J Infect Chemother 25 (2019) 318–321

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: http://www.elsevier.com/locate/jic

Note

The prevalence of low serum free testosterone and the short-term effect of anti-retroviral therapy in male Japanese treatment-naïve HIV patients☆

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ARTICLE INFO

Article history:
Received 9 July 2018
Received in revised form
24 August 2018
Accepted 8 September 2018
Available online 4 October 2018

Keywords:
Anti-retroviral therapy
Free testosterone
HIV
Hypogonadism

ABSTRACT

Objectives: The prevalence of hypogonadism in HIV patients is still a matter of debate. Today, serum free testosterone (fTST) is thought to be more important than serum testosterone in the diagnosis of hypogonadism in patients with HIV. This study aimed to determine the prevalence of low fTST levels and the effects of anti-retroviral therapy (ART) on fTST levels in treatment-naïve male Japanese patients with HIV.

Methods: Patients who visited Teikyo University Hospital, Japan between 2010 and 2016 were enrolled. Patients' fTST levels were evaluated twice with a radioimmunoassay in the morning, at the onset of ART and one year later. Clinical factors were also reviewed. The patients were divided into two groups ('hypogonadism' and 'normal') based on Japanese criteria. To determine factors related to low fTST in treatment-naïve patients, the Mann-Whitney *U* test and a multiple-regression analysis were used. Changes in fTST levels after ART initiation were evaluated with a paired *t*-test.

Results: Data from 25 patients were collected. Their median age was 36.0 years, and the median fTST level was 8.00 pg/ml in the treatment-naïve state. Thirteen patients (52%) were in the hypogonadism group. Low levels of fibroblast growth factor 23 were significantly related to low fTST levels. After the start of ART, fTST levels increased significantly (median 8.00 interquartile range [6.40–9.70] to 9.60 [7.60–13.10] pg/ml, $p = 0.0081$).

Conclusions: Subnormal fTST levels occurred frequently among the present study patients in treatment-naïve settings. Free testosterone levels in patients with HIV were significantly increased one year after the start of ART.

Hypoonaism was recognized as being relatively common early in the HIV epidemic. Low levels of testosterone in male HIV patients may be associated with a variety of symptoms and signs including weight loss, low bone mineral density, and so on [1]. In early studies from the 1980s and early 1990s, 30–50% of symptomatic male HIV patients had low testosterone levels, which were strongly correlated with weight loss and low CD4 cell counts [2,3]. Today, HIV therapy is initiated at an earlier stage of HIV infection than in the past, and the prevalence of hypogonadism is thought to be lower. In fact, Araujo et al. recently showed that HIV status was not associated with a risk of testosterone deficiency [4]. However, many previous studies only reported total testosterone levels, which might inaccurately reflect free bioactive testosterone concentrations because of increased sex hormone-binding globulin (SHBG) concentrations among men with HIV infection [5]. Additionally, the prevalence of testosterone deficiency in the HIV population was not well defined, since slightly different criteria were adopted in different studies [6,7]. Therefore, it remained unclear whether testosterone deficiency was prevalent in HIV patients in the current situation. Lachâtre et al. recently showed that hypogonadism was prevalent in HIV patients based on the levels of serum free

Abbreviations: SHBG, sex hormone-binding globulin; fTST, serum free testosterone; INSTI, integrase strand transfer inhibitor; ART, anti-retroviral therapy; FGF23, serum fibroblast growth factor 23; TDF, tenofovir disoproxil fumarate; FTC, emtricitabine; ABC, abacavir; 3TC, lamivudine.

☆ All authors meet the ICMJE authorship criteria.

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https://doi.org/10.1016/j.jiac.2018.09.007
1341-321X/

testosterone (fTST) [8]. However, this study population was relatively small. A larger number of clinical trials is needed to validate this result.

In addition, data on changes in fTST levels after the initiation of potent antiretroviral therapy are sparse, especially in recent clinical settings in which an integrase strand transfer inhibitors (INSTIs) are used in the anti-retroviral therapy (ART) regimen [9–11]. The purpose of this analysis was to determine the prevalence of a low fTST level in Japanese male HIV patients without previous ART therapy and to analyze the effect of antiretroviral agents on serum fTST levels.

