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혈청내 섬광면역측정법에 의한 활성적 Testosterone과 Dihydrotestosterone의 농도

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=국문초록=

Concentrations of Bioavailable Testosterone and Dihydrotestosterone Determined by Luminescence Immunoassay in Serum

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혈액에서 생물학적 활성을 나타내는 (bioavailable) steroid hormone은 주로 비결합형(free form)과 알부민 결합형(albumin-bound form)으로 구성된다. 특히 Testosterone (T)과 5 alpha-Dihydrotestosterone (DHT)의 활성적 분획이 전체의 T, DHT 양에 비해 생리적 현상과 보다 잘 일치하는 것으로 알려지고 있다. 본 연구는 섬광면역측정법(Luminescence immunoassay, LIA)으로 혈청내 활성적 T 및 DHT의 농도의 측정에 이용하고져 하였다.

항체는 T- 또는 DHT-3-CMO-BSA를 항원으로 토끼에 면역주사하여 얻었다. 추적자는 T-3-CMO, DHT-3-CMO에 aminobutylethylisoluminol(ABEI)를 부착시켜 사용하였다. 항체중 IgG분획을 Protein-A-Sepharose CL-4B로 분리한 후 Immunobead(Bio-Rad)에 부착시켜 Solid-phase LIA를 실시하였다.

본 연구에서 LIA는 정확도(accuracy), 정밀도(precision), 감도(sensitivity), 교차반응도(specificity)등을 조사하고, 기존의 방사면역측정법(RIA)과 비교하여 만족할만한 결과를 얻었다. 혈청내 T및 DHT의 활성적 분획의 농도를 측정한 결과는 다음과 같았다. T의 경우는 남성에서 T의 전체량의 33% 이상으로 7.1±1.5nmol/l, 여성에서는 26% 이상으로 0.28±0.05nmol/l이었다. DHT의 활성적 분획은 남성의 경우 601.7±85.8pmol/l, 여성의경우 52.4±19.9 pmol/l이었다.

이상의 결과를 보아 본 연구에서 이용된 LIA는 혈청내 활성적 농도를 측정하기에 충분하다고 사료된다. 또한 이 방법을 이용하여 여성의 Androgenicity 및 남성 정소기능등의 제어방법에 응용될 수 있을 것으로 판단된다.

INTROCUCTION

Testosterone in serum is bound largely to either sex-hormone-binding globulin (SHBG, approximately 44% in males and 66% in females)or albumin(approximately 50% in males and 30% in females) and also corticósteroid-binding globulin. Only 1-3% of T is in a protein unbound (i.e. free) state. It has been pos-

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tulated that the small portion of unbound sex steroids(free androgens, FA) is hormonally and metabollically active, and that the steroids-SHBG serves as a biologically inert reservoir in which the hormone is protected fromcatabolismor excretion(Anderson, 1974, ; Dunn et al., 1981). The free testosterone (FT) which is independent of the changes in SHBG would appear to be a more appropriate analyte for investigation of androgeneicity than total testosterone (TT). The concentrations of T in saliva are also considered to reflect free T levels in blood (James and Baxendale, 1982 ; Baxendale and James, 1983).The determination of salivary androgens may therefore provide the direct measures of physiologically active levels in blood with non-invasive, easy and multiple sampling techniques (Gaskell et al., 1986 ; Gould et al., 1986 ; Johnson et al., 1987). However, recent studies suggest that the bioavailable T (BT ; free and albumin bound T)appears to correlate much better with androgen activity than does serum T (Total T) or free T (Cumming and Wall, 1985 ; Manni et al., 1985 ; lNankin and Calkins, 1986). Measuremet of total T may therefore be misleading for the investigations of androgen studies.

Dihydrotestosterone(DHT) is the most potent endogenous androgen and responsible for the differentiation of male external genita-lia and also an intermediate in androgen biotransformation. But the changes of free DHT(FDHT) and bioavailable DHT (BDHT) in serum and saliva of the male or female reproductive state remain uncertain.

Direct radioimmunoassays(RIA) of total serum T and FT are commercially available but not DHT RIA (Wilke and Utley, 1987). In the present study, we developed chemiluminescence immunoassays(LIA) for the measurements of bioavailable DHT and T in serum and saliva.

