Shiraz E-Med J. January 2014; 15(1): e19734. DOI: 10.17795/semj19734

Published online 2014 January 15. **Research Article**

Hypogonadism and Associated Factors in Patients With HIV Infection Based on Total and Free Testosterone

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Received: November 5, 2013; **Revised:** December 15, 2013; **Accepted:** April 3, 2014

Background: The prevalence of hypogonadism and associated related factors among a group of human immunodeficiency virus positive (HIV+) patients in Shiraz, Iran, were examined based on free testosterone (FT) and total testosterone (TT) levels.

Objectives: In this study we planned to determine the prevalence of hypogonadism among a group of men with an HIV infection in Shiraz, Iran. Measurements of free and total testosterone levels and their association with some related factors were determined, in order to suggest new approaches in harm reduction strategies.

Materials and Methods: A total of 237 males, HIV-positive patients were recruited based on convenience sampling, from May to October 2010. All patients provided their informed consent, and blood samples were collected after an overnight fast in order to measure free testosterone concentrations, HGB, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prolactine (PRL) levels.

Results: A total of 180 (75%) subjects were hepatitis C virus (HCV) positive, and 23 (9.7%) subjects were HBS-positive. Based on free testosterone (FT) levels 62.8% subjects had hypogonadism, and with total testosterone (TT) levels 59.6% subjects had hypogonadism. The results showed that FT levels were associated with; age, methadone use (OR = 1.74, CI: 0.97–3.1), LH (OR = 0.91, CI: 0.87-0.95), HGB (OR = 0.788, CI: 0.69-0.89), body mass index (BMI) (OR = 0.88, CI: 0.79-0.98) and PRL (OR = 1.18(CI: 1.09-1.28), but it had no significant association with highly active antiretroviral therapy (HAART), smoking, hepatitis or reaching the AIDS stage. On the other hand, TT had a significant association with BMI, LH, PRL, and HGB.

Conclusions: The prevalence of hypogonadism in the participants was high. It is recommended that a hypogonadism examination should be included in surveillance programs for HIV-positive men.

Keywords: Hypogonadism; Testosterone; HIV; Acquired Immunodeficiency Syndrome

1. Background

Hypogonadism is defined as low serum testosterone levels, together with more than one of the following clinical manifestations: sexual dysfunction, fatigue, depression, anemia, loss of libido, weight loss, or muscle loss (1, 2). Several studies have revealed that hypogonadism is a prevalent and important endocrinology abnormality among patients infected with the human immunodeficiency virus (HIV) (3, 4).

The cause of hypogonadism in HIV-positive patients is not clearly known and a number of cofactors can intensify it, in addition, some studies have proposed that hypogonadism has multi-factorial causes, including: aging (5), anemia (6), diabetes (7), malnutrition (8), common acute and chronic illnesses (9), weight loss (10), invasion of glands by HIV, or other pathogens like hepatitis (11), smoking, using drugs, such as opiates, megestrol acetate, methadone (12), and steroids (13), as well as progression to AIDS stages (14). Occasionally hypogonadism may contribute to primary testicular etiology, termed 'primary hypogonadism'. As a result of abnormalities in the testis, serum levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), secreted from the gonadotrophs of the anterior pituitary, are increased. However, in the majority of patients, hypogonadism is the result of inadequate stimulation from the gonadotrophs, which is called 'hypogonadotropic hypogonadism' or 'secondary hypogonadism'. In this type of hypogonadism, pituitary gland LH and FSH levels are inappropriately normal or low (15). In hypogonadism patients, prolactine levels may be elevated and sometimes they can predict hypogonad-

Implication for health policy/practice/research/medical education:
Research into the prevalence of hypogonadism among men with an HIV infection, may suggest new approaches in the development of harm reduction strategies.

ism independently (16). Highly active antiretroviral therapy (HAART) may have a protective effect on the development of hypogonadism (17).

