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Assessment of Sex Hormones and Gonadotropin Levels in Alzheimer Patients

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Abstract

Backgrounds: Increasing age is the most significant risk factor for Alzheimer's disease and depletion of sex hormones is an important consequence of normal aging. This study aimed to investigate the serum level of sex hormones and gonadotropins in patients with Alzheimer's disease in comparison with the control group. **Materials and Methods:** This case-control study was conducted between October 2010 and November 2011 in Shiraz Mottahari Clinic. Fifty-one patients with Alzheimer's disease and 49 age-matched volunteers without dementia participated in this survey. Both groups were evaluated by two neurologists according to DSM- IV criteria. Blood samples were taken after 12 hours fasting to measure serum levels of estradiol, testosterone, gonadotropins and sex hormone binding globulin (SHBG). **Results:** Eighteen females and 33 males in the patient group, and 23 females and 26 males in the control group participated. There were no significant differences between the two groups regarding their gonadotropins, estradiol, free androgen index and body mass index, but the mean level of SHBG in patients was significantly higher than the control group (P=0.03). In addition, male patients had a higher total testosterone mean compared to male subjects in the control group (P=0.02). **Conclusion:** Our findings regarding testosterone levels in males of two groups were contrary to some of the previous surveys in this area. Moreover, we found higher levels of SHBG in patients compared to the control subjects. Further investigation is needed to define whether and how changes of sex hormones can affect brain health and vulnerability to Alzheimer's disease. **[GMJ.2015;4(4):139-45]**

Keywords: Alzheimer's Disease;Sex Hormones;Gonadotropins;Sex Hormone Binding Globulin

Introduction

Alzheimer's disease (AD), as the most common form of dementia, is a progressive neurologic disorder that results in memory loss, global cognitive dysfunction and functional impairment. Costs arising from the consequences of this disease in the United States have totaled $ 100 billion in year that will undoubtedly increase until 2050 [1, 2]. While AD pathogenesis has been linked to oxidative stress, inflammation and neuronal dysfunction, none of them alone can explain the wide spectrum of abnormalities in these patients. Since female gender has been proposed

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as a risk factor for AD, a couple of hypotheses have been suggested regarding the effect of sex hormones on cognition in the elderly men and women [3, 4].

Although several studies have been conducted to date in this area, we still do not know what effects sex hormones and aging will have on cognitive functions. For example, Geerlings and colleagues in their study showed that in postmenopausal women who did not receive hormone replacement therapy (HRT), there were higher levels of estradiol with a higher risk of dementia [5]. Furthermore, the results of the Women's Health Initiative Memory Study (WHIMS) showed that treatment with sex hormones did not reduce the rate of progression of cognitive decline in women with dementia and in fact it even increased its risk [6]. Moreover, the surveys conducted in men with Alzheimer also revealed that androgens may play an important role in the pathogenesis of AD [7-9].

On the other hand, loss of sex steroids leads to increased gonadotropin levels following menopause or andropause. Mounting studies in humans and in-vitro surveys demonstrated luteinizing hormone (LH) receptors in brain and particularly hippocampus region. Additionally, increased levels of LH in the neurons of patients who are in the first stages of the disease can predict the risk of neuronal degeneration and their death. Thus, it has been suggested that LH may have an important role in cognitive function as well [10-13]. Interestingly, an alternate hypothesis that may explain the lack of efficacy of HRT in older post-menopausal women is the inability of HRT to provide efficient negative feedback on gonadotropins and especially on LH [4, 14, 15].

In this regard, separating the roles of sex hormones and gonadotropins on cognition is important to understand the impact of hypothalamo-pituitary-gonadal (HPG) axis dysfunction on cognitive decline in AD.

This study aimed to investigate whether there are differences in the serum sex hormones and gonadotropins levels between patients with Alzheimer's disease and cognitively normal controls.

Materials and Methods

Sample Size Calculation

In primary analyses on 10 individuals (five from each group) and based on the difference in the mean levels of the two groups (diff=5.7) and their shared standard deviation (SD=10) at the error level of alpha=0.05 and power=80%, the number of participants was determined as 49 in each group.

Subjects

Between October 2010 and November 2011, fifty one patients and 49 volunteers aged 55 years or older were included in this study as patient and control groups, respectively. They were evaluated by two neurologists according to DSM- IV criteria (the Fourth edition of diagnostic and statistical manual of mental disorders) and diagnosed as having Alzheimer's disease or not demented. The subjects in both groups referred consecutively to senior investigator of the study in Mottahari Clinic affiliated to Shiraz University of Medical Sciences. Liver and renal function tests, serum electrolyte levels, Wright, 2- mercaptoethanol (2-ME) and thyroid function tests were requested for all the participants before enrollment in the study. All patients underwent a brain computed tomography (CT) or magnetic resonance imaging (MRI).