MATERIALS AND METHODS

One hundred and eighty four men (aged 25-52 yrs) and 154 normal or pregnant women (agee, 25-66 yrs) had participated in the present study. All were healthy, not taking medicines, had no known diseases, and had normal screening physical examinations. The young men (n=96) were all stuydents under 24 years old and fives were proven fathers (28-37 yrs).

The same single serum and matched saliva were obtained between 0800 and 0900 h. Saliva samples from all the subjects were collected by the participants directly into sam-ple tubes and stored at -40℃ until arsayl.

Serum free T was measured by two different method : Firstly, the modifed method of Vlahos et al., (1982), a centrilfugal ultrafiltration method was used. Between-batch coefficient of variations (BV) were 12.5% at 2.4 pmol/land 8.7% at 42.4 pmol/l. Briefly, serum samples were pre-incubated with highly purified 1,2,6,7-tritium labelled testosterone ^{3}HT, 10 0,000 dpm, 4.5 pmol, specific activity, 80-100 Ci /mmol) or 1,2,4,5,6,7-tritium labelled 5-alpha dihydrotestosterone, ^{3}HDHT, spec. ect., 110-150 Ci/mmol). For this purpose, 10 ul of purified tracer and 30 ul of HEPES (1 mol/l, pH 7.4) were addded to 0.6 ml of serum samples, and then mixed gently to equilibrate to 37℃ for 30 min. The radioactivity in 50 ul of serum solution was measured in duplicate and the remaining sample was pipetted into the reservoir of MPS-1 centrifugal ultrafiltration device using YMB membranes (Amicon Corp., GmbH). After the device was capped and placed into a prewarmed angle rotor, the tubes were centrifuged at 1100 xg for 10 min. Ultrafiltrate (200 to 300 ul) in the filtrate cup were obtained and 50 ul alinquots in duplicate were taken for radioactivity measurement. The FTconcentration in undiluted serum was calculated as follows : Diluted % freeT= (cpm of ultrafiltrate/cpm of dilyted)X100 ; Undiluted % free T=diluted % free/D, where D is the dilution factor (D=ca 1.6=(total volume (serum+buffer+tracer)/volume of se-

rum). The absolute free T is then calculated, after the total concentration is determined by indirect LIA as follows : Undiluted free T= (undiluted % free/100)X total T. The second method was Coat-A-Count free testosterone kit (Diagnostic Products Corporation, DPC), using a solid-phase RIA with iodine-125 labelled T analog method for direct estimation. The FT intra- and inter-assay varitions were 6.8% and 5.9%, respectively, based on analyses performed in triplicate.

Bioavailable androgens were estimated using 50% ammonium sulfate precitation of SHBG using the method described by Manni et al., (1985) with minor modifications. Briefly, tracer amounts of tritium labelled T or DHT were added to serum aliquots. An equal volume of saturated solution of ammonium sulfate (at final concentration of 50%) was added to precipitate TeBG, testosterone estrogen binding globulin with its bound steroid. Sepatation of this SHBG bound fraction was done by centrifugation at 200 xg for 30 min at 4°C using the above devices. The percentage of the labelled steroid remaining in the supernatant (the free and albumin-bound fractions was then calculated (normal range, 25-72% in men and 12-28% in women). This non-SHBG bound T or DHT concentration was obtained by multiplying the total steroid concentration determined by total androgen LIAs. The intra-and interrassay variations were 1.7% and 10.1% for non-SHBG-androgens. The results multiplied by the serum androgens were reploted as absolute non-SHBG-androgens concentration. The measurement of SHBG for DHT in serum were done by products of apibioMerieux GmbH or of Farmous Diagnostical, Turku, Finland. Serum albunin was mea-sured colorimetrcally with bromocresol green(Peters et al., 1982) or by some modification of RIA kits using double antibody method of DPC albumin kits.

For LIA, aminobutylethylisoluminol(ABEI) was synthesized according to the procedure of Schroeder et al.,(1978) for light emitting substance and coupled to DHT-or T-3-CMO by a mixed anhydride method as described for proteins by Erlanger et al.,(1957). The tracers were identified by FD-mass-and UV-spectrometry (Kreysing, Yoon, and Nieschlag, 1988).

The antibodies were generated against DHTor T-3-CMO-BSA in rabbits. The IgG fraction was collected using affinity chromatography : The antisera were applied to a Protein A-Sepharose CL-4B (Pharmacia) eolumn eluted woth physiocally buffered saline (PBS pH 7.2). Then the bound IgG was eluted from the gel with 0.1 mol/l glycine-HCl buffer (pH 3.0). For coupling the IgG fractions to an immunobead matrix, the glycine-HCl buffer was replaced by a phosphate buffered saline (pH 6.3) by ultrafiltration through an Amicon filtering system. Then 10 mg IgG were coupled to 200 mg Immunobead matrix according to the procedures of a carbodiimide method (Bio-Rad).

