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Original Article

Relationship Between Depression Anxiety Stress Scale (DASS) and Urinary Hydroxyproline and Proline Concentrations in Hospital Workers

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Objectives: Although increased reactive oxygen species (ROS) is caused by stress accelerates collagen degradation, there was no data on the relationship between stress and urinary hydroxyproline (Hyp) and proline (Pro), a good marker of collagen degradation. The purpose of this study was to evaluate the relationship between depression, anxiety, and stress (DAS) and concentrations of urinary Hyp and Pro.
Methods: 97 hospital employees aged 20 to 58 were asked to fill out comprehensive self-administrated questionnaires containing information about their medical history, lifestyle, length of the work year, shit-work and DAS. Depression Anxiety Stress Scale (DASS) was applied to evaluate chronic mental disorders. Urine samples were analyzed using High Performance Liquid Chromatography (HPLC) with double derivatization for the assay of hydroxyproline and proline.
Results: The mean value of Hyp and Pro concenturation in all subjects was 194.1±113.4 μmol/g and 568.2±310.7 μmol/g. DASS values and urinary Pro concentrations were differentiated by sex (female > male, $p < 0.05$) and type of job (nurse > others, $p < 0.05$). In the stepwise multiple linear regressions, urinary Hyp and Pro concentrations were influenced by stress (Adjusted $r^2 = 0.051$) and anxiety and job (Adjusted $r^2 = 0.199$), respectively.
Conclusions: We found that stress and anxiety were correlated with urinary Hyp and Pro concentrations. To identifying a definite correlation, further study in large populations will be needed.

Key words: Depression, Anxiety, Stress, Hydroxyproline, Proline
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INTRODUCTION

Most people experience depression, anxiety, and stress (DAS) in their lifetime. There have been numerous studies on pathophysiological effects like hormonal change that were caused by DAS. Activation of nervous system caused by psychological stimuli like DAS promotes the excretion of catecholamine such as epinephrine and norepinephrine [1]; and steroid hormones such as cortisol [2-3] in the adrenal gland. However, if mental stimuli disappear, hormones return to normal range values quickly. Therefore, stress hormones are not suitable biomarkers for chronic stress.

There have been many studies on association between stress and reactive oxygen species (ROS) as a pathophysiological change. Anxiety increased secretion of plasma norepinephrine as a stress hormone, which in turn increased ROS formation by mononuclear cells [4]. In addition, high anxiety level significantly increased the generation of ROS in the peripheral blood lymhpocytes, granulocytes and monocytes [5]. In recent study, elevated ROS caused by DAS augmented concentration of 8-hydroxy-2'-deoxyguanosine, a biomarker of aging [6].

Depression has been likened to a state of "accelerated aging" and depressed individuals have a higher incidence of aging diseases [7]. Besides, ROS can cause both a decrease in fibrillar collagen synthesis and an increase in matrix metalloproteinase (MMP) activities [8]. Moreover, in recent years, it has become clear that ROS serves an important role as signaling molecules that regulate many genes, including MMPs [9-12]. hydroxyproline (Hyp) and proline (Pro) are especially abundant in collagen and give rigidity to collagen molecules [13]. It is well known that Hyp and Pro, highly correlated with collagen degradation, is excreted into urine [14].

Several published research papers found that DAS is concerned with collagen metabolism causing hormonal

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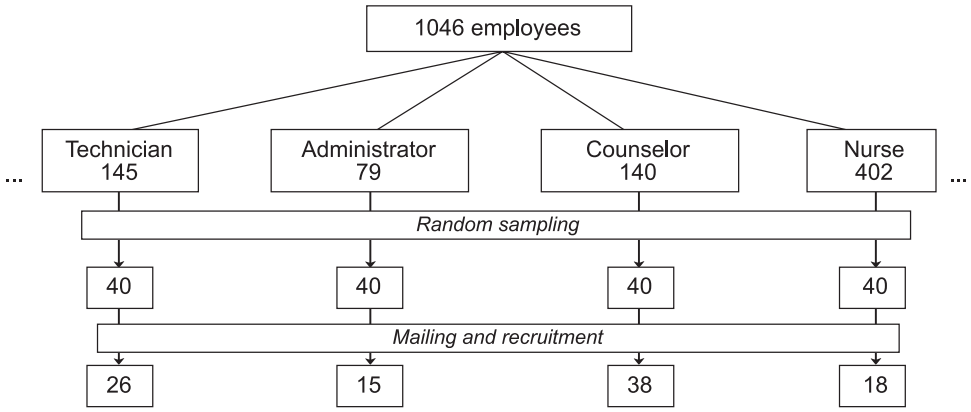

Figure 1. Sampling process of the study.

change, ROS increase, and increased activation of MMPs. However, there was no data on the relation between DAS and urinary Hyp and Pro. Therefore, the purpose of this study was to identify whether Hyp and Pro, good markers of collagen degradation could be biological DAS indicators analyzing correlation between DAS and urinary Hyp and Pro.

