

Full Length Research Paper

Sex hormones and free androgen index in non-diabetic male hemodialysis and renal transplanted patients

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Ageing and diabetes mellitus (DM) are known to affect sex hormones. Several studies have reported on sex hormones in male hemodialysis patients (HDPs) and renal transplant recipients (RTRs). None of those studies had excluded the effect of either ageing or DM, which might be one of the reasons why those studies had contradicting results. The aim of this study was to calculate free androgen index (FAI), assess the level of sex hormones in non-diabetic male (NDM) HDPs and RTRs whose ages were below 50 years, and to compare our findings with those of the others. In this study, NDM HDPs and RTRs had equal total testosterone (TT) level that was not significantly different from that of the controls; however, their FAI was significantly lower than that of the controls. Levels of luteinizing hormone (LH), follicular stimulating hormone (FSH), estradiol, prolactin, and sex hormone binding globulin (SHBG) were higher in those two patients groups than in the control group. In conclusion, NDM HDPs and RTRs had TT level that was not different from that of the controls, but they had abnormally low FAI level and abnormally elevated levels of LH, FSH, estradiol, prolactin, and SHBG.

Key words: Free androgen index, sex hormones, hemodialysis, transplantation.

INTRODUCTION

Endocrine abnormalities are a common feature of end stage renal disease (ESRD) (Schaefer et al., 1992; Clodi et al., 1998). While hypothalamic-pituitary-gonadal axis

(HPGA) alterations in men with ESRD are well known and being characterized by: low testosterone (T) levels, high serum prolactin and estradiol values; discordant data exist on male gonadal function in RTRs (Albaaj et al., 2006; Karakitsos et al., 2006; Palmer, 1999). Normalization of estradiol, prolactin and T levels after renal transplantation was reported by Samojlik et al. (1992). Adequate recovery of sexual and reproductive function was revealed in most renal transplant patients studied by De Celis and Pedron-Nuevo (1999) and Saha et al. (2002). Partial improvement of gonadotropins levels was reported early after renal transplantation in some studies conducted by Ishikawa et al. (1996), Prem et al. (1996) and Akbari et al. (2003). Persistent abnormalities of the HPGA function in RTRs were reported by De Besi et al. (1988), Nieszporek et al. (1989), Talbot et al. (1990), Chan et al. (1992) and Shamsa et al. (2005). However, none of these studies has excluded old patients or diabetic ones which might be the reason why

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Abbreviations: DM, Diabetes mellitus; HDPs, hemodialysis patients; RTRs, renal transplanted patients; FAI, free androgen index; NDM, non-diabetic male; TT, total testosterone; LH, luteinizing hormone; FSH, follicle stimulating hormone; ESRD, end-stage renal disease; SHBG, sex-hormone binding globulin; HPGA, hypothalamic-pituitary-gonadal axis; T, testosterone; CsA, Cyclosporine A; GFR, glomerular filtration rate; CMIA, chemiluminescent microparticle immunoassay method; SD, standard deviation; ANOVA, analysis of variance; IQR, inter-quartile range; GnRH, gonadotropin-releasing hormone; Epo, erythropoietin.

these studies had contradicting results.

Harman et al. (2001) proved in a large longitudinal study on 890 blood samples withdrawn from men, that age per se lowers both total (TT) and bioavailable circulating T levels at a relatively constant rate. In an earlier study, Vermeulen et al. (1972) concluded that mean plasma T levels remained within the same range from the age of 20 until the age of 50 years after which it started to decrease; therefore, only subjects between the age of 20 and 50 years were included in this study.

Grossmann et al. (2008) conducted a cross-sectional survey of adult males with DM (574 type 2 DM patients and 69 type 1 DM ones) but without established hypogonadism and/or T replacement therapy. In that survey, 43% (249 patients) and 57% (326 patients) of all men with type 2 DM were found to have low TT and free T levels. According to this study and others, (Fukui et al., 2007; Laaksonen et al., 2004; Stellato et al., 2000) we considered DM as a potential confounding factor that should be eliminated to delineate the sole effect of dialysis and renal transplantation on sex hormones; therefore diabetic subjects were also excluded in this study.

