The relationship between serum sex steroid levels and heart rate variability parameters in males and the effect of age

Erkeklerde serum cinsiyet steroidleri ile kalp hızı değişkenliği verileri arasındaki ilişki ve yaşın etkileri

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Objectives: We evaluated the relationships between sex steroid levels and heart rate variability (HRV) parameters.

Study design: The study included 114 male subjects (mean age 46.6±11.3 years) presenting to our department for cardiologic evaluation. Hormonal analysis included serum levels of luteinizing hormone, prolactin, total testosterone (TT), free testosterone, estradiol (E2), and dehydroepiandrosterone sulfate (DHEA-S). Parameters of HRV were derived from 24-hour Holter monitoring. The associations between serum sex steroid levels and HRV parameters were investigated in three age groups (20-39 years; 40-59 years; >60 years).

Results: All the participants had normal biochemical results. The three age groups were similar in terms of anthropometric measurements. Among sex steroids analyzed, only serum DHEA-S level was significantly different among the groups (p=0.026), showing a decreasing trend with age. In the evaluation of HRV, all parasympathetic activities decreased (for HF_n, pNN50, and rMSDD: p=0.001, p=0.000, and p=0.000, respectively), while only LF/HF among sympathetic activities increased (p=0.000) with age. Partial correlation analysis with control of age and waist circumference showed that TT and DHEA-S were positively correlated with HF_n (parasympathetic parameter), and were in negative correlation with $LF/HF_{24\ hours}$ and global sympathetic index (GSI) (sympathetic parameters). Serum E2 level was negatively correlated with the parasympathetic parameter of rMSSD, and positively correlated with $LF/HF_{24\ hours}$ and GSI. Among serum sex steroids, DHEA-S was the most correlated parameter with autonomic functions.

Conclusion: Our results showed positive correlations between androgens and parasympathetic activity and between estradiol and sympathetic activity in men, independent from anthropometric factors.

Key words: Androgens; dehydroepiandrosterone; electrocardiography, ambulatory; estradiol; gonadal steroid hormones; heart rate; male; testosterone.

Amaç: Bu çalışmada cinsiyet steroidleri ile kalp hızı değişkenliği (KHD) verileri arasındaki ilişkiler araştırıldı.

Çalışma planı: Çalışmaya, kardiyolojik açıdan değerlendirme için başvuran 114 erkek hasta (ort. yaş 46.6±11.3) alındı. Hormon analizlerinde serumda luteinize edici hormon, prolaktin, total testosteron (TT), serbest testosteron, östradiol (E2) ve dehidroepiandrosteron sülfat (DHEA-S) düzeyleri ölçüldü. Yirmi dört saatlik Holter kayıtlarından KHD parametreleri hesaplandı. Serum cinsiyet steroidleri ile KHD değerleri arasındaki ilişkiler hastalar üç yaş grubuna (20-39 yaş; 40-59 yaş; >60 yaş) ayrılarak incelendi.

Bulgular: Biyokimyasal sonuçlar katılımcıların tümünde normal bulundu. Antropometrik ölçümler açısından üç yaş grubu benzerlik gösterdi. İncelenen cinsiyet steroidleri içinde sadece serum DHEA-S düzeyi üç yaş grubu arasında anlamlı farklılık göstererek (p=0.026) yaşla azalma eğilimi sergiledi. Kalp hızı değişkenliği değerlendirmesinde, parasempatik aktiviteyi gösteren tüm veriler yaşla anlamlı düşüş gösterirken (HF_n, pNN50 ve rMSDD için sırasıyla p=0.001, p=0.000 ve p=0.000), sempatik aktivite göstergeleri arasında sadece LF/HF oranı yaşla artış gösterdi (p=0.000). Yaş ve bel çevresi ayarlı kısmi korelasyon analizinde, TT ve DHEA-S parasempatik aktivite göstergelerinden HF_n ile pozitif ilişkili; sempatik aktivite göstergelerinden $LF/HF_{24\ saat}$ ve global sempatik indeks (GSİ) ile negatif ilişkili bulundu. Serum E2 ise parasempatik parametre olan rMSDD ile negatif, $LF/HF_{24\ saat}$ ve GSİ ile pozitif ilişki gösterdi. Cinsiyet steroidleri arasında otonomik fonksiyonlarla en ileri ilişkiyi gösteren DHEA-S idi.

