COVID-19 Patients With Altered Steroid Hormone Levels Are More Likely To Have Higher Disease Severity

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Abstract

Purpose: This study aims to evaluate the correlations between the severity of the disease and serum steroid levels by analyzing the serum steroid levels in COVID-19 patients with different levels of disease progression and the control group.

Methods: Morning plasma Aldosterone, 11-deoxycortisol, Androstenedione, 17- hydroxyprogesterone, Dihydrotestosterone (DHT), Dihydroepiandrostenedion (DHEA), Corticosterone, Dihydroepiandrostenedion sulfate (DHEAS), Estrone, 11- deoxycorticosterone, Cortisol, Corticosterone, Androsterone, Pregnenolone, 17- hydroxypregnenolone levels were measured in 153 consecutive patients were grouped as mild, moderate, and severe based on the WHO COVID-19 disease severity classification and the control group. Steroid hormone levels were analyzed at once with a liquid chromatography-tandem mass spectrometric method (LC-MS/MS).

Results: In our study, all of the steroids except DHT and DHEAS were statistically significantly higher in the patients' group than in the control group ($p>0.001$). Also, DHEA was an independent predictor of the disease severity with COVID-19

Conclusions: Our study reveals that altered steroid hormone levels have a negative impact on COVID-19 prognosis by simultaneous measurement of steroid hormones levels with LC-MS/MS. Also, adrenal insufficiency due to COVID-19 is an endocrinological pathology that should be followed up.

Introduction

Coronavirus disease (COVID-19), caused by Coronavirus 2 (SARS-CoV-2) emerged in Wuhan, China, in December 2019 and spread to other countries and even continents rapidly within a short period of time. At the present time, it has affected over 500,000,000 individuals, more than 6,200,000 lives in over 200 countries worldwide [1]. COVID-19 has a wide range of clinical spectrum from asymptomatic to life threatening infection. It has been revealed that most of the cases (81%) were mild, while 14% of cases were severe and only 5% critical. The overall case-fatality rate for COVID-19 was 2.3% [2]

Although the pathogenesis is not clear enough yet, it is known that SARS-CoV-2 enters lung cells by using Angiotensin-converting enzyme 2 (ACE2) as a receptor. Both membrane bound (mACE2) and soluble ACE2 (sACE2) are integral parts of the renin-angiotensin-aldosterone system (RAAS). mACE2 is expressed in many organs' arterial and venous endothelial cells, including adrenal glands. Also, SARS-CoV-2 is identified in adrenal glands in autopsy studies on patients who died from SARS in 2003 [3].

The adrenal gland is a multifunctional organ that produces the steroid hormones and neuropeptides. It is shown that there are about 50 steroid hormones synthesized in adrenal cortex. Control of adrenal steroid hormon synthesis is complex, including ACTH and Angiotensin II. sACE2 binds to the angiotensin II receptor on the adrenal glands, stimulating the release of the mineralocorticoid aldosterone. SARS-CoV-2 has also the potential to activate RAAS and the secretion of aldosterone [4]. SARS-CoV-2 expresses some

amino acids that mimic the host adrenocortical hormone ($ACTH_{1-39}$). The first 24 amino acids ($ACTH_{1-24}$) are preserved in different mammalian cells. But 26th, 29th, 31st, 33rd, 37th, and 39th amino acids of mammalians' ACTH have antigenic properties. SARS-CoV-2 has amino acids that resemble these significant ACTH amino acids residues. Due these amino acids, antibodies against the SARS-CoV-2 destroy the ACTH of the host and thus preventing mainly the increase in cortisol levels [2]. From this point of view, dynamics of adrenal steroidogenesis including glucocorticoids, mineralocorticoids and androgens might be affected in patients with SARS-CoV-2.

In the critical illness conditions including COVID-19, serum cortisol levels increase by activating the hypothalamic-pituitary-adrenal (HPA) axis. And also cortisol metabolism and cortisol binding protein levels decrease [3–6]. Systemic (i.e. intravenous or oral) corticosteroid therapy is strongly recommended for 7 to 10 days in patients with severe and critical COVID-19 in the guideline released by the World Health Organisation (WHO) on Sep 2, 2020 [7]. Cytokine storm emerging by COVID-19 in the host is also prevented by using corticosteroids [2, 8].

