Practical Laboratory Medicine 24 (2021) e00204

Contents lists available at ScienceDirect

Practical Laboratory Medicine

journal homepage: www.elsevier.com/locate/plabm

Correlation of age and sex with urine dehydroepiandrosterone sulfate level in healthy Thai volunteers

Karnpimon Krorakai [a], Siriporn Tontipattananon [a], Rattikorn Leemahanil [a], Supannika Klangphukhiew [a], Chathaya Wongrathanandha [b], Akarin Phaibulpanich [c], Rina Patramanon [a,d,*]

[a] Department of Biochemistry, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand
[b] Department of Community Medicine, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
[c] Department of Statistics, Faculty of Commerce and Accountancy, Chulalongkorn University, Bangkok, Thailand
[d] Protein and Proteomics Research Center for Commercial and Industrial Purposes (ProCCI), Khon Kaen University, Khon Kaen, Thailand

ARTICLE INFO

Keywords:
Dehydroepiandrosterone sulfate
Urine
Age
Sex

ABSTRACT

Objective: Dehydroepiandrosterone sulfate (DHEAs), a prohormone secreted by the adrenal gland, plays a role in the synthesis of sex hormones, namely, androgen and estrogen. It has been found that the amount of DHEAs is correlated with age, although most studies have focused on the correlation of serum DHEAs levels with age and sex. Thus, this noninvasive, cross-sectional study aimed to investigate the correlation of urine DHEAs levels with age and sex in healthy Thai volunteers aged 20–80 years.

Methods: DHEAs levels were measured in 178 healthy volunteers using electrochemiluminescence immunoassay and then normalized by creatinine. Multiple regression was performed to determine the correlation of urine DHEAs levels normalized by creatinine with age and sex.

Results: The normalized DHEAs levels are correlated with age group for both sexes. Moreover, an increasing trend in DHEAs levels was found in the age group 20–29 years, and the DHEAs level peaked at the age group 30–39 years before declining with advancing age. Based on the multiple regression analyses, the significance of the interaction term ($P < 0.05$) indicates that both age and sex significantly contribute to the prediction of ln (DHEAs/Creatinine). Our fitted model implies the following: as age increases by 1 year, DHEAs/Creatinine is expected to decrease by 3.63% in females and by 2.18% in males.

Conclusion: This study reports more data on clinical reference value of urine DHEAs levels in healthy volunteers. Our result demonstrates urine DHEAs levels are associated with age and sex and decline by 2–3% a year.

1. Introduction

The prohormone dehydroepiandrosterone (DHEA) and its sulfate conjugate (DHEAs) are mainly produced by the adrenal glands and are the most abundant circulating steroid hormones in humans. This prohormone serves as an initial substance in the synthesis of sex steroid hormones, namely, androgens and estrogens [1]. DHEAs has been thought to be a biomarker of aging because its serum level

* Corresponding author. Department of Biochemistry, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand.
E-mail address: narin@kku.ac.th (R. Patramanon).

https://doi.org/10.1016/j.plabm.2021.e00204
Received 12 June 2020; Accepted 13 January 2021

peaks in young adulthood and significantly decreases with aging (2%–3% per year) [2–4]. Recently, DHEAs have attracted considerable attention because the decline in serum DHEAs levels with advancing age is related to age-related diseases or with the deterioration of physiological functions [5] such as cognitive function [6], depression [7], cardiovascular disease [8], osteoporosis [9], and cancer [10]. In addition, DHEAs is often known as the "fountain of youth" [11]. Some publications have reported that DHEA supplementation could prevent or ameliorate age-related physical and memory impairment as well as improve one's well-being [12–15].

The most relevant researches have investigated the correlation of serum DHEAs levels with age and sex and presented for use as a clinical reference value. These studies have reported the remarkable changes in serum DHEAs levels throughout one's lifetime. After birth, DHEAs levels decrease dramatically and remain considerably low until the age of 6 years, and then increase continuously during adrenarche because the cells in the zona reticularis start to divide in preparation for the reproductive age [16,17]. In adulthood, the DHEAs levels peak at the age of 17–19 years in females and at the age of 20–29 years in males and then progressively decline with aging [18]. These studies thus demonstrated a negative correlation between serum DHEAs concentration and age (20–80 years old) in both sexes. Moreover, DHEAs levels were higher in males than in females in all age groups [3,19,18].

Although some studies have shown that serum DHEAs concentrations were positively correlated with urine DHEAs excretion [20, 21], a few studies have reported on the correlation of urine DHEAs levels with age and sex, particularly with a specific age group [21–23]. Moreover, the correlation of age and sex with urine DHEAs levels has not yet been investigated in any non-western populations. Thus, to fill this research gap, this work investigates the DHEAs levels in urine samples of healthy Thai volunteers aged 20–80 years and determine their correlation with age and sex. This study may serve as a baseline study for future studies in this field.

2. Subjects and methods

2.1. Research design

This work is an observational study, and clinical information were collected from healthy volunteers and from those who have had a health checkup at the Department of Family Medicine and at the Health Checkup Center for Going Abroad, Faculty of Medicine Ramathibodi Hospital, Mahidol University, in Bangkok, Thailand. The data collected were presented according to age and sex as described by Ryun-Sup Ahn and co-authors [24].

