



ORIGINAL RESEARCH ARTICLE

# Effect of temporary examination stress on ceruloplasmin, copper, and iron levels in sera of academic students

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## Abstract

**Objective:** Examination stress is a common and widespread phenomenon for students. So the main aim of this study was to check the effect of temporary examination stress on some biochemical parameters in sera of academic students.

**Methods:** A total of 55 apparently healthy undergraduate students were enrolled in this study. Blood samples were collected two times from each student, at a written exam (E-day) and at the normal day (N-day). The serum Cp concentration and Cp ferroxidase activity were measured by spectrophotometry, while total serum copper and iron ions were measured by atomic absorption technique. The results were compared between E-day and N-day.

**Results:** Significantly decrease ( $P < 0.05$ ) were found in the serum Cp concentration, Cp ferroxidase activity, and total serum copper for all students. The total serum iron was significantly decreased ( $P < 0.05$ ) in the female group.

**Conclusion:** Cp ferroxidase activity was significantly decreased in E-day compared with N-day in response to releasing iron that accumulates in the liver. It may be an indicator of increasing oxidative stress in the sera of students in examination stress; also serum copper was significantly decreased in all undergraduate students in this comparison in response to antioxidant role of Cp.

**Keywords:** examination stress, ceruloplasmin, academic students, copper and iron

## Introduction

Stress would simply define as the pressure or force that is exerted by a body. According to psychologists, stress is the demand. It possesses on an organism and how the organism attempts to adapt or cope with the specific demands<sup>1</sup>. Stress is a necessary and unavoidable concomitant of daily living, necessary because without stress human would be a listless and apathetic creature, and unavoidable because it relates to any external event, be it pleasurable or anxiety producing<sup>1</sup>. Stress occurs with an interaction between individuals and the sources of demand within the environment and is affected by one's perception of the stressor either as adaptive or threatening to one's resources<sup>2</sup>. A stressor is any real physical, social, or psychological event or stimulus that causes human bodies to react or respond<sup>3</sup>. It may promote physiological or behavioral disturbances or both. A person's response towards stress depends on

whether an event is appraised as a challenge or a threat<sup>4</sup>. Several factors play a fundamental role in determining the nature and consequences of the stress response. These factors include inherent features of a given type of stressor as well as the conditions under which the stressor is encountered<sup>5</sup>. Examination stress refers to the pressure or stress that is experienced by students to perform well in final school or undergraduate examinations and competitive college entrance examinations<sup>6</sup>. Having examination stress is a common and widespread phenomenon for students<sup>7</sup>. Up to 30–50% of students has test-induced anxiety problems<sup>8</sup>. Every student aspires to pursue academic success to achieve respect, family pride, and social mobility<sup>9</sup>. The test scores of students may decrease due to anxiety under pressure<sup>10</sup>, meanwhile if test anxiety is eliminated, student grades will increase<sup>11</sup>, also showed that test anxiety is significantly correlated with low academic performance<sup>12</sup>. A mild degree of stress and strain can sometimes be beneficial. For example, often feeling mild stressed when carrying out a project or assignment compels us to do a good job,

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focus better and work energetically<sup>13</sup>. Ceruloplasmin (Cp) is one of the antioxidant proteins with 1046 amino-acid residues; it is an abundant  $\alpha_2$ -glycoprotein with 7–8% carbohydrate content<sup>14</sup>, containing over 95% of the copper found in the plasma of all vertebrate species<sup>15</sup>, containing 6–7 copper atoms per molecule and four potential sites for asparagine-linked glycosylation. It is the only blue copper oxidase of human and animal species<sup>16,14</sup>. Cp is synthesized and secreted exclusively by the liver<sup>17</sup> although, the kidneys<sup>18</sup>, lungs<sup>19</sup> and lymphocytes as apportion<sup>15</sup>. It has been implicated in iron metabolism mostly because of its catalytic oxidation of Fe (II) to Fe (III) by its ferroxidase activity<sup>20</sup>. Also, it catalyzes the reduction of an oxygen molecule with the formation of water, without releasing intermediaries potentially toxic ( $O^{2-}$ ,  $H_2O_2$ )<sup>21</sup>. Additionally, Cp plays an essential role in the transport of copper<sup>22</sup>, and it acts as an antioxidant enzyme by inhibiting iron-dependent lipid peroxidation, and formation of hydroxide from hydrogen peroxidase by its ferroxidase activity<sup>23</sup>.