Sonuç: Çalışmamızın bulguları, antropometrik faktörlerden bağımsız olarak, erkeklerde androjenlerin parasempatik aktivite ile, östradiolün ise sempatik aktivite ile ilişkili olduğunu göstermektedir.

Anahtar sözcükler: Androjen; dehidroepiandrosteron; elektrokardiyografi, ambulatuvar; östradiol; gonodal steroid hormonu; kalp hızı; erkek; testosteron.

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Heart rate variability (HRV) and its computed components are noninvasive, reliable, and popular indicators for assessing the activities of the autonomic nervous system and are used for indirect evaluation of autonomic functions.[1-7] Recent studies have shown that factors such as gender, sex steroid levels, age, and anthropometric features may also influence autonomic functions.[1-6] The relationship between serum sex steroid levels and autonomic functions in both genders has become an attractive area of research.

Testosterone regulates sexual maturation in fetal and pubertal periods, and determines the sexual characteristics and function in adulthood.[8,9] There are few studies in the literature regarding the effect of sex steroids levels on autonomic function and HRV. The effect of testosterone on the autonomic neural ganglion, particularly on the control of perineal reflexes and nerve transduction pathways has been described in recent years, and providing evidence that testosterone affects parasympathetic responses and facilitates the baroreflex response in autonomic function.[10-13] These findings indicate that serum sex steroids, especially testosterone, have effects not only on sexual differentiation, but also on autonomic functions of the cardiovascular system.

Animal studies have shown that serum estradiol level also influences autonomic functions.[14,15] In human studies, peripheral fat tissue, serum leptin level, and testosterone/estradiol ratio have been shown to affect sympathovagal tonus in males.[16-18] These findings suggest a primary and/or a secondary role for sex steroids in autonomic function.

The aim of the present study was to investigate the effect of age on the relationship between sex steroid levels and autonomic functions. For this purpose, we utilized HRV as an indicator of autonomic functions.

PATIENTS AND METHODS

Patients. A total of 114 male patients aged between 20 and 79 years (mean 46.6±11.3 years) were enrolled into the study. All patients were admitted to the Cardiology Department for general health examination and were evaluated with a detailed history and physical examination. Height, weight, waist, and hip measurements were made; body mass index (BMI) and waist/hip ratio were calculated, and arterial pressures were recorded. The study was approved by the local ethics committee. Detailed information was given to all the patients, and written informed consent was obtained from all the participants.

Exclusion criteria included the following clinical or laboratory features: cardiac disease such as congenital heart disease, left ventricle hypertrophy or dilatation, cardiac arrhythmias or conduction problems; systemic diseases such as diabetes mellitus and hypertension, neurological problems, psychiatric diseases, mixed connective tissue disorders, endocrinopathy such as thyroid pathology and hypogonadism; abnormal liver and renal function tests, hyperlipidemia, smoking or alcohol use, use of medications for chronic disorders, and obesity (BMI >28 kg/m^2).

The patients were divided into three age groups as follows: 20-39 years (group 1), 40-59 years (group 2), and >60 years (group 3). There were 43 patients in group 1 (mean age 33.9±4.9 years), 46 patients in group 2 (mean age 47.8±3.2 years), and 25 patients in group 3 (mean age 66.6±4.5 years).

Biochemical and hormonal analysis. Laboratory work-up involved a detailed biochemical analysis (SMA-24) and complete blood count. Additionally, luteinizing hormone, prolactin, total testosterone (TT), free testosterone (FT), estradiol (E2), and dehydroepiandrosterone sulfate (DHEA-S) were assayed. Non-fasting blood samples were drawn between 09.00 and 10.00 hours. Serum hormone levels were determined using the electrochemiluminescence immunoassay with the Roche Elecys 2010 immunoassay analyzer and Roche kit (Roche Diagnostics, Indianapolis, Indiana, USA).