2.2. Eligibility criteria

The inclusion criteria include: 1) at least 20 years old; 2) non-smoker for a year; 3) non-drinker for three months; 4) did not take any

Table 1
Clinical parameters used as inclusion criteria for participants aged 35 years and above.

List	Clinical parameters	Unit	Reference value
1	Body mass index (BMI)	kg/m^2	18.0–26.9
2	Blood pressure (BP)		
	Systolic blood pressure (SBP)	mmHg	<159
	Diastolic blood pressure (DBP)	mmHg	<99
3	Heart rate (HR)	/min	40–140
4	Complete blood count (CBC)		
	Hemoglobin (Hb)	g/dL	13.0–17.9
	Hematocrit (Hct)	%	37–52
	White blood cell (WBC) count	$/mm^3$	3700–10,000
	Neutrophil percentage (PMN)	%	35–80
	Lymphocyte percentage (Lym)	%	20–50
	Monocyte percentage (Mono)	%	2–9
	Eosinophil percentage (Eo)	%	0–9
	Platelet smear		Adequate
	Platelet count	$/mm^3$	138,000–400,000
	Red blood cell morphology		Normochromic Normocytic
5	Fasting blood sugar (FBS)	mg/dl	70–125
6	Uric acid	mg/dl	<8.0
7	Renal function test (RFT)		
	Blood urea nitrogen (BUN)	mg/dl	5–29
	Creatinine (Cr)	mg/dl	0.5–1.4
8	Liver function test (LFT)		
	Serum glutamate oxaloacetate transaminase (SGOT)	U/L	<60
	Serum glutamate pyrophosphate transaminase (SGPT)	U/L	<60
	Alkaline phosphatase (ALP)	U/L	<200
	Albumin (Alb)	g/dl	≥30
9	Lipid profile (LP)		
	Cholesterol (CHO)	mg/dl	<260
	Triglyceride (TG)	mg/dl	<300
	Low density lipoprotein (LDL)	mg/dl	<190
	High density lipoprotein (HDL)	mg/dl	>30

medicine or supplement for a week; 5) with a body mass index of 18.0–26.9; 6) when aged 35 years and above, the volunteers' clinical parameters must fall within the reference values (Table 1), which were adopted from Park and co-worker [25], although having all of the listed parameters checked is not necessary; and 7) free of the following chronic diseases: diabetes mellitus, hypertension, hypotension, renal disease, liver disease, cardiovascular diseases, hypercholesterolemia, cancer, osteoporosis, scleroderma, Huntington's disease, chronic obstructive pulmonary disease, asthma, neurodegenerative disease, and Alzheimer's disease. Participants will be excluded if they withdraw from the study.

This study was approved by the Ethical Committee of the Faculty of Medicine Ramathibodi Hospital, Mahidol University. All subjects gave their written informed consent.

2.3. Urine sample collection and analysis

Urine samples of the healthy volunteers were collected in plastic bottles, aliquoted in 2 mL Eppendorf tube, and stored at −20 °C. The amount of urine DHEAs was normalized by creatinine to account for the variations in urine concentration [26]. DHEAs concentrations were measured by electrochemiluminescence immunoassay on the Roche Cobas e601 (Roche Diagnostics, Switzerland), and creatinine was determined by enzymatic assay using an Architect Ci16200 (Abbott, USA).

2.4. Statistical analysis

A descriptive method was used to describe the frequency, median, and interquartile range (IQR) for each of the age groups and genders. Calculations and analyses were performed using SPSS for Windows (version 22.0, SPSS, Inc., Chicago). Multiple regression was used to assess the correlation of urine DHEAs levels normalized by creatinine with age and sex as explanatory variables. P-values < 0.05 were considered statistically significant.

3. Results

3.1. Descriptive summary

Of 227 volunteers who agreed to participate in this study, 178 met the inclusion criteria. Among them, 58 (32.58%) were male and 120 (61.2%) were female. The volunteers were then divided into five age groups: 20–29, 30–39, 40–49, 50–59 and ≥60. For participants 35 years old and above, all had got BMI, BP, HR and RFT checked (Table 2). Regarding individual parameters, CBC and LP were tested in most of the subjects. There were less subjects who had LFT and Uric acid results, and only one had FBS tested. For multiple parameters tested in each subject, 76.3% had BMI, BP, HR, CBC and RFT tested and 59.0% had BMI, BP, HR, CBC, RFT and LP tested. There was no subjects with complete results of the clinical parameters.

Table 3 and Fig. 1 show the normalized DHEAs levels in urine samples of the male and female volunteers. The normalized DHEAs levels apparently correlated with age group for both sexes. Moreover, the DHEAs levels showed an increasing trend in the age group 20–29 years, peaked in the age group 30–39 years, and then decreased with advancing age. However, outliers were found in the data.