Sex steroids are produced mainly by the gonads and also in the adrenal glands. Hovewer, the adrenal cortex also makes small amounts of sex hormones but this only becomes important if overproduction is present in particular. It is shown that there are differences between males and females in terms of COVID-19 morbidity and mortality. Male patients have poor prognosis than females. This might be attributed to differences in levels of sex steroid hormones. Estrogen and progesterone hormones may provide protection to females against COVID-19 through their immunomodulatory and cardioprotective roles [9].

In the light of this information, the blood levels of steroids might be a quite complex situation, especially in COVID-19, which can show a critically serious course. According to our literature review, there were no studies in which all of the steroids used in clinical diagnosis and treatment in COVID-19 patients were analyzed at once with a liquid chromatography-tandem mass spectrometric method (LC-MS/MS). And also, in most studies, steroids were analyzed by non-extraction methods. These methods have poor sensitivity, and specificity, and are susceptible to interference. These problems have been overcome with LC-MS/MS method to obtain more reliable results for this study.

This study aims to evaluate the correlations between the severity of the disease and serum steroid levels by analyzing the serum steroid levels in COVID-19 patients with different levels of disease progression and the control group. In this way, we aim to contribute to the literature by obtaining data for therapeutic strategies about SARS-CoV-2 related endocrinopathies and especially COVID-19, which has a severe clinical course.

Material And Methods

Subjects

The institutional review board (Ethics Board, Ankara City Hospital) approved the study on the basis that it complied with the declaration of Helsinki and that the protocol followed existing good clinical practice guidelines (No. E1-22-2296). COVID-19 patients who were followed by Ankara City Hospital Infectious Diseases Clinic, with confirmed diagnosis by detecting SARS-CoV-2 RNA in oro-nasopharyngeal swab samples, included in this study. These patients were grouped as mild, moderate, and severe based on the WHO COVID-19 disease severity classification. According to this classification, patients without evidence of pneumonia or hypoxia were included in the mild group, while patients with signs and symptoms of pneumonia but no signs of severe pneumonia were considered as moderate. Patients with pneumonia and any of the following; > 30 breaths/min; severe respiratory distress; or SpO2 < 90% at room were included in the severe patient group. In addition, blood samples taken from healthy volunteers were used as a control group. There were no gender discrimination and no age limit for the people included in the study None of the female patients are under hormone replacement therapy and has premature menopause history.

For the patient group, those who had negative SARS-CoV-2 RNA test, those who had been diagnosed with hypoadrenalism or endocrine disease before COVID 19, and those who received systemic glucocorticoid and diuretic therapy; for comparison of sex steroids, those who were after menapouse; for the control group, those who have a diagnosed chronic disease and those who do not volunteer to participate in the study were not included in this study.

Sample Collection and Laboratory Analysis

Blood samples were collected at 08:00 h by venipuncture into EDTA tubes for Aldosterone, 11-deoxycortisol, Androstenedione, 17- hydroxyprogesterone, Dihydrotestosterone (DHT), Dihydroepiandrostenedion (DHEA), Corticosterone, Dihydroepiandrostenedion sulfate (DHEAS), Estrone, 11- deoxycorticosterone, Cortisol, Corticosterone, Androsterone, Pregnenolone, 17- hydroxypregnenolone analysis. After centrifugation, plasma aliquotes were separated, frozen, and stored at – 80°C until the analysis day. Laboratory tests were carried out using LC-MS/MS method.

