〈Original Article〉

Blood oxidative stress levels and urinary dopamine values reflect the impact of academic stress on students

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Summary A national examination is compulsory for students aiming to be medical technologists. A medical technologist's license is essential for employment in hospitals and medical institutions, and the study time required to achieve it is lengthy. In this study, we researched the influence of academic stress based on the relationship between oxidative stress, and urinary dopamine as a biomarker. The subjects were 30 healthy students. We collected blood and urine prior to the 55th national examination for medical technologist. As an oxidative stress monitor, we measured the blood and urine levels of a derivatives-reactive oxygen metabolites (d-ROMs) test. A correlation was found between the blood d-ROMs levels three days before the national examination and the examination scores (r=-0.487); there was also a correlation between the urinary dopamine and d-ROMs levels (r=0.445). These results suggest that d-ROMs are eliminated through the kidney, and that dopamine, which regulates the renal plasma flow rate, plays an important role in the regulation of oxidative stress.

Key words: Academic stress, Oxidative stress, d-ROMs, Urinary dopamine

1. Introduction

A national examination is compulsory for students seeking to be medical technologists. The examination is difficult for most students. Since a medical technologist's license is required for employment in hospitals and medical institutions, they must work hard to pass the examination. Such academic stress results in oxidative stress in vivo, leading to various oxidative stress-related conditions. In this study, we researched the influence of academic stress in vivo based on the relationship between the values of oxidative stress, which was evaluated using a derivatives-reactive oxygen metabolites (d-ROMs) test[1)], established by Alberti et al. and the score on a national examination. Many studies have reported the relationship between this biomarker and exercise stress or various disorders[2-4)]. We collected blood and urine samples from 30 students who were studying to pass the national examination, and investigated whether d-

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ROMs may be used as a biomarker of academic stress.

2. Materials and Methods

1. Subjects and samples

The subjects were 30 students belonging to the Department of Clinical Technology, Fujita Health University. We collected 20 μl of blood through fingertip puncture and 10 ml of urine at any time during the three weeks prior to the 55th national examination for medical technologist. Three days before the examination, 20 μl of blood was also collected. The self marking results of each subject were declared as a national examination scores on which the national examination order was based. Written informed consent was obtained from all subjects, and the study was in compliance with our university rules for human experimentation.

2. Measurement of blood d-ROMs, urinary d-ROMs and urinary dopamine

For an oxidative stress monitor including hydroperoxides, a precursor of the chain initiation of lipid hyperoxidation[5], the whole blood and casual urine levels of d-ROMs were determined using a special measurement device (FRAS4, H&D Inc., Italy). This test was a measure of the equilibrium between free radical production and antioxidant defense. Hydroperoxides are converted into radicals that an oxidize chromogen substrate (N,N-diethyl-para-phenylendiamine) and that can be detected through spectrophotometric procedures at 505 nm[1]. In this method, 1 unit (Carratelli units: U.CARR) represents 0.08 mg/100 mL H_2O_2, equivalent to the concentration of hydroperoxides. The normal range of d-ROM in whole blood is 250 to 300 U.CARR. Values outside this range indicate a modification of the prooxidant /antioxidant ratio[1]. Urinary creatinine was measured using the enzyme colorimetric method (SRL Inc.; Tokyo). The urinary dopamine was measured using the HPLC (L-7000, Hitachi, Ltd.; Tokyo) method (SRL Inc.; Tokyo). Urinary d-ROMs and dopamine levels were compared by dividing them by the urinary creatinine concentration.

3. Statistical analysis

Statistical evaluations of the data were performed using Pearson's product-moment coefficient of correlation. A statistical level of $p < 0.05$ was considered significant.

3. Results

The blood d-ROMs levels determined three weeks before the national examination was 243±62 U.CARR. Three days before it was 247±50 U.CARR, thus showing no significant difference both levels. The urinary d-ROMs level was 0.021±0.021

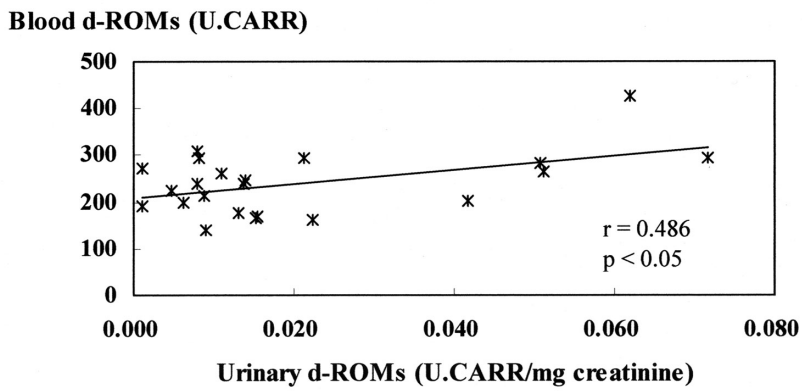

Fig. 1 Correlation between urinary d-ROMs and blood d-ROMs
A significant correlation exists between blood d-ROMs and urinary d-ROMs, indicating that the former are eliminated to urine.