This study was a cohort analysis of a study on bone mineral density in HIV patients and was approved by the Ethics Committee of Teikyo University School of Medicine (13-1390). HIV patients who visited the outpatient clinic of Teikyo University Hospital, Tokyo, Japan, between April 2010 and October 2016 were enrolled. Written, informed consent was obtained from all study participants. Data on age, body mass index, history of drug use, conditions at the outpatient clinic, lumbar spine and femoral neck bone mineral densities, serum CD4-positive lymphocyte count, plasma HIV-RNA concentration, serum albumin, aspartate transaminase, alanine aminotransferase, alkaline phosphatase, serum creatinine, serum calcium, serum phosphorus, serum 1.25-dihydroxyvitamin D3, 25-hydroxyvitamin D, serum fibroblast growth factor (FGF)23, serum intact parathyroid hormone, serum bone-specific alkaline phosphatase (BAP), urinary N-terminal telopeptide/creatinine, urinary calcium/creatinine, urinary phosphorus/creatinine, smoking status, and status of hepatitis B and/or hepatitis C infections were collected before the start of ART. Patients also underwent fTST measurements with an analog ligand radioimmunoassay in the morning, before initiation of ART. The patients were divided into 2 groups (hypogonadism and normal) based on cut-off values (fTST <8.50 pg/ml: mean −2*standard deviation of 20 to 29-year-old men from the study of healthy men in Japan) [12]. The levels of fTST, FGF23 and albumin were also measured one year after the start of ART. The results are expressed as medians [interquartile range], unless otherwise indicated. To determine risk factors for low fTST in treatment-naïve patients, the Mann-Whitney *U* test and multiple regression analysis were used. Changes in fTST levels after the start of ART were assessed by the paired *t*-test. To identify the influence of each type of anti-retroviral agent on changes in fTST levels after the start of ART, Student's t-test was used. To identify the influence of clinical backgrounds on changes in fTST levels after the start of ART, Spearman's Rank correlation coefficient was used. All P values were two-sided and were considered significant at $P < 0.05$. StatFlex version 6 software (Artech Co., Osaka, Japan) was used for analysis.

Twenty-five patients were followed-up. No patients had histories of injection and tranquilizer drug use. No patients visited our outpatient clinics to get a blood test after staying up all night. The clinical data of all patients before the start of ART are shown in Table 1. Thirteen patients (52.0%) were in the hypogonadism group. Univariate analysis showed that old age, low FGF23 level, and high urinary phosphorus/creatinine were significantly related to a low fTST level. Multivariate analysis showed that FGF 23 was the only significant factor. All patients were given two nucleotide reverse transferase inhibitors as backbone drugs and darunavir/ritonavir or an INSTI as a key drug. In all, 16 (64.0%) patients were treated with tenofovir disoproxil fumarate (TDF)/emtricitabine (FTC), and 9 (36.0%) patients were treated with abacavir (ABC)/lamivudine (3TC) as backbone drugs of the ART regimen. Thirteen (52.0%) patients were treated with darunavir/ritonavir, and 12 (48.0%) patients were treated with INSTIs (2 patients raltegravir, 7 patients elvitegravir/cobicistat, and 4 patients dolutegravir) as key drugs of the ART regimen. The overall changes in fTST after the start of ART in each patient are shown in Fig. 1. After the start of ART, fTST levels increased significantly from 8.00 [6.40–9.70] to 9.60 [7.60–13.10] pg/ml ($p = 0.0081$). The change in fTST was 1.20 [0.00–4.50] pg/ml. With respect to the backbone drugs, the change in fTST was 1.05 [0.10–3.78] pg/ml in patients treated with TDF/FTC and 2.00 [0.00–4.50] pg/ml in patients treated with ABC/3TC; the difference was not significant ($p = 0.4103$). With respect to key drugs, the

Table 1
Clinical data of the study population before the start of anti-retroviral therapy.