Steroid hormone analysis by LC-MS/MS

Steroid hormone levels were measured using the Eureka LC-MS/MS kit (Eureka Lab Division, Chiaravalle, Ancona, Italy) with AB Sciex 6500 QTRAP ™ (Sciex, Concord, ON, Canada). In this method briefly, the sample was centrifuged at 14.000 rpm for 10 minutes after adding 1000 µl of deproteinization solution containing the internal standard. The supernatant was removed and dried by an evaporator followed by 100 µL diluting solution addition centrifugation was done at 14.000 rpm for 5 minutes. The transferred supernatant into the vial was injected into the LC system. Agilent Zorbax RRHD Eclipse Plus C 18 (50 x 2.1 mm. 1.8 um) was used as an analytical column. 6 levels of calibrators, 2 levels of controls, and internal standard solution containing Aldosterone-d7, Cortisol-d4, and Testosterone-d3 were used. The limit of quantification (LoQ) was 0.005ng/mL for 17-hydroxyprogesterone, Androstenedione and Cortisol; 0.05 ng/mL for DHEA, Corticosterone and Androsterone; 0.007 ng/mL for 11- deoxycortisol and Deoxycorticosterone; 0.01 ng/mL for Aldosterone and Estradiol; 0.03 ng/mL for Dihydrotestosterone and

Estrone; 0.02 ng/mL for Pregnenolone and 17- hydroxypregnenolone; 0.002 ng/mL for Testosterone and Progesterone; 10 ng/mL for DHEAS.

Statistical analysis

Statistical analyses were performed using the SPSS 26.0. The Kolmogorov-Smirnov test was performed to check the normality of the variables. Descriptive analysis was presented using mean ± SD for normally distributed variables and median (IQR) for non-normally distributed variables. Laboratory data were compared between the groups using the Kruskal-Wallis test for nonparametric variables and ANOVA test for parametric variables. Comparisons for categorical variables were executed using the chi-square test. For the multivariate analysis, the possible factors identified with univariate analyses were further entered into the logistic regression analysis to determine independent predictors of the severity of the disease. The Hosmer-Lemeshow goodness of fit test was used. The odds ratio (OR) was calculated for significantly associated variables. Statistical significance was defined as $p < 0.05$.

Results

A total of 153 patients were included in this study. The median age was 43 ± 17 years, and the male: female ratio was 86:67. Forty-three patients (28.1%) were in severe group, sixty patients (39.2%) were in moderate patients group, and fifty patients (32.7%) were in mild group. There were 5 males and 28 females in the control group. We intentionally excluded patients admitted to the ICU to avoid the many confounding factors that may affect the adrenal function in the ICU setting. All the patients had positive COVID-19 real-time polymerase chain reaction test results. In the patient group, 11 patients had hypertension, 5 patients had diabetes mellitus (DM), 6 patients had DM and hypertension, 1 patient had lung disease and hypertension, 2 patients had coronary artery disease, DM, and hypertension, 1 patient had hypertension and renal disease, 1 patient lung disease and hypertension, chronic kidney disease, 1 patient coronary artery disease and hypertension.

In our study, all of the steroids except DHT and DHEAS were significantly higher in the patients' group than in the control group ($p > 0.001$). DHT levels were not significantly different among all groups. DHEAS levels in the moderate disease group were the highest among all groups. But corticosterone, 11-deoxycorticosterone, 17- hydroxypregnenolone, and 21- deoxycortisol levels were significantly lower in the severe disease group compared to the mild disease group ($p < 0.001$). Cortisol, corticosterone, 11-deoxycorticosterone, and 17- hydroxypregnenolone levels were significantly lower in the severe disease group compared to the moderate disease group ($p < 0.001$). Cortisol levels were significantly higher in the moderate disease group compared to the mild disease group ($p < 0.001$) (Table 1)