For the extraction of androgens 1 ml of serum and 3ml of saliva were extracted with 5ml of diethylether twice. DHT and T fractinos were isolated using celite column(Radioassay System Laboratory Ltd, California). For LIA, redissolved residues of the extracted samples, tracer, and Immunobead suspended in physiological saline supplemented with 1g /1bovine IgG were added. Total volume was 0.3ml. After incubation at 4°C overnight or 3 hours at 37°C, the immunogen-bound immunobead was washed twice with 1ml of 0.02 % Tween 20, and then suspended in 250 ul NaOH (2 mol/l). After incubating at 60°C, for 1 hour the luminescent reaction was stårted by adding 100ul hydrogen peroxide (0.15%) and measured for 10 sec in a Berthold LB 950 or Picolite 6100 luminometer.

The concentrations of androstenedione and DHT were determined by the kits of api bioMerieux GmbH, after the steroids were isolated using commercial columns under nitrogen gas pressure by the procedurtes recommended.

Table 1. Performance data of the LIAs for DHT and T.

Factors	DHT-LIA (n=27)	T-LIA (n=24)
Slope (b)	2.11±0.14	2.34±0.92
Correlation(r)	0.96±0.003	0.97±0.007
Intercept(y, nmol/l)	5.26±0.78	5.46±0.93
Sensitivity(pg/tube)	1.70±0.09	3.90±0.03
Accutacy		
a.	2.90±0.40	2.80±0.06
b.	9.80±0.90	9.50±1.70
c.	14.30±2.80	16.10±2.10
Intraassay variation(nmol/l)	2.30±0.03 (CV=8.7%)	21.70±1.30 (CV=6.0%)
Interassay variation(nmol/l)	2.50±0.30 (CV=12.0%)	22.20±3.40 (CV=15.3%)

*Sera of charcoal-stripped serum pool were spiked with a(3.0 nmol/l), b(10.0 nmol/l), and c (15.0 nmol/l) respectively

Table 2. Cross reactions of related steroids in developed LIAs

Compounds	DHT-LIA	T-LIA
5α-dihydrotestosterone	100%	38%
Testosterone	23%	100%
5α-androstan-3α, 17β-diol	7.4%	7.0%
Androstenedione	4.6%	1.5%
Androsterone	2.1%	1.6%
Androstenediol	2.7%	2.7%
Cortisol	<0.001%	<0.001%
Progesterone	<0.01%	<0.01%
Estradiol-17β	<0.01%	<0.01%
Dehydroepiandrosterone	<0.001%	<0.001%

* The percentages of the doses giving 50% inhibition of the ABEI-labelled tracers compared to the initial bindings were determined.tracerscompared to the initial bindings.

Statistical analyses of the data obtained from normal subjects were done by Student's t test, but the data which was not normally distributed in the samples were performed using the Mann-Whitney "U" test. The correlations between two different variables were calculated by linear regression analyses. All results are expressed as mean ± SD unless otherwise stated.

RESULTS

1) Validation of indirect chemiluminescence immunoassays for DHT and T in comparison to RIA for both hormones

Accuracy and precision were determined for the solid-phase LIAs by repeated measurements of native and of androgens-supplemented human female serum samples, with T- and DHT-concentrations ranging from 1.5 to 36.2 nmol/l. The results for the intra-and inter-assay variations are given in Table 1. The correlations between expected(X) and observed(Y) values in DHT-and T-LIA were

r=0.989 and r=0.988 respectively. The regression curves are described as Y=0.94X+0.9 for DHT and Y=1.01X+0.6 for T.

The sensitivity defined as the minimal detection limit of an assay, which is the least concentrations of unlabelled DHT or T (mean of 10 standard curves-2SD). These sensitivities, the least amounts for DHT or T LIAs were 1.7 pg/tube or 3.9 pg/tube.

Specificity has been calculated by determining the doses giving 50% inhibition of the tracer compared to the binding of the zero standand and summarized in Table 2. There were no differences between the LIA and RIA.