## Materials and Methods

A total of 55 apparently healthy undergraduate students (30 males, 25 females) from College of Science specialized in mathematics, physics, geology, and biotechnology departments, affiliated to the University of Baghdad, and also from Al-Esraa college, a specialty from medical analyzes department were participated in this study. The age of these students ranged from 19 to 24 years ( $20.4 \pm 1.32$ ), and BMI mean values was with ( $24.20 \pm 2.6 \text{ kg/m}^2$ ). All undergraduate students were subjected to a personal interview using specially designed questionnaire format including full history with detailed information. The menstruation day was recorded for females; females during and before one week of menstrual cycle were excluded. Blood samples obtained from the undergraduate students for 2 days; the first day was in the morning of written exam (E-day) and the second day was after 7–15 days after exam, in the morning normal day (N-day). These undergraduate students were sitting in a relatively quiet laboratory place, and they rested for 5–15 min before blood sample taken, and then 5 ml of venous

blood samples were collected, centrifuged after clotting, and the clear serum was separated and stored at  $-20^\circ\text{C}$  in Eppendorf tubes until the time of assay. In the normal day, the collecting of samples from undergraduate students with abnormal feeling was postponed to another day.

## Determination of Cp ferroxidase activity, total protein, and Cp concentration

Cp ferroxidase activity was measured according to Erel by the end point measurement method<sup>24</sup>. To calculate the Cp ferroxidase-specific activity, total protein concentration was determined by Modified Lowry's method<sup>25</sup>. Cp concentration has been determined using Holmberg Laurell method<sup>26</sup>.

## Determination of serum copper and iron

Serum copper and iron was determined using digestion method with flame atomic absorption technique<sup>27</sup>.

## Statistical Analysis

The data were analyzed by Duncan's multiple range test at ( $P < 0.05$ ) was accepted as statistically significant, using the SPSS version 21.0 with depending *t*-test analysis.

## Results

To study the effect of examination stress on the studied parameters, the parameters were measured in the sera of all undergraduate students and according to gender comparing E-day with N-day, and to check the variation between male and female the results of each day (E-day and N-day) were compared.

As shown in Table 1, there were significant decreases ( $P < 0.05$ ) in the mean values of Cp concentration and Cp ferroxidase activity with its specific activity in sera of all undergraduate students. Depending on gender, only female group showed a highly significant decrease ( $P < 0.01$ ) in these parameters, while there were non-significant values in the male group in this comparison. Depending on each day (N-day or E-day) there were significant differences values in Cp concentration and Cp ferroxidase activity in

**Table 1 Comparison mean values and  $\pm$ SD of ceruloplasmin concentration and ceruloplasmin ferroxidase activity with its specific activity for all undergraduate students and separated gender**

Parameters	Pairs	All (N = 55)	P-value	Male (N = 30)	P-value	Female (N = 25)	P-value
Cp ferroxidase activity (U/L)	E-day	5.02 $\pm$ 2.93	<0.05	4.68 $\pm$ 2.58	N.S	5.22 $\pm$ 3.02	<0.01
	N-day	6.22 $\pm$ 3.06		4.84 $\pm$ 1.87		7.04 $\pm$ 3.37	
Cp ferroxidase Sp.ac (U/g)	E-day	0.59 $\pm$ 0.317	<0.05	0.564 $\pm$ 0.335	N.S	0.593 $\pm$ 0.312	<0.05
	N-day	0.8 $\pm$ 0.431		0.606 $\pm$ 0.038		0.941 $\pm$ 0.473	
Cp concentration (g/dL)	E-day	7.37 $\pm$ 2.62	<0.05	7.83 $\pm$ 2.58	N.S	7.09 $\pm$ 2.66	<0.01
	N-day	8.45 $\pm$ 2.85		8.66 $\pm$ 2.61		9.02 $\pm$ 2.96	

both groups in favor to females except Cp ferroxidase activity at E-day group, was showed non-significant differences as listed in Table 2.

Total serum copper and iron ions were measured in all undergraduate students and depending on gender in E-day comparing with N-day as illustrated in Table 3. The results were indicated significant decreases ( $P < 0.05$ ) in serum copper while serum iron was showed non-significant decreases for all students. Depending on gender serum copper and iron were revealed significant decreases in female group, while in males group, there were non-significant values in serum trace elements in this comparison.

As results listed in Table 4, significant differences in serum copper and iron levels in E-day group in favour to males while in N-day, the results were appeared significant differences only in serum copper, in favour to males also.