Due to the small sample sizes and the non-normal nature of the data, nonparametric test on equality of median was chosen to compare median urine DHEAs and DHEAs/Creatinine between the two genders. The result shows that the median urine DHEAs and DHEAs/Creatinine between male and female groups are not significantly different for age groups 20–29, 30–39, and 40–49, while the significant difference in urine DHEAs/Creatinine is being shown in the age groups 50–59 and the significant difference in urine DHEAs is

Table 2
Clinical parameters for volunteers 35 years old and above.

Clinical parameters	Volunteers≥35 (n = 139)		
	Female (n = 99)	Male (n = 40)	Total (%)
Individual parameters checked			
BMI	99	40	139 (100)
BP	99	40	139 (100)
HR	99	40	139 (100)
CBC	73	33	106 (76.3)
RFT	99	40	139 (100)
LFT	43	20	63 (45.3)
LP	84	29	113 (81.3)
Uric acid	19	9	28 (20.1)
FBS	0	1	1 (0.7)
Multiple parameters checked			
BMI + BP + HR + CBC + RFT	73	33	106 (76.3)
BMI + BP + HR + CBC + RFT + LP	60	22	82 (59.0)
BMI + BP + HR + CBC + RFT + LP + LFT	30	14	44 (31.7)
BMI + BP + HR + CBC + RFT + LP + LFT + Uric acid	10	7	17 (12.2)
BMI + BP + HR + CBC + RFT + LP + LFT + Uric acid + FBS	0	0	0 (0)

Table 3
DHEAs and DHEAs/Creatinine levels in the male and female populations clustered into age groups.

Age group	Male (n = 58)			Female (n = 120)			*P*-value[a]	*P*-value[b]
	n	DHEAs (ng/mL)	DHEAs/Creatinine	N	DHEAs (ng/mL)	DHEAs/Creatinine		
20–29	11	1498.00 (2183.0)	0.0051 (0.0038)	9	1632.00 (3532.2)	0.0039 (0.0060)	1.000	0.370
30–39	13	1819.10 (2921.4)	0.0050 (0.0085)	24	2015.50 (5224.0)	0.0043 (0.0061)	0.570	0.904
40–49	8	1081.05 (1978.0)	0.0028 (0.00433)	28	1736.50 (4484.8)	0.0034 (0.0034)	0.229	0.688
50–59	13	1830.00 (1950.0)	0.0036 (0.0028)	25	889.00 (1286.0)	0.0017 (0.0015)	0.494	0.040[c]
≥60	13	1555.00 (1068.5)	0.0016 (0.0016)	34	571.50 (622.8)	0.0012 (0.0001)	0.041[c]	0.928

Data represent the median (IQR).
[a] *P*-value for testing equality of median DHEAs between male and female groups.
[b] *P*-value for testing equality of median DHEAs/Creatinine between male and female groups.
[c] *P*-values < 0.05.

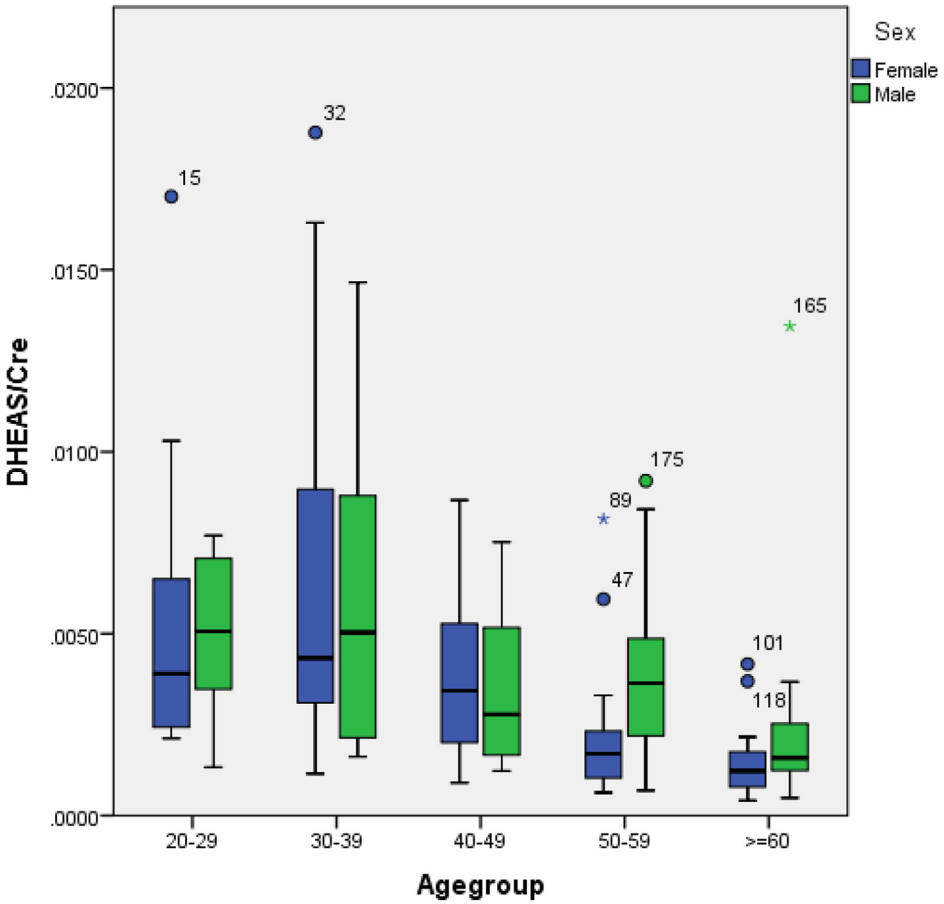

Fig. 1. Box plots of the DHEAs/Creatinine in urine samples of the male and female volunteers clustered into age groups. The box extends from the 25th percentile to the 75th percentile, with a horizontal line at the 50th percentile (median).

being shown in the age groups >60.