Correlations of LIA to RIA : For the further validations serum samples (n=86) were determined by LIA and RIA. The coefficient of correlations between the LIA values for CHT or T and their RIAs were r=0.977 (n=87) and r=0.930 (n=142) respectively. The regression curves gave the linear equation Y(LIA values)=1.02 X(RIA)+4.42 for DHT and Y=0.97+0.51 for T.

2) Determinations of bioavailable and free DHT or T in sera and saliva

A number of different procedures have been tested to compare the concentrations of free DHT and T in serum samples (Table 3), with the equilibrium dialysis method being used as the reference method. This table sh-

Table 3. Comparison of the methods to determine free testosterone in serum

Method	Normal serum from	
	Men (n=44)	Women (n=51)
Equilibrium dialysis	614.8±483 (295~680)	21.2±1.0 (15~32)
Gel-filtration	716.0±50.5 (470~815)	23.2±2.1 (19~28)
Ultrafiltration	585.0±19.8 (245~710)	26.1±2.4 (16~30)
DPC Kit	91.70±18.1 (43~210)	8.70±1.9 (2~13)

The DPC kit is a Coat-A-Count free testosterone kit of Diagnostic Products Corporation.
Ultrafiltration was done using MPS-1 centrifugal ultrafiltration devices with YMB membrane.
The equilibrium dialysis method is taken as the reference method.

Table 4. Intra-and inter-assay variation of salivary T and free T in serum to assess the assay quality using three quality control(QC) samples

	intraassay variation (CV,%)	interassay variation (CV,%)
Salivary T (pmol/l)		
Sample k1 (n=20)	43.7±7.9(18.1)	42.5±8.7(20.5)
Sample K2 (n=20)	181.5±19.6(10.8)	179.1±30.4(17.0)
Sample K3 (n=20)	319.2±25.9(8.1)	325.1±43.7(13.4)
Serum free T (pmol/l)		
Sample K1 (n=18)	6.8±0.7(10.3)	5.4±1.1(20.4)
Sample K2 (n=18)	45.6±2.8(6.1)	42.4±3.7(8.7)
Sample K3 (n=18)	147.6±8.9(6.0)	150.3±11.6(7.7)
Serum total T (nmol/l)		
Sample K1 (n=15)	1.2±0.08(6.7)	1.1±0.09(8.2)
Sample K2 (n=15)	9.8±0.4(5.5)	10.3±0.8(7.8)
Sample K3 (n=15)	28.5±2.3(8.1)	26.4±2.4(9.1)

owed a range for one method in the parenthesis and the mean values ± 1 SD. The different methods for measuring free T concentration agreed quite well. The DPC kit showed a strong negative bias range where the upper limit is lower than the means repor-ted by the developed ultrafiltration method in the present study.

The mean recoveries of $89.6 \pm 15\%$ were obtained when 50,200, 350pmol/l of andro-gens were added to charcola-stripped saliva pull. The precisions of salivary T and serum FT and TTmeasurements are assessed fromintra- and inter-assay analyses on replicates of pooled samples. Results are given in Table 4. Sensitivities using triplicate estimates were 20 pmol/l for salivary T assay and 5.8 pmol/l for serum FT and 13.5 pmol/l for serum total T assays respectively.

The values of salivary, serum free, and serum T obtained in normal males and females are given in Table 5. A good correlation ($r=0.87$, $n=158$, $p<0.01$) was obtained between concentration (pmol/l) of FT in serum and salivary T in both normal subjects. These samples covered a wide range of 20~415pmol/l for salivary T and 16~710 pmol/l for serum FT. A significant correlation was obtained in the 131 healthy subjects between serum T and TT ($r=0.92$, $p<0.01$) and a FT/TT ($r=0.98$, $p<0.01$, $Y=3.8x+2.3$). The salivary T concentration expressed as a % of the total serum T was $1.1 \pm 0.3\%$ in healthy male, 3.5 ± 2.0 percent in the healthy female. These values were significantly different ($p<0.01$).

The percentages of T and DHT distribution between the SHBG, albumin, and free forms are summarized in Table 6. The free DHT percentages of the serum total DHT values in male serum samples were 2.15 ± 0.20 (0.8 ~2.54) % and were slightly higher than those in females, comparing with calculated FDHT levels. There was no significant difference between serum FT in healthy nale and in female, expressed as a percentage of the total T serum values. The percentages of bioavailable T and DHT in female were significantly lower than those in male ($p<0.01$). The percentages of SHBG-bound and DHT in female serum were also significantly highr than those in male ($p<0.05$).