Inclusion criteria were diagnosis of Alzheimer's disease according to DSM-IV criteria and age of 55 years or more. Exclusion criteria were a history of gastric surgery, neoplastic disorders, thyroid dysfunction, removal of the gonads (ovaries in women and testes in men), liver and kidney diseases or using medications with potential effects on sex hormone or gonadotropin levels (such as sex hormones, steroids, thyroid hormones and anticonvulsants), and evidence of multi-lacunar infarction, normal pressure hydrocephaly, brain mass, subdural hematoma or any gross pathological findings in neuroimaging except for cortical and hippocampal atrophies.

Data Collection

Venous blood samples were collected in the early morning after an overnight fasting.

Serum separation was performed within one hour of venipuncture and kept frozen at -70°C until assayed. For all subjects, height and weight (wearing light clothing without shoes) were measured and body mass index (BMI) was calculated as body weight in kilogram divided by the height in square meter.

Hormonal assays included: serum follicle-stimulating hormone (FSH), LH, total testosterone (T), estradiol and sex hormone binding globulin (SHBG). The free androgen index (FAI) was calculated as 100× (Total testosterone/ SHBG). All hormonal assays were performed in Endocrinology and Metabolism Research Center of Namazi Hospital. Serum FSH ((FSH-IRMA, BioSource, Europe S.A., Nivelles Belgium), LH (LH-IRMA, BioSource, Europe S.A., Nivelles Belgium) and SHBG (SHBG-IRMA, IMMNOTECH, Czech Republic) were measured with immunoradiometric assay.Serum testosterone (RIA-Testosterone, direct IMMUNOTECH, France) and estradiol (RIA-Estradiol IMMUNOTECH, France) were measured with radioimmunoassay. The intra- and inter-assay coefficients of variation were 1.5-2.7% and 2-5.3% for FSH, 1-5% and 3.3-5.7% for LH, ≤14.8% and ≤ 15% for testosterone, ≤12.1% and ≤11.2% for estradiol, 3.8% and 7% for SHBG, respectively.

Ethical Considerations

The review board and ethics committee of Shiraz University of Medical Sciences approved the study protocol, and informed consents were taken from all participants.

Statistical Analysis

The data were analyzed using SPSS software version 16. Mean values for age, BMI and blood levels of the variables were compared among patients and controls using independent t-test and their correlation by Pearson Chi-square test. In addition, where desired variable did not have normal distribution, the Mann-Whitney test was used. P≤ 0.05 was considered as statistically significant.

Results

In this case-control study, 51 patients with Alzheimer's disease (18 women and 33 men) and 49 control individuals (23 women and 26 men) were enrolled. The mean age of the patients and controls was 73.05±8.17 and 72.59±7.96 years, respectively. There was no statistically significant difference between both groups regarding their age (P=0.1) and BMI (P=0.1). Demographic characteristics of both groups are presented in Table 1.

The mean level of total testosterone in females of both groups did not reveal a statistically significant difference (P= 0.3), but the mean testosterone levels in the men of the patient group were significantly higher than those in the control group (P= 0.02)Also, estradiol, LH, FSH and FAI means in males and females of both groups did not differ significantly (Tables 2 and 3).Serum levels of SHBG in 16 females and 33 males of the patient group and 19 females and 22 males in the control group were measured. The mean of the control group was 64.52 ± 28.44 nmol/L (median=73.50) and in patient group it was 77.49± 28.90 nmol/L (median=78.50). SHBG mean value was significantly higher in patients compared with the control group (P= 0.03). Meanwhile, the mean levels of FAI between males and females in both groups showed no significant differences.

Table 1. Demographic Characteristics of Patient and Control Groups

Variables	Control Group	Patient Group	P Value
Female	23	18	0.1
Male	26	33	
Age (year)	72.59±7.96	73.05±8.17	0.1
BMI(Kg/m²)	24.16±4.57	22.81±4.08	0.1

Measurements are expressed as mean ± SD, P≤ 0.05 is significant.
BMI: Body Mass Index

Table 2. Total Testosterone, Free Testosterone, Estradiol and Gonadotropins Mean in Control and Patient Females

	Control Females	Patient Females	P Value
Total Testosterone (ng/ml)	0.45±0.36	0.35±0.17	0.3
Estradiol (pg/ml)	21.13±20.58	22.87±17.17	0.7
FSH (mIU/ml)	31.43±10.23	18.12±8.76	0.8
LH (mIU/ml)	26.54±16.98(20.70)	30.52±16.70(30.35)	0.4
FAI(range)	1.50±1.20	0.52±0.24	0.07

FSH: Follicle Stimulating Hormone, LH: Luteinizing Hormone, FAI: Free Androgen Index

Table 3. Total Testosterone, Free Testosterone, Estradiol and Gonadotropins Mean in Control and Patient Males