	All (n = 25)	Low (n = 13)	Normal (n = 12)	P (univariate)	P (multivariate)
Age (years old)	36.0 [30.8–50.5]	43.0 [33.8–54.3]	31.5 [25.0–44.0]	0.04118*	0.09199
BMI	22.4 [20.7–24.3]	22.5 [18.9–23.6]	22.3 [21.7–24.4]	0.54963	
Lumbar BMD	0.90 [0.85–1.01]	0.87 [0.84–0.99]	0.93 [0.87–1.03]	0.41156	
Femoral BMD	0.76 [0.71–0.83]	0.72 [0.68–0.83]	0.76 [0.74–0.84]	0.23136	
CD4 (/μl)	289 [219–396]	275 [163–330]	346 [243–419]	0.30129	
HIV copy x103	36.0 [13.3–67.5]	36.0 [16.4–102]	32.5 [12.5–49.0]	0.53147	
AST (IU/L)	23.0 [19.8–27.3]	22.0 [18.8–25.8]	24.0 [20.5–31.5]	0.41366	
ALT (IU/L)	19.0 [14.8–27.0]	19.0 [12.0–27.0]	20.0 [15.0–40.0]	0.54824	
ALP (IU/L)	268 [194–290]	273 [246–295]	211 [178–276]	0.18257	
Serum Cr (mg/dl)	0.82 [0.71–0.89]	0.78 [0.65–0.90]	0.84 [0.74–0.89]	0.5862	
Ca (mg/dl)	8.90 [8.70–9.30]	8.90 [8.58–9.05]	9.15 [8.75–9.40]	0.09545	
P (mg/dl)	3.50 [3.20–3.80]	3.50 [3.18–3.73]	3.60 [3.30–3.90]	0.38249	
Cystatin C (mg/dl)	1.03 [0.89–1.19]	1.03 [0.85–1.23]	1.02 [0.91–1.18]	0.90769	
1.25VitD (ng/ml)	46.7 [38.3–62.3]	44.9 [36.2–62.3]	52.1 [38.9–61.8]	0.58649	
25OHvitD (ng/ml)	19.0 [13.8–26.3]	17.0 [14.0–23.8]	22.0 [10.5–27.0]	0.64307	
FGF23 (pg/ml)	44.0 [36.8–56.0]	39.0 [33.8–44.3]	52.5 [41.0–57.5]	0.03855*	0.03468*
PTHin (pg/ml)	40.0 [31.0–49.0]	42.0 [33.5–49.0]	32.5 [27.0–47.5]	0.32717	
BAP (IU/L)	11.9 [9.20–15.2]	12.0 [8.40–14.1]	11.8 [9.45–15.9]	0.56777	
freeTST (pg/ml)	8.00 [6.40–9.70]	6.70 [3.58–7.58]	9.90 [9.25–11.5]	0.00002*	
NTx/Cr (nmolBCE/mmolCr)	32.5 [29.7–43.7]	32.5 [30.7–45.3]	32.5 [29.2–36.6]	0.4964	
urin Ca/Cre x10-1 (g/Cre)	0.80 [0.50–1.20]	0.80 [0.50–1.13]	0.75 [0.45–1.35]	0.93476	
urin P/Cre (g/Cre)	0.37 [0.24–0.56]	0.49 [0.34–0.82]	0.27 [0.16–0.43]	0.03376*	0.1054
Smoking	11 (44%)	6 (46.2%)	5 (41.7%)	0.97267	
HBV	7 (28%)	3 (23.1%)	4 (33.3%)	0.6728	

BMI: body mass index, BMD: bone mineral density, AST: aspartate transaminase, ALT: alanine aminotransferase, ALP: serum alkaline phosphatase, Cr: creatinine, Ca: serum calcium, P: serum phosphorus, 1.25VitD: serum 1.25-dihydroxyvitamin D3, 25OHvitD: serum 25-hydroxyvitamin D, FGF23: serum fibroblast growth factor 23, PTHin: serum intact parathyroid hormone, BAP: serum bone-specific alkaline phosphatase, fTST: free testosterone, NTx/Cr: urinary N– terminal telopeptide/creatinine, urin Ca/Cre: urinary calcium/creatinine, urin P/Cre: urinary phosphorus/creatinine, HBV: hepatitis B virus.
* Significant difference ($p<0.05$).