3.2. Correlation of DHEAs/Creatinine levels with age and sex

A multiple regression was performed to determine the correlation of the urine DHEAs levels normalized by creatinine (the independent or the response variable) with the two predictor variables: sex (using dummy variables coded as 1 for female and 0 for male) and age.

Fig. 2(A) and (B) show the histograms of DHEAs/Creatinine and ln (DHEAs/Creatinine) in urine samples (ng/mL), respectively, for each sex. The histograms for DHEAs/Creatinine show highly positively skewed for both the males and females with a few outliers. The

Shapiro-Wilk tests were also performed to assess the normality of DHEAs/Creatinine. The result (P-value < 0.001) confirms that DHEAs/Creatinine significantly deviate from a normal distribution for both genders. Therefore, the ln (log base e) transformation of DHEAs/Creatinine was applied, and the Shapiro-Wilk test P-values are 0.163 and 0.765 for female and male groups, respectively, suggest that ln (DHEAs/Creatinine) is normally distributed. Therefore, in our multiple regression model, we used the ln transformation of DHEAs/Creatinine as our independent variable.

A regression model with interaction showing the relationship between age and sex (Table 4) significantly contributes to the prediction of ln (DHEAs/Creatinine), implying that age and sex are the significant predictors. However, the coefficients presented in Table 4 suggest that age is a more significant predictor than sex. The R^2 value was 0.193, indicating that 19.3% of the variance in ln (DHEAs/Creatinine) could be explained by the model.

The model can be written as follows: estimated ln (DHEAs/Creatinine) $= -4.741 + 0.513$xSex $- 0.022$xAge $- 0.015$ Sex x Age. As seen in Fig. 3, we can obtain separate models for each sex as follows:

For female (Sex $= 1$), estimated ln (DHEAs/Creatinine) $= -4.228 - 0.037$xAge.

For male (Sex $= 0$), estimated ln (DHEAs/Creatinine) $= -4.741 - 0.022$xAge.

We may interpret the coefficients of the slopes as follows: For females, as age increases by 1 year, ln (DHEAs/Creatinine) is expected to decrease by -0.037 units or the DHEAs/Creatinine is expected to decrease by $100\times$ (1−exp (−0.37)) = 3.63%. For males, as age increases by 1 year, ln (DHEAs/Creatinine) is expected to decrease by -0.022 units or the DHEAs/Creatinine is expected to decrease by $100\times$ (1−exp (−0.22)) = 2.18%.

4. Discussion

In this work, we analyzed urine DHEAs in participants from five age groups with ten-year intervals. The relationship of urine DHEAs was studied in normal healthy subjects because many health conditions possibly affect DHEAs levels. Several studies have demonstrated

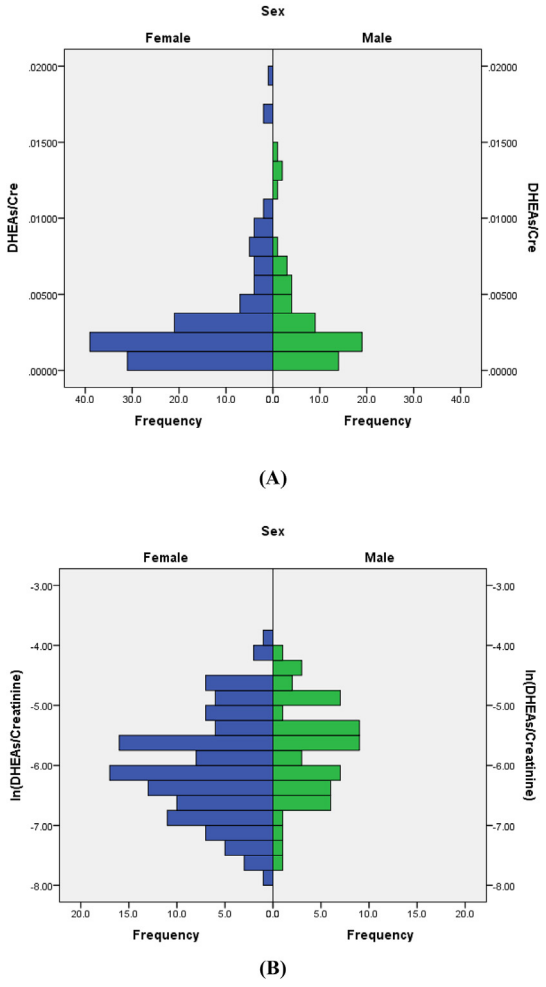

Fig. 2. Scatter plots showing the histograms for (A) DHEAs/Creatinine and (B) ln (DHEAs/Creatinine) in urine samples (ng/mL) of the male and female populations.