	Control Males	Patient Males	P Value
Total Testosterone(ng/ml)	5.48±3.12	7.02±2.81	0.02
Estradiol(pg/ml)	37.76±32.81	49.60±9.12	0.1
FSH(mIU/ml)	18.06±10.15	17.88±8.84	0.9
LH(mIU/ml)	9.11±7.50(7.40)	11.64±9.46(7.90)	0.2
FAI (range)	9.30±2.61	10.22±4.67	0.1

FSH: Follicle Stimulating Hormone, LH: Luteinizing Hormone, FAI: Free Androgen Index
FSH: Follicle Stimulating Hormone, LH: Luteinizing Hormone, FAI: Free Androgen Index

Discussion

In this study, although the serum levels of gonadotropins and FAI showed no statistically significant difference in both groups, the total testosterone in male patients revealed significantly greater values than cognitively normal men. Also, people with Alzheimer's disease had higher amounts of SHBG compared with the control group.

Moreover, in our study, there was no significant difference between women and men of both groups regarding their estrogen levels, while several studies have already been carried out and conflicting results regarding the effects of estrogen in Alzheimer's disease have been proposed [5, 6, 16-18].

Our finding of higher total testosterone in male patients was inconsistent with some results of previous studies in this field, which demonstrated an inverse relationship between testosterone levels and the risk of AD [7-9]. Among studies consistent with ours was Pennanen's study which showed higher total and free testosterone levels in AD patients; however, in our study, free androgen index level was not significantly different in both groups [19]. Almeida *et al*. also reported higher levels of FAI in those with AD, although the difference was not statistically significant [20]. Moreover, in another study the authors found that testosterone levels were only higher in the controls who were apolipoprotein E allele epsilon4 carriers [21].

One of the reasons proposed to explain lower testosterone levels in prior reports was dysfunction of HPG axis which naturally occurs during aging [4, 7]. Some studies have also stated that testosterone decline increases the amyloid serum level and accelerates its depo-

sition in the brain and disrupts behavioral performance of hippocampus [8-10]. Meanwhile, levels of LH and FSH have been shown to be significantly increased in AD patients compared to controls in some but not all studies and in a recent study, LH levels were correlated with amyloid-β levels [10,11]. Therefore, it has been suggested that increased levels of gonadotropins in the process of aging can be associated with the development and progression of AD [7, 12-14]. In our investigation, there was no significant difference regarding the levels of gonadotropins in men and women of both groups. This finding, in addition to higher testosterone levels in our male patients, provides no support for hypogonadism as an important mechanism to be considered in the pathogenesis of AD or is at least somehow reflective of an underlying pathological process. We assumed at least 3 reasons to explain our findings. First, the feedback mechanisms regulating hormone levels may be dysfunctional. On the other hand, the feedback mechanisms may be fully functional, but the brain is trying to resist AD by increasing the testosterone level via enhancing its production. It is also possible that the activity of aromatase enzyme which converts testosterone to estrogen has been increased in the brain and peripheral tissues of AD patients. Consistent with this idea, are genetic studies that suggest CYP19 gene which encodes aromatase enzyme has functional alterations leading to changed expression or activity of this enzyme in AD. In addition, it has been shown that brain injury in rats rapidly up-regulates aromatase enzyme expression in glial cells at the injury site, suggesting that aromatase may exert neuro-protective effects through increased local estrogen levels [22, 23]. In this regard Twist *et al*., in a study on brain estradiol and testosterone levels in AD patients, showed that males with AD had higher brain estradiol levels [24]. Therefore, if serum levels of testosterone have any influence on its brain levels, then more testosterone is converted to estrogen and the feedback mechanisms merely try to increase the production of testosterone to meet the demand.As was stated earlier in our study, sex hormone binding globulin (SHBG) level in patients was significantly higher than that of the control group and since a part of the hormone that is not attached to globulin is active, SHBG may be the main factor controlling the balance between active and inactive forms of sex hormones. Indeed, increased levels of SHBG have been linked to an increased risk of dementia in both men and women with AD [7, 25, 26]. Experimental studies have shown that SHBG is also produced in brain and is needed to enable some steroids. Additionally, it has been suggested that in Alzheimer's patients, there are probably defects in regulating the secretion and production of SHBG [27]. Thus, another possibility is that the difference in SHBG levels by changing the balance of free testosterone levels causes conflicting results about the relationship between hormones and dementia [28]. Finally, it seems important that the impact of increased SHBG levels as a confounding factor, which binds to sex steroids be considered for determining the efficacy of sex hormone replacement treatment.

Conclusion

This study provided no support for hypothesis of disproportionally decreased levels of testosterone in AD. Also, we recommend that aspects thought to be secondary players such as SHBG and aromatase which regulate bioavailability or production of steroids may potentially have a direct impact on cognition. In this regard, further research conducted on elucidating the specific role of these secondary players is particularly important when interpreting the results of clinical trials using sex steroid replacement.

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Conflict of Interest

The authors declare that they have no competing interests for this study.

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