METHODS

I. Participants

The study subjects were drawn from Ajou University Medical Center (AUMC) in South Korea. Among 1046 employees, four representative types of job were selected such as medical technician (145 persons), administrator (79 persons), counselor (140 persons) and nurse (402 persons). Random numbers were assigned to each 766 individuals. 40 individuals in each type of job were selected by systematic sampling. A total of 160 individuals were got a letter from investigators and recruited. Finally, 97 male and female workers aged 20 to 58 years participated voluntarily (Figure 1).

II. Questionnaires

All participants filled out a comprehensive self-administrated questionnaires requesting information on their medical history (osteoporosis, cardiovascular diseases, inflammatory diseases, musculoskeletal diseases, and other diseases), lifestyle, length of the work year, status of shit-work and DASS. Job description of participants was medical technician (26 persons), administrator (15 persons), counselor (38 persons) and nurse (18 persons). To measure depression, anxiety, and stress, DASS was used. DASS was based on depression, anxiety, and stress. DASS-Depression is characterized by low positive affects, loss of self-esteem and incentive, and a sense of hopelessness (absence of positive affect); DASS-Anxiety is characterized by autonomic arousal and fearfulness (psychological hyper arousal); and DASS-Stress is characterized by persistence tension, irritability, and a low threshold for becoming upset or frustrated (negative affects). DASS has been primarily applied to nonclinical samples, and provided strong support for the internal consistency and convergent and divergent validity of its three scales [15]. Each three DAS scales contains 14 items. Subjects are asked to use 4-point severity scales to rate the extent to which they have experienced each state over the past week. Scores for DAS are calculated by summing the scores for the relevant items. DASS reliability and validity were already published in previous studies [11-14]. In this study, the internal consistency of the DASS subscales was considerably high, which is consistent with previous studies [15-18]. All subjects were asked to fill out the DASS questionnaire, which takes approximately 10 minutes, about 1 hour before the completion of their daily work. Urine samples were collected after they completed questionnaires.

III. Urine Analysis

Concentrated hydrochloric acid (1 mL) was added to urine (1 mL). The mixture was hydrolyzed at 120°C for 16 hours in a screw-capped vial using a heating block and then left standing at room temperature for 30 min. The resultant mixture (200 uL) was transferred to a vial and mixed with 2 mol/L sodium carbonate (500 uL) and then mixed with borate buffer (0.1 mol/L, pH 8.0, 1 mL) and OPA (0.4% in acetonitrile:borate buffer (0.1 mol, pH 8.0)

= 1:1500 uL). After standing for 20 min at room temperature, 500 uL of the OPA-treated mixture was passed through a Bond-Elut C18 column and the column was then washed with borate buffer (500 uL) and the washings were mixed. The mixture was used for the derivatization reaction with Dansyl-Cl (1.5 mmol in acetone, 300 uL) at 70°C for 10 min. The reaction mixture was mixed well with dichloromethane (2 mL) and then centrifuged at 3000 rpm for 10 min before an aliquot (10 uL) of the aqueous layer was subjected to HPLC. The Hyp and Pro control samples were treated in the same way as the urine samples except for overnight heating.

The HPLC system (Hewlett Packard Series 1050, USA) consisted of two pumps, an auto injector, and a 1064 A fluorescence detector. An Eclipse XDB-C18 column (250 ×4.6 mm i.d., 5 um Hewlett Packard) was connected with a gradient system of (A) phosphate buffer (25 mmol/L, pH 3.0)-(B) acetonitrile. The elution program consisted of an isocratic elution of 20% B for 5 min, followed by linear gradient elution from 20 to 30% of B for 5 min, followed by linear gradient elution from 30 to 70% of B for 10 min, finally followed by a stepwise decrease to 20% B to re-equilibrate the column for 10 min.