According to the Iranian Ministry of Health and Medical Education, in Iran, a total of 27 041 cases had been identified up to September 2013 (18). Today, with regard to harm reduction strategies, many countries including Iran use methadone for addiction therapy. Based on the previous study, despite the fact that the methadone maintenance treatment (MMT) program has developed in Iran as one of the main methods of harm-reduction, it may cause hypogonadism in patients with an HIV infection (12). Nevertheless, hormonal testing is not routinely performed in the national surveillance program for HIV patients, and policy makers have no current plans to make any changes in the HIV surveillance program after implementing MMT in Iran. Although a total testosterone (TT) assay is widely available and inexpensive to perform, in community practice the choice of testosterone parameter to measure the detection of hypogonadism is still being debated (19).

2. Objectives

In this study we planned to determine the prevalence of hypogonadism among a group of HIV infected men in Shiraz, Iran, based on free and total testosterone levels and their association with some related factors, in order to suggest some new approaches in harm reduction strategies.

3. Materials and Methods

All participants had enrolled in either; voluntary counseling and testing (VCT) services or MMT centers, affiliated with the Shiraz University of Medical Sciences in Shiraz, Southern Iran. In total, 278 men with HIV positive infection were selected for the study, based on convenience sampling between May and October 2010.

The study's objectives were explained to all the participants and written informed consent was obtained from each subject prior to commencement of the study. Then, the questionnaires, including socio-demographic and HIV-related variables, were completed by all participants. Next, the patients were asked to give serum samples for morning serum FT and TT levels, LH, FSH, and prolactine.

In this study, the serum TT concentration was measured through a direct method radioimmunoassay (Immunotech, Marseille, France). Inter-assay variation was $\leq 15\%$ and the normal range was 3.0 to 12 ng/mL. In addition, FT was measured with an enzyme-linked immunosorbent assay (ELISA) (DRG Instruments, Marburg, Germany). The inter-assay variation was $\leq 10\%$ and the normal range was 4.5 to 42.0 pg/mL. In addition, prolactine was measured by an immune radiometric assay (Immunotech, Irma Kit, Czech Republic). The inter-assay variation was $\leq 8.0\%$, and the normal range was 1.0-18.0 ng/mL. Finally, LH was measured by an immune radiometric assay (Immunotech, Irma Kit, Czech Republic). The inter-assay variation was $\leq 3.7\%$ and the normal range was 0.05-10 IV/L.

From a sample of 278 HIV infected males, 28 (10%) patients completed the questionnaires, but they did not refer for serum sampling at the beginning of the study, and six (2.1%) subjects with systemic diseases, including; cirrhosis, chronic renal failure, chronic pulmonary disease, and diabetes mellitus, which may contribute to hypogonadism, were excluded. As hyper-prolactinemia produces hypogonadism by interfering with the secretion of gonadotropin-releasing hormones, five (1.8%) subjects with hyper-prolactinemia (serum prolactine level above 18 ng/mL), were also excluded from the study. In addition, we excluded two (0.71%) patients with hypergonadism (TT above 12 ng/mL). Finally, 237 (85%) participants were enrolled in this study. We defined hypogonadism patients by TT levels of ≤ 348.3 ng/dL (12.1nmol/L), and FT levels of ≤ 70.0 pg/ML (243 pmol/L).

The data were analyzed using the Statistical Package for the Social Sciences (SPSS) for Windows, Version 18.0. We carried out a simple descriptive analysis and logistic regression. The study protocol was approved by the Research Ethics Committee of Shiraz University of Medical Sciences.