## Discussion

To our knowledge, no work has dealt with the study of Cp concentration, Cp ferroxidase activity with its specific activity, as well as copper, and iron concentrations in examination stress. Table 1 shows significant decreases ( $P < 0.05$ ) in Cp ferroxidase activities with its specific activities for all undergraduate students. A recent study indicated that psychological stress causes

increasing of non-transferrin bound iron (NTBI)<sup>28</sup>, and NTBI is avidly taken up by the liver<sup>29,30</sup>, an increasing in (NTBI) in the bloodstream may cause the decrease in the Cp ferroxidase activity, however, Cp is normally required to convert ferrous iron to the ferric form that is required for binding to transferrin. The blood with the shorter storage time should preferentially be used when Cp activity is low<sup>31</sup>. Also, the antioxidant activity of Cp decreases with increasing transferrin saturation by iron. Increased transferrin saturation and decreased unsaturated iron-binding capacity may be occurred consequent to oxidative stress and then further promote oxidative stress by decreasing serum antioxidant buffering against redox-active iron. Harris and Hubel<sup>32,33</sup> found that iron accumulation in the liver may be due to aceruloplasminemia in mice model, and it can be released when these mice were treated with Cp. Thus, it can be said that the accumulation of NTBI and free iron in the liver may due to psychological stress lead to serum Cp decrease its activity by leaving bloodstream to the liver to prevent converting of remaining  $Fe^{2+}$  to  $Fe^{3+}$  because there is little amount or no free transferrin in the blood stream to bind with iron. On the other hand, free transferrin that avidly taken up by the liver and free irons also accumulate in the liver, hence Cp oxidizing  $Fe^{2+}$  to  $Fe^{3+}$  by binding it to transferrin or ferritin. Finally, the ferritin-Fe complex

**Table 2 Mean values and  $\pm$ SD of ceruloplasmin concentration and ceruloplasmin ferroxidase activity with its specific activity comparing males with females in N-day and E-day group**

Parameters	Male E-day	Female E-day	P-value	Male N-day	Female N-day	P-value
Cp ferroxidase activity (U/L)	4.68 $\pm$ 2.58	5.22 $\pm$ 3.02	N.S	4.84 $\pm$ 1.87	7.04 $\pm$ 3.37	<0.05
Cp ferroxidase.sp.ac (U/g)	0.606 $\pm$ 0.335	0.593 $\pm$ 0.312	<0.01	0.564 $\pm$ 0.038	0.941 $\pm$ 0.473	<0.05
CP Concentration (g/dL)	7.83 $\pm$ 2.58	7.09 $\pm$ 2.66	<0.05	8.66 $\pm$ 2.61	9.02 $\pm$ 2.96	<0.05

**Table 3 Mean values and  $\pm$ SD concentration of copper and iron in E-day compared with N-day for all undergraduate students and separated gender**

Parameters	Male E-day	Female E-day	P-value	Male E-day	Female E-day	P-value
Cu (mg/dL)	0.357 $\pm$ 0.235	0.291 $\pm$ 0.219	<0.05	0.382 $\pm$ 0.285	0.364 $\pm$ 0.210	N.S
Fe (mg/dL)	1.573 $\pm$ 0.346	1.003 $\pm$ 0.207	<0.05	1.68 $\pm$ 0.442	1.288 $\pm$ 0.19	$P < 0.05$

**Table 4 Mean values and  $\pm$ SD concentration of copper and iron comparing males with females depends on days (N-day and E-day)**

Parameters	Pairs	All (N = 55)	P-value	Male (N = 30)	P-value	Female (N = 25)	P-value
Cu (mg/dL)	E-day	0.316 $\pm$ 0.22	<0.05	0.357 $\pm$ 0.23	N.S	0.291 $\pm$ 0.21	<0.05
	N-day	0.371 $\pm$ 0.238		0.382 $\pm$ 0.28		0.364 $\pm$ 0.21	
Fe (mg/dL)	E-day	1.33 $\pm$ 0.46	N.S	1.57 $\pm$ 0.346	N.S	1.00 $\pm$ 0.207	<0.05
	N-day	1.47 $\pm$ 0.340		1.68 $\pm$ 0.442		1.288 $\pm$ 0.19	

stored in the liver and transferrin-iron complex (TF-Fe) back to the blood stream to continue its vital role. The above discussion is summarized in Figure 1.

As shown in Table 1, there were significant decreases in Cp concentrations in E-day comparing with N-day. The decreasing of Cp concentration may be due to its ferroxidase activity in response to binding transferrin and ferritin to iron in the liver. From Table 1, it can be noted that a non-significant decrease in Cp concentration in male undergraduate students, while the female Cp concentration was significant comparing E-day with N-day.