Table 4
Summary of multiple regression model (n = 178).

Variable	B	SE	*P*-value
Intercept	−4.741	0.2766	0.000
Sex	0.513	0.3446	0.136
Age	−0.022	0.0057	0.000
Sex × Age	−0.015	0.007	0.034

$R^2 = 0.193$, F = 13.896, $P < 0.001$.

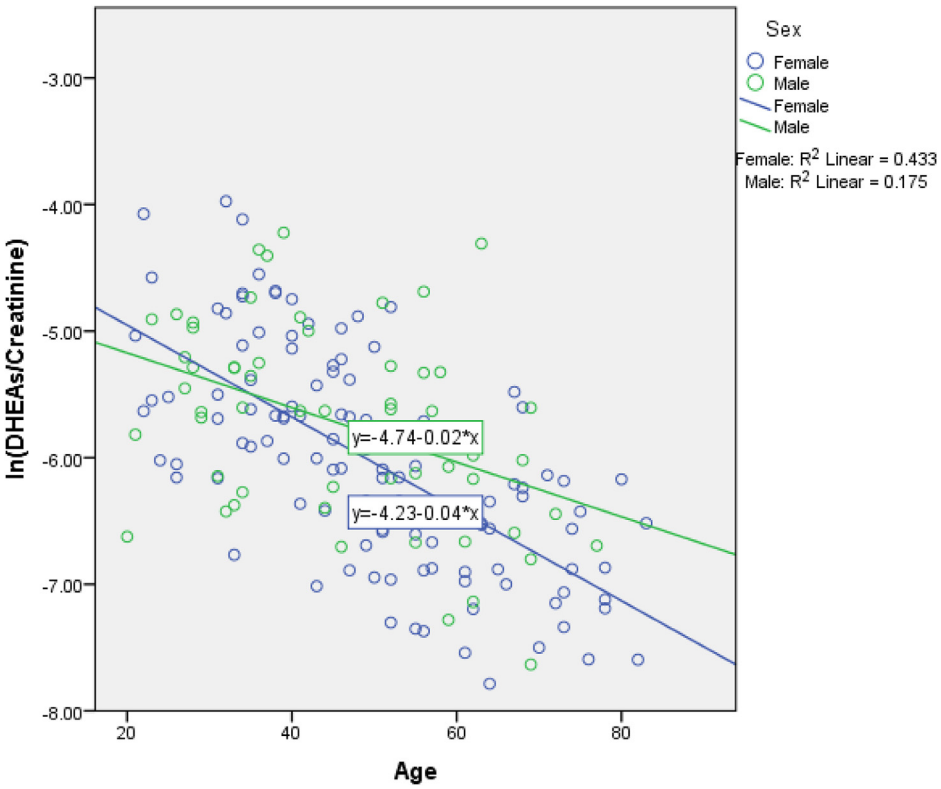

Fig. 3. Scatter plots of ln (DHEAs/Creatinine) in relation to age with fitted regression lines for the male and female populations.

that low DHEAs levels are associated with the risk of having testosterone deficiency syndrome in men, as well as with cardiac diseases, sleeplessness, Alzheimer's diseases, and cognitive impairment [1,5]. Furthermore, smoking and drinking affect DHEAs level, that is, serum DHEAs level increases with smoking and alcohol intake ($P < 0.001$) [27].

Some non-invasive studies have investigated the variations in the urine DHEAs levels in individuals younger than 20 years in order to formulate hypotheses on the mechanism of adrenarche [22,28,29]. However, only a few studies have reported on the normal range of urine DHEAs levels, whereas no data on the correlation of age and sex with urine DHEAs levels in healthy Thai population have been reported. This study showed that the urine DHEAs levels of healthy Thai volunteers vary in different age groups (20–80 years), and the highest levels were found in the age group 30–39 years. In the age group consisting of individuals 40 years and older, the DHEAs levels decreased and then slightly declined with advancing age. Thus far, no study has investigated the correlation between urine DHEAs level and a wide age range; however, Jia et al. reported that the level of 24-h urine 17-ketosteroid sulfate conjugates (DHEA, DHEAs, epi-androsterone-3-β-sulfate, androsterone-3-α-sulfate, and etiocholanol-17-one-3-α-sulfate) normalized by creatinine was negatively correlated with age (30–70 years) for both sexes, and it was highest in individuals aged 30–39 years [23].

A few researches have investigated the relationship between the DHEAs levels in serum and urine. Pratt et al. found that DHEAs levels were highly correlated with urinary excretion of adrenal androgen, with DHEA and DHEAs as the major secretory products (r = 0.82) [28]. The plasma DHEAs concentrations in 14 adult volunteers were positively correlated (r = 0.66) with urine DHEAs excretion [20]. Similarly, another study revealed that plasma DHEAs concentrations were positively correlated with urine DHEAs excretion (r = 0.574) [21]. Several studies have shown an inverse association between serum DHEAs levels and age. In adulthood, DHEAs level peaks at the age of 17–19 years in female and at the age of 20–29 years in male and then declines with advancing age [18]. Moreover, the serum DHEAs concentrations were negatively correlated with age (20–80 years) in both sexes [3,19,18]. These patterns differ from the

current result wherein urine DHEAs peaked in the age group 30–39 years for both sexes and then decreased with advancing age.