Serum TT may not always reflect the exact androgen status of a subject. If two men, for example, have TT concentration of 20 nmol/L, and one has SHBG concentration of 20 nmol/L while the other has SHBG concentration of 80 nmol/L, then they will have entirely different FAI. The first will have an FAI of 100% (that is, $20 \times 100/20$) while the second will have an FAI of 25% (that is, $20 \times 100/80$). T bound to SHBG is considered as biologically inactive (Laaksonen et al., 2004); therefore an estimate of the non-SHBG-bound fraction expressed as FAI is a more reliable measure of androgen status.

MATERIALS AND METHODS

Subjects

This study included 49 NDM HDPs, 44 NDM RTRs, and 63 age-matched NDM controls. HDPs underwent regular polysulfone lowflux dialysis (Hemoflow F 7 HPS, Fresenius AG) three times a week (4 h/per session). RTRs were on regular immunosuppressive medications: prednisolone from 5 to 10 mg/day, mycophenolate mofetil from 1 to 2 g/day, and cyclosporine A (CsA) from 150 to 450 mg/day or tacrolimus from 2 to 8 mg/day, according to the donor/recipient compatibility.

Inclusion criteria

- (A) For the three groups: (i) Male gender (ii) Age between 20 to 50 years.
- (B) For HDPs: (i) Frequency of dialysis 3 times/week (ii) Duration of dialysis > 6 months.
- (C) For RTRs: (i) Well functioning graft (that is, glomerular filtration rate (GFR) > 60 ml per min) (ii) age of the graft is \geq 6 months.

Exclusion criteria

- (A) For the three groups: (i) DM, (ii) testosterone replacement

therapy, (iii) History of endocrinological disease, (iv) History of liver disease.

(B) For RTRs: (i) Graft rejection (that is, no features of rejection in graft biopsy, based on Banff criteria).

Demographic data and treatment regimen were collected from the patients' files.

Biochemical investigations

After obtaining ethical approval and informed written consents from each participant, morning (pre-dialysis in HDPs and before the next dose of CsA or tacrolimus in RTRs) venous blood was withdrawn to determine the level of hemoglobin, blood sugar, albumin, urea, creatinine, electrolytes, TT, FSH, LH, estradiol, prolactin, trough levels of CsA or tacrolimus, and SHBG.

TT, FSH, LH, estradiol, prolactin, SHBG, and tacrolimus were assayed by Architect i2000 analyzer (Abbott Laboratories, Chicago, IL) that employed Chemiluminescent Microparticle Immunoassay (CMIA) method. CsA level was determined by AxSYM (Abbott Laboratories, Chicago, IL) monoclonal whole blood assay which utilized Fluorescence Polarization Immunoassay. Hemoglobin was assayed by The ADVIA 2120 Hematology system (Dade Behring Inc, Newark, DE) using cyanide-free hemoglobin method. The rest of the tests were performed using RxL Chemistry Analyzer (Dade Behring Inc, Newark, DE).

Statistical analysis

All statistical analyses were carried out using SPSS PC+ version 13.0 statistical software and version 11.5.1.0 MedCalc software. Normality of the distribution of measured variables was assessed by Kolmogorov-Smirnov and Shapiro-Wilk tests at an α level of 0.05.

Normally distributed variables were described using mean \pm standard deviation (SD) and compared across the three groups using Analysis of Variance (ANOVA). Contrast testing was used to detect the pair of the normally distributed variable that had a significant mean difference. Variables that were found by normality testing to have significant departure from normality were expressed using median and inter-quartile range (IQR).

Medians of the later type of variables were compared across groups using Kruskal-Wallis test followed by Pairwise analysis testing to determine the pair with significant median difference. For correlation studies, Pearson correlation test was used. *p-value* of < 0.05 was considered as statistically significant.