4. Results

4.1. Characteristics of the Study Participants

Among the 237 participants who were enrolled in the study, the mean age of the participants was 37.4 ± 7.4 years (range 22-63 years), and the largest age subgroup was 30-39 years (54.1%). All of the subjects were male and 84.2% were unemployed. Slightly fewer than half of the participants 106 (44.7%) were single, and 88 (37.1%) were married. Moreover, 54 (22.7%) of the patients had developed AIDS, while 180 (75.9%) subjects and 23 (9.7%) subjects, were HCV-positive and HBS-positive, respectively. According to the weight status categories associated with body mass index (BMI) ranges for adults; 15.1% of the subjects had a BMI below 18.5 (underweight), 51.1% were between 18.5 and 24.9 (normal), 7.6% were between 25 and 29 (overweight), and approximately 0.4% had a BMI above 30 (obese). Almost 194 (82%) subjects smoked cigarettes and 97 (41%) were active clients in the MMT program. There were 32 (13.5%) participants who were injection heroin users, 33 (13.9%) used opium, 8 (3.4%) used marijuana and other drug users, and 11 (4.6%) subjects belonged to others or missing data.

4.2. Hypogonadal vs. Eugonadal

According to the TT levels, 35.4% of the patients had a serum level below three or were hypogonadal, whereas 59.6% had a serum TT level between 3 and 12 ng/mL and were eugonadal. On the other hand, 62.8% and 30.8% of

the patients were hypogonadal (FT level below 4.5 pg/mL) and eugonadal (FT between 4.5 to 42 pg/mL). Among the hypogonadal patients defined by total testosterone serum levels, 23.8% had primary hypogonadism, and 76.2% had secondary hypogonadism. Considering free testosterone serum levels, 30.8% and 69.1% of the patients had primary and secondary hypogonadism, respectively. Comparison of hypogonadal and eugonadal patients (Tables 1 and 2).

Table 1. Comparison Between Hypogonadal and Eugonadal Subjects Based on Total Testosterone [a,b]

-	Hypogonadal (n=84)	Eugonadal (n=133)	P-value	OR (95%CI)
Age, y	37.6 ± 6.6	37.7 ± 7.1	0.88	0.99 (0.95-1.03)
20-29	6 (7.1)	10 (7.5)	-	-
30-39	44 (52.4)	71 (53.4)	-	-
40-49	23 (27.4)	34 (25.6)	-	-
50-60	6 (7.1)	9 (6.8)	-	-
BMI, kg/m^2	20.38 ± 2.7	21.7 ± 4.03	0.02	0.88 (0.79-0.98)
<18.5	16 (19)	12 (9)	-	-
18.5-24.9	47 (56)	67 (50.4)	-	-
25.0–29.9	4 (4.8)	15 (11.3)	-	-
30>	67 (79.8)	1 (0.8)	-	-
FSH, IU/L	4.3 ± 2.8	4.9 ± 3.1	0.152	0.93 (0.84-1.02)
LH, IU/L	6.8 ± 5.9	10.7 ± 7.8	< 0.0001	0.91 (0.87-0.95)
PRL, ng/mL	8.3 ± 4.2	6.2 ± 2.9	< 0.0001	1.18 (1.09-1.28)
HGB	12.9 ± 2.3	14.1 ± 2.1	< 0.0001	0.788 (0.69-0.89)
Smoking	-	-	0.64	0.86 (0.47-1.58)
Yes	71	103	-	-
No	9	28	-	-
Unknown	4	2	-	-
Methadone	-	-	0.67	1.12 (0.64-1.96)
Yes	46	55	-	-
No	37	77	-	-
Unknown	1	1	-	-
Hepatitis	-	-	0.14	2.3 (CI:0.73-7.5)
Negative	1	10	-	-
Positive	66	90	-	-
Unknown	17	33	-	-
AIDS	-	-	0.912	1.03 (0.54-1.98)
Yes	23	34	-	-
No	45	69	-	-
Unknown	16	30	-	-
HAART	-	-	0.50	1.23 (0.64-2.44)
Yes	22	26	-	-
No	52	77	-	-
Unknown	10	30	-	-

[a] Data are presented in Mean ± SD or No. (%).
[b] Abbreviations: BMI, Body Mass Index.