Table 1
Comparison of steroids between groups

	Severe Group (n = 43)	Moderate Group (n = 60)	Mild Group (n = 50)	Control Group (n = 33)	*p
AGE	57 ± 15	15 (21)	30 (14)	27 (14)	> 0,001[a,b,d,e,f]
ALDOSTERONE pg/mL	85,3 ± 33,4	93,5 ± 32,7	110,5 (75,3)	7,74 (16,4)	> 0,001[a,b,c]
11 DEOXYCORTISOL**	93,9 (224)	497 (538)	489 (512)	13,5 (14,4)	> 0,001[a,b,c]
ANDROSTENEDIONE**	783 (1362)	1221 (964)	1246 (1284)	138 (130)	> 0,001 [a,b,c]
17- OH- PROGESTERONE **	315 (542)	625 (649)	532 (417)	26 (49,2)	> 0,001[a,b,c e]
DHEA ug/L	1,25 (2,56)	6,05 (4,10)	4,43 (4,34)	4,49 (4,85)	> 0,001[a,b,c]
CORTISOL ug/dL	79,3 (154)	149 (204)	46,6 (73,7)	12,1 ± 6,02	> 0,001[a,b,c,e,f]
CORTICOSTERONE **	760 (1423)	3382 (3769)	2052 (1834)	168 (243)	> 0,001[a,b,c,d,e]
11-DEOXYCORTICOSTERONE **	66,5 (81)	542 (697)	794 ± 482	3,06 (3,81)	> 0,001[a,b,c,d,e]
ANDROSTERONE ug/L	484 ± 195	544 ± 236	494 ± 245	0,62 (1,06)	> 0,001[a,b,c]
PREGNENOLENE **	329 (561)	523 ± 199	531 (462)	111 (134)	> 0,001[a,b,c]
17-OH-PREGNENOLONE **	14,4 (22,7)	35,6 (43,8)	41,6 (100)	75,9 (132)	> 0,001[a,b,d,e]
DHEAS ug/dL	144 (436)	436 (447)	148 (227)	97,6 (62,2)	> 0,001[b,e,f]
21-DEOXYCORTISOL **	35,3 ± 10,8	40,9 ± 12,1	43,9 ± 12,7	1,34 (1,73)	> 0,001[a,b,c,d]

Abbreviations: DHEA: Dihydroepiandrostenedion; DHEAS Dihydroepiandrostenedion sulfate
Normal value as mean±standart deviation, Non Normal value as Median (IQR); ** indicates : ng/dL

*p values indicate; a=significant difference between severe group and control group; b= significant difference between moderate group and control group; c= significant difference between mild group and control group; d= significant difference between severe group and mild group group; e= significant difference between severe group and moderate group; f= significant difference between moderate group and mild group

Sex steroid hormones were compared in each gender group seperately. Estradiol, estrone and progesterone levels in the control group were lower than in all of the disease groups ($p < 0.001$). Although estrone and progesterone levels in COVID-19 patients were higher than the levels obtained in the control group, this difference were statistically important only in the mild and moderate group in comparison to the conrol group ($p < 0.001$) (Table 2). There were only five men in the control group. Therefore, comparisons were done among the disease groups for male patients. Testosterone levels in the moderate disease group were the highest among all groups for these patients (Table 3).

Table 2

Comparison of sex steroids in females

	Severe Group (n=8)	**Moderate Group (n=32)**	**Mild Group (n=41)**	**Control (n=26)**	***p**
ESTRONE pg/mL	299 ± 150	580 ± 262	542 (386)	55,6 (71,3)	>0,001 [b,c]
ESTRADIOL pg/mL	675 ± 361	557 (418)	400 (314)	66,1 (52,2)	>0,001[a,b,c]
PROGESTERONE ug/L	1,23 ± 1,01	1,20 (2,02)	1,31 (2,82)	0,12 (1,55)	>0,001[b,c]
Normal value as mean±standart deviation, Not Normal value; Median (IQR); *p values indicate; a=significant difference between severe group and control group; b= significant difference between moderate group and control group; c= significant difference between mild group and control group.					

Table 3

Comparison of sex steroids between male patients groups

	Severe Group (n = 31)	**Moderate Group (n = 39)**	**Mild Group (n = 16)**	***p**
TESTOSTERONE ng/dL	1114 (1781)	3555 (3133)	1708 (148)	< 0,001[a,b]
DIHYDROTESTOSTERONE pg/mL	393 ± 182	371 ± 152	400 ± 141	> 0,05
Normal values as mean ± standart deviation, Nonnormal values as median (IQR)				
*P values indicate; a = significant difference between severe group and moderate group; b = significant difference between moderate group and mild group.				