For the relationship between DHEAs levels and gender, serum DHEAs levels were higher in males than females in all age groups [3,19,18]. Some study reported that serum DHEAs values were higher in men than in women until the ninth decade of life, but statistically significant sex difference was observed only in the aged group of 70–89 year (P-value < 0.0001) [19]. For non-invasive study, Jia et al. studied the correlation between DHEAs in 24-h urine and gender. Their analysis found that 24-h secretion amount of DHEAs in urine has significantly higher in males than the same age of females (P-value $= 0.0046$) [23]. In this study, the result demonstrates that there was no sexes difference in median DHEAs and DHEAs/Creatinine for the age groups 20–29, 30–39, and 40–49, while the significant difference in DHEAs/Creatinine was observed in the age groups 50–59 and the significant difference in DHEAs was shown in the age groups >60. The results are limited by the small sample sizes so an increase in the sample sizes would provide significant results. Due to our limitations, not all subjects 35 years and above were tested to complete all clinical parameters adopted from Park et al. [25] They were checked for laboratory parameters ordered by their physicians on their visits. However, most of them had got BMI, BP, HR, RFT, CBC and LP results.

The results from the multiple regression showed that the DHEAs levels normalized by creatinine correlated with age and sex ($P < 0.05$). Our fitted model implies that as age increases by 1 year, DHEAs/Creatinine is expected to decrease by 3.63% in females and by 2.18% in males. Similarly, serum DHEAs levels significantly decrease with age (2%–3% per year) [2–4]. Therefore, urine DHEAs measurement could be a non-invasive method to be employed in studies on aging and age-related diseases.

Conflict of interest

Potential conflict of interest exists:

Bottom of Form.

We wish to draw the attention of the Editor to the following facts, which may be considered as potential conflicts of interest, and to significant financial contributions to this work:

The nature of potential conflict of interest is described below:

No conflict of interest exists.

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Funding

Funding was received for this work.

All of the sources of funding for the work described in this publication are acknowledged below:

This study was funded by the National Innovation Agency; Silicon Craft Technology PLC, Thailand; the Center of Excellence on Medical Biotechnology [grant number CD-61-005]; the S&T Postgraduate Education and Research Development Office; the Office of Higher Education Commissionand the Science Achievement Scholarship of Thailand. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

No funding was received for this work.

Intellectual property

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

Research ethics

We further confirm that any aspect of the work covered in this manuscript that has involved human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

IRB approval was obtained (required for studies and series of 3 or more cases).

Written consent to publish potentially identifying information, such as details or the case and photographs, was obtained from the patient(s) or their legal guardian(s).

Authorship

The International Committee of Medical Journal Editors (ICMJE) recommends that authorship be based on the following four criteria:

1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND.
2. Drafting the work or revising it critically for important intellectual content; AND.
3. Final approval of the version to be published; AND.
4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of

the work are appropriately investigated and resolved.

All those designated as authors should meet all four criteria for authorship, and all who meet the four criteria should be identified as authors. For more information on authorship, please see http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html#two.

All listed authors meet the ICMJE criteria. We attest that all authors contributed significantly to the creation of this manuscript, each having fulfilled criteria as established by the ICMJE.

One or more listed authors do (es) not meet the ICMJE criteria.

We believe these individuals should be listed as authors because:

[Please elaborate below].

We confirm that the manuscript has been read and approved by all named authors.

We confirm that the order of authors listed in the manuscript has been approved by all named authors.

Contact with the editorial office

The Corresponding Author declared on the title page of the manuscript is:

Rina Patramanon.

This author submitted this manuscript using his/her account in editorial submission system.

We understand that this Corresponding Author is the sole contact for the Editorial process (including the editorial submission system and direct communications with the office). He/she is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs.

We confirm that the email address shown below is accessible by the Corresponding Author, is the address to which Corresponding Author's editorial submission system account is linked, and has been configured to accept email from the editorial office of the journal of Practical Laboratory Medicine: narin@kku.ac.th.

Someone other than the Corresponding Author declared above submitted this manuscript from his/her account in editorial submission system:

We understand that this author is the sole contact for the Editorial process (including editorial submission system and direct communications with the office). He/she is responsible for communicating with the other authors, including the Corresponding Author, about progress, submissions of revisions and final approval of proofs.

Acknowledgments

We would like to acknowledge the financial support extended by the National Innovation Agency; Silicon Craft Technology PLC, Thailand; the Center of Excellence on Medical Biotechnology [grant number CD-61-005]; the S&T Postgraduate Education and Research Development Office; the Office of Higher Education Commission and the Science Achievement Scholarship of Thailand.