U. CARR/mg creatinine, while the urinary dopamine concentration was 0.559±0.118 mg/mg creatinine.

There was a correlation between the blood and urinary d-ROMs levels (r=0.486, p <0.05)(Fig. 1) as well as a correlation between the urinary dopamine and d-ROMs levels (r=0.445, p <0.05)(Fig. 2). We compared the subjects' order on the national examination with their blood d-ROMs levels assessed three weeks earlier, and found no correlation (r=0.168: Figure not shown). However, there was a positive correlation between their order and the blood d-ROMs levels three days before the examination (r=0.532, p <0.01)(Fig. 3). In addition, the blood d-ROMs level three days before it was negatively correlated with the examination score (r=-0.487, p <0.05). On the other hand, a positive correlation existed between the urinary dopamine concentration and the examination score (r=0.567, p <0.01)(Fig. 5). There was no correlation between the examination score and the excretion values to the urine of d-ROMs, norany correlation between it and the examination order (r=0.210 and -0.251: Figure not shown).

4. Discussion

We have examined academic stress using stress

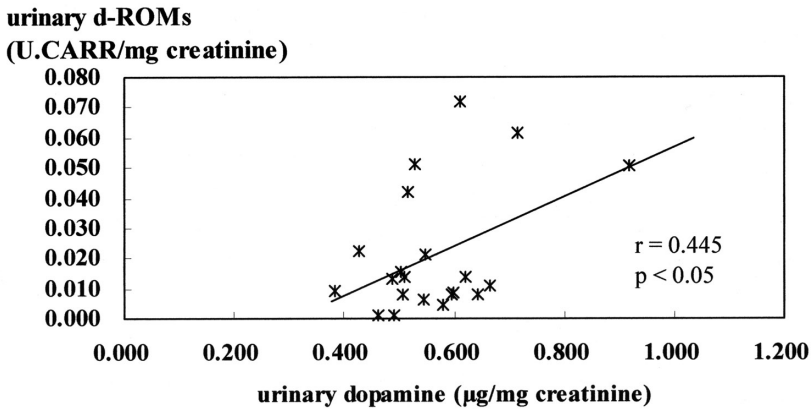

Fig. 2 Correlation between urinary dopamine and urinary d-ROMs.
A significant correlation exists between urinary dopamine and urinary d-ROMs, implying that if there is much elimination of dopamine in the urine, there is also an elimination of d-ROMs to the urine.

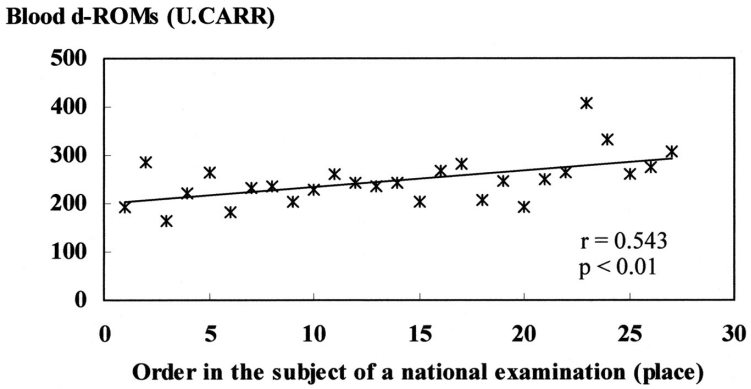

Fig. 3 Correlation between the order of a national examination for medical technologist and blood d-ROMs levels three days before the examination.
A significant correlation existed between the order of a national examination and blood d-ROMs levels, r=0.543 (p <0.01).

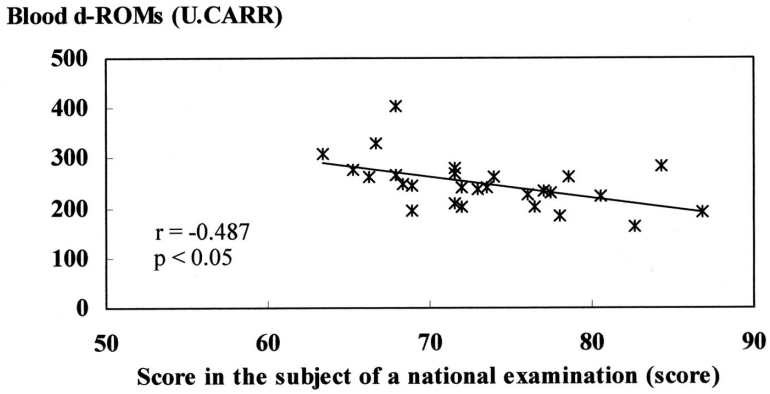

Fig. 4 Correlation among scores on a national examination for medical technologist and blood d-ROMs levels during the previous three days.
Significantly correlation between examination scores and blood d-ROMs levels, r=-0.487 ($p < 0.05$).