The binary logistic regression model included DHEA, 11- deoxycorticosterone. It was statistically significant with $\chi 2 = 4.946$; $p < 0.001$. The model correctly classified 95.7% of the cases. These increased parameters, especially DHEA, were independent predictors of the disease severity with the likelihood ratios shown in Table 4.

Table 4
Odds ratios and coefficients of binary logistic regression analysis of factors associated with disease severity

						95% C.I.for EXP(B)	
	B	S.E.	Wald	Sig	Exp(B)	Lower	Upper
DHEA (ug/L)	,321	,131	5,967	,015	1,379	1,065	1,784
11-DEOXYCORTICOSTERONE (ng/L)	,023	,007	11,553	,001	1,010	1,037	1,038
Hosmer Lemeshow GFT: p = 0.763; Model: $\chi 2 = 4.946$; p < 0.001; Percentage Correct = 95.7%.							

Discussion

COVID-19, the ongoing pandemic, has still issues that need to be clarified to manage the disease and its consequences despite of extensive research and several large studies describing the clinical, biochemical, and radiological features. Steroid hormones are commonly classified as glucocorticoids, mineralocorticoids, and sex steroid hormones. Analyzing and monitoring these hormones provides useful information in terms of diagnosis, follow-up, and prognosis of diseases [10]. For this purpose, we analyzed sex steroids, glucocorticoids, and mineralocorticoids simultaneously with LC-MS/MS, a sensitive and specific method, in COVID-19 patients with different disease severity and healthy individuals. According to our literature review, these hormones were evaluated simultaneously in COVID-19 for the first time. Analyzing steroids, whose production is related to each other, in all individuals at the same time, allowed the hormones to be evaluated together and to obtain more reliable data.

SARS-CoV-2 viral entry requires two host proteins: the angiotensin-converting enzyme-2 (ACE2) and the transmembrane protease, serine2 (TMPRSS2). SARS-CoV-2 enters many organs, including endocrine organs, through ACE2 receptors and creates a clinical picture in various spectrums that can change depending on the individual's immune system, age, gender, concomitant diseases, and other unexplained reasons, from mild to severe, and even cause death. Transcription of the TMPRSS2 gene and ACE2 receptor activation are positively regulated by androgens. It is accepted that this situation contributes to the more common and worse prognosis of COVID 19 in men [11, 12]. In our study, we found that the highest androgen levels were in patients with moderate COVID-19. However, we found that as the severity of the disease increased, steroid levels decreased. We attributed this situation to the possibility of high viral load interfering with hormone production, as in the study of Bermejo-Martin et al. in which they compared plasma viral load with disease severity and serum parameters [13].

There are also opinions supporting that the differences in the severity and mortality of COVID-19 between the sexes are due to immunological and especially hormonal differences, apart from the entry of SARS-CoV-2 into the cell [9, 11]. It has been observed that COVID-19 alters spermatogenesis and testosterone production. In a study by Montaño et al., a negative correlation was found between the severity of COVID-19 and testosterone levels[11] (8). In our study, we also found low testosterone levels in severely ill

COVID-19 patients, but we did not find dihydrotestosterone (DHT) levels differently. DHT is a metabolite that results from the reduction of testosterone by 5α-reductase. DHT is a more potent agonist of androgen receptors than testosterone. But its half-life in plasma is shorter than that of testosterone and its levels reflect the local expression of 5α-reductase. Therefore, we think that we could not find DHT levels different in patients with different clinical courses. Due to these differences in studies, there are opinions supporting that innate immunity and genetic errors are more important than the sex steroid levels of individuals in the critical course of COVID-19 [14].

It is assumed that female sex hormones are potent immunomodulators, and therefore, COVID-19 has a milder clinical course in women [15]. However, in our study, we found lower sex steroid hormone levels in patients with a more severe COVID-19 clinical course. In the comparisons we made according to genders, sex steroids were lower in severe cases than in other patient groups. This may be due to the down-regulation of the gonadal axis by cytokines in patients with acute, severe disease [16]. The results of our study also support this situation.