RESULTS

Clinical features and biochemical investigations for the three groups are summarized in Table 1. Age, systolic, and diastolic blood pressure were the only normally distributed variables in this study; therefore, ANOVA and contrast testing were used to compare their means across the three groups. The other variables had non-Gaussian distribution and their corresponding medians were compared between groups using Kruskal-Wallis and Pairwise analysis. It was clear from Table 1 that there was no significant age difference between the three groups. That table also showed some expected differences in blood pressure and routine biochemical investigations between the groups. TT level difference was not significant across the three groups ($p = 0.4$) while

Table 1. Clinical features and biochemical investigations of included subjects with their respective statistical comparison.

One-way analysis of variance (ANOVA)	Controls	Hemodialysis patients	Renal transplanted patients	P – value (two-tailed)
Clinical features (mean ± SD)				
Age in years	36.7 ± 7.0	38.2 ± 6.6	36.5 ± 6.3	0.6
Age of the graft in years	-	-	4.6 ± 4.8	-
Duration of dialysis in years	-	8.2 ± 5.2	-	-
Systolic blood pressure*	121.2 ± 5.1	133.2 ± 10.3	128.6 ± 11.8	< 0.001
Diastolic blood pressure*	80.6 ± 2.2	86.0 ± 5.2	84.6 ± 8.6	0.01
Biochemical investigations (median; IQR)				
Kruskal-Wallis test				
Urea (mmol/L)*	4.6; 1.3	22.3; 18.6	8.6; 4.7	< 0.001
Creatinine (µmol/L)*	79.0; 13.0	985.0; 652.0	127.0; 45.0	< 0.001
Potassium (mmol/L)	4.2; 0.7	4.1; 1.9	4.2; 0.6	0.4
Sodium (mmol/L) ‡	140.0; 5.0	137.0; 3.0	139.5; 5.0	< 0.001
Albumin (g/L)*	42.0; 5.0	38.8; 5.0	39.5; 5.0	< 0.001
Hemoglobin (g/L)*	15.0; 1.0	11.8; 2.2	13.25; 2.4	< 0.001
Total testosterone (nmol/L)	15.4;6.2	17.4;10.9	15.1;9.9	0.4
SHBG (nmol/L)**	24.2; 13.0	33.8; 20.7	31.1; 17.5	0.01
FAI% ¶	60.4; 25.5	48.4; 33.8	53.7; 34.6	0.03
FSH (IU/L)**	2.16; 1.9	4.4; 6.7	4.7; 5.4	< 0.001
LH (IU/L)*	2.95; 1.9	7.1; 7.8	5.02; 4.4	< 0.001
Prolactin (mIU/L)*	178.6; 83.0	429.5; 287.0	288.7; 128.9	< 0.001
Estradiol (pmol/L)*	67.8; 30.0	122.0; 54.8	98.5; 47.5	< 0.001

SD, standard deviation, IQR, inter-quartile range, *: median score is significantly different among each of the three groups, **: controls median score is significantly lower than those of hemodialysis and renal transplanted patients while there was no significant difference between the later two groups, ‡: median score of hemodialysis patients is significantly lower than those of controls and renal transplanted ones while there was no significant difference between controls and renal transplanted patients, ¶: controls median score is significantly higher than those of hemodialysis and renal transplanted patients while there was no significant difference between the later two groups.

SHBG level was significantly ($p < 0.01$) higher in hemodialysis and renal transplanted patients than in the controls with no significant difference of its level between the later two groups. FAI was significantly ($p = 0.03$) higher in the control group compared to the two patients groups (hemodialysis and renal transplanted patients) with no significant difference between patients groups (Figure 1). Significant correlations between TT and FAI (Figure 2) were found in controls ($p < 0.01$, $r = 0.39$), HDPs ($p < 0.001$, $r = 0.59$), and RTRs groups ($p < 0.01$, $r = 0.42$). SHBG was significantly correlated with TT in controls ($p < 0.001$, $r = 0.44$) and RTRs ($p < 0.01$, $r = 0.45$) groups, but not in HDPs ($p = 0.1$, $r = 0.2$) group. A significant correlation was found between estradiol and CsA levels ($p = 0.02$, $r = 0.57$). In RTRs group, creatinine level was correlated with prolactin level ($p = 0.02$, $r = 0.34$). Such correlation was not found in controls ($p = 0.2$, $r = -0.2$) or HDPs ($p = 0.8$, $r = 0.02$) groups.