Table 2. Comparison Between Hypogonadal and Eugonadal Subjects Based on Free Testosterone [a]

Variables	Hypogonadal (n=149)	Eugonadal (n=73)	P-value	OR (95%CI)
Age, y	38.3 ± 6.9	35.9 ± 6.5	0.016	1.05 (1.010-1.107)
20-29	9 (6)	8 (11)	-	-
30-39	78 (52.3)	42 (57.5)	-	-
40-49	38 (25.5)	18 (24.7)	-	-
50-60	13 (8.7)	2 (2.7)	-	-
BMI, kg/m^2	20.6 ± 2.8	22.2 ± 4.4	0.009	0.87 (0.79-0.96)
< 18.5	24 (16.1)	5 (6.8)	-	-
18.5-24.9	74 (49.7)	42 (57.5)	-	-
25.0-29.9	9 (6)	11 (15.1)	-	-
30 >	-	1 (1.4)	-	-
FSH, IU/L	4.6 ± 3.1	4.8 ± 2.9	0.669	0.98 (0.89-1.07)
LH, IU/L	8.4 ± 7.1	11.7 ± 8.1	0.041	0.96 (0.93-0.99)
PRL, ng/mL	7.5 ± 3.8	6.04 ± 3.04	0.006	1.13 (1. 03 – 1. 23)
HGB	13.2 ± 2.2	14.6 ± 1.9	< 0.0001	0.7 (0.6-0.82)
Smoking	-	-	0.20	0.68 (0.38-1.2)
Yes	124	54	-	-
No	19	19	-	-
Unknown	6	0	-	-
Methadone	-	-	0.042	1.74 (0.97-3.1)
Yes	79	24	-	-
No	69	48	-	-
Unknown	1	1	-	-
Hepatitis	-	-	0.24	1.9 (0.63-6.0)
Negative	5	5	-	-
Positive	110	51	-	-
Unknown	34	17	-	-
AIDS	-	-	0.40	0.75 (0.38-1.4)
Yes	36	21		
No	82	36	-	-
Unknown	31	16		
HAART	-	-	0.39	1.34 (0.67-2.67)
Yes	31	18	-	-
No	95	41	-	-
Unknown	23	14	-	-

[a] Data are presented in Mean ± SD or No. (%).

According to Tables 1 and 2, BMI, LH, and HGB were related to hypogonadism for both TT and FT. Nevertheless, a significant association was only observed between FT and age, as well as methadone. Furthermore, increasing age and lower body mass index were associated with hypogonadism. By increasing one unit of LH and HGB, a protective effect can be seen, which means that anemia, or lower HGB and lower LH levels, can cause hypogonadism. On the other hand, increasing the PRL serum level could induce hypogonadism. According to levels of free testosterone, the patients who received methadone maintenance therapy might have been affected by hypogonadism. There was no relationship between hypogonadism and HAART, smoking, hepatitis, and AIDS.

5. Discussion

In the present study, the prevalence of hypogonadism

among people with an HIV infection was high based on FT and TT. This difference could be due to abnormalities of the testosterone binding to sex hormone binding globulin (SHBG). Assessing the androgen levels in HIV-infected patients is a much more reliable method than measuring levels of (FT), which is not bound to SHBG. In fact, higher SHBG levels in HIV-positive patients may cause an increase in (TT) levels, but not (FT) (3, 4), however, the most widely accepted parameters to establish the presence of hypogonadism is the measurement of serum TT (20). On the other hand, some studies have proposed that FT is the preferred method for the detection of hypogonadism (19). We found BMI, LH, PRL and HGB to be the most important risk factors for inducing hypogonadism based on total testosterone, in addition to these factors, age and methadone can decrease (FT) and cause hypogonadism.

The results showed that as age increased, the total serum testosterone concentration decreased slightly; similarly, the results of one study has showed that serum free testosterone falls more rapidly in older patients and patients who are at risk of HIV infection also had low androgen levels as well. Similar to these studies, our study revealed a relationship between increasing age and hypogonadism based on FT. Although the mean age of the participants was 37.4 + 7.4 years, the risk of hypogonadism increased by 0.05% per year with increasing age, although age was not considered as one of the risk factors for inducing hypogonadism based on a TT assessment.