In an autopsy study conducted on patients with SARS, it was shown that the virus reached the hypothalamus directly through the hematogenous route or directly through the cribriform plate and was destroyed by the identification of the SARS genome in the hypothalamus [3]. In a postmortem study of COVID-19 patients, no significant pathology in adrenal glands was found but areas of necrosis/infarction were seen in one out of the nineteen examined pituitaries [17]. In addition, the hypothalamic-pituitary-testicular axis is also suppressed in acute diseases [12]. In the study of Alzahrani et al., in which they investigated the effect of COVID-19 on the HPA axis, they found no difference between the median values of cortisol in patients with COVID-19 infection of different severity but found low cortisol levels in patients with severe infection [18]. In our study, there were more patients at each COVID-19 patient group than in the study of Alzahrani et al. We found significant differences between the median levels of cortisol and many other steroids between the groups at each severity level, but in severe cases, our steroid levels were low like the mentioned study. In an autopsy study conducted on patients with COVID-19, they observed signs of necrosis, cortical lipid degeneration, hemorrhage, and focal gland inflammation in the adrenal glands associated with infection [19]. This explains the low level of adrenal steroids in patients with severe COVID-19, which we demonstrated in our study.

DHEA is synthesized by zona reticularis of the adrenal cortex from 17 α-hydroxypregnenolone on ACTH stimulation. This androgenic steroid is sulfated to form dehydroepiandrosterone sulfate in the adrenals and peripheral tissues. DHEAS has immunostimulatory effect. DHEA has been found to be more sensitive to HPA axis stimulation than cortisol [20, 21]. However, its relationship with the severity of COVID-19 has not been evaluated. We found that DHEA and DHEAS levels were higher in patients with pneumonia due to the immunostimulatory effect, while they decreased as the severity of the disease increased due to suppression of the HPA axis as the disease progressed. With logistic regression, we determined that with each unit increase in DHEA level, there may be a 1.379 (1.065–1.784)-fold risk increase for COVID-19 disease severity.

Both the cytopathic effect of SARS-CoV-2 virus on the adrenal cortex and secondary adrenal insufficiency caused by ACTH destruction due to the homology of ACTH with viral RNA by antibodies against SARS-CoV-2 have been shown in COVID-19 patients. In addition, stress-induced cortisol increase may be prevented by inadvertent degradation of ACTH by antibodies formed against viral particles [3, 12]. In a study by Gonen et al., they found the frequency of adrenal insufficiency to be 8.2%. They found Antipituitary antibodies (APA) in 3 of these patients who developed adrenal insufficiency and antihypothalamic antibodies (AHA) in 1 of them. They found the cortisol level to be high regardless of the severity of the COVID-19 disease [22]. In our study, cortisol levels were high in patients with moderate severity, but cortisol levels decreased in the group with severe COVID-19 based on the WHO classification for COVID-19. In addition, in many other publications such as Gonen et al., cortisol level was analyzed by electrochemiluminescence immunoassay (ECLIA). We think that one of the reasons for the inconsistency with our results may be due to the difference in the analysis method.

The strength of our study is that the method we used for hormone analysis is sensitive and specific in particularly measuring low hormone concentrations. However, the inability to perform menstrual cycle questioning in female patients is a limitation of our study. In addition, the control group was not matched with the patient group in terms of gender.

In conclusion, this research paper reveals that altered steroid hormon levels has a negative impact on COVID-19 prognosis by smiltaneous measurement of steroid hormones' levels with LC-MS/MS. Also adrenal insufficiency due to COVID-19 is an endocrinological pathology that should be followed up. By analyzing the steroid hormon levels in with sensitive and specific methods for especially patient's follow-up, more reliable data will be obtained, and a positive contribution can be made to patient management with early diagnosis and treatment of the pathology that may occur.

Declaration

The authors have no relevant financial or non-financial interests to disclose.