The absolute concentrations of albumin-bound, SHBG-bound and bioavailable T and DHT are shown in Table 7. There were significant correlation between the values of bioavailable T and DHT and their total T and DHT : $r=0.91(n=95)$, and $r=0.89(n=75)$ for DHT. As shown in this table, the absolute values of female serum BT and BDHT were much higher than the limits levels of both assays.

Table 5. Values of salivary T and serum free T or DHT in men and women

Samples	Male (n=90)	Female (n=90)
Salivary T (pmol/l)	290.0±65.1 (70~415)	33.0±15.4 (20~62)
Serum FT (pmol/l)	585.0±19.8 (245~710)	26.1±2.4 (16~30)
Total T (nmol/l)	21.5±5.1 (10.5~33.6)	1.1±0.3 (0.4~2.3)
Serum FDHT (pmol/l)	19.9±1.2 (10.5~36)	*ND
Total DHT (nmol/l)	1.8±0.3 (0.7~9.7)	0.2±0.03 (0.2~1.4)

*ND ; not detectable.
Serum FT and FDHT were determined by the modified mothod of Vlahos et al., (1982) using a centrifugal ultrafiltration through YMB membranes of MPS-1 devices.

Table 6. Distribution of androgens between the SHBG-, albumin-, and non-protein bound fraction

	Male (n=50)	Female (n=50)
Testosterone		
Free T (%)	1.80±0.1	1.90±0.7
Albumin-bonud T (%)	31.20±8.41	24.01±5.44
SHBG-bound T (%)	67.76±3.99	74.62±4.63
Bioavailable T (%)	33.08±6.71	25.68±4.67
(Free+albumin-bound)		
Dihydrotestosterone		
Free DHT (%)	2.15±0.20	1.88±0.32
Albumin-bound DHT (%)	31.14±5.36	24.63±4.78
SHBG-bound DHT (%)	66.57±2.95	74.02±7.93
Bioavailable DHT (%)	33.43±4.60	25.41±4.92
(Free+albuimn-bound)		

*p < 0.01, compared the values of male sample with the corresponding values of female.

Table 7. Concentrations of bioavailable (free+albumin-bound) and SBHG-bound androgens in serum

Hormones	Male (n=75)	Female (n=95)
Totale testosterone(nmol/l)	21.50±5.1	1.1±0.3
Albumin-bound T(nmol/l)	6.71±1.65	0.26±0.04
SHBG-bound T(nmol/l)	14.62±0.86	0.82±0.05
Bioavailable T(nmol/l)	7.10±1.48	0.28±0.05
(free+albumin-bound)		
Albumin-bound DHT(pmol/l)	570.52±101.5	50.11±11.60
SHBG-bound DHT(pmol/l)	1201.00±58.1	148.92±18.56
Bilavailable DHT(pmol/l)	601.74±85.8	52.44±19.9
(free+albumin-bound)		

All values of the female samples were significantly lower than those of male samples ($p<0.001$, by Student's t-test). The bioavailable androgens were determined in the supernatant after treatment of ammonium sulfate (at final concentration of 50%) to precipitate the SHBG fractionx, using a modified methods of Manni et al., (1985).

DISCUSSION

The present study reevaluated again by applying the LIA techniques to determine the salivary, serum free, and serum total DHT and T and also their bioavailable fractions, utilizing the labelled tracers DHT or T-3-CMO-ABEI. The indirect LIA for T has been already stablished by Kreysing, Yoon and Nieschlag (1988). The indirect DHT LIA using high performance liquid chromatography has been reported (Yoon et al., 1987) but the more convenient and simpler methods are needed to assay a large number of samples. Both assay systems have been validated using the usual criteria of sensitivity, precision, specificity, and parallelism etc. The overall performances were very similar to those of RIA. Thus the present study shows that the developed LIA systems appear to be suitable for use in researches and clinical assays for male and female samples in order to determine the bioavailable and free androgens in serum and saliva.

The main problems in developing highly sensitive direct LIA methods for use in the crude biological samples probably arise from the interferences with light-producing reac-

tion (Kohen et al., 1982 ; Pazzagli et al., 1982). Direct LIA assays have shown reported to show a matrix effect. The mean values obtained for extract method were close to the expected ones, but results from recovery experiments with charcoal-stripped polycythaemic sera and with spiked buffer samples increasing BSA content showed a large variations. In addition, the cross reactivity by the similar steroids compromises the accuracy of specific steroid determination in both system. Because the highly specific antisera including monoclonal antibodies only for DHT or T determinations are not available and satisfactory until now, the direct LIA could not be estableished.