DISCUSSION

In this study, serum TT level of HD group was not

significantly different from that of the control group (Table 2) which contradicts some previous studies that showed that HDPs usually have low TT level compared to controls (Albaaj et al., 2006; De Vries et al., 1984; Fukui et al., 2007; Lim and Fang, 1976). This contradiction may be, as will be discussed in more details later, attributed to the difference between this study and the others in terms of subjects' mean duration of dialysis, their treatment, or the exclusion of diabetics in this study.

HDPs' SHBG level was significantly higher than that of the controls while FAI of HDPs was significantly lower than that of the controls (Table 1) which indicated that most of T molecules were bound to SHBG and not biologically active. This finding highlighted the importance of using FAI to assess patient's androgenic status in combination with TT measurement rather than relying solely on the later especially in situations where SHBG is abnormally elevated.

Comparing LH level of HDPs with that of the controls demonstrated the significantly elevated level of this hormone in HD group (Table 1). Elevated LH level in HDPs has been found by many researchers and has been

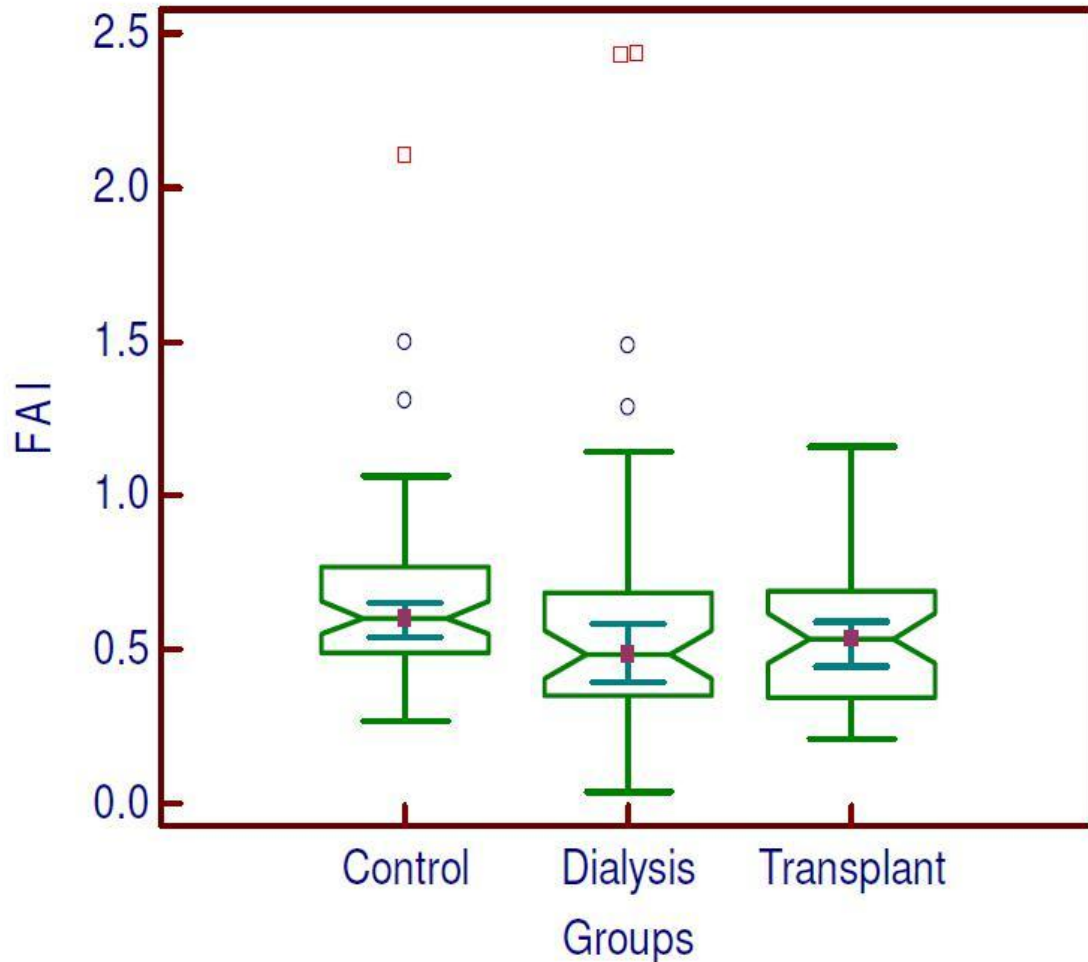


Figure 1. FAI in the three groups. *Line within the box* represents the median, the *two horizontal lines* contained within the box and surrounding the median are the upper and lower limits of 90th confidence interval of the median, *lower border of the box* represents 25th percentile, *upper border of the box* represents the 75th percentile, *lower and upper whiskers* represent the minimum and the maximum limits of the range, respectively. *Blue-circle dots* represent outlying points (that is, values larger than the upper quartile plus 1.5 times the interquartile range), and *red squared dots* represent extreme values (that is, values larger than the upper quartile plus 3 times the interquartile range).