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Figures

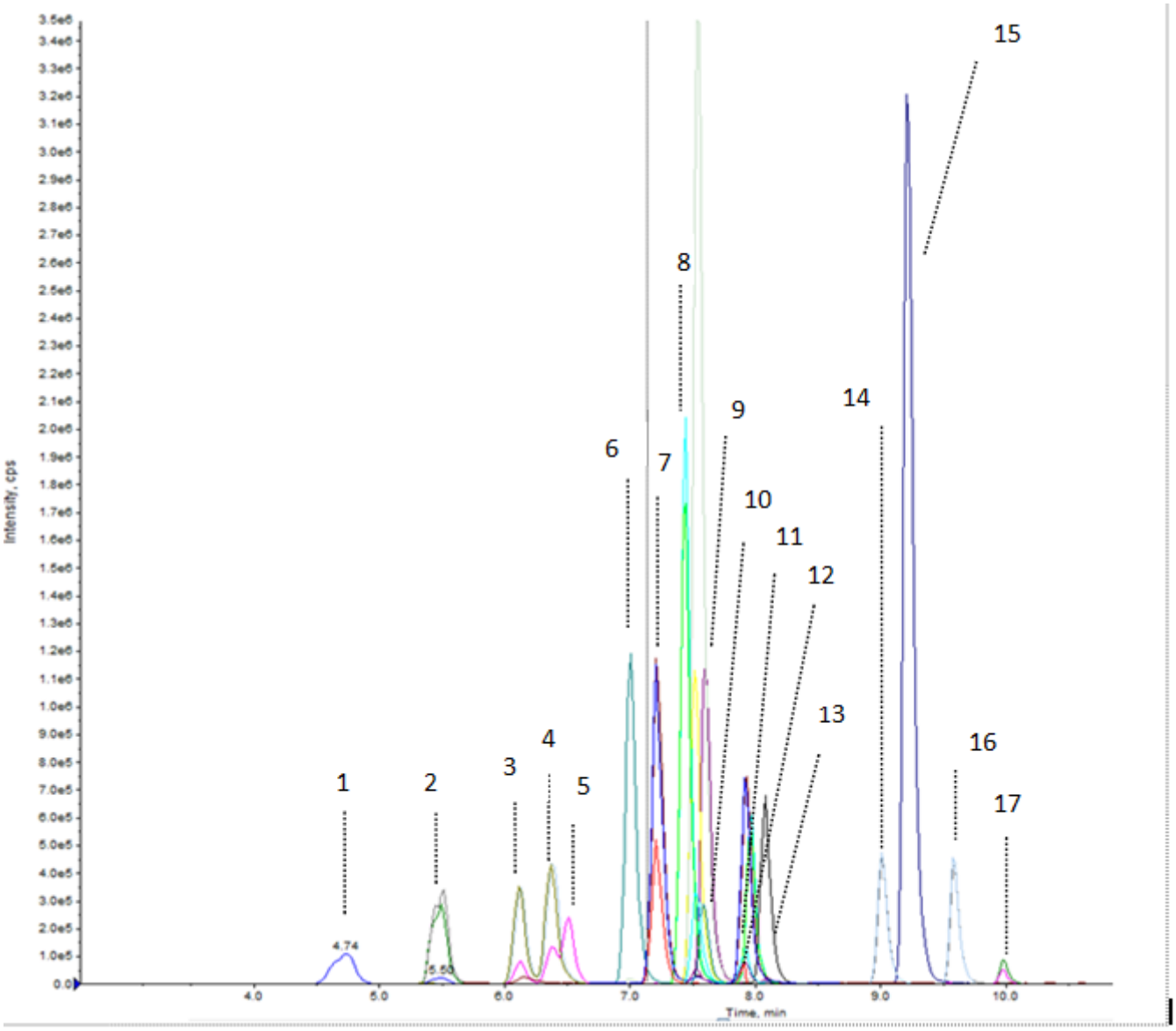

Figure 1

LC-MS/MS chromatogram for steroid hormones in mix solution.
Numbers indicate the following metabolites. 1: Aldosterone; 2: Cortisol; 3: 21- deoxycortisol, 4: Corticosterone, 5: 11- deoxycortisol, 6: Androstenedione, 7: Estrone, 8: 11- deoxycorticosterone, 9: Testosterone 10: Estradiol, , 11: Dehydroepiandrosterone, 12: 17- hydroxyprogesterone, 13: 17-hydroxypPregnenolone, 14: Dihydrotestosterone, 15: Progesterone, 16: Androsterone, 17: Pregnenolone.