Cardiologic evaluations. Patients were taken to a silent and quiet test room at 20°C and were allowed to rest for 15 minutes in the supine position at the beginning of the test. Then, arterial blood pressure was measured and 12-channel ECG recordings were obtained. Additionally, all the patients were evaluated with posteroanterior chest radiography, exercise stress test (Quinton 4500 treadmill, Seattle, Washington, USA), and color Doppler echocardiography (GE Vivid 7 Pro, General Electric, Florida, USA). Finally, a Holter device was affixed and the starting time was set to record second sensitivity.

Measurement of 24-hour HRV. Heart rate variability parameters were derived from the recordings of 24-hour Holter monitoring and analyzed with the Del Mar-Impresario System (Del Mar-Impresario Medical Systems, Irvine, CA, USA). Data were evaluated using the HRV standards recommended by the Task Force Report in 1996.[7] Time-domain HRV variables included rMSSD (square root of the mean of the sum of the squares of differences between successive RR intervals) and pNN50 (percentage of differences between successive RR intervals that are greater than 50 msec).

Table 1. Clinical characteristics, steroid hormone levels, and autonomic function test results of the three age groups (Mean±SD)

	Group 1 (20-39 years) (n=43)	Group 2 (40-59 years) (n=46)	Group 3 (>60 years) (n=25)	*p**
Clinical findings				
Body mass index (kg/m^2)	25.9±1.7	25.5±1.9	25.9±1.5	0.447
Waist circumference (cm)	95.9±10.3	98.2±10.2	96.6±11.8	0.579
Hip circumference (cm)	91.4±6.7	92.5±7.5	92.2±7.8	0.809
Waist/Hip ratio	1.0±0.1	1.0±0.1	1.0±0.1	0.578
Steroid hormone levels				
Luteinizing hormone (mIU/ml)	4.6±2.0	4.1±1.6	4.5±2.7	0.573
Prolactin (ng/ml)	11.2±5.3	10.4±3.4	12.7±7.3	0.213
Testosterone (ng/ml)	4.7±1.1	4.6±1.5	3.9±1.3	0.076
Free testosterone (pg/ml)	10.2±5.2	9.1±3.5	8.7±4.5	0.370
Estradiol (E2) (pg/ml)	32.6±12.9	33.4±13.8	34.3±16.2	0.887
Total testosterone/E2	0.17±0.10	0.16±0.10	0.15±0.01	0.648
Free testosterone/E2	0.37±0.30	0.33±0.20	0.32±0.20	0.668
Dehydroepiandrosterone-sulfate (μg/ml)	246.3±73.3	217.3±75.9	197.3±71.5	**0.026**
Autonomic function tests				
Parasympathetic activities				
HF_n	221.7±168.2	174.2±162.0	76.8±59.9	**0.001**
pNN50 (%)	10.7±6.3	8.4±7.3	3.7±2.8	**0.000**
rMSSD (msec)	52.1±17.3	40.5±15.9	32.9±7.8	**0.000**
Sympathetic activities				
LF_n	237.3±169.9	279.1±100.3	281.9±152.7	0.326
LF/HF	2.8±2.3	2.6±2.1	5.9±5.6	**0.000**
Global sympathetic index	7.9±4.5	9.2±5.7	9.9±5.3	0.292

p*: One-way ANOVA test; HF_n: Normalized high frequency power; LF_n: Normalized low frequency power; pNN50: Percentage of differences between successive RR intervals that are greater than 50 msec; rMSSD: Square root of the mean of the sum of the squares of differences between successive RR intervals.