been attributed to different potential mechanisms such as: pituitary response to primary hypogonadism (or to the presence of LH receptor inhibitors) (Dunkel et al., 1997) and decreased clearance of this hormone (Mitchell et al., 1994). In our HDPs, LH level had no significant correlation neither with creatinine nor urea, which made the possibility of decreased clearance less likely and was in favor of its increased production. High HDPs' LH level concomitant with low FAI is a finding that pointed towards its increased production as a physiological response to the low biologically active testosterone (that is, reflected by low FAI). However, failure of fully normalizing FAI signified the possibility of the presence of a combined pathology (that is, pituitary-hypothalamic failure in addition to the testicular one). In other words, the pituitary secreted high LH level but yet failed to secrete it in a high

enough level to drive more T production to overcome that relative T deficiency (caused by the high SHBG level) to normalize FAI. We also found that FSH level of HDPs was significantly (Table 1) higher than that of the controls. Both LH and FSH are under control of gonadotropin releasing hormone (GnRH), but the control of FSH secretion is more complex since inhibin B, a hormone produced from testicles which level is proportional to spermatogenesis, has a negative feedback effect on pituitary FSH secretion. Elevated HDPs' FSH level, pointed towards the possibility that these patients had impaired spermatogenesis which would not be surprising since impaired gametogenesis in HDPs has been reported in several reports (Handelsman and Dong, 1993; Shiraishi et al., 2008). However, without measuring inhibin B and semen analysis, we could not