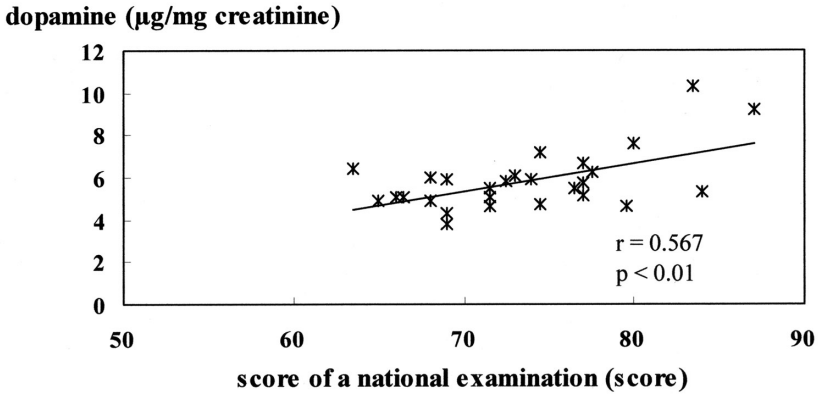

Fig. 5 Correlation between urinary dopamine and score of a national examination.
A significant correlation existed between urinary dopamine and scores on a national examination.
Subject with copious eliminations of dopamine scored highly on the examination.

biomarkers including d-ROMs[6-8]. We also found that diet was an etiological factor related to academic stress[8]. If a regular life rhythm is maintained, oxidative stress may not be enhanced. However, the blood d-ROMs level may not depend on stress-related changes in dietary contents alone[8]. In this study, we investigated the urinary elimination of dopamine, which regulates the renal blood flow rate[10-12], using the urinary elimination of d-ROMs as a biomarker[9]. As presented in the "Results" section, the correlation coefficient between the blood d-ROMs level immediately before the national examination for medical technologist and score was high. This can be explained as follows: 1) D-ROMs, including hydroperoxides in blood, are eliminated in urine[9] (Fig. 1). Therefore, if blood d-ROMs, which increase in the presence of academic stress[6], are thoroughly eliminated in urine, its blood level may not increase; 2) As dopamine increases the renal blood flow rate, the urinary elimination of d-ROMs is higher among those in whom the urinary dopamine level is increased (Fig. 2). Based on the relationship between these biomarkers, the urinary elimination of d-ROMs may be low in subjects showing a low urinary dopamine level. In addition, the blood d-ROMs level increases with the rise in academic stress; and 3) An elevation in the grade of

oxidative stress may increase mental fatigue, reducing learning volition. Therefore, a correlation was observed between the scores on a national examination and d-ROMs levels (Fig. 4). Briefly, those results suggest that dopamine, which regulates the renal plasma flow rate[10-11], also plays an important role in the regulation of oxidative stress. On the other hand, with previous subjects we have measured BAP (Biological Antioxidant Potential) test (Diacron International, Italy) as an antioxidant potential. The BAP test allows us to evaluate the plasma antioxidant biological potential as the capacity of the plasma sample to reduce ferric ions to ferrous ions. Such a biological antioxidant potential is attributable to the major component of the plasma barrier to oxidation (vitamin C, vitamin E, uric acid, bilirubin etc.)[13-14] Our results showed no relation between the BAP test and examination results. However we have recognized that the values of both d-ROMs (U.CARR) and BAP (mmol/L) were significantly correlation in samples taken before an examination. In addition, Since dopamine is a central nerve transmitter, the learning ability may depend on its level (Fig. 5). However, as this issue could not be analyzed in this study, it should be futher examined in a future study.

We concluded that preparations for a national examination produces academic stress, inducing oxidative stress in vivo. We estimated the influence of academic stress on the physical condition based on the d-ROMs levels, including hydroperoxide and dopamine. Dopamine may regulate the urinary elimination of blood d-ROMs, thus influencing the blood d-ROMs levels. The d-ROMs levels immediately before a national examination were correlated with the examination score, suggesting the importance of maintaining a lifestyle that minimizes oxidative stress.

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