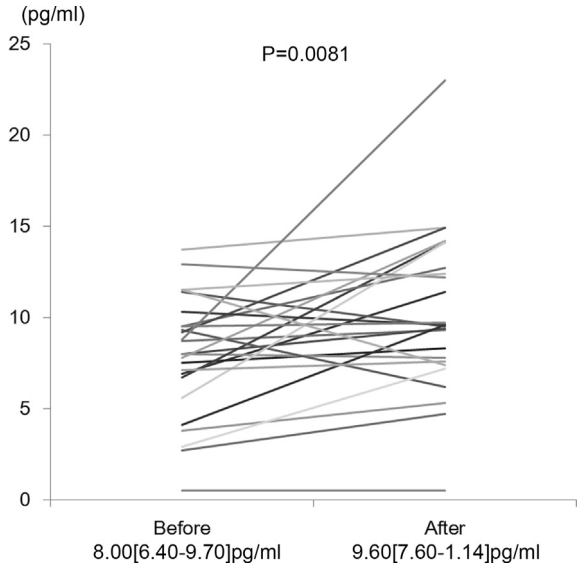

Fig. 1. Changes of free testosterone (fTST) after the start of anti-retroviral therapy (ART) are shown. There are significant changes after the initiation of ART (8.00 [6.40–9.70] to 9.60 [7.60–11.4] pg/ml P = 0.0081).

change in fTST levels was 1.50 [0.20–5.70] pg/ml in patients treated with INSTIs and 1.00 [0.05–3.53] pg/ml in patients treated with protease inhibitors; there was also no significant difference between these two groups (p = 0.4034). We examined the clinical factors involved in the change of fTST. Low levels of serum fTST before starting ART was significantly related to the change of fTST (r = −0.4761, p = 0.0187). The changes in fTST after the start of ART in each group (the hypogonadism group and normal group) are shown in Fig. 2. There was definitely a significant increase of fTST levels only in the hypogonadism group.

In the present study, subnormal fTST levels occurred frequently among Japanese male treatment-naïve HIV patients. In men who started potent antiretroviral therapy, fTST levels increased significantly after commencement of treatment. The serum fTST levels increased more after HIV treatment, especially, in patients who had lower serum fTST levels before ART initiation. The fTST levels were measured directly with a radioimmunoassay method. Testosterone binds to a specific plasma protein (SHBG) and binds weakly to nonspecific proteins such as albumin. Because SHBG concentrations increased among men with HIV infection, fTST is now thought to be more reliable for diagnosing hypogonadism [5]. Radioimmunoassay and calculation methods have been used to measure fTST levels. The calculated fTST level is determined from total testosterone, SHBG, and albumin levels using mathematical formulae, and it is the method that has been mainly used. It was also used in a recent study of fTST in HIV patients [8]. However, in Japan, measurement of fTST only by radioimmunoassay is permitted by health insurance, and the serum fTST level measured by the radioimmunoassay method is used in the criteria for hypogonadism. The numerical values for fTST measured by the radioimmunoassay method are approximately one-eighth of the values obtained by the calculated fTST method. However, Moreno et al. showed a strong correlation between fTST measured by the radioimmunoassay method and the calculated fTST in a clinical population of ambulatory men [13]. We believe that the present results accurately show the status of hypogonadism in HIV patients because unique Japanese criteria based on measurement of fTST levels by radioimmunoassay were used to diagnose.

In the treatment-naïve setting, multivariate analysis showed that only a low FGF23 level was a significant risk factor for a low fTST level. However, FGF23 level was not significantly changed after ART initiation [44.0 (37.5–56.0) to 36.0 (33.0–50.0) pg/mL, p = 0.0817], and the change of FGF23 after ART was not correlated with that of fTST.

FGF23 is produced by bone and promotes the excretion of urinary phosphate. FGF23 is thought to affect bone metabolism. It has been reported that TDF promoted FGF23 production and caused a low serum phosphate level [14]. However, in the present study, there was no significant evidence of a relationship between the serum FGF23 level and the use of TDF, and FGF23 was also not significantly related to urinary phosphate (data not shown). The mechanism by which FGF23 affects fTST is unknown. However, we believe this result is clinically valuable, because this implies that FGF23 may have other potential roles. Indeed, the relationship