Power spectral (frequency) analysis of HRV was also performed using a fast Fourier transform to break down the time series to its underlying periodic function. Total power (TP) was defined as the energy in the heart period power spectrum from 0 to 0.40 Hz frequency. Low frequency (LF) and high frequency (HF) powers were defined as the energy in the heart period power spectrum between 0.04 and 0.15 Hz and 0.15 and 0.40 Hz, respectively. The LF/HF ratio was calculated. Finally, LF and HF were also measured in normalized units, which represent the relative value of each power component in proportion to the total power minus the very low frequency (VLF) component. Normalized LF (LF_n) was calculated as LF power in normalized units [LF/(total power - VLF) x 100], and normalized HF (HF_n) as HF power in normalized units [HF/(total power - VLF) x 100].[7]

As a marker of sympathovagal balance, global sympathetic index (GSI) was calculated with the following formula [(VLF+LF)/HF].[19]

Statistical analysis. All statistical analyses were performed using the SPSS software package, version 11.5. Data were expressed as mean ± standard deviation (SD). One-way ANOVA followed by an adjusted post-hoc Bonferroni test was used for the evaluation of differences in serum sex steroid levels, HRV, and anthropometric values between the three groups. The effect of sex steroid levels on HRV values was assessed using the Pearson correlation test and partial correlation analysis. Additionally, Student's t-test was used to compare normogonadotropic (eugonadotropic) and hypogonadotropic patients. A p value of less than 0.05 was accepted as statistically significant.

RESULTS

All the patients enrolled into the study had normal biochemical results. Body mass index, waist/hip ratio, serum sex steroid levels, and HRV recordings according to age groups are shown in Table 1. There were no significant differences between the three groups in terms of anthropometric features (p>0.05).

There was no significant decrease in serum TT and FT levels with increasing age. Similarly, TT/E2 and FT/E2 ratios did not differ with age. However, serum

Table 2. Correlations between serum sex steroid levels and HRV parameters after controlling the effects of age

	HF_n		LF_n		$LF/HF_{24\ hours}$		$HF_{24\ hours}$		GSI		rMSSD	
	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*
Total testosterone	0.283	**0.040**	-0.346	**0.011**	-0.310	**0.024**	0.382	**0.005**	-0.351	**0.010**	-0.019	0.895
Free testosterone	-0.033	0.850	0.191	0.265	0.052	0.762	-0.059	0.732	-0.103	0.549	0.003	0.985
Estradiol	0.319	0.071	0.293	0.098	0.390	**0.025**	-0.255	0.152	0.347	**0.048**	-0.370	**0.034**
DHEA-sulfate	0.606	**<0.001**	0.258	0.055	-0.556	**<0.001**	0.589	**<0.001**	-0.605	**<0.001**	0.258	0.052

DHEA: Dehydroepiandrosterone; HF_n: Normalized high frequency power; LF_n: Normalized low frequency power; GSI: Global sympathetic index; rMSSD: Square root of the mean of the sum of the squares of differences between successive RR intervals.

DHEA-S level showed a significantly decreasing trend with age (p=0.026), primarily due to the difference between group 1 and group 3 (p=0.029).

In the evaluation of HRV, parasympathetic activities decreased and sympathetic activities increased with age. All parasympathetic activities showed significant decreases with age (for HF_n, pNN50, and rMSDD: p=0.001, p=0.000, and p=0.000, respectively), while only LF/HF among sympathetic activities showed a meaningful increase with age (p=0.000).

Correlation analysis showed positive correlations between parasympathetic parameters and serum TT and FT levels and negative correlations between parasympathetic parameters and E2 level. In contrast, both TT and FT levels were negatively and E2 was positively correlated with sympathetic activities. However, none of these correlations were statistically significant after controlling the effects of age and waist circumference.

We performed partial correlation analysis to determine the correlations between serum sex steroid levels and HRV parameters after controlling the effects of age (Table 2). This analysis showed that TT and DHEA-S were positively correlated with the parasympathetic parameter of HF_n; and for sympathetic activities, TT was in negative correlation with LF_n, $LF/HF_{24\ hours}$, and GSI, while DHEA-S was in negative correlation with $LF/HF_{24\ hours}$ and GSI. There was no correlation between serum FT levels and HRV parameters. Serum E2 level was negatively correlated with the parasympathetic parameter of rMSSD, and positively correlated with the sympathetic parameters of $LF/HF_{24\ hours}$ and GSI. Among serum sex steroid variables, DHEA-S was the most correlated with autonomic functions (Table 2).