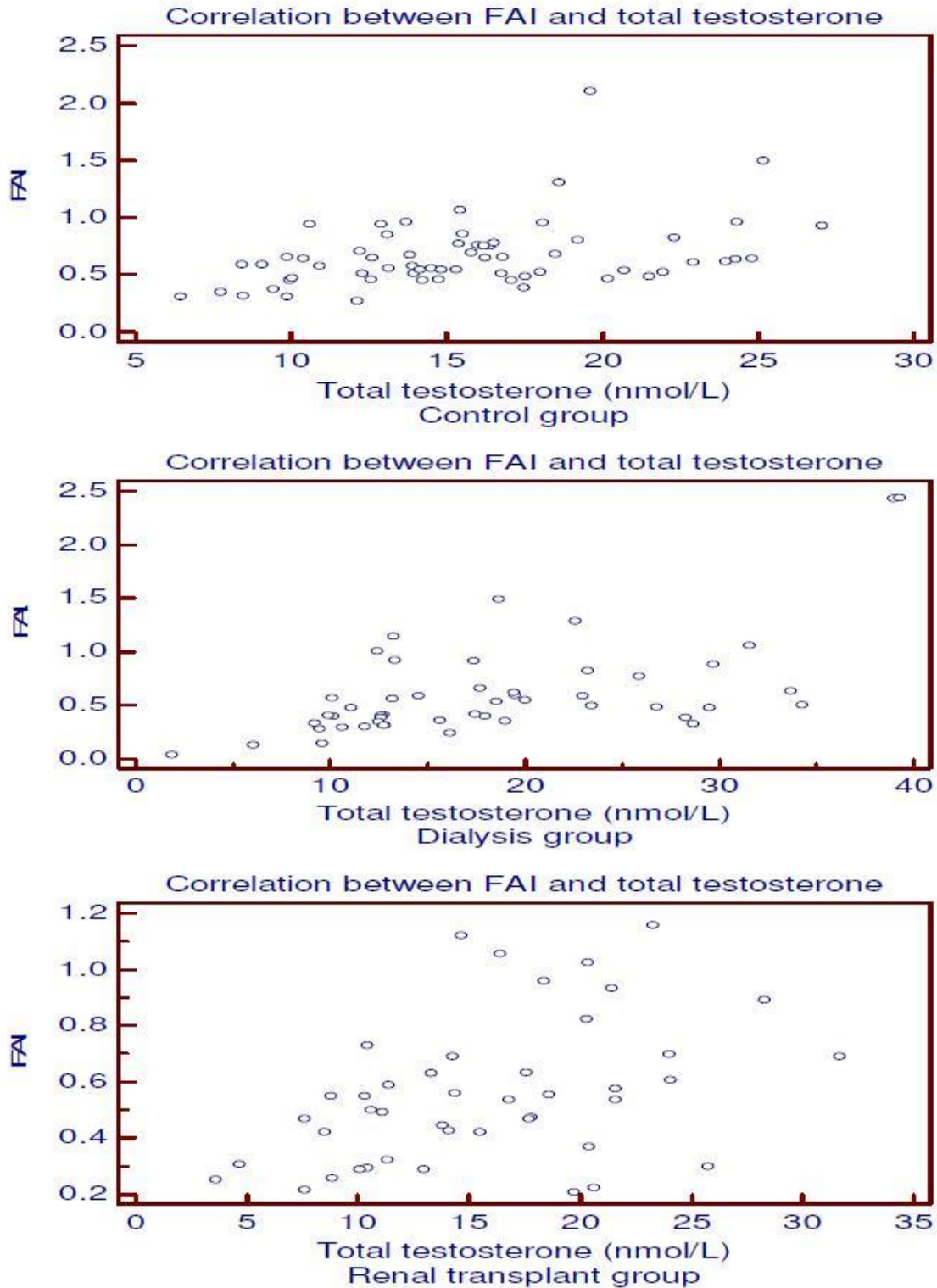


Figure 2. Correlation between FAI and total testosterone in the three groups.

confirm this possibility.

HDPs' estradiol level was significantly higher than that of the controls'. Estradiol elevation in HD group is not likely

to be due to its retention, for its level did not correlate with creatinine level of this group. Its increased level due to increased gonadal production or aromatization is more

likely. We have no reason to think of an increased T aromatization in HDPs, but with the elevated level of HDPs LH, one cannot help but wonder if this high LH level caused estrogen elevation directly by increasing its testicular production.

Prolactin level of HDPs was significantly higher than that of the controls and its level did not correlate with neither creatinine nor urea level, making the possibility of its autonomic overproduction more likely. The exact mechanism by which prolactin is overproduced in ESRD is unknown.