IV. Statistical Analysis

Cronbach's alphas were calculated for each of the DASS subscales to evaluate the internal consistency. The characteristics of the study subjects were presented in Table 1. Mean differences of DAS, Hyp, Pro were tested according to the categories of sex, job description, smoking status, and alcohol drinking status using *t-test* and ANOVA. To assess the variables affecting Hyp and Pro, stepwise multiple linear regressions were conducted.

Table 1. Characteristics of study subjects

Characteristics	n	% or Mean±SD
Age	97	34.1±7.6
Sex		
Male	37	38.1
Female	60	61.9
Job description		
Technician	26	26.8
Admistrator	15	15.5
Counselor	38	39.2
Nurse	18	18.6
Smoking		
No	79	81.4
Yes	18	18.6
Alcohol drinking		
No	82	84.5
Yes	15	15.5

SD: standard deviation.

Independent variables in each model were age, sex, job description, depression, anxiety, and stress. All *p*-values were two-tailed, with a *p*-value of less than 0.05 considered to be of statistical significance. All statistical analyses were conducted using PASW Statistics 17.0 (SPSS Korea Datasolution, Chicago, USA).

RESULTS

Internal consistency of the DASS subscales was considerably high, with Cronbach's alphas of 0.91, 0.85 and 0.90 for the depression, anxiety, and stress subscales, respectively. Characteristics of the study participants are presented in Table 1. There were 37 male (38.1%) and 60 female (61.9%) participants. Mean Hyp and Pro of all subjects were 194.1±113.4 umol/g creatinine (n=92) and 568.2± 310.7 umol/g creatinine (n=97),

Table 2. Comparison of mean values of the depression anxiety stress scale (DASS) and hydroxyproline and proline concentrations according to sex, job description, smoking and alcohol drinking

Characteristics	DASS (Mean±SD)			Urine (Mean±SD, mmol/g creatinine)	
	Depression	Anxiety	Stress	Hyp	Pro
Sex					
Male	4.62±5.07**	5.97±5.51**	9.49±7.25**	186.50±128.06	453.34±218.73*
Female	8.10±6.61	8.77±5.52	12.83±6.46	198.52±104.76	639.06±338.36
Job description					
Technician	3.79±5.06**	5.79±6.28	8.71±7.33**	198.24±149.00	452.14±260.88**
Admistrator	5.46±4.81	7.85±5.83	12.54±7.63	166.00±49.62	485.04±170.65
Counselor	6.84±6.20	8.41±5.63	11.57±6.02	197.55±89.34	602.28±279.91
Nurse	11.39±6.54	8.89±4.50	15.0±6.62	201.67±139.35	783.11±413.66
Smoking					
No	5.33±4.64	7.11±5.80	12.17±7.95	203.26±159.55	547.28±473.04
Yes	7.10±6.57	7.84±5.64	11.42±6.72	192.15±102.39	572.99±264.39
Alcohol drinking					
No	7.10±6.51	7.94±5.74	11.98±7.08	189.29±117.49	588.82±327.50
Yes	5.00±4.60	6.40±5.14	9.27±5.68	223.17±81.84	455.57±158.88

Hyp: hydroxyproline, Pro: proline, SD: standard deviation.
*p<0.01, **p<0.05.

Table 3. Stepwise multiple linear regression analysis for Hyp and Pro concentrations

Dependent variable	Independent variable	β	SE	*p-value*	Adjusted r^2
Hyp	Stress	4.023	1.665	0.018	0.051
Pro	Nurse	237.619	72.975	0.002	0.199
	Anxiety	17.992	5.047	0.001	

Independent variables: age, sex, job description (nursing vs. others (reference)), depression, anxiety, stress.
SE: standard error, Hyp: hydroxyproline, Pro: proline.

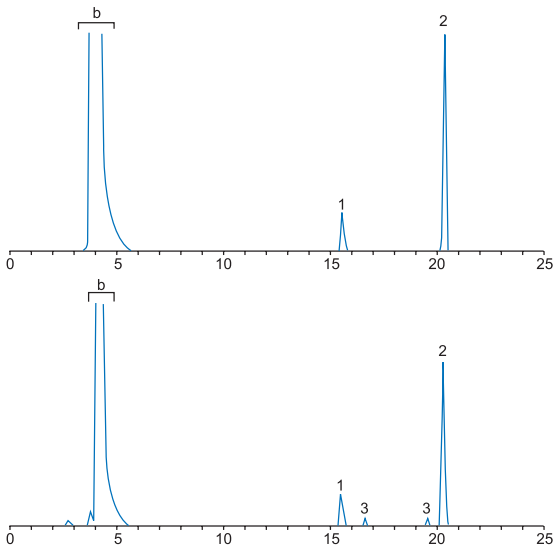

Figure 2. Chromatogram obtained from (A) standard solution and (B) urine according to the procedure for urine analysis.