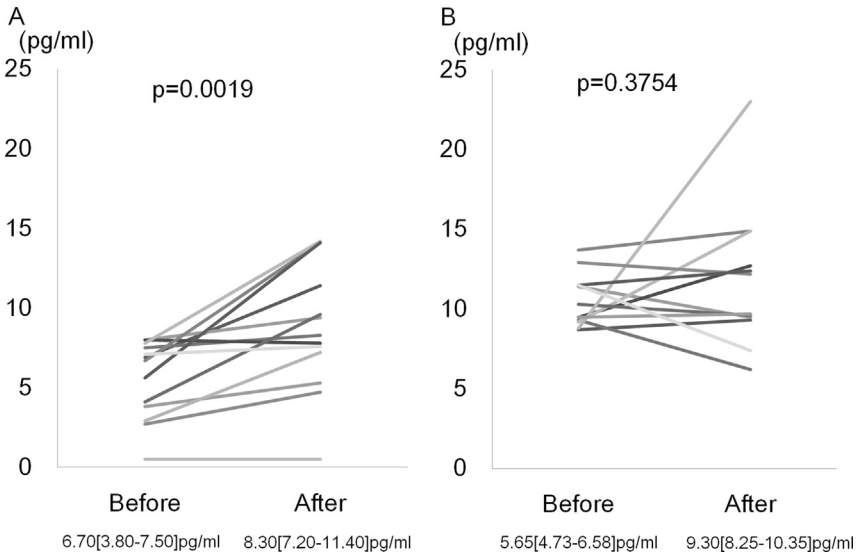

Fig. 2. Changes of free testosterone (fTST) after the start of anti-retroviral therapy (ART) in hypogonadism group (A) and normal group (B) are shown. There are significant changes after the initiation of ART in the hypogonadism group (6.70 [3.80–7.50] to 8.30 [7.20–11.40] pg/ml, P = 0.019). On the other hand, in normal group, there are no significant changes (5.65 [4.73–6.58] to 9.30 [8.25–10.25] pg/ml, P = 0.3754).

between FGF23 and cardiovascular diseases was proven recently, which also implies that FGF23 has other potential roles [15].

The serum fTST level was significantly elevated after initiation of ART in the present study. According to previous reports, opinions were divided about whether ART could increase the serum testosterone level [10,11]. However, these reports were published in 2007. Anti-retroviral agents were developed, and the INSTIs as a new type of anti-retroviral drugs were invented and have been used since 2011, and they recently became the most important key drugs for HIV infection. This work showed that the recent anti-retroviral agent regimen improved the status of hypogonadism. To assess the effect of nutrition status on the change of serum fTST levels, we checked the serum albumin level and analyzed the change of serum albumin after stating ART. The nutrition status of all study patients was almost normal, even in pre-ART settings, and there were no significant changes in the serum albumin levels [median 4.30 [4.10–4.50] to 4.50 [4.20–4.60] mg/dl, P = 0.0853].

The prevalence of HIV-infected patients with low fTST in this study was higher than in other previous reports. It is well known that there is a significant tendency toward age-related decreases in total testosterone in many countries. Also, in Japan, there is a tendency toward age-related decreases in total testosterone, though there is no statistical significance. A consistent result showing age-related decreases in free T was obtained in a number of several reports from cross-sectional studies. The percentage of age-related decrease is known to be significantly higher in free T than in total T in Japan [12]. The cause of this may be that SHBG levels increase with age. In addition, HIV infection itself can increase SHBG levels [5]. So, it is possible that hypogonadism in Japanese people more directly depends on the increase of the serum levels of SHBG binding testosterone, and the prevalence of HIV-infected patients with low fTST in Japan was higher than that in the US and European countries.

The sample size was small in the present study. A large prospective cohort study is required to fully show the prevalence of testosterone deficiency and the effect of ART on fTST levels in HIV patients. In addition, we could not show the symptoms related to hypogonadism in our study because this study is sub-analysis of the study of bone mineral density in Japanese HIV patients, and we had never collected the data of those symptoms at the time we measured serum free testosterone and bone mineral density in each patients. Nonetheless, this is the first study to show the relationship between fTST and FGF23. We also believe this study is clinically valuable because fTST levels were measured directly to determine the prevalence of hypogonadism in treatment-naïve HIV patients, and the recent anti-retroviral agent regimen were found to improve the status of hypogonadism in HIV-infected individuals.

Conflicts of interest

None.

Acknowledgements

Nothing to declare.

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