Based on the TT levels, we divided the patients into two groups as hypogonadotropic (TT <3.1 ng/ml, n=15) and eugonadotropic (TT ≥3.1 ng/ml, n=99). The two groups significantly differed in terms of age, serum TT and DHEA-S levels, all the parasympathetic activities, and sympathetic parameters of LF/HF and GSI (Table 3).

Correlation analysis for the relationship between sex steroid levels and HRV in the hypogonadotropic group did not show significant correlations with parasympathetic activity. In the eugonadotropic group, E2 level showed positive correlations with GSI (r=0.549, p=0.012) and $LF/HF_{24\ hours}$ (r=0.550, p=0.012), and DHEA-S level showed negative correlations with GSI (r=-0.539, p=0.002) and $LF/HF_{24\ hours}$ (r=-0.532, p=0.002) after controlling the effects of age and waist circumference.

DISCUSSION

In this study, we found that TT and DHEA-S were positively correlated with parasympathetic and negatively correlated with sympathetic activities. While there was no significant association between serum FT level and HRV parameters, serum E2 level was negatively correlated with parasympathetic and positively correlated with sympathetic activities. Among the serum sex steroids, DHEA-S exhibited the most significant correlations with autonomic functions after controlling the effects of age. After controlling the effects of age and waist circumference, there was no correlation between TT, FT, E2 and HRV parameters. When we classified the patients into two groups based on the TT levels, we observed similar results in the normogonadotropic group. Partial correlation analysis showed that E2 and DHEA-S were significantly correlated with autonomic functions in the normogonadotropic group after controlling the effects of age and waist circumference. However, sex steroid levels and HRV parameters were not correlated in the hypogonadotropic group.

There are few clinical studies investigating the relationship between HRV and serum sex steroid levels. Such studies mainly focused on testosterone, reporting not only that testosterone was positively correlated with parasympathetic activity, as in our study, but also it was neuroprotective. In addition to its reproductive effects, testosterone has regulatory effects on neural maturation and function, especially of the pelvic neurons.[11,20,21]

Table 3. Patient characteristics, steroid hormone levels, and autonomic function test results based on the serum total testosterone levels (Mean±SD)

	Hypogonodotropic group (n=15)	Eugonadotropic group (n=99)	*p**
Characteristics			
Age (years)	59.9±10.4	44.5±12.3	**0.000**
Body mass index (kg/m²)	26.2±1.6	25.7±1.7	0.329
Waist/hip ratio	1.0±0.1	1.0±0.1	0.830
Steroid hormone levels			
Luteinizing hormone (mIU/ml)	5.3±3.3	4.2±1.8	0.494
Prolactin (ng/ml)	9.8±3.4	11.5±5.4	0.300
Testosterone (ng/ml)	2.5±0.7	4.8±1.2	**0.000**
Free testosterone (pg/ml)	9.2±4.2	8.5±5.2	0.263
Estradiol (E2) (pg/ml)	34.6±15.4	33.1±13.8	0.703
Total testosterone/E2	0.3±0.2	0.2±0.1	**0.000**
Free testosterone/E2	0.3±0.2	0.3±0.2	0.428
Dehydroepiandrosterone-sulfate (μg/ml)	193.0±78.3	228.5±74.7	**0.045**
Autonomic function tests			
Parasympathetic activities			
HF_n	107.9±116.7	180.3±160.7	**0.035**
pNN50 (%)	3.4±2.8	8.9±6.8	**0.000**
rMSSD (msec)	33.9±11.9	44.6±16.9	**0.019**
Sympathetic activities			
LF_n	306.7±98.3	258.0±156.1	0.152
LF/HF	5.3±5.1	3.3±2.8	**0.026**
Global sympathetic index	12.4±6.1	8.4±4.9	**0.008**

p*: Student's t-test; HF_n: Normalized high frequency power; LF_n: Normalized low frequency power; pNN50: Percentage of differences between successive RR intervals that are greater than 50 msec; rMSSD: Square root of the mean of the sum of the squares of differences between successive RR intervals.