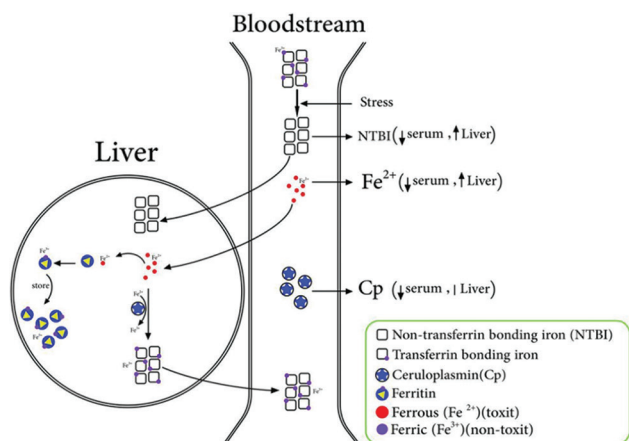
Table 2 shows non-significant decreases in Cp ferroxidase activity with its specific activity in male undergraduate students' group in E-day comparing with N-day while Cp ferroxidase activity with its specific activity showed highly significant decreases in female students' group in this comparison. These results can be described that the females were more stressed at examination situation<sup>34</sup>. Also, Table 2 shows significant differences in serum Cp ferroxidase activity with its specific activity in E-day and N-day groups in favor of females.

There were significant decreases in serum copper for all undergraduate students (Table 3). Table 3 reveals a significant decrease in serum copper in female students in E-day comparing with N-day while there was non-significant decrease in the male group. According to the literature, over 95% of human copper is associated with the Cp as a non-dialyzable fraction and the remaining 5–10% of plasma copper is believed to be fairly loosely attached to albumin and histidine<sup>35</sup>. This result is in line with Cp results. Alloway was reported that the students with high level of serum copper had a cognitive problem as well as low academic achievement<sup>36</sup>. Lam was also confirmed the association between high serum copper levels and low cognitive performance in older adults<sup>37</sup>, while Salustriwas found that copper may be linked to working memory through its effects on attention<sup>38</sup>, and Zecca<sup>39</sup> was indicated that copper tends to accumulate to a higher level in brain areas associated with

attention, that means, decreasing of serum copper may be a type of response to improvement of working memory.

In a recent study, grouping was observed that the students with high level of serum copper scored lower working memory than with the normal level of serum copper<sup>40</sup>. Additionally, the mechanism of cognitive impairment leads to low-working memory and high examination stress related to high copper levels. That may be due to copper toxicity to brain function, especially, the redox-active Cu(II) facilitates amyloid- $\beta$  peptide (A $\beta$ )-mediated oxidative damage to brain cells and thus potentiates neurotoxicity exhibited by A $\beta$ <sup>41</sup>.

As listed in Table 3, there were non-significant decreases in serum iron in all undergraduate students in E-day comparing with N-day. Table 3 indicates non-significant decrease in male undergraduate students, while serum iron significantly decreases in female students at this comparison. The decreasing of serum iron at examination stress is in line with Zhao who found that the psychological stress exposure could decrease the serum iron level, serum Hb, bone marrow iron and significantly inhibition in erythropoiesis<sup>42</sup>. Also, a recent study improved that the psychological stress in rat model increased liver iron concentration and decreased serum transferrin saturation<sup>28</sup>. These results can be confirmed that the decreasing of serum iron should be compensated. Meanwhile, increasing iron storage may stimulate hepatic fibrogenesis<sup>43</sup>. Also, accumulation of iron in the liver can lead to the formation of hydroxyl radicals and hydroxyl anions via the Fenton Reaction ( $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH\cdot + OH^-$ ), and increasing liver iron levels can also generate peroxy/alkoxy radicals due to  $Fe^{2+}$ -dependent lipid peroxidation<sup>44</sup>. However, Cp by its ferroxidase activity may play an essential role to scavenge iron toxicity. Transferrin receptor 1 and 2 protein hepcidin and ferroprotein play a significant role in transport and regulate iron<sup>45</sup>. Table 4 shows significant decreases in serum iron comparing males and females in E-day and also N-day. Decreasing of serum iron in females compared to males may be due females are more stressed at examination situation than male students and the female students have a higher level of anxiety in the multiple-choice examination<sup>46–48</sup>.



**Figure 1** The discussion of Cp ferroxidase activity results.

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### Competing interests

None

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