Peaks: 1=Hyp, 2=Pro, 3=unknown, b=reagent blank. Concentration: (A) Hyp = 91.5 umol/l, Pro = 243.2 umol/L; (B) Hyp = 82.5 umol/L, Pro = 123.3 umol/L.
Hyp: hydroxyproline, Pro: proline.

respectively. 79 participants were non-smokers (81.4%) and 18 smokers (18.6%). 82 (84.5%) respondents did not drink on the day immediately before the questionnaire day and 18 (18.6%) respondents did (Table 2). Respondents did not suffer from osteoporosis, cardiovascular diseases, inflammatory diseases, musculoskeletal diseases (Data not shown).

The mean values of the DAS and Hyp and Pro concentrations were compared with the general characteristics of the participants. DAS values and urinary Pro concentrations in females were remarkably higher than in males ($p<0.05$). However, there were no significant differences in DAS and Hyp and Pro concentrations by smoking status and alcohol drinking status (Table 2).

After adjusting for covariates, Table 3 presented the results of stepwise linear regression analysis for Hyp and Pro. Urinary Hyp concentrations were related to stress ($\beta = 4.023$, $p = 0.02$) with 0.051 of adjusted r^2. Pro concentrations were related to nurse ($\beta = 237.619$, $p = 0.002$) and anxiety ($\beta = 17.992$, $p = 0.001$), with 0.199 of adjusted r^2.

DISCUSSION

Although many studies on the relationship between acute stress and hormonal change s have been published, studies on psycophysiological changes including aging caused by chronic stress are limited. We investigated whether Hyp and Pro is available as a physiological indicator of chronic stress using a cross-sectional data.

Regarding urine analysis, we applied improved precise method than a previous published method [19]. Typical chromatograms were obtained from standard solution and urine under the described conditions is shown Figure 2(A) and (B), respectively. Peaks 1 and 2 corresponding to Hyp and Pro eluted 15.5 and 20.2 min, respectively. The within-day and day-to-day relative standard deviations (RSD) for urinary Hyp and Pro were 4.54 and 4.84 %, 4.97 and 5.61 %, correspondingly. The detection limits (signal-to-noise $\approx$ 3) of both Hyp and Pro were 10 nmol per injection (corresponding to about 10 umol/L each in urine).

Urinary Hyp and Pro concentrations of this study subjects were 194.1$\pm$113.4 umol/g creatinine (n=92) and 568.2$\pm$310.7 umol/g creatinine (n=97), respectively. 97 subjects were 34.1$\pm$7.6 years old and 61.9% was female. Job descriptions were technician, administrator, counselor, and nurse. DAS mean values and Pro was significantly different according to sex and job description. There are some ambiguities reporting of association of urinary Pro concentration and sex. In studies on the difference of urinary Hyp and Pro according to age and sex, in 20 to 40 years old, differences were weak; while, in 50 years old or more, females had higher concentration of Hyp and Pro than males [19,20]. In this study, females aged 23 to 47 were included. Therefore, in the regression model results, age and sex were excluded. Shift work was not included in regression models because 17 shift workers were all female (nurse). In 37 females, differences of DAS and urinary Hyp and Pro according to shift work were not significant (Data not shown). In stepwise multiple linear regression tests, urinary Hyp concentration was influenced by stress, about 5.1% and urinary Pro was influenced by anxiety and job description (Nurse vs. others), about 19.9%, respectively. Since DASS is a screening tool for diagnosis of chronic mental disorders, DAS scores need to be defined as normal or

abnormal as independent variables. However, DAS could not be included as categorical variables due to small abnormal cases.

In summary, we tried to find a relationship between chronic mental disorders and urinary Hyp and Pro concentrations. Pro was correlated with anxiety but Hyp was weakly correlated with stress. Further evaluations are needed to verify the relationship between DAS and urinary Hyp and Pro.

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CONFLICT OF INTEREST

The authors have no conflicts of interest with the material presented in this paper.

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