References

[1] S.S.C. Yen, G.A. Laughlin, Aging and the adrenal cortex, Exp. Gerontol. 33 (1998) 897–910, https://doi.org/10.1016/S0531-5565(98)00046-1.

[2] N. Orentreich, J.L. Brind, J.H. Vogelman, R. Andres, H. Baldwin, Long-term longitudinal measurements of plasma dehydroepiandrosterone sulfate in normal men, J. Clin. Endocrinol. Metab. 75 (1992) 1002–1004, https://doi.org/10.1210/jcem.75.4.1400863.

[3] F. Labrie, A. Bélanger, L. Cusan, J.L. Gomez, B. Candas, Marked decline in serum concentrations of adrenal C19 sex steroid precursors and conjugated androgen metabolites during aging, J. Clin. Endocrinol. Metab. 82 (1997) 2396–2402, https://doi.org/10.1210/jcem.82.8.4160.

[4] Y. Nishizaki, A. Kubo, I. Kuwahira, R. Tanino, T. Ogawa, N. Ishii, A proposal research for new health check-up items focusing on the future super-aging society in Japan, Heal. Eval. Promot. 38 (2011) 537–546, https://doi.org/10.7143/jhep.38.537.

[5] M.A. Flynn, D. Weaver-Osterholtz, K.L. Sharpe-Timms, S. Allen, G. Krause, Dehydroepiandrosterone replacement in aging humans, J. Clin. Endocrinol. Metab. 84 (1999) 1527–1533, https://doi.org/10.1210/jc.84.5.1527.

[6] G. Valenti, L. Ferrucci, F. Lauretani, G. Ceresini, S. Bandinelli, M. Luci, G. Ceda, M. Maggio, R.S. Schwartz, Dehydroepiandrosterone sulfate and cognitive function in the elderly: the InCHIANTI study, J. Endocrinol. Invest. 32 (2009) 766–772, https://doi.org/10.3275/6438.

[7] G. Zhu, Y. Yin, C.L. Xiao, R.J. Mao, B.H. Shi, Y. Jie, Z.W. Wang, Serum DHEAS levels are associated with the development of depression, Psychiatr. Res. 229 (2015) 447–453, https://doi.org/10.1016/j.psychres.2015.05.093.

[8] T.T. Wu, Y. Chen, Y. Zhou, D. Adi, Y.Y. Zheng, F. Liu, Y.T. Ma, X. Xie, Prognostic value of dehydroepiandrosterone sulfate for patients with cardiovascular disease: a systematic review and meta-analysis, J. Am. Heart Assoc. 6 (2017) 1–14, https://doi.org/10.1161/JAHA.116.004896.

[9] M.A. Ghebre, D.J. Hart, A.J. Hakim, B.S. Kato, V. Thompson, N.K. Arden, T.D. Spector, G. Zhai, Association between DHEAS and bone loss in postmenopausal women: a 15-year longitudinal population-based study, Calcif. Tissue Int. 89 (2011) 295–302, https://doi.org/10.1007/s00223-011-9518-9.

[10] Y. Aslan, Ü. Tekdoğan, A. Tuncel, M.B. Uzun, E. Karabulut, A. Atan, Serum dehydroepiandrosterone sulfate usage for early detection of prostate cancer in men with serum prostate specific antigen level between 2.5 and 4.0 ng/ml: a pilot study, Turk. J. Med. Sci. 38 (2008) 399–404.

[11] E.E. Baulieu, Dehydroepiandrosterone (DHEA): a fountain of youth? J. Clin. Endocrinol. Metab. 81 (1996) 3147–3151, https://doi.org/10.1210/jcem.81.9.8784058.

[12] E.-E. Baulieu, G. Thomas, S. Legrain, N. Lahlou, M. Roger, B. Debuire, V. Faucounau, L. Girard, M.-P. Hervy, F. Latour, M.-C. Leaud, A. Mokrane, H. Pitti-Ferrandi, C. Trivalle, O. de Lacharriere, S. Nouveau, B. Rakoto-Arison, J.-C. Souberbielle, J. Raison, Y. Le Bouc, A. Raynaud, X. Girerd, F. Forette, Dehydroepiandrosterone (DHEA), DHEA sulfate, and aging: contribution of the DHEAge study to a sociobiomedical issue, Obstet. Gynecol. Surv. 55 (2000) 695–696, https://doi.org/10.1097/00006254-200011000-00018.

[13] W. Arlt, F. Callies, J.C. van Vlijmen, I. Koehler, M. Reincke, M. Bidlingmaier, D. Huebler, M. Oettel, M. Ernst, H.M. Schulte, B. Allolio, Dehydroepiandrosterone replacement in women with adrenal insufficiency, N. Engl. J. Med. 341 (1999) 1013–1020, https://doi.org/10.1056/NEJM199909303411401.

[14] P.J. Hunt, E.M. Gurnell, F.A. Huppert, C. Richards, A.T. Prevost, J.A.H. Wass, J. Herbert, V.K.K. Chatterjee, Improvement in mood and fatigue after dehydroepiandrosterone replacement in Addison's disease in a randomized, double blind trial, J. Clin. Endocrinol. Metab. 85 (2000) 4650–4656, https://doi.org/10.1210/jc.85.12.4650.