Wranicz et al.[22] showed increased parasympathetic activity in patients with high testosterone levels after acute myocardial infarction (MI), suggesting that testosterone might influence autonomic cardiac functions. Several studies reported an association between higher levels of testosterone and higher levels of HRV parameters reflecting parasympathetic tone.[12,13] It was also assumed that testosterone was a strong supporter of baroreflexes in males. Moreover, serum bio-T levels were found lower in men with coronary artery disease compared to healthy counterparts, suggesting that bio-T levels affected HRV parameters.[23]

In our study, there were positive correlations between serum TT and FT levels and parasympathetic HRV. Furthermore, we observed a higher parasympathetic activity in eugonadotropic patients compared with hypogonadotropic patients. However, after controlling the effects of age and waist circumference, correlations between serum TT and FT levels and HRV parameters disappeared. Although obesity and waist circumference are independent factors determining autonomic functions,[24] we consider that obesity is also a linking factor between serum sex steroids and HRV. Some studies have shown that the level of aromatase activity may be effective on heart rate variability and baroreflex sensitivity,[25] and that aromatase genotype may affect serum E2 levels in males.[26] For this reason, we consider that serum E2 level might be a more stable factor to be in correlation with autonomic functions.

The effect of E2 on autonomic function has not been clarified. There are reports that increasing concentrations of E2 in the central nervous system may cause some autonomic functional activity.[14,15] Elevated serum E2 levels have been shown in men after MI compared to men with only coronary artery disease without MI history.[22] In animal studies, injection of intrathecal E2 into the autonomic function-regulating area resulted in increased parasympathetic activity.[27,28] However, increased E2 concentration in the insular cortex caused increased sympathetic responses.[29] The diverse effects of E2 may be explained by differing concentrations of E2 receptors in individual localizations.

There have been limited studies on the association between cardiac function and serum DHEA-S levels. Serum DHEA and its sulfated derivative have

been extensively studied for their potential anti-aging effects. Serum DHEA levels decline with age in humans, suggesting that it may be important in the aging process.[30,31] Alwardt et al.[32] found that exogenous DHEA-S supplementation was capable of reversing left ventricular stiffness and fibrosis secondary to aging, with a paradoxical increased right ventricular stiffness in young mice. In the Massachusetts Male Aging Study, an inverse relationship was reported between heart disease and serum DHEA-S levels.[33] In our study, serum DHEA-S levels decreased with age, but DHEA-S had a more prominent effect on HRV than gonadal steroids. This finding might be explained by the existence of cardiac receptors for DHEA-S identified in previous studies.[32] On the other hand, Imrich et al.[26] showed that DHEA-S had regulatory effects on sympathoadrenal activity and was positively correlated with reduced sympathoadrenal response to hypoglycemia. Although this study was performed in women, it can be predicted that there are major regulatory effects of DHEA-S on autonomic functions in males, as well. Although the mechanisms of DHEA-S effects on sympathovagal balance have not been elucidated, this action can be attributed to specific receptors and the effect of DHEA-S on the brain.[34]

In the present study, there are two major limitations. First, the number of hypogonadotropic patients was rather low (n=15) to draw precise conclusions about the dual effect of testosterone on HRV. Second, bio-T level was not measured and its effect on HRV could not be assessed.

In conclusion, serum sex steroids may have important effects on cardiac autonomic function in addition to their effects on reproductive function. While physiological levels of androgens were positively related with parasympathetic activity, estrogens were positively related with sympathetic activity in men. In contrast, decreased androgen levels in aging males have controversial effects on autonomic function. However, adrenal androgens seem to be more important for cardiac autonomic control. Further large-scale studies may shed light on the effect of adrenal androgens on autonomic functions.

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