to the control group. It may be due to the effect of normal bicarbonate concentrations on testosterone concentrations as well as generally improved nutritional status of these patients (Tables 1, 2) [9,11,13].

Our results suggest that concentrations of testosterone and, especially – free testosterone in the HD and the CRF patients may be lower than in the PD patients and the healthy subjects (Table 1). One of the possible reasons for this phenomenon

may be acidosis, as shown in our work. The catabolic impact of acidosis may explain an adverse influence of renal failure on testosterone concentration and survival of patients (Tables 2, 3 and Figure 1) [20]. On the other hand, recently published electronically work by Bello AK et al. does not relate the quality of life and survival rate to free testosterone and acidosis [24]. Influence of acidosis on testosterone concentration is not as convincing. It was found that the bicarbonates within the 24–26 mmol/l in the serum are best for reducing the progression of kidney disease. Lower mortality from cardiovascular reasons in patients without acidosis was observed as well [17,18]. There are no clinical studies on the effects of acidosis on the concentration of free and total testosterone. Another cited work by Carrero et al. performed in 120 hemodialyzed patients showed the relationship of testosterone levels and mortality [21].

The quality of life in patients with renal failure decreases in many aspects [4,12,25]. These problems occur before the beginning of dialysis treatment and do not normalize during the dialysis therapy. Complaints that occur in the first place are lowered energy and libido. Interviews with CRF patients show that the factor which impairs life quality the most is sexual dysfunction. The lowered testosterone concentration is usually assessed at values less than 300 ng/dL (3 ng/ml) [25,26].

In men with CRF testosterone deficiency contributes to worsening or progression of cardiovascular disease [27]. In this group of patients, concentrations of testosterone show inverse correlation with the concentrations of inflammatory markers such as CRP, IL-6, and fibrinogen [21,28]. Lowered testosterone concentrations are associated with a higher activation of endothelium [29]. In studies by Carrero et al. and Gungor et al. there was an inverse correlation between testosterone concentration and age as well as leptin concentration while testosterone concentration was proportional to albumin concentration as a marker of malnutrition, and concentrations of creatinine as a marker of muscle mass [3,21]. Patients with CRF and low concentrations of testosterone were more often cachectic [21]. In our work we do not show an influence of inflammatory state and malnutrition on testosterone concentration in patients with CRF (Table 2). We found a significant association between mortality and age and even stronger association between mortality and decrease of free testosterone concentrations in all groups of patients (Tables 4, 5). This may be a result of the described multidirectional impact of testosterone – these statements are an important value of the work, but need to be confirmed in large studies.

Men with CKD who are on dialysis and with reduced testosterone levels often have diagnosed diabetes, cardiovascular disease, hypertension, high levels of phosphorus, PTH, CRP and have diagnosed vascular vasodilatation associated with

reduced blood flow [21,30]. In HD patients, decreased testosterone levels are associated with increased arterial wall thickness, increased atherosclerosis and also reduced diastole associated with blood flow [29].

One of the main reasons for mortality in HD patients is anemia. In patients with end stage renal disease (ESRD) and decreased concentrations of testosterone greater number of hypochromic erythrocytes and higher demand for erythropoietin (EPO) was detected [21,28]. In HD patients, administration of testosterone suppresses the development of anemia, while in the CRF patients administration of testosterone may be helpful in the prevention of anemia [12,31,32]. In the study [33] it was shown, that androgens probably stimulate erythropoiesis because demand for EPO in HD men is lower than in HD women [33]. Future studies may show how androgens increase erythropoiesis. In our study we do not find the association between anemia and testosterone concentrations. However, the construction of the study did not aim at detecting this association (Table 3).

In HD patients androgens increase sensitivity to EPO [34], while in patients on peritoneal dialysis increase of Hb after using androgens was similar to increase after treating with EPO [4,35]. In older people, both in men and women low concentrations of testosterone were associated with higher risk of anemia development [25].

We've found that higher BMI in our study groups accompanies decreased testosterone concentration, which is similar to the results of other authors [3] (Table 2). In patients with ESRD, lowered concentrations of testosterone are related to decreased body mass [28,36,37]. In patients with decreased concentrations of testosterone symptoms of protein energy wasting (PEW) syndrome were more frequent and these patients were characterized by lowered muscle strength [28,37]. In men with renal failure, both end-stage as well as chronic, disturbances in anabolic-catabolic balance are observed, and deficiency of testosterone increases catabolism in that group of patients [38,39].

The accuracy of free testosterone calculation with one of many intermediate methods is not entirely perfect. These formulas are criticized, and the results vary with age [40,41]. Moreover SHBG increases with age too, which may also affect the free fraction of testosterone [1,40,41]. Renal failure has a complex influence on testosterone metabolism, development of cardiovascular diseases and also has an influence on quality of life of patients with renal failure. Studying concentrations of testosterone allows prediction of patient survival, and it seems that free testosterone concentrations are no less important than age of patients (Table 5) [42].

There are no similar studies in literature, and the results need to be proved on a larger group, with direct measurement of free testosterone concentration assessing the prognostic value of the levels of testosterone.

Conclusions

Total and free testosterone levels were lower in HD and predialysis than in healthy patients. Free testosterone level may

predict long-term survival better than age. Total and free testosterone levels are lower in metabolic acidosis and total and free testosterone levels were positively associated with HCO₃ level.

Statement

The study was performed after obtaining acceptance of the Ethics Committee of the Medical University of Warsaw dated 30.06.2005.

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