[15] M. Panjari, S.R. Davis, DHEA therapy for women: effect on sexual function and wellbeing, Hum. Reprod. Update 13 (2007) 239–248, https://doi.org/10.1093/humupd/dml055.
[16] E. de Peretti, M.G. Forest, Pattern of plasma dehydroepiandrosterone sulfate levels in humans from birth to adulthood: evidence for testicular production*, J. Clin. Endocrinol. Metab. 47 (1978) 572–577, https://doi.org/10.1210/jcem-47-3-572.
[17] L.N. Parker, J. Sack, D.A. Fisher, W.D. Odell, The adrenarche: prolactin, gonadotropins, adrenal androgens, and cortisol, J. Clin. Endocrinol. Metab. 46 (1978) 396–401, https://doi.org/10.1210/jcem-46-3-396.
[18] D.G. Young, G. Skibinski, J.I. Mason, K. James, The influence of age and gender on serum dehydroepiandrosterone sulphate (DHEA-S), IL-6, IL-6 soluble receptor (IL-6 sR) and transforming growth factor beta 1 (TGF-β1) levels in normal healthy blood donors, Clin. Exp. Immunol. 117 (1999) 476–481, https://doi.org/10.1046/j.1365-2249.1999.01003.x.
[19] G. Ravaglia, P. Forti, F. Maioli, F. Boschi, M. Bernardi, L. Pratelli, A. Pizzoferrato, G. Gasbarrini, The relationship of dehydroepiandrosterone sulfate (DHEAS) to endocrine-metabolic parameters and functional status in the oldest-old. Results from an Italian study on healthy free-living over-ninety-year-olds, J. Clin. Endocrinol. Metab. 81 (1996) 1173–1178, https://doi.org/10.1210/jcem.81.3.8772596.
[20] T. Remer, K. Pietrzik, F. Manz, Measurement of urinary androgen sulfates without previous hydrolysis: a tool to investigate adrenarche. Validation of a commercial radioimmunoassay for dehydroepiandrosterone sulfate, Steroids 59 (1994) 10–15, https://doi.org/10.1016/0039-128X(94)90038-8.
[21] R.H. Straub, C. Weidler, B. Demmel, M. Herrmann, F. Kees, M. Schmidt, J. Schölmerich, J. Schedel, Renal clearance and daily excretion of cortisol and adrenal androgens in patients with rheumatoid arthritis and systemic lupus erythematosus, Ann. Rheum. Dis. 63 (2004) 961–968, https://doi.org/10.1136/ard.2003.014274.
[22] C.J.H. Kelnar, C.G.D. Brook, A mixed longitudinal study of adrenal steroid excretion in childhood and the mechanism of adrenarche, Clin. Endocrinol. 19 (1983) 117–129, https://doi.org/10.1111/j.1365-2265.1983.tb00750.x.
[23] Q. Jia, M.F. Hong, Z.X. Pan, C. Ritter, S. Vance, M. Cortes-Guzman, S. Cherniske, Quantification of urine 17-ketosteroid sulfates and their age correlations, Spectroscopy 16 (2002) 171–181, https://doi.org/10.1155/2002/835987.
[24] R.S. Ahn, Y.J. Lee, J.Y. Choi, H.B. Kwon, S. Il Chun, Salivary cortisol and DHEA levels in the Korean population: age-related differences, diurnal rhythm, and correlations with serum levels, Yonsei Med. J. 48 (2007) 379–388, https://doi.org/10.3349/ymj.2007.48.3.379.
[25] J.H. Park, B.L. Cho, H.T. Kwon, C.M. Lee, Developing a biological age assessment equation using principal component analysis and clinical biomarkers of aging in Korean men, Arch. Gerontol. Geriatr. 49 (2009) 7–12, https://doi.org/10.1016/j.archger.2008.04.003.
[26] N.I. Bahr, R. Palme, U. Möhle, J.K. Hodges, M. Heistermann, Comparative aspects of the metabolism and excretion of cortisol in three individual nonhuman primates, Gen. Comp. Endocrinol. 117 (2000) 427–438, https://doi.org/10.1006/gcen.1999.7431.
[27] T. Nagaya, Y. Kondo, T. Okinaka, Serum dehydroepiandrosterone-sulfate reflects age better than health status, and may increase with cigarette smoking and alcohol drinking in middle-aged men, Aging Clin. Exp. Res. 24 (2012) 134–138, https://doi.org/10.1007/BF03325159.
[28] J.H. Pratt, A.K. Manatunga, M.A. Wagner, J.J. Jones, F.J. Meaney, Adrenal androgen excretion during adrenarche. Relation to race and blood pressure, Hypertension 16 (1990) 462–467, https://doi.org/10.1161/01.HYP.16.4.462.
[29] T. Remer, K.R. Boye, M.F. Hartmann, S.A. Wudy, Urinary markers of adrenarche: reference values in healthy subjects, aged 3-18 years, J. Clin. Endocrinol. Metab. 90 (2005) 2015–2021, https://doi.org/10.1210/jc.2004-1571.