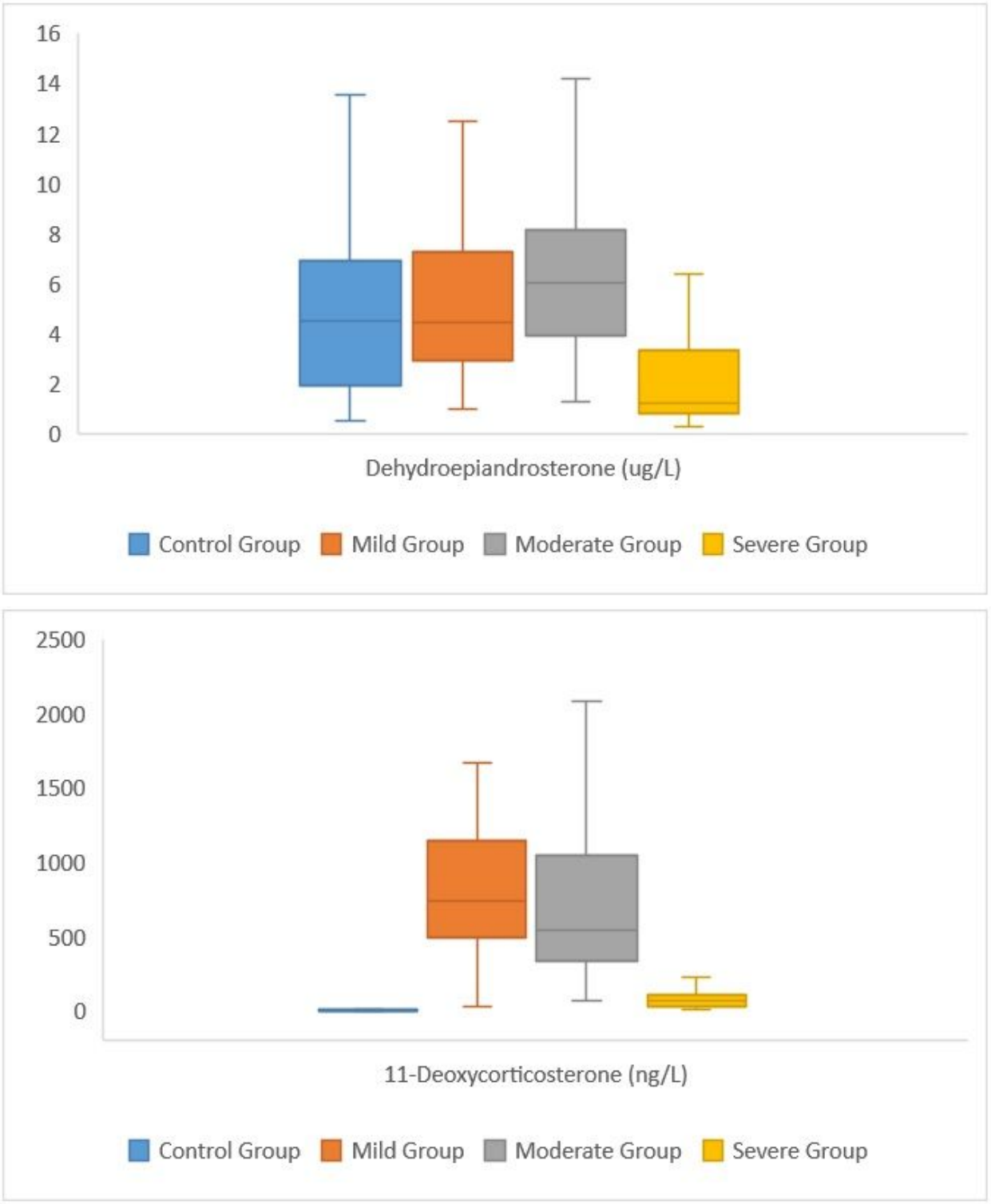

Figure 2

Distributions of Dehydroepiandrostrone, 11-Deoxycorticostrone levels between groups