Similar to another study, the present study also revealed a correlation between BMI and hypogonadism (21); since for each unit increase in BMI, the probability of getting hypogonadism was reduced by 13%, this means that weight loss can cause hypogonadism, therefore appropriate diets for these patients should be take into consideration.

Although the MMT program in HIV-seropositive IDUs is associated with harm reduction and health promotion behaviors, several studies have suggested that methadone can cause hypogonadism (5, 22). Methadone shares the ability to stimulate prolactine release along with phenothiazine and butyrophenone narcoleptics. Although approximately half of the patients were chronic methadone users, only 3.5% of the patients with hyperprolactinemia used it. As a consequence, methadone may contribute to secondary hypogonadism. In this study, the patients who used methadone were 74% more likely to have hypogonadism, rather than hyperprolactinemia. More studies should be conducted in order to determine the unknown effects of methadone on hypogonadism. However, the patients who are assigned to HIV harm reduction programs are less likely to use illicit opiates and more likely to adhere to antiretroviral medications during their treatment. As a consequence, they have lower addiction severity scores and are less likely to engage in high risk behaviors. Therefore, one of the most important reasons for applying hormonal evaluation in surveillance programs for these patients could be the distinctive use of MMT in HIV patients in Iran.

Before the introduction of HAART, patients with HIV were commonly diagnosed with hypogonadism. Today, data continue to reveal an increased prevalence of hypogonadism among patients with HIV, although the rates are not as high since the introduction of HAART (1). A protective effect of HAART was not found in this study.

According to a previous study, about one out of three participants with HIV suffer from chronic hepatitis C (23). In this study, more than two thirds of the HIV-positive men had a HCV infection. This might be due to the fact that HCV-HIV co-infection is high according to some studies that have been carried out in Iran (24, 25). In general, patients with cirrhosis can develop hypogonadism (26); however, the study findings showed no significant relationship between chronic hepatitis B and C, without cirrhosis and hypogonadism. For that reason, according to the results of this study, chronic hepatitis without cirrhosis is not an acceptable reason for routine testing of testosterone.

Tobacco use has been associated with a lower risk of hypogonadism (27). Nevertheless, due to the fact that the patients of the current study used cigarette along with methadone, the protective effects of cigarette could not be detected.

Some studies have revealed that hyperprolactinemia frequently induces hypogonadism in men (28). In our study, 5.9% of HIV-positive patients had hyperprolactinemia. We analyzed prolactine separately and the results suggest that hypogonadism has a significant relationship with hyperprolactinemia; with each unit increase in serum prolactine, the probability of hypogonadism decreased by 13%.

The results of another study suggest that hemoglobin increases significantly in a linear, dose-dependent manner in both younger and older men in response to graded doses of testosterone (29). In this study, with each unit increase in Hb, a 0.3% decrease was observed in the probability of hypogonadism. Regarding the elevation of FSH and LH in hypogonadism patients, the results showed a significant difference in eugonadal and hypogonadal patients based on LH.

In this study, the participants were selected from VCT and MMT, the only two places where HIV/AIDS patients are referred in order to use appropriate services, but obviously the sample cannot be representative of the entire HIV/AIDS patients. Since the highest HIV-seropositive patients are likely to be taking medication, including antidepressants, the possible drug interactions that may arise, such as their effects on sexual function and sexual hormones, should be taken into consideration. Another limitation in this study is the missing data for some variables, like HAART or AIDS status, and patients' methadone use. Consequently, this would have affected some of the study's results, and we suggest that future randomized clinical trials would pro-

vide better investigations of these risk factors. In Iran, to the best of our knowledge, hypogonadism and its associated factors in males with an HIV infection has not been previously investigated. This study was the first study to compare two scales for detecting hypogonadism, and we also compared some risk factors in hypogonadal and eugonadal patients using two different methods. Moreover, we proposed that sexual dysfunction should be considered as one of the most important factors of HIV/AIDS and that may be beneficial in enhancing HIV/AIDS clinical care programs. In particular, we hope our study encourages other researchers to determine guidelines for best clinical care programs and to find essential aspects of care for the wellbeing of people with an HIV infection.