Concentration of steroid hormones in saliva are considered to reflect the free levels in blood plasma (James and Baxendale, 1982). Previous reports showed that the level of male salivary T is in the range of 270~420 pmol/l (Walker et al.,1980 ; Wang et al.,1981 ; Baxendale and James, 1983 ; Gould et al., 1986 ; Johnson et al.,1987). However, with the female saliva samples, there is a wide discrepancy in the previous reports with values ranging from 14~215 pmol/l, except the data of Landman et al., (more than 677 pmol/l). Table 5 shows the present results for male salivary T lie within the range. The female salivany T lie at the lowere end of the range,but agreed well in the range of 14~52 pmol/l with those reported by other researchers (Gould et al., 1986 ; Jo-hnson et al., 1987).

To measure FT, a number of different precedures have been developed, with the equilibrium dialysis method being used as the reference method. The DPC kit using antibody coated tube method is very simple, very attractive.and highly desirable but the kit reports a FT range where upper limit is lower than the mean measured by other method. Cheng et al. (1986) and Wilke and Utley (1987) reported similarly a negative mean bias (-76%) for the DPC method compared with the reference method. The miniature steady-state gel filtration through Sephadex-200 column has removed a lot of the tedium and procedures as the direct measurement does. The determined values by this method agreed well with the previous reports (Wheelera and Nanjee, 1985). The present study using Amicon filtration system looks attractive eventhough Vlahos et al. (1982) reported excessive leakage in 5% of the devices. These FT values are similar to those in the previous report (Nanjee and Wheeler, 1985). Johnson et al. (1987) reported that there is a good correlation ($r=0.83$, $p<0.01$, $n=194$) between salivary and serum free T in matched serum and saliva samples. The present result also showed a good correlation ($r=0.87$, $p<0.01$, $n=158$) between them. Thus the present study confirmed that salivary T measurement has potential advantages over serum with regard to ease of collection of samples, multiple specimen collection, non-invasive and employing established LIA techniques. But it does appear to overestimate the free T and saliva could be easily contaminated.

It has been generally known that 98% of androgens in blood are bound to three plasma proteins : SHBG, albumin and corticosteroid binding protein, and that levels of SHBG are lower in male primate than in female ones. Table 6 shows the little higher percentages of T and DHT and no differences between in man and women when these results are compared to those of Anderson (1974). Because FT is independent of the changes in SHBG, it would appear to be more appropriate analyte for the investigation of androgenicity than total T. It has been reported that the affinity of SHBG for DHT is 1.2 to 1.3 times higher than that for T and 4 times higher than that for estradiol (Anderson, 1974). The present result also agreed well with this assumption.

Bioavailabel T(BT) includes circulating FT and large proportion (55%) of albumin-bound T. The SHBG-bound T appears to dissociate too slowly to be biologically active

for the target tissues (Cumming and Wall, 1985 ; Manni et al., 1985). Recently it has been reported that BT in serum are decreased in aged men and that BT is more sensitive indicator for this decline than are total T or serum free T (Nankin and Calkins, 1986). Present result shows that the percentages of BT and BDHT in male ate more than 33 % of serum total values, while those in female are more than 24%, and that thesevalues are within the standard ranges and more suitable to determine with normal routine assays. We found also that serum BT and BDHT concentrations were significantly lower in old peoples (data not shown, i.e. 10.8±0.6 nmol/l for BT, n=15, 60 to 66 years) than in young men(20-40 year of age, 15.7±2.1 nmol/l for T, n=93). Manni et al. (1985) reported BT to range between 2.60 to 10.47 nmol/l values. These are very similar to those of our result.

On the other hand, Harman and Nankin (1985) reported no statistically significant changes in BT with aging. But lower serum FT in old man and in the impotent men are reported by Koritnik and Marschke (1986). These kinds of discrepancy should be stidoed more carefully.

In conclusion, the present study shows that the measurement of biologically available and serum free androgens by our established LIA could be used as a research method for male physiology and also for the monitoring the androgenicity of female, and also could be clinically useful, reliable and routinely applicable method. On the other hand, the direct measures of physilolgically active steroid levels and more appropriate for the androgen monitoring systems.

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