So far our findings of high LH, FSH, prolactin, and estradiol levels agree with most of the published results. When it comes to our finding of normal TT, our study contradicts almost all previous studies which have described low or in the low normal range TT level in the mentioned earlier, this discordance could be attributed to the difference between our subjects and those included in those studies in regards to mean duration of dialysis and medications. Another possible cause of this discordance is the exclusion of diabetics in this study. The previous studies, as we mentioned earlier, included diabetics or at least did not mention their exclusion. As mentioned previously, DM per se can decrease T level. It is also known that DM is the most common cause of ESRD, so it is very likely that those previous studies had more diabetics in their patients group compared to the controls, which made their patients' mean TT lower than that of the controls. This relatively high TT can also be attributed to the good nutritional status of our HDPs reflected by their relatively high albumin and hemoglobin levels compared to the usually low levels found in HDPs.

Hemoglobin levels of our HDPs were maintained at the higher recommended level (that is, 11 to 12 g/dl) (KDOQI, 2007) by regular erythropoietin (Epo) treatment which is also a highly suspected source of the disagreement between our findings and the others'. Reviewing the literature revealed that some researchers investigated Epo effect on sex hormones with controversial results. Some studies reported a significant increase in TT after Epo treatment (Lawrence et al., 1997; Ramirez et al., 1992) while others had reported insignificant change (Kokot et al., 1995; Steffensen and Aunsholt, 1993). Those studies that proved its effect also had some controversy in terms of the hormones affected, elevating or decreasing effect, and the duration after which the effect occurred. Some of those who have proved its effect on sex hormones have also mentioned that its effect on the same hormone differed according to the duration of treatment (that is, it elevated a certain hormone during the first few months of treatment, after which that effect disappeared).

Unfortunately, there was a lack of standardization between those studies regarding the duration of treatment after which the effect should be measured. Although some researchers have suggested a direct action of erythropoietin on Leydig cells (Foresta et al., 1994), the exact mechanism by which erythropoietin might

might elevate T level is still not clear. There is no agreement between studies on the effect of Epo on SHBG, gonadotropins, estradiol, and prolactin. It is also controversial as to whether these endocrinological changes are solely the result of correction of anemia or a direct effect of erythropoietin (Foresta et al., 1994; Kokot et al., 1995; Kuwahara et al., 1995). In this study, we found no correlation between hemoglobin level with SHBG or any sex hormone, which goes against the hypothesis that anemia correction plays direct role in changing sex hormones level. We did not find any significant correlation between Epo dose and SHBG level or any sex hormone level, either. However, lacking such correlation in this study does not preclude its existence especially when we take into consideration that our HDPs had different durations of dialysis. Since there is a possibility of Epo effect varying with time, the best way to study the correlation between its dose and sex hormones level is to recruit HDPs having the same duration of Epo treatment (that is, which we did not do in this study) and test for the presence of such correlation.

When it came to RTRs, these patients had TT level that was not significantly different neither from that of the HDPs or from that of the controls. When we calculated RTRs FAI, it was not significantly different from that of HDPs, but significantly lower than that of the controls (Table 1 and Figure 1). Like the situation in controls and HDPs, FAI significantly correlated with TT of RTRs (Figure 2), which is not surprising since TT represents the numerator of FAI (that is, $FAI (\%) = TT \times 100 / SHBG$). This strong correlation between FAI and TT among the three different groups supports the substitution of TT by FAI for the assessment of patient's androgenic status, since TT is highly affected by SHBG while FAI is not.

RTRs SHBG level was significantly higher than that of the controls (Table 1) but not significantly different from that of HDPs. This finding and the finding of low RTRs' FAI, again indicated that most of TT molecules were bound to SHBG and not physiologically active. It also re-emphasized the importance of using FAI to assess patient's androgenic status and not to only rely on TT to do so.