In this study, we tried to find the relationships between hypogonadism and related risk factors, and the results showed that they differed based on the type of testosterone measure used. One of the most important findings of this study was the high prevalence of hypogonadism among HIV-positive men. The implementation of harm reduction programs in Iran has set the stage for a large number of HIV positive patients to become prone to hypogonadism. Hence, it is recommended that hypogonadism examination is included in surveillance programs; in addition, testosterone replacement therapy could be performed for HIV-positive men.

Acknowledgements

We greatly appreciate the efforts of the patients who participated and our colleagues at the VCT and MMT centers who supported our research.

Authors' Contribution

All authors have participated equally in this study.

Financial Disclosure

The authors declare no conflict of interest.

Funding/Support

The work was funded by the Vice-Chancellery for Research, Shiraz University of Medical Sciences (grant No: 87-4527).

References

1. Crum NF, Furtek KJ, Olson PE, Amling CL, Wallace MR. A review of hypogonadism and erectile dysfunction among HIV-infected men during the pre- and post-HAART eras: diagnosis, pathogenesis, and management. *AIDS Patient Care STDS.* 2005;**19**(10):655–71.
2. Petak SM, Baskin HJ, Bergman DA, Dickey RA, Nankin HA. AACE clinical practice guidelines for the evaluation and treatment of hypogonadism in adult male patients. *Endocr Pract.* 1996;**8**(6):440–53.
3. Dobs A. Role of testosterone in maintaining lean body mass and bone density in HIV-infected patients. *Int J Impot Res.* 2003;**15 Suppl 4**:S21–5.
4. Dobs AS, Dempsey MA, Ladenson PW, Frank PB. Endocrine disorders in men infected with human immunodeficiency virus. *The American Journal of Medicine.* 1988;**84**(3):611–6.
5. Klein RS, Lo Y, Santoro N, Dobs AS. Androgen levels in older men who have or who are at risk of acquiring HIV infection. *Clin Infect Dis.* 2005;**41**(12):1794–803.
6. Roberson DW, Kosko DA. Men living with HIV and experiencing sexual dysfunction: an analysis of treatment options. *J Assoc Nurses AIDS Care.* 2013;**24**(1 Suppl):S135–45.
7. Blick G. Optimal Diagnostic Measures and Thresholds for Hypogonadism in Men With HIV/AIDS: Comparison Between 2 Transdermal Testosterone Replacement Therapy Gels. *Postgraduate Medicine.* 2013;**125**(2):30–9.
8. Engelson ES, Rabkin JG, Rabkin R, Kotler DP. Effects of Testosterone upon Body Composition. *Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology.* 1996;**11**(5):510.
9. Grinspoon SK, Bilezikian JP. HIV disease and the endocrine system. *N Engl J Med.* 1992;**327**(19):1360–5.
10. Rietschel P, Corcoran C, Stanley T, Basgoz N, Klibanski A, Grinspoon S. Prevalence of hypogonadism among men with weight loss related to human immunodeficiency virus infection who were receiving highly active antiretroviral therapy. *Clin Infect Dis.* 2000;**31**(5):1240–4.
11. Perez I, Moreno T, Navarro F, Santos J, Palacios R. Prevalence and factors associated with erectile dysfunction in a cohort of HIV-infected patients. *Int J STD AIDS.* 2013;**24**(9):712–5.
12. Amini Lari M, Parsa N, Marzban M, Shams M, Faramarzi H. Depression, Testosterone concentration, sexual dysfunction and methadone use among men with hypogonadism and HIV Infection. *AIDS Behav.* 2012;**16**(8):2236–43.
13. Laudat A, Blum L, Guechot J, Picard O, Cabane J, Imbert JC, et al. Changes in systemic gonadal and adrenal steroids in asymptomatic human immunodeficiency virus-infected men: relationship with the CD4 cell counts. *Eur J Endocrinol.* 1995;**133**(4):418–24.
14. Jain N, Mittal M, Tripathi A, Verma S, Dandu H, Gutch M. An observational study of endocrine disorders in HIV infected patients from north India. *Journal of HIV and Human Reproduction.* 2013;**1**(1):20.
15. Radhakutty A, Wittert GT. Hypogonadism in men: how to evaluate and when to trea. *MODeRn MeDICIn.* 2013;**38**(6):47–50.
16. Collazos J, Esteban M. Has prolactin a role in the hypogonadal status of HIV-infected patients? *J Int Assoc Physicians AIDS Care (Chic).* 2009;**8**(1):43–6.
17. Cotter AG, Powderly WG. Endocrine complications of human immunodeficiency virus infection: hypogonadism, bone disease and tenofovir-related toxicity. *Best Pract Res Clin Endocrinol Metab.* 2011;**25**(3):501–15.
18. *Iranian Ministry of Health. AIDS epidemic.* 2013. Available from: http://aids.behdasht.gov.ir/index.aspx?siteid=328&pageid=50511.
19. Carnegie C. Diagnosis of hypogonadism: clinical assessments and laboratory tests. *Rev Urol.* 2004;**6 Suppl 6**:S3–8.
20. Moreno-Perez O, Escoin C, Serna-Candel C, Portilla J, Boix V, Alfayate R, et al. The determination of total testosterone and free testosterone (RIA) are not applicable to the evaluation of gonadal function in HIV-infected males. *J Sex Med.* 2010;**7**(8):2873–83.
21. Saad F, Haider A, Doros G, Traish A. Long-term treatment of hypogonadal men with testosterone produces substantial and sustained weight loss. *Obesity (Silver Spring).* 2013;**21**(10):1975–81.
22. de la Rosa RE, Hennessey JV. Hypogonadism and methadone: Hypothalamic hypogonadism after long-term use of high-dose methadone. *Endocr Pract.* 1996;**2**(1):4–7.
23. Sherman KE, Rouster SD, Chung RT, Rajicic N. Hepatitis C Virus prevalence among patients infected with Human Immunodeficiency Virus: a cross-sectional analysis of the US adult AIDS Clinical Trials Group. *Clin Infect Dis.* 2002;**34**(6):831–7.
24. Alavian SM, Adibi P, Zali MR. Hepatitis C virus in Iran: Epidemiology of an emerging infection. *Arch Iranian Med.* 2005;**8**(2):84–90.
25. Khani M, Vakili MM. Prevalence and risk factors of HIV, hepatitis B virus and hepatitis C virus infections in drug addicts among Zanjan prisoners. *Arch Iranian Med.* 2003;**6**(1):1–4.
26. Van Thiel DH, Lester R, Sherins RJ. Hypogonadism in alcoholic liver disease: evidence for a double defect. *Gastroenterology.*

1974;**67**(6):1188–99.
27. Crum-Cianflone NF, Bavaro M, Hale B, Amling C, Truett A, Brandt C, et al. Erectile dysfunction and hypogonadism among men with HIV. *AIDS Patient Care STDS.* 2007;**21**(1):9–19.
28. Carter JN, Tyson JE, Tolis G, Van Vliet S, Faiman C, Friesen HG. Prolactin-screening tumors and hypogonadism in 22 men. *N Engl J Med.* 1978;**299**(16):847–52.
29. Coviello AD, Kaplan B, Lakshman KM, Chen T, Singh AB, Bhasin S. Effects of graded doses of testosterone on erythropoiesis in healthy young and older men. *J Clin Endocrinol Metab.* 2008;**93**(3):914–9.