LH level of RTRs was higher than that of the controls and yet significantly lower than that of HDPs (Table 1). LH elevation in this group cannot be attributed to its retention as all RTRs had good kidney function reflected by $GFR > 60$ ml per min and no correlation was found between its level and those of creatinine or urea. Pituitary increased secretion of LH is more likely. As we explained earlier, LH is secreted from pituitary gland as a response to the decrease of T level. It is possible that RTRs had a mild degree of primary hypogonadism limiting testicular ability to secrete enough T to normalize FAI and there is also failure of hypothalamic-pituitary system to over secrete LH to normalize FAI (that is, the same combined pathology found in HDPs). The next question to be asked now is why would RTRs have primary hypogonadism? Although CsA had been shown to decrease T production

in vitro (Seethalakshmi et al., 1992) and tacrolimus was believed to have the same effect on T production (Kantarci et al., 2004); we could not attribute RTRs' primary hypogonadism to immunosuppressants, since neither CsA nor tacrolimus level had any significant correlation with TT. Another possibility is a permanent partial Leydig cell damage or fibrosis, due to the increased oxidative stress and toxins retention, which occurred during dialysis. This possibility of permanent Leydig cell damage is supported by the work of other researchers who reported interstitial fibrosis in HDPs' testicles and considered that as an irreversible damage (Holdsworth et al., 1977; Prem et al., 1996; Shiraishi et al., 2008). It is worth mentioning here that although RTRs had elevated LH level, it was not as high as HDPs' LH level, this could be due to Epo effect in HDPs (as we mentioned earlier, some studies found that Epo increased LH level) or due to corticosteroidal inhibitory effect on pituitary gland in RTRs. Corticosteroids are known to suppress GnRH release and consequently decrease LH secretion (MacAdams et al., 1986).

The high estradiol level of RTRs could not be attributed to its retention since all RTRs in this study had normally functioning graft not to mention that estradiol level in these patients did not correlate with their creatinine level which left us with three possibilities: the possibility of its increased production, the possibility of its decreased metabolism, and the possibility of the presence of a combination of increased production and decreased metabolism.

In males, a small portion of estradiol is secreted directly from testicles as a response of direct stimulation by LH. RTRs showed, as discussed earlier, mildly elevated level of LH which might have contributed to their elevated estradiol level, however, no significant correlation was found between estradiol and LH level in RTRs which is against this possibility. Surprisingly, we found a significant positive correlation between estradiol and CsA level. This finding pointed out to the role of CsA treatment in the elevation of estradiol level in RTRs. Both CsA and estradiol are metabolized by cytochrome P3A4 (CYP3A4), a subfamily of the human microsomal enzymes cytochrome P450 (Kelly and Kahan, 2002; Schaefer et al., 1992).

Accordingly, it made sense to attribute RTRs' estradiol elevation to a combination of its inhibited metabolism by CsA and to its increased testicular secretion due to the elevated LH in those patients (the third possibility).

Niwa et al. (2007) proved that although tacrolimus is metabolized by CYP3A4, it did not have a clinically important interaction with other CYP3A4 substrates. This could explain why no significant correlation between tacrolimus level and estradiol level was detected in our RTRs. Keeping in mind what has been reported by previous studies on Epo effect on hormones, it makes sense to hypothesize that Epo treatment in HDPs could have played a part in elevating their estradiol to a level that was higher than that of the RTRs.

Prolactin level was significantly higher in RTRs than in the controls, yet significantly lower than that of HDPs (Table 1). Like estradiol, Epo treatment in HDPs could have played a part in making their prolactin level higher than that of the RTRs. It is worth mentioning here that in addition to CsA and tacrolimus, mycophenolate mofetil effect on sex hormones was also investigated. No significant correlation was found between the dose of this later drug and any other studied sex hormone. Reviewing the literature revealed that no study has been conducted to test such an effect, and therefore, we had no data to compare this finding with.

At the end, this study showed that when the effect of DM and ageing were excluded, male RTRs and HDPs (at least those on Epo treatment) had TT level that was not significantly different from that of normal individuals, but their FAI was significantly low. They also had significantly elevated LH, FSH, estradiol, prolactin, and SHBG levels. These hormonal abnormalities were due to the nature of the disease and most probably due to medications taken by each group (that is, Epo in HDPs and immunosuppressants in RTRs). Due to the inherent limitations of a cross-sectional study, we were not able to follow up RTRs to study the effect of reducing immunosuppressant dose on their hormonal pattern.

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